SEWANA
(South Europe, West Asia and North Africa)
Durum Research Network

International Center for Agricultural Research in the Dry Areas

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SEWANA (South Europe, West Asia and North Africa) Durum Research Network

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Foreword

Durum and bread wheats form the staple component of the diets of millions of people and the basis of farming systems throughout much of the world. Durum is perhaps best known for its use in pasta on an international scale; however, it is also an important food for rural communities, forming the basis of *couscous*, *burghul*, *frike* (roasted green grains) and home-baked flat-bread. About 90% of the world's durum wheat is grown in the Mediterranean region, particularly in drier areas.

The International Center for Agricultural Research in the Dry Areas (ICARDA) has a mandate for the improvement of durum and bread wheats in West Asia and North Africa (WANA), in collaboration with the International Maize and Wheat Improvement Center (CIMMYT), based in Mexico. Much of the durum area in WANA is still planted with landraces selected and maintained by farmers over numerous generations, though high-yielding varieties from modern plant-breeding programs, with improved tolerance to drought and other stresses, are beginning to be adopted by farmers. CIMMYT/ICARDA's durum-breeding research in WANA emphasizes the use of landraces in the breeding program to provide a source of tolerance to drought, cold, heat, diseases, insects and viruses; the development of breeding methods that are better adapted to the Mediterranean region, and therefore more efficient than commonly used methods; and, the development of research networks in the Mediterranean region.

The SEWANA (South Europe, West Asia and North Africa) Integrated Durum Research Network brings together durum researchers from the WANA region with their counterparts in southern Europe. Networking enables scientists with similar interests to discuss common problems and possible solutions, share resources and expertise, and thus increase the effectiveness of research.

The SEWANA meeting at ICARDA's headquarters in Aleppo in March 1995 brought together durum scientists from Algeria, Egypt, Lebanon, Libya, Morocco, Syria, Tunisia, Turkey and Yemen in WANA, with counterparts from France, Italy and Spain in southern Europe, and others from Belgium, Bulgaria, Canada, China and the USA. The papers presented at the meeting are included in these proceedings. They are a reflection of the vast amount of durum research conducted worldwide, and how that is benefited by the SEWANA Network.

These proceedings are offered not only as a record of this important international meeting, but also to promote further cooperation and collaboration in durum research.

Prof. Dr Adel El-Beltagy
Director General, ICARDA

Introduction

The importance of durum as a major food in the Mediterranean region is well established. ICARDA, in collaboration with the NARSs of WANA and South Europe, and CIMMYT, has been working for almost 17 years toward the major goal of improving durum yield and production in West Asia and North Africa.

A systematic breeding approach for the Mediterranean dryland has laid the foundation for the development of the productive and stress tolerant durum (PASTD) varieties. The current achievements made, show improved tolerance to drought, cold, and heat; improved resistance to the main diseases and insects occurring in the region; and better grain quality over the last decade. The improvements in these traits heralded significant yield advances over the local durum varieties and the introduced high yielding varieties in WANA region. Durum breeding efforts have been expanded through the joint ICARDA/CIMMYT's collaboration with NARSs of WANA and South Europe.

In the midst of this productivity advances in dryland and improved quality; and along with the strong and dynamic ongoing collaboration among the participating programs in SEWANA integrated durum research network, we felt that to further expand the productivity of durum in the Mediterranean region and reinforce the ongoing joint works, there was a need to hold a workshop to further develop and integrate the knowledge accumulated during the last five years in the durum breeding, abiotic and biotic stresses, use of landraces and wild relatives, double haploid techniques, grain quality, and molecular markers techniques. To achieve this, we invited the durum researchers collaborating in the SEWANA integrated durum research network and interested scientists for a four-day international workshop, 20–23 March 1995, at Aleppo, Syria.

Selected papers from the collaborating scientists and others are presented to provide an updated information on durum research. Discussion focused on developing and consolidating the activities of the network. For the different activities presented during the workshop, collective information assembled has been and presented here. The network activities will continue to be on voluntary basis, flexible, and based on the priorities et by the collaborating institutions, agreed activities among durum researchers in SEWANA, and the other collaborating institutions outside the region.

Durum Breeding Research to Improve Dryland Productivity in the Mediterranean Region

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Abstract

More than 85% of the world durum production area is located in the Mediterranean basin. Manufacturing and marketing of durum products are also concentrated in the region. Growing conditions are ideal for producing a grain quality suitable for the various products (pasta, couscous, burghul, durum bread) which are the main foods in the basin. Consequently, the majority of durum research is conducted in Mediterranean countries. Until recently, the various Mediterranean durum research programs tended to work independently. A collaborative program was initiated by the CIMMYT/ICARDA durum breeding program located at ICARDA, Aleppo, Syria, in collaboration with durum breeding programs in West Asia and North Africa and various southern European universities. The objectives of this durum network are: to develop productive genetic materials combining high grain quality with resistance to the main abiotic and biotic stresses in the region; to use the available genetic variations found in local landraces and wild relatives; and to incorporate the use of DNA molecular markers in durum breeding and selection. Drought, cold, and terminal stresses are the major abiotic stresses limiting durum yield. Selection for abiotic stress resistance, the complex nature of traits, biotic stresses, and boron toxicity are discussed in this paper.

Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.)is grown on 10% of the world wheat area. It occupies approximately 11 million ha in the Mediterranean basin (Table 1). Rainfall and temperature in the Mediterranean dryland show large and unpredictable fluctuations from year to year (Nachit 1989). Drought and extreme thermal stresses (cold and heat) affect growth at various stages of development. In the same growing season and growth stage, either drought and cold or drought and heat can occur. Diseases, insects, and viruses attack durum wheat in this region.

Durum wheat grain is included in most dishes and food products consumed in the Mediterranean basin. Traditional products made from durum wheat grain are burghul, frike, couscous, pasta, and durum breads (Nachit 1992). Durum grain consumption is very high (especially in rural areas), from 150–200 kg per person per year. The most important durum wheat products are summarized in Table 2.

Table 1. Area and average grain yield for durum wheat grown in Mediterranean North Africa and West Asia.

Moisture	Area (x 1000 ha)	Percentage	Grain yield (t/ha)
Usually under stress	3,500	41	0.63
Frequently under stress	2,400	27	0.98
Sometimes under stress	2,500	29	1.23
Rarely under stress	500	3	2.12

Table 2. Food products made of durum wheat in WANA.

Product	Consumption (%)
Two-layered flat bread	20.0
Single-layered flat bread	10.0
Raised bread	20.0
Burghul	14.5
Pasta	12.0
Couscous	14.0
Frike	5.0
Other foods	4.5

Selection for abiotic stress resistance is laborious because of the complex nature of the traits involved and because specific regional adaptation requires different genes in different sites and years. Additionally, biotic stresses (nematode, virus, root rot, Hessian fly) and micro-nutrient deficiency or toxicity (boron toxicity, zinc deficiency) occur widely and often limit durum yield and production (Nachit and Ketata 1987).

In 1983 the international durum breeding program at ICARDA initiated a collaborative program with durum breeding programs in West Asia and North Africa. This program also included the following southern European institutions: Tuscia University, Viterbo, Italy; ENSA/INRA, Montpellier, France; University of Paris, Sud, France; and Barcelona University, Spain. In 1984, a joint durum breeding program with CIMMYT was established. The durum program at ICARDA plays a major role in coordinating durum research in the Mediterranean region—Southern Europe, West Asia, and North Africa (SEWANA). In 1991, the network was further extended to include other collaborators such as Cornell University, Ithaca, USA, and Laval University, Quebec, Canada (Nachit and Baum 1993).

The Mediterranean basin is rich in durum landraces and wild relatives. In the CIMMYT/ICARDA durum breeding program, landraces and wild relatives possessing novel traits are evaluated and used to improve durum varieties in the Aleppo and SEWANA durum breeding programs (Duwayri and Nachit 1989; Elang and Nachit 1991). Conventional crosses, *in vitro* culture techniques, stress-resistance tools, and molecular markers are used to generate durum germplasm for immediate use by durum scientists in the region (Nachit et al. 1993; Pecetti and Nachit 1993). Techniques which produce double haploids are studied to hasten the development of populations for genetic studies and to map qualitative and quantitative trait loci for important agronomic traits.

The objectives of the CIMMYT/ICARDA durum network are: (1) to develop productive durum genetic material combining high grain quality with resistance to the main abiotic and biotic stresses encountered in the Mediterranean region; (2) to use the available genetic variation found in durum local landraces and wild relatives; and (3) to use DNA molecular markers in durum breeding.

Agro-ecological Zones of Durum Wheat in the Mediterranean Region

In the Mediterranean region, drought may occur alone or in combination with extreme thermal stress at various stages of crop development. Furthermore, even when the crop is grown under optimum moisture, occasional periods of stress due to dryness or temperature extremes may occur during the growing season. Irrigation is limited, and in many areas nonexistent. Complete crop loss can result from frost damage at anthesis or from sirocco (hot, dry wind) damage during anthesis and grain filling. Temperature extremes (i.e., cold, frost, and heat) can affect grain yield directly and also aggravate the effect of drought (Nachit et al. 1988). Therefore, the genetic manipulation of plants to improve productivity and stability by increasing abiotic stress resistance is the only practicable solution (Nachit and Ouassou 1988).

Lack of moisture remains the dominant feature of the dryland in WANA. Erratic precipitation adds to the hazard of limited rainfall, and makes it more difficult to develop cultivars adapted to this environment. In addition, durum wheat yield in the Mediterranean region is curtailed by biotic stresses, poor crop management, and weed control inefficiency.

This paper gives long-term averages for precipitation, evapotranspiration, minimum and maximum temperatures, soil productivity, mineral deficiency or toxicity, and frequency and extent of disease and insect damage in the main durum growing areas of the region. Three major agro-ecological zones have been identified (according to prevailing abiotic and biotic stresses) with the aim of developing specific germplasm containing the required resistance genes (Nachit 1992).

Continental Areas with Low Winter Temperatures

This zone covers approximately 55% (6.0 million ha) of the area sown with durum wheat in the Mediterranean basin. Biotic stresses are the major constraints. However, spells of cold, frost, and sirocco also affect durum yield.

The major abiotic production constraints are:

- Drought
- Winter kill and frost at anthesis
- Terminal stress (drought and heat)

Site-to-site and year-to-year variables are very large. The following biotic stresses are also endemic to the zone:

- Yellow rust
- Common bunt
- *Septoria tritici*
- Tan spot
- Sunni pest
- Stem sawfly

Wheat stem sawfly and sunni pest usually cause crop damage in the dry season, while yellow rust and common bunt dominate during the cool and wet seasons. This environment is found in the continental areas of Morocco, Algeria, Tunisia, Syria, Turkey, and Iraq.

Temperate Areas with Mild Winters

This zone covers about 35% (4.0 million ha) of the land cultivated with durum in the region, located mainly in the coastal and southern latitudes of North Africa.

The major abiotic stresses in this zone are:

- Drought
- Terminal stress (heat and drought)

The biotic stresses are:

- *Septoria tritici*
- Tan spot
- Leaf rust
- Hessian fly
- Dryland and foot rots

Hessian fly and dryland and foot rots usually occur in the dry seasons, while leaf rust and *Septoria tritici* are more common in wetter seasons.

High Altitude Areas with Severe Cold Winters

This area includes the high plateaus of Anatolia and the Atlas, and Syro-Lebanese Mountains. The major abiotic and biotic constraints of this zone are:

- Cold
- Drought
- Boron toxicity
- Yellow rust
- Leaf and stem rust
- Common bunt
- Tan spot
- BYDV

Breeding Methodology

Since drought is the dominant stress factor limiting durum production, the CIMMYT/ICARDA durum breeding program has developed a strategy that aims at breeding improved germplasm resistant to drought as well as cold and heat, and with responsiveness to improved conditions when they occur.

Our research work (Nachit and Jarrah 1986; Nachit et al. 1988) has shown that selection efficiency is greatest when selection is made: (1) in the environment in which the varieties will be commercially grown; (2) for a combination of resistance to abiotic stresses and yield potential; and (3) for adaptation to variable environmental conditions.

The basis of our breeding work is thus to select durum populations and advanced lines that are resistant to abiotic and biotic stresses, and to test for yield stability and productivity under Mediterranean dryland conditions. The cornerstone of this strategy is the introgression of resistance genes from landraces and wild relatives to cultivated durums, and the utilization of contrasting and representing environments in the Mediterranean basin.

Utilization of Landraces and Wheat Relatives

Mediterranean landraces possess desirable traits, lacking in other materials, such as resistance to drought and cold, early growth vigor, long peduncle, and high tillering. Our results on the use of durum wheat landraces in the hybridization program show that substantial progress can be achieved in developing improved cultivars for dry areas.

Besides landraces, wheat relatives such as *Triticum turgidum* subsp. *dicoccoides, Triticum monococcum* subsp. *monococcum*, *Aegilops* spp., etc., provide valuable sources for widening the genetic base of durum and improving its resistance to abiotic and biotic stresses. Further, wild relatives conferring abiotic and biotic stress resistance are being crossed with adapted durum genotypes, and recombinant lines developed. Introgression of alien chromatin into *T. durum* will be monitored. For this purpose, repetitive species-specific probes will be generated for the relevant *Aegilops* species (*Ae. speltoides*, *Ae. ovata*, etc.) and other wild relatives (Nachit 1991, 1992, 1993). All advanced populations are tested in labs and fields in SEWANA. Information on field performance, resistance, and physiological and morphophysiological traits is used for QTL analysis (Nachit et al. 1993).

A selection of landraces and improved genotypes showing a wide range of responses to abiotic and biotic stresses and grain quality parameters is analyzed at ICARDA and Cornell. DNA markers, from cDNA or genomic libraries and cDNA libraries, specific to abiotic and biotic stress responses are being used. By employing multivariate analyses (correspondence analysis, canonic correlation, discriminating analysis), possible associations between quantitative trait polymorphisms and molecular markers are estimated. True linkages will be subsequently confirmed in the mapping population.

Optimum Utilization of Test Sites

Selection takes place at five sites:

- Tel Hadya (35° N) has an annual average rainfall of 330 mm, cold winters, and moderate terminal stresses.
- Breda (35° N) has an annual average rainfall of 270 mm, cold winters, and severe terminal stresses.
- Lattakia (35° N) has an annual average rainfall of 784 mm, mild winters, and severe disease pressure.
- Terbol (32° N) has an annual average rainfall of 550 mm and high input conditions.
- Yabrud (32° N) has an annual average rainfall of 200 mm and severely cold winters, drought, and terminal stresses.

Because an important part of our work, particularly in the early stages, is carried out at research stations, we have developed a stress-screening technique that simulates several different environments at one site (Nachit 1983; Nachit and Ketata 1986). The technique involves growing the same germplasm using staggered sowing dates (early, normal, late, and summer). The germplasm is thus subjected to different stresses (cold, drought, terminal stress, and heat) according to date of sowing.

At Tel Hadya, durum wheat germplasm is grown under four environmental conditions:

- Early sowing (mid October) with supplemental irrigation (450 mm, including rainfall) to simulate a crop cycle of long duration and favorable growing conditions.
- Normal sowing date under dryland conditions, representing Mediterranean continental dryland conditions.
- Late sowing (early April) to simulate a short growing season with severe terminal stress (drought and heat).
- Summer sowing (early July/mid October) to test for high temperature conditions during all growth stages.

Early sowing subjects plants to cold damage (winter kill) during tillering, frost during anthesis, and attacks of yellow rust and *Septoria tritici*. Late sowing subjects plants to terminal heat and drought stress, particularly during grain filling. Late sowing increases attack by aphids, BYDV, and Hessian fly. Summer sowing subjects plants to extreme high temperatures and sirocco.

Breda is a dry site with cold winters and a high natural infestation of wheat stem sawfly. Yabrud experiences severe cold, drought, and terminal stresses. Lattakia is a high rainfall site, used for testing resistance to diseases under natural and artificial infestation, particularly *Septoria tritici* and BYDV. Terbol is used during the winter to test for yield potential and resistance to yellow rust and cold, and during the summer to screen for resistance to heat as well as stem and leaf rusts.

The testing sites/environments provide two interacting selection gradients for rainfall and temperature. This double selection gradient encompasses the main abiotic and biotic stresses in the three major agro-ecological zones.

Selection Procedures

Before developing selection procedures for abiotic stresses, it is crucial to determine the frequency of occurrence of a particular stress and its timing in relation to crop development in each agro-ecological zone. In our approach, all early segregating populations are subjected to the stresses in the above-mentioned environments with the aim of identifying the populations that do particularly well in certain environments and are not sensitive to the stresses of the other environments.

The pedigree method of selection is used to select individual plants from the populations that are selected across all sites/environments. The bulk method is used extensively to select among populations during winter and summer cycles. This presents the double advantage of testing more crosses at several sites/environments. Promising bulked segregating populations are shared with the various NARSs and tested in several agro-ecological zones of WANA. With this selection procedure, it is possible to identify at an early stage those populations that combine productivity and stability with tolerance to biotic and abiotic stresses and are heterogeneous for a number of important characteristics in populations reaching the advanced testing stage.

Results of selection work under contrasting environments show that genotypes that do well under favorable conditions do not necessarily do so under less favorable conditions. It may be difficult to select for genotypes with high yield potential in dryland environments, but it is far more difficult to select for moisture-stress tolerance under high-input environments. It appears that selection under extreme environmental conditions alone (too favorable or too dry) is not an efficient way of identifying cultivars for the Mediterranean drylands. This region is characterized by high year-to-year variability and an unpredictable alternation between favorable and less favorable seasons. Therefore, breeding cultivars which combine yield ability with stress tolerance and yield stability is a prerequisite (Nachit 1989).

Stress-associated Traits

Plant characterization is another tool used to improve breeding work (Nachit and Jarrah 1986). We have found that earliness, fertile tillering, spike fertility, peduncle length, and early plant vigor are associated with higher grain yield under drought conditions. Most of our improved lines show desirable values for these traits (Nachit 1988). An analytical approach is likely to be more useful for areas with severe moisture stress. It relies on the plant's different adaptive mechanisms in a stress environment, with the possibility that breeding and selection for these adaptations will contribute to growth and yield under stress. In the Mediterranean drylands, fertile tillering ability is by far the most potent predictor of durum grain yield under moisture stress (Nachit 1990, 1991, 1992, 1993). Trait contribution estimates have shown that fertile tillering can account for more than 30% of the total variability in grain yield, whereas spike fertility and earliness account for 5.3 and 4.1% of the total variability in grain yield, respectively.

Further, within the SEWANA network, measurements have been taken for relative water content, osmotic adjustment, isotopic carbon discrimination, canopy temperature, chlorophyll fluorescence, chlorophyll a, chlorophyll b, total chlorophyll content, osmolyte accumulation (soluble sugars, proline, etc.), root parameters (volume, weight, deepness, number), leaf anatomy and morphology, boron toxicity, zinc deficiency, and heat and cold damage at different vegetative and reproductive stages (Nachit 1993; Dib et al. 1994).

Multilocational Testing

Although the delimitation of agro-ecological zones in WANA decreases the genotype–environment (GE) interaction, it does not necessarily eliminate it. This is because the year-to-year and site-to-site variations within a zone can still be very important. This makes it imperative to look for cultivars possessing an acceptable degree of consistency of superior performance (commonly called stability) across a series of environments within an agro-ecological zone.

Cultivar performance thus reflects the interaction of genetic and environmental factors. The performance of genotypes or crosses may vary in different environments, in which case the genotype is said to interact with the environment. Results from this project, as well as other work, show the overwhelming evidence of GE interactions. For an efficient breeding program in a given region it is important to know the causes and the nature of GE interactions (Nachit et al. 1992; Nachit et al. 1993). Multilocational testing provides the data for assessing the consistency of cultivar performance. It also enables the identification of cultivars that combine desirable traits such as resistance to various diseases and insects with tolerance to drought, cold, and heat. These cultivars provide good sources of parental material for the hybridization program.

Use of Molecular Markers and Double Haploid Techniques

Molecular mapping is being carried out for the durum population Jennah Khetifa × Cham 1, and the backcross populations Omrabi 5/*T. dicoccoides*//Omrabi 5, and Korifla/*T. dicoccoides*//Korifla. Linkage analysis, particularly with respect to quantitative trait loci (QTL), will be performed at ICARDA, CIMMYT, Tuscia University, and Cornell University. Markers related to drought resistance, temperature-extreme resistance, and grain quality are being developed (Sorrells et al. 1995). Sequences such as dehydrin, gliadin, and glutenin genes will be used to screen for abiotic stresses and grain quality. Identified sequences will be applied to the mapping populations to determine their location and relationship to other DNA markers.

The development of haplomethods is a useful step for several biotechnological applications. Genetic map construction is facilitated by the rapid development of completely homozygous lines. Several methods are currently being investigated, such as anther culture, microspore culture, induced gynogenesis through intergeneric crosses, and *in vitro* selection at Sud University in Paris.

Breeding for Abiotic Stress Tolerance

Drought-tolerance Breeding

In the drier zones, emphasis is placed on resistance to drought, heat, winter kill, frost at anthesis, and pathogens and insects specific to dry areas, e.g., root rot, common bunt, wheat stem sawfly, and Hessian fly (Nachit 1994). Genetic stocks have been developed with combined resistance to drought and cold (winter kill). The number of advanced lines with consistent and high yield is steadily increasing, reflected by an increased number of lines released in the Mediterranean region.

The progress achieved in drought-resistance breeding is exemplified in Omrabi 3 and 17. These lines originate from a cross between a Middle East landrace (Haurani) and a CIMMYT high-yielding variety (Jori C69). Because of their high performance under both stress and favorable conditions, they were included in the farmers' field verification trials for low and high rainfall areas of Syria and Lebanon. Omrabi 3 has been released as Cham 5 for dry areas in Syria. Omrabi 17 is adapted to low rainfall areas with continental climates. Korifla (released in Algeria, and, under the names of Cham 3 and Petra, in Syria and Jordan) is adapted to dry areas with mild winters. These results confirm earlier findings in which yield and stress resistance were successfully combined (Nachit and Ouassou 1988).

Breeding for Tolerance to Cold and Frost

In continental Mediterranean dryland and high-altitude areas, durum wheat is often damaged by cold at the vegetative stage and/or by frost at anthesis. Cold and frost incidence increases at higher latitudes and altitudes.

Cold damage reduces dry matter production and spikes per unit area, whereas spring frost damage impairs spike fertility. In the continental Mediterranean drylands, more than 44% of grain yield variability is explained by the number of fertile tillers (Nachit 1990, 1991, 1992, 1993).

Breeding for Resistance to Heat and Terminal Stresses

High temperatures during grain filling cause crop damage through premature desiccation, kernel shriveling, and reduction of grain yield. Intermittent high temperature during the growing season also increases water stress. This phenomenon is found in southern latitudes and mild winters. Durum wheat grown in light-textured soils and/or land that is not fallowed is particularly prone to moisture and high temperature stress.

Testing for heat and premature desiccation stresses is described as follows. Heat screening is carried out under summer sowing at Terbol and Tel Hadya, where maximum temperatures are above 30 °C and minimum temperatures do not go below 20 °C during the vegetative stage of plant development. Late sowing at Tel Hadya simulates stress caused by high evapotranspiration demand and premature desiccation. The temperature rises from mid-April onward, causing high evapotranspiration, and normally coincides with a rapid decrease of rainfall (Nachit and Ketata 1987; Acevedo et al. 1990).

Field screening for heat and premature-desiccation resistance is made difficult because of uncontrollable temperatures and the confounding effects of other stresses, particularly drought. However, if the objective is to develop cultivars for a target environment where drought and heat often occur simultaneously, the confounding effects need not be emphasized. This technique has been used for the last decade, and the results are rewarding, as evidenced by the performance in warm areas (Nile Valley and Arabian Peninsula) of durum wheat lines identified through heat and premature desiccation screening.

Breeding for Biotic Stress Resistance

Disease Resistance

The many diseases affecting durum wheat in cold winters areas include: yellow rust (*Puccinia striiformis)*, loose smut (*Ustilago tritici*), and common bunt (*Tilletia foetida*, *Tilletia caries*). In agro-ecological zones with mild winters the important diseases are *Septoria tritici*, leaf rust (*P. recondita*), stem rust (*P. graminis*), barley yellow dwarf virus (BYDV), root rot (*Fusarium* and *Choliobolus sativus*), and powdery mildew (*Erysiphe graminis*).

The most economical and practical way to avoid loss by disease is through the incorporation of disease resistance. This holds particularly true for marginal areas with low input agriculture. However, changes in crop management practices may have a dramatic effect on the development of diseases. For example: early sowing, often recommended by agronomists, favors the development of foliar diseases, particularly yellow rust and *Septoria tritici*; late sowing can increase attack by leaf rust, stem rust, or Hessian fly; and monoculture and minimum tillage increase the inoculum potential of diseases that reside on crops over the summer (Nachit et al. 1985; Mamluk et al. 1986; Nachit 1992; Mergoum et al. 1993; Mamluk and Nachit 1994).

Selection for resistance exploits indigenous races of pathogens to create artificial inoculation. Multilocational resistance screening and testing in "hot spots" is also carried out to detect other races of disease. The screening of advanced generations and materials for leaf and stem rusts in Mexico plays an important role in identifying broad resistance to these diseases. However, the local races for all three rusts are more virulent, thus rust resistance found at CIMMYT in Mexico is not effective in WANA.

Through intensive breeding, increased resistance has been achieved for yellow rust, stem rust, leaf rust, *Septoria tritici*, common bunt, and BYDV. Resistance genes for the different diseases are incorporated into durum wheat genotypes carrying resistance to drought, cold, and heat. Most of the advanced genotypes included in the international nurseries and trials now have a better combination of resistance to biotic and abiotic stresses than local and previously developed varieties.

Insect Resistance

Durum production in the rainfed areas of the Mediterranean region is beset by many insects, accounting for annual yield losses of 5–14%. In some areas the losses are even higher, e.g., in Morocco Hessian fly (*Mayetiola destructor*) can cause 80% crop loss in dry areas. Hessian fly, wheat stem sawfly (*Cephus pigmaeus*), and sunni pest (*Eurygaster integricens*) are found primarily in rainfed areas. Aphid is primarily a problem in the favorable areas of the southern latitudes, although any wheat grown under irrigation or in high-to-moderate rainfall areas may also be heavily infested.

Most of the newly-developed durum genotypes exhibit medium to high resistance to wheat stem sawfly under natural infestation. Resistance to the wheat stem sawfly is apparently not restricted to stem solidness. However, several durum landraces from Morocco were found to possess solid stems and are now used in the crossing program for wheat stem sawfly resistance (Nachit 1990, 1992, 1993). Resistance to wheat stem sawfly from different sources with different mechanisms combines to develop stable resistance. Screening for resistance to aphid and Hessian fly is carried out with the national programs of Egypt and Morocco. For the first time, durum cultivars with resistance to Hessian fly have been produced, thanks to a joint program with the NARSs of Morocco and ICARDA, and are now proposed for release. The loss due to Hessian fly in Morocco is valued at US$ 150 million annually.

Breeding for Improved Grain Quality

Identification of desirable quality parameters and incorporation into improved germplasm are given high priority in the CIMMYT/ICARDA durum wheat project (Williams et al. 1989; El Haramein et al. 1993; Nachit et al. 1995; Impiglia et al. 1995). The most often-used quality test parameters are: protein content (%), vitreousness, sedimentation test (SDS), carotene content, grain size, the gliadin 45 band, low molecular weights, and, recently, PCR primers.

Crosses are made to increase industrial and nutritional qualities of stress-tolerant germplasm. WANA landraces are the best source for local products, while *T. dicoccoides* are used to increase grain protein content (Nachit 1990, 1991, 1992, 1993). Vitreousness screening using zero nitrogen and irrigation (Nachit and Asbat 1987) is now generating promising results. This technique discriminates between genotype quality traits and enables better selection of genotypes with high grain quality.

Conclusion

Since it is improbable that more land can be put under cultivation, production increases have to come from improved technology, including development of suitable varieties, better cultural practices, and weed control. Results from experimental stations, on-farm research trials, and large-scale testing in several countries such as Syria point to the possibility of at least doubling current yield in most of the Mediterranean region.

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Breeding Activities on Drought and Cold Tolerance in Algeria

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Abstract

Environmental stresses are very common in North Africa. In Algeria, weather stresses from drought and cold combine in the large cereal production area; heat stress is an important factor in the high plateaux. The introduction of new agronomic practices and new cultivars is the main way to improve cereal production in these environments. Strategies include alternating the selection of genetic material from many contrasting locations and testing advanced lines at several sites. The selection for different environments is based on yield and its associated traits, such as number of spikes per square meter, number of kernels per head, and 1,000 kernel weight. Results show that correlations between yield and associated characters exist, agronomic factors are very important, and cultivars respond differently in different environments. Some cultivars are widely adapted and others perform well only in specific environments.

Introduction

In many areas of the world, crop production is limited by a number of climatic, edaphic, and biotic factors. The ability of a crop cultivar to perform reasonably well in rainfall-variable and drought-stressed environments is an important trait for the stability of production under North African dryland conditions, because the annual precipitation is highly variable and droughts are frequent. Drought tolerance in the spring germplasm pool of cereal cultivars is a prerequisite for acceptance by farmers in dry areas. Restricted water supply is a widespread stress, often occurring in combination with other stresses (low winter temperature, heat during grain filling, and lack of macro- and micro-nutrients), and with biotic stresses (diseases and insect pests).

The productivity of the crop in Algeria is also limited by frost in winter and early spring. Yields vary greatly among years and locations, reflecting the variation in seasonal rainfall and other adverse factors.

Agro-ecologic Characterization of the Different Environments

Algeria, unlike its North African neighbors, is a very large country, with a total land area of 2,380,000 km^2. Its topographical and bioclimatic characteristics—

ranging from the humid areas in the north, where intensive cultivation prevails, and the arid plateau in the south—permit a variety of landscapes and agricultural systems. The southern plateau is intersected by plains, and consequently agricultural activity differs from east to west. Drought, either dry fall or dry spring, occurs in almost every region.

There are three large agroclimatic zones:

- The humid zone includes coastal and near-coastal regions. From the center of the country eastwards, average annual rainfall exceeds 600 mm and is relatively well distributed.
- The semi-arid zone is made up of a number of distinct areas including the Tell Plains and the northern border of the High Plains. Average annual rainfall varies between 350 and 550 mm but distribution is regular.
- The sub-arid zone has low rainfall and is agro-pastoral, with a hilly terrain (high plateaux). Average rainfall varies between 200 to 350 mm with continental temperatures prevailing (30–40 days of frost) and more than 40 days of sirocco winds per year.

Breeding Strategies under Rainfed Conditions

A number of durum landraces and cultivars developed by ICARDA and CIMMYT were selected for their ability to perform well in adverse environments and are used as parents for yield improvement in crosses made at El Khroub Station. Alternating the selection of segregating populations in many contrasting locations—such as El Khroub in the interior plains and Setif, Tiaret, or Ouled Hamla in the high plateaux—and testing advanced lines at several other locations helps to identify cultivars adapted to unpredictable environments.

Our strategy is to screen under the stress conditions of the target environment, make crosses that perform well in the given environment, test in multilocational trials, and characterize and identify plant material adapted to different types of stresses. Grain yield over locations and years is the most reliable approach for identifying drought-resistant and responsive cultivars in dry areas.

This strategy increased yield more than 30% over landraces Mohamed Benbachir and Bidi 17 at Ouled Hamla.

General Overview of the Program

- Breeding for cold- and drought-tolerant material.
    - Crosses and selections in the segregating material.
    - Selection among other material in the observation nurseries.

  - Multilocational yield trials.
  - National adaptation trial network.
- Morphophysiological characterization of the existing durum material.
- Analysis of morphophysiological traits in durum and wild relatives.

Results

In 1993/94 at El Khroub and Tiaret, 47 simple crosses, 20 backcrosses, and some 60 other crosses were made between landraces and adapted genotypes.

Crosses that are performing well across all zones are:

- Waha/Tell
- Bidi 17/Waha
- Hedba 3/Gdo vz619
- Hedba 3/Polo
- Polo/Gdo vz578//Swan

Many selections were from segregating material from local crosses or from ICARDA (F_2 continental durums).

Different adaptation patterns can be suggested based on the various studies undertaken to select lines or cultivars adapted to each environment (national yield trials). Some cultivars show wide adaptation (Waha, Sahel, Vitron and Zb/Fg) whereas others show good results in specific environments only (Mexicali for coastal areas and Polo/Zb for the high plateau). None of the other local material performed well.

These results indicate that good genotypes selected under favorable environments can be used in environments with stress conditions.

Conclusion

Increasing crop production in stressed and fluctuating environments is indeed possible, provided that we face the challenge in an imaginative and innovative way. The improvement of crop production in extreme agro-ecological environments is urgently needed and scientifically challenging. The results achieved so far have been on the whole discouragingly limited. The alternative approaches and new techniques used by some scientists and institutions are showing slow but hopeful progress.

Quality Characters of Durum Wheat in Lebanon

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Abstract

In Lebanon, both durum and aestivum wheat are used in the manufacture of burghul (75%) and other products such as smid, macaroni, couscous, moughrabie, and frike. Two types of burghul are available on the market: red and white. Hardness, vitreousness, and protein content are important characters for the durum industry. Durum flour is mixed with low-quality flour for making local bread. Haurani is the preferred variety for white burghul, and Salamouni Red and Bekaii are the preferred red. For burghul making, the grains are first cleaned, then boiled in water until they become soft (without reaching the disintegration stage). The cooked grains are sun-dried to reduce the moisture content to 10–12%. The grains are tempered to separate the barn from the burghul. After drying, the grains are milled and broken into 1–2 mm diameter particles. In kishk preparation, the burghul is soaked in yogurt until it becomes soft. Yogurt is added daily until complete fermentation and high acidity are reached. The burghul–yoghurt mixture is cut into small pieces (5–10 cm diameter), quick dried, and milled with a kishk mill into flour. In moughrabie preparation, large particles of burghul are rolled slowly with flour, and water is added to form 5 mm balls. The smid procedure is similar to burghul, but with fine grinding. For frike, premature wheat is charred in fire. The desiccated grains are threshed, dried, and coarsely milled.

Introduction

Durum wheat grain plays an important role in the diets of the Middle East and North Africa. Lebanon has a long tradition of growing durum. Traditional durum production and processing has resulted in the manufacture of several products made with high-quality grain. Although the industry is presently restricted to products of local interest, there is a large scope for expanding the durum agro-industry to cover a broader range of products.

About 4,000 t of wheat are used for the burghul industry and other products each year. A survey carried out in 1994 on wheat varieties grown in Lebanon revealed the following. In the Bekaa Valley, most varieties were new improved types such as Jori, Stork, and Sebou, along with traditional varieties such as Salamouni Red, Bekaii, and Haurani. In South Lebanon, Salamouni White, Salamouni Red, Bekaii, and Haurani were found. In Akkar, Salamouni White, Salamouni Red, Bekaii, Senator Cappeli, and Jori dominated.

Several varieties were tested in the Farmers' Field Verification Trials, carried out from 1992 to 1994 (Table 1). These varieties are now undergoing large-scale testing in Lebanon.

Table 1. Grain yield (kg/ha) of new durum varieties tested in Farmers' Field Verification Trials (1992–1994)

Variety	1992	1993	1994	Average yield
Stork	3,340	4,123	2,843	3,435
Sebou	3,350	4,216	2,571	3,379
Omrabi 17	3,480	3,993	3,309	3,594
Omlahn	3,730	3,462	2,310	3,167
Waha	3,950	4,339	3,066	3,785

Wheat Use

In Lebanon, both durum and aestivum wheat are used in the manufacture of burghul (75%) and other products such as smid, macaroni, couscous, moughrabie and frike. Two types of burghul are available in the market: red and white. A short survey of the industry in the Bekaa shows that 75% of the wheat used for burghul is red, and 23% is white. For this reason, millers use the red æstivum variety.

Desirable Qualities for Burghul

Hardness and vitreousness

This character is very important in the industry. Soft wheat requires a high flour percentage. Hard wheat requires a low flour percentage, so it is used in producing burghul. Durum flour is mixed with low-quality flour for local bread making. The range for burghul is 85–90%, with the rest made up of flour and bran. A high protein content is also important for nutrition.

Good taste

Some varieties have a good taste and are consistent enough for local food products. White burghul, prepared from *T. durum* cv *Haurani*, has the best taste among all varieties due to its high carotene content. Salamouni Red and Bekaii have the best taste among red varieties. These varieties almost always command high prices in the local market, sometimes more than double other varieties.

Measurements taken on sedimentation and baking strength may not be totally applicable for durum wheat characters, but do reflect the comparative value of durums and aestivums. In a recent study at the American University of Beirut, Olaby (1994) carried out a cluster analysis to detect quality characters for burghul making. He found an overlap between aestivum and durum in characters desired for kebbe and falafel making. The traditional varieties used in burghul production are hard aestivums and traditional durums.

Wheat Industry

Durum and Hard Red aestivum wheat are used in Lebanon for the production of burghul, macaroni, smid, moughrabie, and frike. Burghul is the most important product, and the biggest industry. Red burghul is more desirable than white because red grain is not found in durum wheat. To find this character millers must turn to traditional aestivum varieties such as Salamouni Red and Hard Red Winter.

A short survey of the five biggest burghul companies in the Bekaa (the main source of burghul production in Lebanon) showed the following:

- The amount of wheat used for burghul production annually is 2,600 t.
- The amount of red burghul produced annually is 1,850 t.
- The amount of white burghul produced annually is 750 t.
- Fine grain is used in dishes such as kebbe, falafel, tabboule, etc., while large grain is used in cooked meals.

Manufacturing

Burghul

After cleaning, the wheat grain is boiled in water until it becomes soft, without reaching the disintegration stage. The boiled grain is then exposed to the sun in a thin layer to reduce the moisture content to 10–12%. At this stage, the grain is milled and broken into 1–2 mm diameter particles. Before processing, the grains are tempered to separate the barn from the grain.

Kishk

This old Lebanese dish is made from burghul. The burghul is soaked in yogurt for one day to make it soft. Yogurt is added for eight days, until complete fermentation and high acidity are achieved. The kishk is then cut into small pieces (5–10 mm diameter), quick dried, and milled in a kishk mill. The product is in the form of flour, and fine sieving is used to extract the large particles. Drying is necessary after the process is complete.

For moughrabie, large particles of burghul are slowly rolled with flour, and water is added to form 5 mm balls. For smid, the same procedure is used as for burghul, but with very fine grinding. For frike, premature durum heads are charred, and the desiccated grain is dried in the sun. Although the durum wheat industry in Lebanon is presently restricted to products of local interest, there is scope for expansion to include a broader range of products, including pasta.

Importance of Durum Wheat in Morocco

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Abstract

Durum is a traditional crop in Morocco and is currently regaining the interest of both farmers and consumers. The area planted to durum varies from 1.1 to 1.3 million ha annually. Yield varies from 800 kg/ha in the unfavorable areas to 1,700 kg/ha in the favorable ones. Fifty five percent of production is in favorable areas, 32% in unfavorable areas, and 11% in mountainous areas. Total annual consumption is 2.5 million tonnes, with a per capita average of 100 kg/year. Drought, heat, and cold are the main abiotic stresses; Hessian fly, dryland root rot diseases, and foliar diseases (rusts, tan spot, septoria, and powdery mildew), viral diseases (BYDV and wheat striate Mosaic), and Russian wheat aphid are the main biotic stresses. Durum lines tolerant to Hessian fly were identified. Numerous crosses with Hessian fly resistant *Aegilops* species and *Triticum timopheevii* subsp. *armeniacum* (Syn. *T. arraraticum*) were made. Advanced ICARDA/INRA lines resistant to Hessian fly were selected. Screening for root rot tolerance/resistance to *Fusarium culmorum* and *Helminthosporium sativum* was carried out. Results for plant emergence, and tiller and spike numbers differed significantly between inoculated and uninoculated plots. Screening and identification of sources of resistance to tan spot and septoria were carried out under various agroclimatic conditions. The Moroccan durum breeding program has focused on the main abiotic and biotic constraints to production. A methodology for resistance to Hessian fly and dryland root rots has been established.

Introduction

Durum wheat is traditionally grown and consumed in Morocco. Figures for production area, yield, and comsumption show that although this cereal suffered from the introduction of bread wheat, it has maintained its importance and is actually regaining the interest of both farmer and consumer. The area planted to durum wheat in Morocco varies from 1.1 to 1.3 million ha/year. This represents about 22% of the area planted to all cereals, which includes barley, bread, durum, and corn (Table 1). About 46% of the durum is grown in the favorable rainfed area (representing 35% of that area), while 41% is in the unfavorable rainfed area (representing 16% of that area). Twenty eight percent of the durum is planted in the irrigated areas (representing 7% of that area), and 11% is grown in the mountainous areas (representing 31% of that area). Yield varies from 800 kg/ha in the most unfavorable areas to 1,700 kg/ha in the most favorable ones. Yield in mountainous area is about 1,400 kg/ha (Tables 2 and 3).

Table 1. Areas planted and production of the four cereals.

Species	Area		Production		Yield
	(1,000 ha)	Percent	(1,000 qx)	Percent	(qx/ha)
Durum wheat	1,165.3	22	16,511.9	25	14.2
Bread wheat	1,270.7	24	19,081.3	29	15.0
Barley	2,420.0	46	27,392.8	41	11.3
Maize	383.0	7	3,486.6	5	9.1
Total	5,339.9	100	66,472.5	100	12.7

Fifty five percent of durum production is carried out in the favorable rainfed areas and 32% in the unfavorable areas. Eleven percent of production is carried out in the mountainous areas (Table 2). The percentage of production per agroclimatic zone (over all cereal productions) varies from 12 to 35% (Table 3).

Table 2. Main durum producing areas of Morocco.

Agroclimatic zone	Area		Production		Yield
	(1,000 ha)	Percent	(1,000 qx)	Percent	(qx/ha)
Favorable	536.4	46	9,326.4	55	17.1
Intermediate	221.4	19	3,099.6	19	14.0
Unfavorable south	186.4	16	1,704.6	10	9.0
Unfavorable west	69.9	6	552.2	3	7.9
Mountain	128.2	11	1,846.1	11	14.4
Irrigated areas	23.3	2	412.4	12	17.7
Total	1,165.6	100	16,941.3	100	14.5

Table 3. Importance of durum in different environments.

Agroclimatic zone	Percent of total area	Percent of total production
Favorable rainfed	35	35
Intermediate rainfed	20	21
Unfavorable south	12	13
Unfavorable east	14	12
Mountain	31	31
Irrigated areas	7	12

Durum yield is similar to that of bread wheat and barley species, widely considered as higher yielding and possessing wide adaptation potential. Durum yield is superior to that of corn (Table 4). Total annual consumption of durum in Morocco is 2.5 millions t. This represents an average per capita consumption of of 90–100 kg/year.

Table 4. Comparison of main cereal yields over the last five years.

Agroclimatic zone	Durum wheat	Bread wheat	Barley	Maize
Favorable	17.4	19.9	16.1	9.0
Intermediate	14.0	15.1	12.5	11.0
Unfavorable south	9.0	6.7	9.3	6.0
Unfavorable east	7.9	8.9	9.7	8.8
Mountain	14.4	15.9	13.1	7.0
Irrigated areas	17.7	16.0	7.5	8.1

Production Constraints

The durum breeding program, located at the regional center of the Institut National de la Recherche Agronomique (INRA), Settat, Morocco, addresses numerous constraints in order to improve durum productivity in Morocco, especially in unfavorable rainfed areas. The importance of each of these constraints varies according to the agro-ecological region. These constraints can be ranked nationally as follows:

1. Yield potential, adaptation, and stability.
2. Drought (in relation to total rainfall and distribution).
3. Heat and cold.
4. Hessian fly.
5. Dryland root rot diseases.
6. Foliar diseases: rusts, tan spot, septoria, and powdery mildew.
7. Grain quality.
8. Viral diseases (BYDV, WSMV).
9. Russian wheat aphid.

Research Facilities and Human Resources in Morocco

The number of researchers working on durum improvement and related fields is increasing as specific constraints are identified. In the past, durum benefited less from research than bread wheat. The major research capabilities and facilities in Morocco are listed below.

Institutions

- INRA.
- Institut Agronomique et Vétérinaire, Hassan II, (IAV-HII), Rabat.
- Ecole Nationale d'Agriculture (ENA), Meknes.
- Several universities and departments. (Rabat, Kenitra Marrakech, Meknes).

Facilities

INRA is the main institution involved in durum breeding research and thus has the most extensive facilities and manpower for basic and applied durum research.

Facilities available at INRA:

- Breeding seed laboratories and storage, located in Settat, Rabat, and Meknes.
- Experimental stations: 10 throughout Morocco.
- Greenhouses: 200 m^2 in Settat, 12 growth chambers in Settat
- Irrigated stations: Deroua, Tassaout, and Sidi El Aidi.
- 1 cereal coordinator/breeder, located in Rabat.
- 2 breeders, located in Settat and Meknes.
- 1 tissue culture specialist, located in Settat.
- 1 entomologist, located in Settat (research also on other cereals).
- 1 virologist, located in Settat.
- 4 pathologists, located in Settat, Rabat, and Meknes.
- 1 quality specialist, located in Rabat.
- 2 physiologists.
- 1 specialist on radio-element mutation breeding.

Cooperating national institutions:

- 2 breeders/tissue culture specialists (IAV-HII).
- 1 biotechnologist (IAV-HII).
- 1 quality specialist (IAV-HII).

- 2 pathologists (IAV-HII, ENA-Meknes).
- Several collaborating biotechnologists, pathologists, quality specialists, physiologists, working on specific research topics associated with students theses (IAV HASSAN II, ENA-Meknes, Universities of Rabat, Marrakech, Rabat, and Meknes).

Program Activities

In addition to the crossing block, segregating material, advanced lines, and preliminary, intermediate, and advanced yield trials, several nurseries and collections are shared with ICARDA, CIMMYT, and other international institutions. Some nurseries are shared with ICARDA. Joint breeding research on the constraints (Hessian fly, root rot) of the temperate Mediterranean agro-ecological environment have been established.

Selections made in international and national yield trials were advanced for further testing. From the Moroccan material, high-yielding and widely-adapted lines were identified, as well as lines tolerant to second generation Hessian fly. A cross with Kyperounda showing tolerance to first generation Hessian fly was identified, desirable because early sowing is recommended. Three advanced lines were selected in our advanced trials and presented this year to the catalogue registration.

Interspecific Crosses

Numerous crosses with *Aegilops* species and *T. armeniacum.* were made and embryo-rescue was successfully carried out. We are in the process of chromosome counting to confirm the interspecificity of these crosses.

Pollination with *Zea mays* pollen to generate haplodiploidization in durum has being tried, but with little success. New facilities have been developed for tissue culture, haplodiploidization, electrophoresis, and protein chain reaction (PCR).

Rust in Durum Wheat

We have started a study on rust races, specifically on resistance genes that might be present in durum wheat (gene postulation study). This program is carried out with IAV-HII, Rabat. Its goal is to increase knowledge about rust resistance in durum.

Evaluation for Russian Wheat Aphid

The Russian wheat aphid is becoming an important problem in Morocco. It started in the northern, high altitude areas and is now observed more in the southern regions. Evaluation of the reaction of the Moroccan cultivars was carried out with advanced durum lines. However, no resistance was found.

Evaluation of BYDV and Striate Mosaic Virus (SMV)

BYDV and SMV viruses are common in Morocco. Research is directed towards finding sources of resistance. In addition, a new virus has broken out in the last two years in irrigated areas (Beni Mellal, Tassaout). Identification and study of this virus are being carried out.

Tan Spot and Septoria

Screening and identification of sources of resistance to tan spot and septoria are being carried out under several agroclimatic conditions. Several lines have been identified and used in the hybridization program to upgrade and diversify the resistance level in Moroccan durum cultivars.

Material suitable to dryland areas, with resistance to Hessian fly, root rot, and tan spot, is being developed. This material is shared with ICARDA, with screening and hybridization carried out by INRA and ICARDA.

The Hessian Fly Resistance Program

Screening one part (1132) of the durum nursery in Jemaa Shaim Experimental Station has shown one durum wheat accession to be resistant. This accession (#829) is, however, late, tall, with low-quality grain. Dead larvae on plants of the 829 accession were developed. All the accessions in this collection were evaluated twice for dead larvae with no resistance found on the remaining accessions.

Parts of the Maghrebian collection under severe attack that were not tested last year (conditions were not favorable in Sidi El Aidi at the time) were tested in Jemaa Shaim and evaluated twice in the field. No resistance (dead larvae) was found, but some accessions were tolerant, standing a longer time than others. However, only a few plants stood up until harvest. Two accessions were harvested for further testing next year, under severe attack enhanced by late planting.

The F_4 families (ICARDA/INRA) resistant to Hessian fly were grown in Jemaa Shaim and Sidi El Aidi stations and in the greenhouse. The percentage of resistant plants necessary for selection was 50%, but most of the resistant families had percentages higher than 75%. At the Sidi El Aidi experimental station, resistant families were harvested and threshed, and will be multiplied (head to row) next year and distributed for multilocational testing. Harvested plants were bulk threshed for further testing and selection next season, because infestation was not severe in Sidi El Aidi.

New simple crosses were conducted, in addition to backcrosses on the F_2 and F_3 generations of durum and resistant bread wheats. A new nursery (340 populations/lines) was sent by ICARDA to be tested for both Hessian fly and root rot.

Root Rot Tolerance/Resistance

A repeat of last year's 1132 study was carried out at Sidi El Aidi. One row was inoculated and one row was used as a check. Checks of the cultivar Marzak and the triticale Juanillo were placed every 20 plots. At Sidi El Aidi, the seed was divided in four one-row plots of 2.5 meters. Two plots were inoculated before planting with *Fusarium culmorum* and *Helminthosporium sativum*. Inoculation was carried out by spraying the fungal solution on the seed, which was dried before planting. Data on emergence and number of tillers and spikes were taken. White head percentages were taken.

Results on plant emergence and tiller and spike numbers differed significantly between inoculated and non-inoculated plots. Crosses among the eleven accessions identified last year as resistant were carried out.

Durum Wheat Breeding Strategy in Syria

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Abstract

The wild relative of durum and its ancestors are still found in Syria. Landraces are still grown (e.g. Haurani and Hamari). However, up to 90% of the durum area in Syria is now covered with productive and abiotic stress-resistant cultivars. During the last five years, Syrian durum production has more than tripled. This is mainly due to the adoption of new varieties, because agronomic management, fertilizers, seed rate, and use of herbicides has not changed during the last 20 years, although the use of supplementary irrigation has increased. Durum is grown in three major environmental zones according to water regime: (1) irrigated and supplementary irrigated areas; (2) high rainfall areas with precipitation above 350 mm/year; (3) low rainfall areas with precipitation between 250 and 350 mm/year. The abiotic stresses are drought and temperature extremes, and the biotic stress are diseases (yellow rust, stem rust, septoria tritici, common bunt, and bacterial strike) and insects (stem sawfly, aphid, sunni bug). In 1977/78, the Cereal Improvement Program of ICARDA and the Syrian Cereal Program of the Syrian Agricultural Scientific Research Directorate, Ministry of Agriculture and Agrarian Reform, developed a joint program to identify cultivars for the Syrian dryland. Several releases of productive and stress-resistant durum cultivars were made, such as Cham 1, Cham 3, and Cham 5.

Introduction

Syria is considered one of the most important habitats for the wild relatives of durum, the ancestors of the current durum genotypes. Many landraces, such as Haurani and Hamari, are still grown in the farmers' fields. However, up to 90% of the durum area is covered with productive and abiotic stress-resistant cultivars. As a consequence, Syrian durum production has more than tripled during the last five years. This is mainly due to the adoption of new varieties, because agronomic management, fertilizer use, seed rate, and herbicide use has not changed during the last 20 years, although there have been changes in the use of supplementary irrigation. But the yield increase is due mainly to the adoption of productive and drought-resistant cultivars by farmers.

Current Status of Durum in Syria

Durum wheat in Syria occupies approximately 85% of the total wheat area. Durum is grown in three major environmental zones, characterized by water regime:

- Irrigated and supplementary irrigated areas area up to 450–500 mm.
- High rainfall areas with precipitation more than 350 mm.
- Low rainfall areas with precipitation between 250–350 mm.

Production in the irrigated and supplementary irrigated areas is stable, but in the rainfed areas it oscillates, particularly in low rainfall areas. This is mainly due to environmental stresses such as:

- Abiotic stresses, particularly drought and temperature extremes. These stresses are related to the following:
    - Low and poor distribution of rainfall during the growing season.
    - High temperatures during grain filling.
    - Cold and frost damage in spring.
- Biotic stresses:
    - Diseases such as yellow rust, stem rust, septoria tritici, common bunt, and bacterial strike.
    - Insects such as stem sawfly, aphids, and sunni bug.

Breeding program activities aim to improve local landraces and develop new durum genotypes as follows:

- Improving performance of local landraces by selection within the landraces and from crosses made or introduced by the national program.
- Creating new durum wheat genotypes characterized by high yield, good quality, and resistance to diseases under irrigated conditions.
- Utilizing wild relatives of durum to develop new genotypes with drought and cold tolerance.

Table 1. Mean performance of durum variety on-farm trials (kg/ha).

Variety	Irrigated	High rain (>350 mm)	Low rain (250–350 mm)	Release date	Source
Gezira 17	6,275			1973	Mutation prg
Bohouth 1	5,981	3,282		1980	NARS
Cham 1	6,831	3,982		1983	ICARDA
Bohouth 5	7,319			1987	National prg
ACSAD 65		3,500		1984	ACSAD
Haurani			1,544		Landrace
Cham 3			1,688	1987	ICARDA

In 1977/78 the Cereal Improvement Program of ICARDA began its activity in Syria in cooperation with the National Syrian Cereal Program in the Agricultural Scientific Research Directorate, Ministry of Agriculture and Agrarian Reform. The major objective of the cooperative program is to increase cereal production in the dry areas through a long-term breeding program involving:

- Exchange of germplasm.
- Exchange of expert visits.
- Training of national human resources.
- Conducting joint on-farm trials and large scale testing.
- Release of productive and stress-resistant cereal cultivars. Table 1 lists the productive and drought-resistant durum cultivars for the dry areas in Syria.

As a result of this collaboration, a new cultivar has been tested, released, and adopted for commercial production in the dry areas. New varieties were developed by ICARDA through the use of adapted landraces in the hybridization program (e.g. Cham 5, a cross between Haurani and Jori C69). The program developed three durum wheat varieties for the different agro-ecological areas in Syria:

- Cham 1, for irrigated, supplementary irrigated, and high rainfall areas.
- Cham 3 and Cham 5, for dry areas with precipitation less than 350 mm/year.

Future Needs

The durum breeding program for dry area must be maintained and sustained. The development of genotypes with the following characters must continue:

- Drought tolerance.
- High yield.
- Good quality.

The current durum breeding program for irrigated and high rainfall areas must be enhanced, and breeding must be carried out for the following traits:

- High yield.
- Good grain quality.
- Disease resistance to yellow rust, stem rust, common bunt, and bacterial strike.

The use of landraces and wild types in the durum breeding program should continue. Training activities at different levels must be sustained. Development of dryland breeding methodologies must continue.

Recent Progress in Durum Breeding in Tunisia

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Abstract

Historically, *Triticum turgidum* subsp. *durum* was the only spices of the genus *Triticum* to be culticated in Tunisia, covering about 800,000 ha, 55% of the area devoted to cereals. Selection was initiated as early as the beginning of this century, and local lines were derived by mass and pedigree selection. Derbassi, Adgini, Agili, Mekki, Hamira, Jenah Khoutifa were the dominant landraces. Chili and Mahmoudi 981 were selected. From crosses between local and Mediterranean landraces, D117, D77 and D522 were selected. By the 1950s, Inrat 69, Badri, and Maghrebi had been released. Through a collaborative project (1967) between Tunisia (INRAT), CIMMYT, and USAID, high-yielding varieties were released (Amel and Maghrebi) for the favorable areas, and later Ben Bachir, Karim, and Razzak. In low rainfall areas, drought is the main limiting factor. Two agro-ecological zones were identified: (1) high yield potential, disease resistance, and good quality are recommended for favorable areas (the variety Khiar was recommended; (2) stable yield with resistance to drought and terminal heat stress are required for the dry areas (the lines Omrabi 3 and Omrabi 5, received from ICARDA, were recommended).

Introduction

Durum wheat is well suited to the drier regions of the Mediterranean basin, and to similar areas in Asia and America. Historically, durum wheat is a very important crop in North Africa. According to Desfontaines, who studied the flora of Algeria and Tunisia in the late 1700s, *Triticum turgidum* subsp. *durum* was the only species of the genus *Triticum* to be cultivated in Tunisia. Later, Boeuf reported in many of his publications that before the colonization by the French in 1881, durum wheat was the only wheat species produced in Tunisia, and bread wheat was present only mixed in durum wheat fields.

Among the cereals, durum wheat is by far the most important in terms of acreage and production. It covers an average of 800,000 ha, about 55% of the area cropped to cereals. Bread wheat and barley are grown only on 10% and 35%, respectively (Anonymous 1994). The important year-to-year variation of cereal production is associated with overall quantities and distribution patterns of rainfall during the growing cycle.

Despite the trend towards increasing yield, the average national durum yield is still low and highly variable. Durum gives slightly lower yields than bread wheat in the north, where the largest bread wheat area is located. Average yields, however, hide big disparities. Yields as high as 80 qx/ha have been obtained under proper cultural practices in the favorable zones of northern Tunisia, while average durum wheat yield is only 20 qx/ha in this part of the country. Durum wheat yields in the semi-arid zones of Kef, Siliana, and Zaghouen affect the national average, as crop failure is frequent in dry years or years with uneven rainfall. This reflects the highly variable environmental and technical conditions under which durum wheat is being produced.

Historical Overview

Cereal breeding began in Tunisia in the early years of the century at the experimental station of the Ecole Coloniale d'Agriculture de Tunisie and, after its creation in 1913, transferred to the Service Botanique et Agronomique de Tunisie. The work began with the existing variability in the local durum wheat populations, the only widely grown wheat species in the country at that time (Boeuf 1932). Local lines, including Derbassi, Adgini, Agili, Mekki, Hamira, Jenah, and Khoutifa, were derived by mass and pedigree selection until 1930. Later, Chili and Mahmoudi 981 resulted in some yield increases.

The first crosses performed, between local types and others from the Mediterranean region, resulted in the release of D117, D77, and D522, which were earlier, gave higher yield, and had better quality characteristics than those used before (Daaloul 1986). Despite their wide adaptation and good quality characteristics, these old varieties showed susceptibility to lodging and disease, were late maturing, and had low yield potential. Remedying these weaknesses was the task of the durum wheat breeding program. By the 1950s, germplasm was introduced to improve earliness, fertility, and disease resistance in local varieties. As a result, three durum wheat cultivars, Inrat 69, Badri, and Maghrebi were released by INRAT. These selections were extensively adopted by farmers in the favorable zone in northern Tunisia, but not in the northwest, where Chili and Mahmoudi 981 were firmly entrenched. Inrat 69 increased yield by 40% over Mahmoudi 981, which is one of its parents (Anonymous 1971).

With the start, in 1967, of the collaborative project between the Tunisian Ministry of Agriculture, CIMMYT, and USAID, high-yielding germplasm began to be introduced, evaluated, and used in crossing programs. Amel and Maghrebi were selected from the segregating material received from CIMMYT and put to farmer use. These two new high-yielding, semi-dwarf, early varieties allowed some intensification of wheat production in the north, along with the use of fertilizers and chemical weed control. Maghrebi yielded an average of 8 qx/ha more than Inrat 69. Nevertheless, these varieties showed some weaknesses in terms of susceptibility to the major wheat diseases. Badri and Amel were particularly susceptible to septoria leaf blotch, leaf rust, and stem rust. Maghrebi and Amel were not competitive with weeds due to their short stature. Their low straw yield was also an argument against their widespread use.

Further progress was achieved with the release of Ben Bachir, Karim, and Razzak. These were intermediate in height and earliness compared to Inrat 69 and Maghrebi (Maamouri et al. 1988). They also showed better resistance to the main wheat diseases. Variety Ben Bachir, although of the right height, was withdrawn because of photoperiod insensitivity, which caused head sterility in very early headings in years with abnormally warm winters. Yields as high as 80 qx/ha were obtained under good cultural practices with Razzak and Karim. Variety Karim was largely adopted by farmers in Morocco.

Substantial increases in the national average yield were obtained as new high-yielding varieties were used. Average national yield increased from 3.40 qx/ha in 1941–1960 to 9.47 qx/ha in 1981–1990.

Constraints

As in all other environments with a Mediterranean climate, cereal production in Tunisia is subject to different types of stress. Drought is by far the most limiting abiotic stress. Only 2–3% of the area planted to cereal receives some additional water. The remainder is rainfed and thus exposed to intermittent water shortages which can occur at any stage of the crop cycle. Below-average crop harvests are associated with prevailing severe drought in one of the main cereal growing regions. To some extent, poor harvests are obtained in drought years in all regions. In the semi-arid zones, drought associated with terminal heat often curtails the grain-filling period and causes grain shriveling leading to poor harvest.

Diseases are less-frequent causes of yield reduction than drought. With the early maturing varieties, stem rust and leaf rust are no longer considered major biotic stresses of wheat. With the widespread use of high-yielding semi-dwarf varieties, other diseases, such as septoria tritici blotch, have become more important, especially in favorable years and under high input conditions. Indeed, overall weather conditions favorable for good crop harvest are also favorable for disease development. According to Harrabi (1991), septoria caused a wheat harvest loss of about 4 million qx in the 1990/91 season, a year where total cereal production reached its highest ever record. Root rot diseases are seen on wheat across all cereal growing areas, more frequently under drought or excessively wet conditions. Their relative importance within each climatic zone and their impact are not well documented. No significant yield losses due to insect pests have been seen so far. However, regular infestations with Hessian fly are observed in isolated sites and therefore should be looked at as a potential problem.

Prospects of Durum Wheat Breeding

Grain yield gains due to breeding decreased with the release of the last high-yielding varieties. This is mainly due to the emphasis given to wide adaptation during selection.

Given the limited area annually grown to cereals, increasing cereal production in Tunisia will rely almost exclusively on crop intensification. Breeding material adapted to each of the main agro-ecological zones is a major goal of the durum wheat breeding program. Two agro-ecological zones have been identified, with somewhat different breeding needs. High yield potential, disease resistance, and good quality are important breeding objectives in the relatively favorable northern part of the country. Highly stable yields with tolerance or resistance to drought and terminal heat stress are key issues in developing varieties adapted to the harsh conditions of the semi-arid part of the country.

Breeding Durum Wheat for Favorable Conditions

Breeding for Yield Potential

Crop intensification in the relatively more favorable rainfed zones of northern Tunisia, as well as in irrigated areas, requires the cultivation of varieties which can give higher yield under optimum inputs. This work is being carried out at the favorable site of Beja through selection from locally developed segregating material and material received from international centers. Yield testing of fixed material is conducted in preliminary yield trials in Beja and selected material is tested in multilocational trials at a network of sites including Beja, Mateur, and Krib, and under supplementary irrigation at Bou Salem. Selection among advanced material integrates agronomic records (plant height, precocity, resistance to lodging, etc.), yield performance compared to the widely cultivated commercial varieties, grain quality parameters (1,000 kernel weight, vitreousness, protein content), and disease scores under natural and/or artificial inoculation in the field.

The work conducted in recent years has resulted in the identification and release of variety Khiar. This variety is recommended for the favorable conditions in the northern part of the country and under supplementary irrigation, where it outyielded Karim and Razzak, the two most widely grown durum varieties (Tables 1, 2, and 3). Khiar has slightly better resistance to septoria tritici than Karim and Razzak.

Table 1. Grain yield (qx/ha) of variety Khiar at experimental sites.

Year	Beja		Bou Salem		Krib		Mateur		Kef	
	Yield	% C	Yield	% C	Yield	% C	Yield	% C	Yield	% C
1988/89	31.19	102.9	29.23	101.1	21.54	86.5	32.88	129.8		
1989/90	13.43	82.3					44.21	87.1	13.37	87.0
1990/91	69.74	103.6	63.09	100.6	38.55	91.7	41.31	93.4	61.93	104.3
1991/92	53.17	102.9	52.45	95.1	53.71	105.0			26.22	105.4
1992/93	68.32	108.7	47.26	103.3	51.98	101.5			32.55	114.2
1993/94	32.13	112.7	55.43	104.2	34.41	105.5			15.45	112.4

% C: Grain yield as a percentage of the Check Razzak in 1988/89, 1989/90, 1990/91, 1992/93 and 1993/94, and the Check Karim in 1991/92.

Table 2. Grain yield (qx/ha) of varieties Khiar, Karim, and Razzak in demonstration trials in 1991/92.

	Beja	Mateur	Jendouba	Bouarada	Zaghouan	Kef	Siliana	Mean
Khiar	53.09	43.23	52.70	41.01	47.89	66.89	52.08	50.98
Razzak	45.22	43.22	45.38	36.52	47.90	56.18	41.83	45.17
Karim	46.92	40.36	54.39	42.72	46.20	63.51	47.58	48.81

Source: Division technique of the Office des céréales.

Table 3. Grain yield (qx/ha) of varieties Khiar, Karim, and Razzak in seed-increase fields in Krib area.

	1990/91	1991/92	1992/93	1993/94
Khiar	45.8	56.9	51.14	34.10
Razzak	40.1	44.8	45.46	21.06
Karim	39.8	43.6	39.72	27.63

Other promising lines tested during the last three years in multilocational trials gave higher yields than the checks. The top yielders were BD 2336, a sister line of Khiar, and a line issued from the cross Razzak/Karim. The average grain yield of BD 2336 over five sites in 1992/93 was 5 qx/ha higher than that of the best check. Moreover, the line Razzak/Karim showed a consistently high 1,000 kernel weight under various environments. Khiar, released for cultivation in favorable conditions, showed a better tolerance to drought than the other commercial varieties under the exceptionally dry conditions of 1993/94 (Table 4). This is proof that drought tolerance can be found in improved high yielding material.

Table 4. Mean yields of varieties Khiar, Karim, and Razzak (1993/94).

Variety	Beja(320)	Krib (360)	Kef (230)	Mean
Khiar	30.71	38.71	12.01	27.14
Razzak	28.71	35.94	11.78	25.47
Karim	28.37	33.39	14.35	25.37

Mean of three yield trials at each site.

( ) mm rainfall from early September to late May.

Breeding for Septoria Resistance

Disease control is part of the optimization of growing conditions to further improve yields in disease-prone environments. Septoria leaf blotch on wheat was described by Viennot-Bourgin (1949) as an endemic disease with negligible importance in Tunisia. The disease became more important with the widespread use of semi-dwarf high yielding varieties (Maamouri 1975; Jerbi and Ghodhbane 1975). Moreover, cultural practices such as continuous wheat cropping, sowing at higher seed rates, early sowing, and the use of high nitrogen amendments to obtain higher yields tend to favor septoria attacks. Previous studies by Jerbi and Ghodhbane (1975) and El Ahmed et al. (1984) show that septoria tritici is more virulent on durum wheat than on bread wheat in Tunisia. Since almost 80% of the durum wheat acreage in northern Tunisia is now planted to susceptible cultivars, breeding resistant durum wheats should curtail septoria breakouts and maintain disease inoculum at a low level in favorable areas where wheat will continue to be grown under relatively high inputs.

The durum wheat breeding program at INRAT started intensive screening in 1990/91 with the aim of identifying suitable sources of resistance. The work conducted over three seasons (1990/91–1992/93) resulted in an increase in the percentage of resistant lines. However, a lesser increase of the percentage of resistant lines with suitable agronomic traits was seen, showing that more resistance is found in agronomically unsuitable backgrounds (Table 5). Selected resistant lines that maintained their relatively better resistance compared to Karim and Razzak are listed in Table 6.

Table 5. Number of resistant lines and selected lines as a percentage of screened lines.

Crop season	Total screened lines	Resistant lines (%)	Selected lines (%)
1990/91	1,056	17	5
1991/92	507	24	6
1992/93	506	28	6.7

Because of the trade-off between good agronomic traits and resistance to septoria, screening should concentrate on problems so that resistant cultivars may be quickly adopted. Yield testing of selected genotypes was undertaken in 1991/92 at the Beja site and in multilocational trials in 1992/93. Results of these yield trials are shown in Table 7.

Table 6. Disease scores of some selected lines under high natural septoria infestation in Beja (1990/91, 1991/92, and 1992/93).

Variety or cross	1990/91	1991/92	1992/93
Krf/Baladia Hamra//Krf	6–4	tr	5–4
BD1457/D73	4–3	6–4	6–4
D86-1-11b-10b=BD2127/BD2125//Karim	4–3	6–4	6–4
D86-162-7b-13b=Karim/Durum24//BD2221/3/Karim	6–3	5–4	5–4
BD2337=GdoVz512/Cit//Ruff/Fg/3/Pin/Gre//Trob	6–5	5–3	4–2
BD2338=SULA"S"//CORM"S"/RUFO/3/SITO"S"	5–6	6–3	6–5
BD2339=STN"S"//YAV"S"/TEZ"S"	6–3	3–1	4–4
BD2340=STN"S"//YAV"S"/TEZ"S"	6–5	4–2	5–4
Karim	8–7	8–8	8–6
Razzak	7–7	8–7	7–6

Table 7. Grain yield of resistant lines with suitable agronomic traits as a percentage of Razzak (%R) and Karim (%K).

Variety	1991/92		1992/93						Mean	
	Beja		Beja		Bou Salem (irrigated)		Krib			
	%R	%K	%R	%K	%R	%K	%R	%K	%R	%K
BD2337	107.9	117.4	107.1	109.6	103.4	109.9	99.7	104.0	104.5	110.2
BD2238	114.5	109.0	94.4	100.2	68.1	71.6	101.3	106.9	94.6	96.9
BD2339	121.0	115.2	93.9	99.7	91.3	95.9	87.7	92.5	98.5	100.8
BD2340	111.1	105.8	89.2	94.8	103.5	108.8	98.3	103.7	100.5	103.3

If high-yielding material with better resistance is released from these lines it should replace or at least be grown alongside the currently used, susceptible cultivars, to avoid septoria epidemics in hot spots. Crosses were made between resistant lines in order to pyramid resistance genes, and adapted material was used to breed well-adapted, resistant cultivars. Segregating populations from crosses with *T. dicoccoides* received from ICARDA in 1993/94 were screened with the aim to widen the genetic base for resistance to septoria leaf blotch. Selected plants will be advanced under high disease pressures during the coming seasons.

Breeding for Grain Quality

Despite the priority given to high grain yield in breeding new durum wheat varieties, quality aspects are always taken into consideration in the selection process. This has resulted in the release of varieties with acceptable grain quality. However, given the changing consumption habits and the diversity of use, quality receives more attention in the breeding program.

Segregating material is systematically checked. Lines with shriveled, non-vitreous grains or with black point are discarded. Seed of lines in yield trials is evaluated for

1,000 kernel weight, test weight, and percent virtuousness. Total protein content, semolina yield, and pasta quality tests are only carried out on promising lines.

The material included in the Tunisian durum crossing block (TDCB) was evaluated for 1,000 kernel weight, test weight, vitreousness, and total protein content in 1993. Table 8 summarizes these results. Unfortunately, the exceptional drought during 1993/94 at Beja hampered evaluation. The work was thus transferred to an irrigated site for 1994/95, where the material was tested under normal and low nitrogen amendments for further confirmation of previous results and selection of yellow-berry resistant material. The durum wheat area under total or partial irrigation is increasing in Tunisia.

Table 8. Thousand kernel weight, test weight, virtuousness, and total protein content of durum wheat material included in the TDCB (Beja 1992/93).

Trait	Mean	Range	Top 10%
1000 kernel weight (g)	41.2	38.5–55.8	48.1–55.8
Test weight (kg/hl)	78.5	75.0–82.5	80.0–82.5
Vitrousness (%)	97.0	90.0–100.0	98.0–00.0
Total protein content (%)	13.9	11.3–16.5	14.8–16.5

Information from various reports was used to choose germplasm with good quality characteristics to be used as parental material to the upgrade grain quality of adapted cultivars.

More precise quality tests will be conducted for better evaluation of the material used. Seed storage proteins will be electrophoretically analyzed to determine their gliadin component profile. Results of this analysis will help better target crosses for the improvement of grain quality in durum wheat.

Breeding Durum Wheat for Dryland Conditions

Although some progress has been achieved for the favorable zones, it remains rather limited for the semi-arid zones of El Kef, Siliana, and Zaghouan, and area representing 25–30% of the national durum wheat acreage (Anonymous 1991). The need for a germplasm adapted to these zones became evident after two consecutive dry seasons, 1987/88 and 1988/89. Improvement of the resistance of wheat to terminal drought and heat was taken as an additional objective in the program. In these areas, although most seasons are dry, favorable years do occur. Therefore, the material developed for these areas should respond to favorable seasons.

The program screens the locally developed material and the germplasm received from ICARDA, CIMMYT, and ACSAD to identify material with the above-mentioned characteristics. The screening includes testing under harsh conditions in different sites in the target area. The program also makes use of local germplasm in crosses with high-yielding material.

Recent work has led to the identification of two new lines, Omrabi 3 and Omrabi 5, received from ICARDA. These varieties seem to show better drought tolerance, especially in years with dry springs. These lines also have good yield potential. Moreover, they meet the farmers' need for straw in the semi-arid zones, where the majority of the farmers are animal owners. Results obtained with these two varieties in dry years are shown in Table 9. Omrabi 3 and Omrabi 5 also performed well across Mediterranean dry areas (Nachit et al. 1991).

Table 9. Grain yield (qx/ha) of varieties Omrabi 3 and Omrabi 5 compared to Razzak and Karim in relatively dry environments.

		Variety			
		Omrabi 3	Omrabi 5	Razzak	Karim
1987/88	Beja (384)	32.41	35.91	32.63	28.92
1988/89	Beja (334)	40.38	45.63	40.79	37.59
1989/90	Beja (327)	11.64	10.58	14.23	14.93
1989/90	Mateur (597[†])	36.25	33.37	43.95	36.72
1989/90	Kef (321)	13.75	9.68	16.87	14.18
1991/92	Kef (354)	43.20	42.90	42.20	
1993/94	Beja (312)		32.02	30.66	30.07
1993/94	Kef (230)	26.7	27.10	22.10	19.20

( ) mm rainfall from early September to late May.
[†] With dry spring.

Omrabi 3 outyielded both old local and improved durum varieties in on-farm verification trials in Kef (Table 10). Omrabi 3 will be proposed for official registration this year and seed increase has started in collaboration with a seed company.

Table 10. Grain yield (qx/ha) of Omrabi 3 compared to old local and improved durum varieties in on-farm verification trials in Kef (1993/94).

Variety	Site	
	Sers	Zâafrane
Ajili	5.1	
Chili	8.6	
INRAT69	7.0	
Karim	6.1	11.4
Razzak		10.7
Khiar	10.1	10.2
Omrabi 3	12.5	16.2

Three advanced lines (issued from crosses with Mahmoudi and Chili, two old local durum varieties) have shown merit under semi-arid conditions and need further confirmation.

Under dry conditions, lines from the crosses Lahn/Gs//Stk, Lahn/Mrb SH, Stn/Mrb SH, Stn/Agia, Gdo512/Cit//Ruff/Fg/3/DWL5023, Gdo512/ Cit//Ruff/Fg/3/Nile, Mrb3/Chen, and Mrb15/Ru had higher grain yield and a better average rank than the national checks.

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Durum Breeding in Turkey

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Abstract

Turkey is a traditional durum country. Durum is grown on 1.8–3 million ha, with production of 2.8–4.1 t/ha per year. Durum is mainly grown in three different zones: the central plateau and transitional zone, the southeast zone, and the coastal zone. These zones contain approximately 50, 30, and 12% of the total durum wheat area, respectively. Major problems are cold and diseases (stripe and stem rust, smut) in the central plateau, septoria tritici stripe and stem rusts in the coastal zone, and drought and stripe and stem rusts in the southeast zone. *Zabrus* spp. and sunni pests are common in the central plateau and southeast, and sawfly in the coastal regions. A number of varieties have been developed in each zone and grown successfully. Average grain yield varies from region to region. It is quite high in the coastal regions (5–7 t/ha), moderate in the central plateau and transitional zones (2–3.5 t/ha), and low in the southeast (1.5–2.5 t/ha). As a source of germplasm for breeding, local varieties, landraces, and wild relatives have been used to overcome obstacles in breeding programs.

Introduction

Durum is grown in the traditional way in only a few areas of the world. It is found in regions with generally low rainfall and is therefore vulnerable to adverse weather conditions. Durum cultivation is thus dependent on the environment, which has a substantial effect on both quality and quantity. Adverse climatic conditions restrict the amount produced and undermine the quality. Annual world durum production varies between 20–30 million t/year (Table 1). This unstable production also has an effect on the durum price in the world market.

Table 1. World durum wheat production (million tonnes).

Year	1993/94	1992/93	1991/92	1990/91	1989/90	1988/89	1987/88	1986/87
EC	8.1	9.0	11.4	7.4	6.5	7.0	7.5	7.2
Turkey	4.2	4.0	5.0	5.5	5.5	4.0	6.0	6.0
Canada	2.9	3.1	4.6	4.3	4.1	2.0	4.0	3.9
USA	2.2	2.6	2.8	3.3	2.5	1.2	2.5	2.7
Others	9.27	9.1	10.6	8.5	8.3	8.2	8.6	9.4
Total	26.6	27.8	34.4	29.0	26.9	22.4	28.6	29.2

Source: Conseil International du Bl (International Wheat Council).

Durum is traditionally an important crop in Turkey, with an annual production of 2.1–6.0 million t (Table 1). Turkey has a suitable environment for durum cultivation, and it has an important production potential. However, during the last two years, production has decreased, resulting in large imports of durum grain.

The total cultivated area in Turkey is 23.9 million ha. Between 17–18 million ha are sown every year. The annual fallow area is 5–6.5 million ha. The area devoted to cereals is nearly 14 million ha, and the wheat area is around 9 million ha. The annual durum area is 1.8–3.0 million ha.

The dominate climates are Mediterranean (with hot and dry summers, mild and rainy winters) and continental (with dry and hot summers, cold winters, low rainfall and humidity and major differences between day and night temperatures). In Turkey, there are several different micro-climates. According to Mizrak (1983), 22 different climatological zones are present.

Turkey is the leading producer of both durum and bread wheat in the Near and Middle East. Other cereals, including barley, rye, oat, spelt wheat (both dicoccum and monococcum), maize, millet, and rice. Average annual production of wheat is 18–20 million t. Recent estimates indicate that approximately 80–85% of the wheat area is devoted to bread wheat and 20–30% to durum.

The durum wheats are grown primarily in three regions: the central plateau and transitional zone, the southeast zone, and the coastal zone. These regions contain approximately 50, 30, and 12% of the total durum area, respectively. In other regions durum is grown on a smaller scale: 6% in the Marmara region, including Thrace, 1% in Black Sea Region, and less than 1% in the northeast. Lesser amounts of durum are grown in the mountainous eastern region. Production is as follows: 50% in the central plateau and transitional zone, 26% in the southeast, 15% in the spring zone, 7% in Marmara and Thrace, and 2% in the remainder.

Durum grain is utilized in Turkey as follows: 850,000 t for bulgur, 360,000 t for pasta, and 75,000 t for other purposes. In 1993, an estimated 60,000 t were exported, in 1994 an estimated 100,000 t, and in 1995 an estimated 150,000 t. To meet the needs of pasta and semolina production companies, Turkey has had to import durum in recent years.

Cold and drought are the major abiotic factors limiting yield. In the high plateaux cold is the major problem, although terminal drought also occurs in some areas. In the southeast, drought is the main constraint. During the winter, durum is affected by cold more than bread wheat, resulting in weaker plants in early spring and, as a consequence, yield reductions. Early spring causes high yield losses.

Durum diseases are rusts, loose smut, common bunt, septoria tritici, barley yellow dwarf virus, root rot diseases, and powdery mildew. The importance of these diseases varies from region to region.

The quality of durum cultivars grown in Turkey needs to be improved. Among the current cultivars, Kunduru 1149 has the highest pasta quality. Kızıltan 91 is the most suitable for bulgur. The others have acceptable quality when grown in a suitable environment. In breeding programs, much more emphasis is now given to quality.

Historical Background

In the early 1930s, durum covered 40–80% of the total wheat area, varying from region to region. In Thrace, it covered 60%, in the southeast 80%, in the central plateau 70%, and in the coastal regions 40% (Tuncer 1964). There were 233 local varieties or populations in 1964, 96 of which were pure durum populations while some were mixed durum and bread wheat populations. The southeast, central plateau, and Thrace had the most durum populations. After the introduction of high-yielding bread wheat varieties, this pattern changed rapidly in favor of bread wheat. More research support was given to bread wheat improvement than to durum. Government price policies were also more in favor of bread wheat. Prices were equal for bread and durum wheats until 1993. Further, as more high-yielding varieties of bread wheat were available, producers preferred it to durum. As a consequence, durum production was limited to marginal areas where bread wheat could not compete.

In the central plateau, many farmers grow durum for their own needs. This production does not go to market and is not counted in the official statistics. In fact, this is still an important production group in the central plateau. These farmers generally grow local varieties or landraces that are mainly facultative and sown in spring.

Durum predominated in Thrace until 1970, when high-yielding bread wheats (such as Bezostaya 1, Kırkpınar 79 and Kate A 1) started replacing it. In the 1960s, 60% of the wheat area was devoted to durum, while in 1991, only a small proportion (approximately 5%) was grown to durum. The regional proportion of the total production has decreased from 30 to 6% (Kün and Ada, 1993). Generally, durum culture is restricted to the more marginal lands, leaving the best lands for the high-yielding bread wheats. Since 1968, the high-yielding bread wheat varieties have largely replaced the lower yielding durum varieties in the coastal regions.

In the southeast, the situation is not unlike the central plateau and Thrace. Due to the lack of high-yielding durum varieties, durum production has decreased rapidly. In the last 20 years durum has lost half its area in this region.

Durum Breeding

Durum wheat breeding has gone through two different stages: before and after the National Wheat Project, which started in 1969 with the goal of integrating wheat improvement and agronomy. Breeding studies on bread wheat and durum started in 1928. Each institute had a separate wheat program until 1969. When the National Wheat Project began, all the institutes working on wheat were involved in the project. Turkey divided wheat improvement into two zones, namely winter and spring, although the institutes located in each work together. Germplasm exchange among the institutes within each zone and across zones has also been initiated. As a result of this joint effort a number of durum varieties have been developed. The varieties released before and after the project are listed in Table 2.

Table 2. Durum varieties released in Turkey.

Cv	Cv	Date
	Before National Wheat Project	
Akba ak 14458		
Akba ak 1523		
Sarı Bursa 1149		
Rahman		
Kunduru 414/44	CRIFC-Ankara	7.10.1963
185-1	TZARI-ESKI EHIR	7.10.1963
Kırmızı 5132	AGR.FAC.-Ankara	7.10.1963
Sarıbursa 7113	AGR.FAC.-Ankara	7.10.1963
Karakılçık 1133	TARI-Edirne	1964
Akba ak 073/44	CRIFC-Ankara	1964
Kunduru 1149	TZARI-ESKI EHIR	26.4.1967
Berkmen 469	CRIFC-Ankara	26.4.1967
Oviachic 65	Sakarya ARI	19.3.1968
	After National Wheat Project	
Dicle 74	Spring zone ARI	13.5.1976
Gediz 75	Soring zone ARI	13.5.1976
Çakmak 79	CRIFC-Ankara	15.5.1979
Tunca 79	TARI-Edirne	15.5.1979
Gökgöl 79	TARI-Edirne	15.5.1979
Diyarbakır 81	Spring zone ARI	29.4.1987
Balcalı 85	AGR.FAC-Adana	26.4.1988
Ege 88	AARI-Izmir	26.4.1988
Creso	Çukurova ARI	20.4.1989
Kızıltan 91	CRIFC-Ankara	1991
Ç-1252	CRIFC-Ankara	1991
Aydın-93	SEARI-Diyarbakır	1994
Fırat-93	SEARI- Diyarbakır	1994

In the 1920s local populations were collected and used as breeding material. Some varieties selected from these populations are still being grown. These varieties are resistant to environmental stresses, tolerant to some diseases, and have good grain quality, and yield stability. After the National Project began, several studies based on these varieties were conducted. Some improvement in yield has been achieved.

High-yielding spring types were used to increase yield. Winter × spring crosses were made and yield increased from 1 to 2 t/ha. Since the late 1980s, old varieties have been used in crossing programs to add adaptation to high-yielding material. Winter × spring × old varieties have been crossed. As a result of this work, yield has stabilized at 3.5 t/ha at the breeding program station and 3 t/ha in the farmer' fields. Further increase is possible with the introduction of high-yielding, disease-resistant germplasm into the breeding program.

Until late 1980s yield was given top priority. Quality and disease resistance were neglected. Since production is now enough for domestic consumption and exportation is gaining importance, quality has started to be an important character in the market. Thus breeding programs have started to conduct quality studies. Old varieties, local populations, landraces, and high-quality varieties are used as sources of germplasm. Disease resistance, however, is another subject. In future breeding work, new varieties should have some certain level of disease resistance. This is especially important in the spring zone. Sources of resistance have been obtained from local populations, wide crosses, and varieties or lines introduced from outside.

Currently, durum breeding studies are carried out in three regions: the central plateau and transitional zone (winter/facultative), the spring zone (spring), and the southeast (spring/facultative). Each institute in these zones has a breeding and agronomy department, and some have a pathology department. The quality lab at CRIFC analyzes the breeding samples of all institutes.

Table 3. Institutes by zone (based on bread wheat breeding).

Winter/facultative	Spring	Spring/facultative
CRIFC-Ankara	AARI- ZM R	SEARI-D Yarbakir
TZARI-ESK EH R	ÇUKUROVA ARI-Adana	BSARI-Samsun[†]
BDIWWRI-Konya	Sakarya ARI-Adapazari[†]	
EAARI-Erzurum[†]	MARI-Antalya[†]	
TARI-Edirne		

[†] The institute has no durum wheat breeding program.

For the purpose of germplasm exchange, an observation nursery is being distributed by the Coordination Center located at CRIFC. Institutes in the winter zone exchange their F_3 generations every year. The Regional Yield Trial of CRIFC has also been distributed.

Since no separate durum program is available, staff working on bread wheat are also working on durum wheat where a durum program is available.

Table 4. Staff involved in wheat improvement in Turkey.

Discipline		Degree	
Breeding	47	PhD	22
Pathology	7	MSc	25
Quality	10	BSc	40
Agronomy	20		
Economy	4		
Total	88	Total	88

Coastal Region (Spring Zone)

This zone includes the Aegean, the Marmara (except Thrace), and Mediterranean regions. The coastal or spring durum growing areas are characterized by relatively high rainfall (500–800 mm) and relatively mild winters. The wheat growing season coincides with the rainy season in November–May. Most of the coastal areas also have irrigation facilities, but these are used for other crops, except in severe drought. Farming in this region is generally mechanized and yields of 5–7 t/ha are possible if proper agronomic practices and cultivars are used. However, high-yielding bread wheat cultivars have been grown on the best lands, and durums are relegated to the marginal lands.

Diseases in this region vary somewhat from one coastal area to another, but in general *Septoria tritici*, stripe rust (*Puccinia striiformis*), and stem rust (*Puccinia graminis* f.sp. *tritici*) are the major diseases. However, these three diseases are seldom epidemic in the same year. Powdery mildew (*Erysiphe graminis*) and leaf rust (*Puccinia recondita*) are frequently severe in the Marmara region, but these diseases seldom cause losses in other areas. Sawfly (*Cephus cinctus*) is present in all coastal areas and sometimes causes considerable damage.

The breeding objective in the spring durum region is to develop high-yielding durums to compete with high-yielding, semi-dwarf bread wheat cultivars. For proper expression of genetic yield potential, the new durum cultivars must also have the proper combination of disease resistance, plant height, straw strength, and maturity.

One of the important considerations in the spring durum program is proper maturity. Because of the rainfall pattern, planting should be finished by mid-November, which is the normal beginning of the rainy season. Planting after this time is not suitable, because once the rain begins, it continues uninterrupted for one or two months. Late planting is associated with reduced grain yield. For this reason, maturity should be adjusted to planting time. This necessitates the development of lines with a long vegetative phase to escape the last spring frost, which occurs in April. But they must mature quickly to escape the terminal drought which starts in June.

Given these objectives, the varieties developed for this region by the breeders were: Dicle 74, Gediz 75, Balcalı 85, Creso, and Ege 88.

Southeast (Spring/Facultative Zone)

This zone includes the southeastern regions and similar parts of Turkey. The southeast is characterized by cool to cold winters, relatively low rainfall (300–450 mm), and very dry, extremely hot summers, frequently with temperatures in the 40–45 °C range. It is divided into three agro-ecological zones. The first zone starts at the Syrian border and goes north, with dry, hot summers and mild winters. The second zone lies in the middle of the region, and has hot, dry summers and cool winters. The third zone is in the north, and has hot, dry summers and cold winters.

A wheat/fallow/wheat rotation is used almost exclusively throughout the region, but the fallow is used mainly for sheep grazing and little attempt is made to conserve moisture. With this management system, yield is entirely dependent on rainfall received during the crop season, and average yield is between 1.5–2.5 t/ha. Due to relatively high temperatures in the spring, and rapid plant development, this region has a low incidence of epidemic disease but in some years stem and stripe rusts can occur. Two insects are common pests in the region: *Zabrus* spp. which eat ungerminated seed or small plants, and sunni pests (*Eurygaster* spp.) which attack the plant in the latter stages of development, sucking the juice of the developing kernel. The only known control system is chemicals.

Currently, 30–40% of this region is devoted to durum production. Even though it has been known as a durum region, production has been decreasing year by year. Durum areas covered 80% in the 1960s and 50–60% in the early 1970s. To reverse this trend, high-yielding durum wheat cultivars adapted to the environmental conditions of the region should be developed.

In the southeast, a large irrigation project (Southeastern Anatolian Project) has been initiated, and will start operations in 1997, with a capacity of approximately 1.7 million ha. In this region, a new crop rotation system will be used. Instead of the wheat/fallow/wheat system, an industrial crops/wheat/vegetables or an industrial crops/wheat system will be tried. To compete with the bread wheat in this system breeding strategies for durum wheat under irrigated conditions must be adjusted. High-yielding, disease-resistant, semi-dwarf varieties will be beneficial for the region.

The breeding program in this region concentrates on developing spring and facultative durum cultivars with some level of cold resistance. The cultivars for this region should also have earliness, drought resistance, and at least moderate levels of stripe and stem rust resistance. Earliness is the most important character because of the hot, dry conditions in spring. Hot, dry winds coming from the Syrian desert cause spike sterility almost every year. These winds generally come in early May, and all late types are affected. Early types can escape the hot winds.

Current varieties grown in the region are Kunduru 1149, Dicle 74, Gediz 75, and Diyarbakır 81. The varieties improved by the institutes working in this zone are Dicle 74, Gediz 75, Diyarbakır 81, Aydın 93, and Fırat 93. The last two were distributed to farmers in 1995.

Central Plateau and Transitional Zone (Winter/Facultative Zone)

This zone accounts for 50% of durum area and production, an amount which was higher in the past. The central plateau and transitional zone is characterized by cold winters and relatively low rainfall (300–450 mm). Climatic variations are large and crop reductions or failures are frequent. The central plateau has less rainfall than the transitional zone. The main durum growing areas in this large region are the northern and eastern sections of the central plateau, and the southern and western sections of the transitional zone. The durums in the region are grown in a wheat/fallow/wheat rotation, except in a few high-rainfall areas in the transitional zone, where annual cropping is practiced. The benefits of fallow systems are well understood by the farmers in the region.

The main diseases in this region are stripe and stem rusts and loose smut. The rusts are generally localized and only rarely reach epidemic proportions. The most recent epidemic was a stripe rust epidemic in 1991. Abiotic stresses in the central plateau include extreme cold, terminal drought, and heat stress at grain filling. In addition, the crop is subject to insect attack (sunni pest and *Zabrus* spp. in the central plateau, and wheat stem sawfly in the transitional zone) and diseases (yellow rust and common bunt). The transitional zone has moderate rainfall and cool or cold winter. The important diseases in this zone are yellow rust, septoria leaf blotch, and leaf rust.

The breeding program for this region concentrates on obtaining improved winter durum types with good levels of winter hardiness or cold tolerance and acceptable grain quality. Even though a certain level of cold tolerance has been achieved, it needs to be increased. The main disadvantages of the currently grown durum cultivars are a low level of disease resistance and medium grain quality (Table 5). Landraces and native populations have been used as sources of cold tolerance and good grain quality. A new project in collaboration with the ICARDA durum wheat program has been started to improve the cold tolerance level.

Stripe and stem rust resistance is the necessary characteristic for winter durum cultivars. Sources of resistance are available in a few landraces, in improved resistance lines from ICARDA, and in some lines from wide crosses. The main obstacle to transferring disease resistance from landraces to improved varieties is yield loss. It is very difficult to isolate segregates with high yield and sufficient winter hardiness. If good, winter-hardy types are developed, then incorporation of winter hardiness to disease-resistant spring or facultative types will be possible. Other characters, such as adaptation, drought tolerance, straw strength, medium height, and earliness, etc., can be incorporated by using top and double crosses.

Table 5. Quality and disease data of selected lines for DCB(1).

Entry	Vitreousness (%)	Protein	Stem rust	Yellow rust
Kunduru 1149	100	14.0	20S	60S
Çakmak 79	96	13.8	30S	60S
Ç1252	97	13.8	15S	40S-MS
KIZILTAN91	97	13.7	20S	40S-MS
MBVD7	100	14.9	20S-MS	60S-MS
9	100	13.4	TMR	20S/40MS-S
12	100	14.1	5S	10MS-S/20MS-S
13	96	14.1	10MS	40S-MS
15	88	12.6	5MR	40S-MS
16	94	13.2		20MS-MR
17	96	12.9	10MS-S	5MS-MR
18	92	13.4	5MS-MR	40MS/50MS
20	96	14.4	TMS	
22	96	14.4	20MS-S	30MS-MR
23	100	14.0	50MS-S	50S-MS

Another major breeding effort for the central plateau and transitional zone is breeding for high and stable yield and a good fit into the cropping rotation. Maximizing yield potential has received widespread attention, but bread wheat has had priority up to the late 1980s. This research lag is now being closed rapidly in order to remain competitive under dryland conditions. The main reason for this lag is the difficulty of working under dryland conditions and the narrow genetic base for important characters. Most winter areas are characterized by large annual and monthly fluctuations in temperature and precipitation. This creates a need for yield stability and increased yield potential.

Depending on the fluctuation in temperature and rainfall, average yield varies greatly from year to year. The difference between the highest vs the lowest average yield per ha is 500 kg/ha, about 40% of the average yield. Yield fluctuations of this magnitude must be eliminated as yield potential is increased.

Since yield in most winter areas depends on precipitation received during the growing period, the fallow system should be carefully applied to conserve moisture. Present results indicate that with the proper wheat/fallow system, and currently grown varieties, it is possible to stabilize minimum yield at about 2.5 t/ha even, with only 290–310 mm of annual rainfall in the central plateau. With 350 mm of rainfall, which is the long-term average in the region, yield raises to 3–3.5 t/ha. Further increases in yield potential will come primarily from new varieties, bred for adaptation to wheat/fallow rotations and the unpredictable moisture patterns of most rainfed areas.

In terms of adaptation, the question is whether to select for drought resistance or water-use efficiency. Currently grown durum landraces are drought resistant, while improved cultivars are much more efficient water users. This is shown by the low yield reductions of landraces and the higher yield reductions of improved cultivars with limited water availability. In fact, selection should be practiced for both water-use efficiency and drought resistance. To do this, physiologists must identify characters which will determine water-use efficiency, especially those which can be visually selected. Characters that affect drought resistance should be determined and provided to the breeders. Characters such as fewer tillers and leaves, leaf rolling, long peduncle, waxy leaf coating, upright leaves, or reduced number of stomata, etc., should be investigated to find out whether they have value or not. Breeders and physiologists can then cooperate to identify other morphological and physiological traits that will enhance water-use efficiency and drought resistance.

In terms of adaptation, the second important issue involves micro-nutrients, especially boron toxicity and zinc deficiency. According to the results of a survey carried out in generally dry areas, soils contain an excess amount of boron. The effect of boron is not great in normal years, but in dry years yield reductions occur. The effect of boron under dry and irrigated conditions is being investigated. Zinc deficiency is another problem experienced under dry conditions. Compared to the boron problem, zinc deficiency has only recently been considered a problem. Both should be evaluated very carefully to solve the adaptation problem in this region.

Table 6. Yield of selected lines from Turkish Durum Wheat Regional Yield Trails (1993/94).

Var. No.	AL	BL	ÇR	HY	ML	YZ	ES	UL	SM	AV	AV[†]
1	236	289	216	326	236	219	455	72	329	264	288
2	264	245	292	319	267	231	494	75	352	282	308
3	306	296	351	381	217	237	573	85	274	302	329
4	261	268	326	358	259	250	455	87	235	278	302
5	266	193	298	322	287	300	564	133	417	309	331
7	252	281	293	298	264	216	533	99	226	274	295
9	265	259	315	338	235	224	427	113	224	267	286
12	244	284	292	269	257	230	513	110	307	278	299
13	261	313	319	350	226	213	515	85	284	285	310
15	256	277	284	328	257	208	497	134	276	279	298
16	240	278	263	356	233	199	463	48	240	258	284
17	240	257	289	277	250	238	431	94	185	251	271
18	226	297	274	321	247	184	544	80	250	269	293
20	276	340	305	281	215	194	474	68	355	279	305
22	208	187	288	329	262	256	442	132	286	266	282
23	274	260	293	289	260	225	472	109	218	267	286

[†] Does not include UL results.

1–5 Checks: 1. Kunduru 1149; 2. Çakmak 79; 3. Ç-1252; 4. Kızıltan 91; 5: Gün 91 (bread wheat cultivar).

In general, water-use efficiency, drought and cold resistance, and micro-nutrients must be considered together. Current durum cultivars improved by the institutes and grown in the region are Kunduru 1149, Çakmak 79, Kızıltan 91, and Ç-1252. The latter are high-yielding cultivars. Ç-1252 has as much cold tolerance as Kunduru-1149.

As cold tolerance is very important in the central plateau, an extensive research program has been initiated in collaboration with the ICARDA/CIMMYT durum program in Aleppo. The first year's results were quite encouraging (Tables 6 and 7). Using these results a crossing block consisting of 57 entries was prepared.

Table 7. Yield of selected lines from Advanced Durum Wheat Yield Trial 51 (1993/94).

Var. No.	Location								
	AL	BL	ÇR	HY	ML	YZ	UL	AV	AV[†]
1	238	280	297	325	222	211	103	240	262
2	262	245	363	272	250	216	96	243	268
3	282	255	321	272	202	243	143	246	263
4	238	228	301	298	152	229	117	223	241
5	269	207	260	342	253	263	141	248	266
7	251	302	291	282	291	230	72	245	274
8	318	290	310	369	273	245	135	277	301
11	249	296	294	308	234	126	119	232	251
18	264	323	368	333	324	204	89	272	303
20	268	307	347	310	256	166	56	244	276

[†] Does not include UL results.

1—5 Checks: 1 Kunduru 1149; 2 Çakmak 79; 3 Ç-1252; 4 Kızıltan 91; 5 Gün 91 (bread wheat cultivar).

The locations of the cold and winter hardiness tests are Haymana (HY), Malya (ML) and Ulas (UL). The coldest is Ulas, followed by Malya and Haymana. Altinova (AL) is used for cold and terminal drought testing. The remainder fall in between these extreme locations.

In Ulas, Ç 1252 (No. 3) yielded as much as the bread wheat check Gün 91 (No. 5). Line No. 8 also yielded as much as these two checks. Kunduru 1149 (No. 1) is a well known and widely-grown variety in the central plateau. Some lines yielded better than Kunduru 1149 under all conditions, including cold and drought.

The lines selected from this trial were also tested for quality and important diseases in the central plateau (Table 4). The results of the quality tests are the averages of all locations. Disease tests have been done in the field by inoculating plants.

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Durum Improvement Program in Libya

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Abstract

Wheat grain accounts for 80% of the daily diet in Libya. Local production is about 520,000 t, which is only half of the national demand. Durum wheat is grown mainly in two major regions: southwest of Tripoli in Jebel Nofosa and in the eastern part of the country in Jebel El-Akhdar. Drought and heat are the main limiting factors. Originally, Mahmoudi and Maghribya were the dominant local varieties. Later, Mexicali 75 was introduced, and recently, Marjawi, Baraka, and Zorda. The later three cultivars have shown significant yield advantage. On average, Marjawi, Baraka, and Zorda outyielded the local check Mexicali 75 by 23, 19, and 12% respectively, with a yield advantage over Mahmoudi and Maghribya was over 50%. Cultivars newly developed for drylands at ICARDA, such as Zahra 5 and Omrabi 5, show better yield advantages in the Libyan drylands.

Introduction

Wheat is the principle staple food in Libya, with wheat products accounting for about 80% of the daily diet. In general, demand exceeds the local production of about 520,000 t/year, and about this amount of durum wheat flour is imported to cover the shortage. Durum wheat is grown in two major regions. The first is located southwest of Tripoli in Jebel Nofosa, at an altitude of 700 meters, with 250–300 mm of rainfall. The second is located in the eastern part of the country at Jebel El-Akhdar, at an altitude of 800 meters, with 400–500 mm of rainfall. These regions are the most suitable areas for durum production in Libya.

In recent years durum production has been affected by severe drought. The barley production program currently dominates the major durum areas because of barley's ability to survive in marginal rainfall regions of about 150–250 mm. Barley also has a dual purpose as it is harvesting in the dry season as hay, or grazed directly by the farmers' flocks.

Research Activities

In the early 1960s research activity started on both durum and bread wheat, focusing mainly on yield improvement. Selection for drought, heat, and disease resistance was also carried out. The agricultural area in Jebel El-Akhdar in the eastern province is approximately 250,000 ha, and the major crop in this area is durum. The soils are red "terra rosa" clays.

Precipitation characteristics are major factors affecting crop production. Both the amount and the distribution pattern of seasonal rainfall are of paramount importance for crop performance. Annual rainfall in this region is variable both spatially and temporally. This region receives an average of 200–600 mm of precipitation annually. Precipitation is highest in the high altitude coastal area (about 400 mm around El-Marj) and decreases sharply inland. Precipitation distribution is characteristically seasonal, with about 75% of the average annual rainfall occurring from November to February, and about 15% in March and April, resulting in increased moisture stress during the late stages of crop development.

Durum wheat is by far the most important cereal crop grown in the region, and under normal circumstances covers an estimated annual area of 120,000–150,000 ha. Until the late 1970s, two durum cultivars, Mahmoudi and Maghribya, dominated the variety spectrum. However, in 1978 an exceptionally dry season resulted in almost total loss of sowing seed from the traditional cultivars. To fill the void, seed of cultivars Mexicali 75, Cocorit, Tanori, and Anahauac was imported from Mexico. Since then, Mexicali 75 had become the dominant variety in the region. Average grain yield obtained by farmers from local varieties is 8–10 qx/ha. Yield has always been considered low. There is scope for improvement in both the cultivar and the cultural practices adopted (Tables 1, 2, and 3).

Durum research activity in Jebel El-Akhdar in the western region (Tables 1, 2, and 3) was intensified by directing efforts towards durum germplasm introduction, testing, selection, evaluation, and promotion of superior lines and genotypes. Each year a considerable number of nurseries from various sources (CIMMYT, ICARDA, ACSAD) were grown, and superior lines were selected. Selected lines then advanced in a progressive evaluation and screening system in stages A, B, C, and D. At subsequent stages, the superior cultivars were evaluated at a number of locations to ascertain their broad and specific adaptations.

Table 1. Average yield of durum cultivars in the western region (1991–1994).

Cultivar	Yield (t/ha)	Percent check	Pedigree
Mexicali	3.9	100	
Zahra 5	4.6	118	Korifla, Cham 3
Baraka	5.2	133	AA(CPE-GZxTC3/BYETC)
Bohooth 103	5.3	136	Chen
Godwa	5.7	146	Cinamon-bursa

Table 2. Best durum cultivars in Maghreb Yield Trial.

Zone	Cultivar
West	Cham 3, Vitron, Chen/Altar 84
East	Cham 3, Waha, Sahel

Table 3. Average grain yield of durum cultivars, Khoms Station (1991–1993).

Cultivar	Grain yield (kg/ha)
Local	
Baraka	5.40
Gara	4.80
Godwa	4.20
Marjawe	3.90
Mexicali	3.80
Zorda	3.60
Aglail Edeeb (landrace)	4.60
Released	
Bohoth 101	4.30
Bohoth 103	5.00
ACSAD 65	4.80
Promising	
ACSAD 299	5.50
Omrabi 5	4.80
Zahra 5	4.60
Zahra 9	4.40
Zahra 1	4.60

As a result of this program, three durum lines with a consistently distinct advantage in yield ability, were released under the names of Marjawi, Baraka, and Zorda. Despite considerable variation in annual rainfall over a number of seasons, and within seasonal distribution patterns, the three cultivars have maintained their yield advantage over the local check (Table 1). On average Marjawi, Baraka, and Zorda outyielded the local check Mexicali 75 by 23, 19, and 12%, respectively, while their yield advantage over the conventional cultivars Mahmoudi and Maghribya was more than 50%.

A study comparing the cultivars formerly grown in the area (Mahmoudi and Maghribya) with newly developed cultivars was conducted over five years. The physiological aspects of yield variation were investigated to gain a better understanding of varietal adaptation and performance. This study confirmed the superior grain yield of the new cultivars compared to the traditional ones. This superiority is accounted for mostly by higher harvest indices in the newly developed cultivars.

The new cultivars generally have larger heads, more numerous grain, and/or larger grain (Tables 2 and 3). They mature much earlier and are shorter in stature.

Cooperation

The agricultural research center programs are cooperating with the international research centers such as ICARDA, CIMMYT, and the NARSs of the Maghreb countries, by exchanging germplasm, participating in traveling workshops, training, scientific visits, and consulting with experts at these centers. More progress has been made with the Maghreb countries by exchanging specific nurseries and conducting mutual experiments to develop high-yielding cultivars tolerant to prevailing environmental stresses. Promising results have been obtained through this collaboration. However, more support is required to upgrade durum program in Libya, particularly training in the various disciplines.

Durum Breeding and Utilization in Egypt

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Abstract

Wheat grain has been the staple food in Egypt for more than 7,000 years. Durum has a long tradition in the Egyptian diet. It is used in the local bread (*shamsi*) and in traditional meals (frike, burghul, and couscous). Total annual pasta consumption is about half a million tonnes. The Sohag milling factory can produce about 50,000 t of semolina per year. The rest is imported from Turkey, Italy, Greece, and the USA. The high-yielding durum varieties in Egypt produce about 100,000 t/year, with an average of 6.5 t/ha from 15,000 ha. The extracted flour could be used to improve local bread. Durum is popularly known as *dakar*. Early work concentrated on selection from local material. Dakar 49 and Dakar 52 were selected. Work on durum improvement has been carried out for the last 30 years. However, the lack of a durum processing industry, particularly milling, has hampered durum adoption and production in Egypt. Despite this constraint, four improved durum varieties (Sohag 1, Sohag 2, Sohag 3, and Beni Sueif 1) had been released by 1991. Under the marginal environments of Upper and Middle Egypt, the irrigated desert, and the rainfed areas, drought and heat stress, low soil fertility, and salinity are the main abiotic stresses. The three common rusts, powdery mildew, aphids, and BYDV are the main biotic stresses. There is a great potential for durum that could be exploited by extensive research in both irrigated and rainfed areas. Further efforts are needed to exploit the full potential of durums in Egypt, especially under rainfed conditions

Introduction

In Egypt, wheat has maintained its role as the primary food crop for more than 7,000 years. Moreover, its straw is an important fodder. Wheat yield has increased gradually over the past four decades. Wheat production increased from 1.3 million t in 1950 to 1.9 million t in 1980, but was far too low to bridge the food security gap. The goal of national self-sufficiency in wheat has been hindered by several factors. Exaggerated increases in wheat utilization have occurred due to heavily subsidized bread prices that result in greater per capita consumption (200 kg/year). Extensive waste has occurred as bread was fed to chickens and livestock. Further, an annual population growth rate of 2.1% between 1965 and 1980, and of 2.6% in the 1980s, was not matched by a similar increase in wheat production. Thus, national production met only 70% of need in 1960 and 21% in 1985.

Therefore, increasing wheat production is an important national goal, necessary to reduce wheat imports, save foreign currency, and provide enough food to meet increasing domestic demand. To face these challenges, an aggressive research program has worked to improve genetic potential, develop new production systems, and introduce wheat to new areas. It was anticipated that high and stable wheat yield could be achieved if the emphasis was directed towards solving the problems of non-availability of appropriate varieties, poor agricultural practices, poor water management, shortage of nitrogen fertilizer and other major and minor elements, late planting, and aphid and disease infestations.

Increasing food demand has led to increased cultivation of wheat under marginal conditions, where drought resistance and heat tolerance are major breeding constraints. Environments with drought and heat stress make up about 80,000 ha along the northwest coast, about 6,000 ha in the New Valley, and about 220,000 ha in the governorates of Upper Egypt. Salinity is also a problem on about 30% of the cultivated area in Egypt.

Since the 1970s, wheat research at the national level has frequently been based on the premise that increasing productivity is a must to reduce the gap between production and consumption. However, this view is far too simplistic. By careful planning of the research and development agenda, it is possible to target the development process towards certain components of higher productivity to meet specific predetermined objectives. New technologies linked with the green revolution help stimulate Egyptian breeders to make marked efforts to attain high productivity. These new methodologies are based on a multidisciplinary, team strategy.

Thousands of varieties and/or accessions have been introduced from several international centers (CIMMYT, ICARDA, FAO). They have been screened for adaptation to the Egyptian environment. Types with desirable characteristics and good yield potential have been identified. This approach has allowed Egyptian scientists to define several semi-dwarf varietal types favored by farmers, providing a source of well-adapted germplasm for current and future breeding work (Appendix A). These new high-yielding varieties (NHVs) are characterized by a stiff stem, required for lodging resistance, and an intrinsic ability to use higher levels of nitrogen fertilizer to obtain high productivity.

Consequently, during the past 15 years, wheat cultivation in Egypt has changed out of recognition. Wheat grain yield per unit area and total wheat production has been on the rise since 1987 (Table 1). The area under wheat increased from 600,000 ha in 1987 to about 900,000 ha in 1992. Average grain yield increased from 4.6 t/ha in 1987 to 5.5 t/ha in 1992.

Table 1. Wheat area, production, and average yield (1980–1992).

	Total Area (million ha)	Average Yield (t/ha)	Production (million tonnes)
1980	0.553	3.2	1.8
1981	0.583	3.3	1.9
1982	0.572	3.5	2.0
1983	0.550	3.6	2.0
1984	0.491	3.7	1.8
1985	0.494	3.8	1.9
1986	0.503	3.8	1.9
1987	0.572	4.7	1.8
1988	0.592	4.8	2.8
1989	0.639	4.9	3.2
1990	0.745	5.5	4.0
1991	0.819	4.8	4.5
1992	0.872	5.3	4.6

Average grain yield in 1991 and 1992 includes the rainfed areas and New Lands.

Table 2. National wheat output, imports, and consumption 1970–1992 (million tonnes).

	Production	Imports	Consumption	Production
1970/71	1.5	2.8	3.7	33.0
1971/72	1.7	2.6	4.1	33.9
1972/73	1.6	2.5	4.3	34.5
1973/74	1.8	2.8	4.6	35.3
1874/75	1.8	3.2	4.9	36.1
1975/76	2.0	3.7	5.1	37.0
1976/77	1.9	3.3	5.4	37.9
1977/78	1.7	4.9	5.9	38.7
1978/79	1.9	5.2	6.4	39.9
1979/80	1.9	4.6	6.6	41.0
1980/81	1.9	5.7	7.1	43.1
1981/82	2.0	5.4	7.4	43.3
1982/83	2.0	5.6	7.7	44.5
1983/84	1.8	6.7	8.1	45.7
1984/85	1.9	6.3	8.1	47.0
1985/86	1.9	6.8	8.7	48.3
1986/87	2.7	7.2	9.9	49.9
1987/88	2.8	6.5	9.3	51.3
1988/89	3.2	7.0	10.0	52.8
1989/90	4.3	7.9	11.3	54.2
1990/91	4.5	6.6	11.1	55.6
1991/92	4.6	5.9	10.5	56.9

These changes led to an increase in wheat self-sufficiency, which reached 43 percent in 1992. The change is also reflected in wheat imports, which reached a maximum of 7.2 million t in 1987 before dropping to 5.9 million t by 1992 (Table 2), despite the fact that both population and wheat consumption have continued to increase.

The modern HYVs have been widely adopted in about 70% of wheat areas. Farmers like their high productivity and good agronomic characteristics, and also their high yield stability. Over time, farmers are more interested in yield stability than in either absolute yield or wide adaptability.

To regulate the alarming population growth rate, the national wheat program (NWRP) is mandated to increase average grain yield by about 25–30% by the end of the century. To accomplish this major goal, the NWRP has the task of promoting high-yielding varieties coupled with the recommended cultural practices at the farmer level throughout all areas under wheat cultivation. New lines with higher biomass, yield components, and harvest index, expected to boost grain yield per unit area, are in the process of being released.

Objectives of the Wheat Program

1. Combine high-yielding characteristics with tolerance to biotic and abiotic stresses.
2. Use appropriate collaborative networks at the national and international levels to test adaptation to different ecological conditions and disseminate promising genetic materials.
3. Develop economically viable technologies that result in sustainable yield increases.
4. Participate in an effective technology transfer program to provide extension agents and farmers with practical information about achieving optimum wheat yields.
5. Improve and maintain high-quality seed to serve at least 50% of the wheat area.

Importance of Durum to the Economy

Durum has a long tradition in the Egyptian diet. It is an important crop in Upper and Middle Egypt. It is a dominant source of local bread (*shamsi*) and a major component in traditional meals (frike, burghul, and couscous). Pasta consumption is estimated at about half a million tonnes annually. High-quality bread wheat with strong gluten makes up about a quarter of total consumption. The Sohag mill can produce about 50,000 t of high quality semolina annually. The rest is imported from Turkey, Italy, Greece, and the USA. High-yielding durum varieties produce about 100,000 t/year, with an average 6.5 t/ha from about 15,000 ha (Table 3). In the rainfed area (100–250 mm), durum, as well as bread wheat, has considerable yield potential (Table 4).

In 1994, the wheat area under rainfed conditions was about 65,000 ha, a figure which could be increased to 100,000 ha. Several studies indicate that durum yield could reach 1–2 t/ha under rainfed conditions. In general, durum grain yield is 5–10% higher than bread wheat in Egypt, with a price about 10% higher. Importing semolina or durum flour would be costly for the pasta industry, as these products are almost twice as expensive as bread wheat flour. Therefore, growing durum and milling it locally is a good economic strategy.

The food gap is not going to disappear overnight. However, it would be better if Egypt were self-reliant in wheat production. Given an extraction rate of about 60% for coarse and fine semolina, about 75,000–100,000 ha of durum wheat has to be grown annually to meet local requirements for the macaroni industry. Not only will the quality of the pasta be improved, but the local bread industry may feel the improvement as well. Down the road, surplus high-quality durum could be exported at a good price, while the lower-priced bread wheat could be imported.

Table 3. Average grain yield (t/ha) of durum compared to bread wheat varieties grown in the farmers' fields (1989–93).

Variety	Year				
	1989	1990	1991	1992	1993
Giza 155	4.14	4.61	4.09	5	2.9
Giza 157	4.71	5.4	4.97	5.63	5.74
Sakha 8	5.09	5.57	5.45	5.33	5.84
Sakha 61	5.31	6.08	5.22		
Sakha 69	5.62	5.96	5.21	5.82	
Giza 163		5.71	5.58	6.07	6.06
Giza 162			5.36	5.57	5.77
Giza 164			4.66	5.54	5.38
Sakha 92			5.19	5.95	5.58
Beni Sueif				6.18	6.75
Stork	5.83	5.47			
Others	3.98	5.08	4.08	4.03	4.85

Table 4. Performance of bread and durum wheat (t/ha) under rainfed conditions along the Northwest Coast.

Variety	Season					
	1990/91 rainfed	1991/92 rainfed	one suppl. irr.	1992/93 rainfed	1993/94 rainfed	one suppl. irr.
		Bread wheat				
Giza 155	0.48	0.65	1.1	0.79	0.19	0.64
Giza 160		0.7	1.5	0.7		1.1
Giza 162		0.6	1.4	0.61	0.21	0.74
Giza 163		0.65	1.3	0.75		1.03
Giza 164		0.57	1.2	0.64		0.92
Giza 165		0.5	1.1	0.57	0.24	0.64
Sakha 8	0.48	0.66	1.3	0.66	0.21	0.66
Sakha 69	0.63	0.8	1.4	0.69	0.21	0.73
Sahel 1		0.85	1.4	0.87	0.38	0.88
Gemmeiza 1		0.79	1.3	0.85	0.31	0.82
Mean	0.53	0.69	1.3	0.713	0.25	0.82
		Durum wheat				
Sohag 1	0.33	0.3	0.5	0.61	0.15	0.4
Sohag 2		0.72	1.4	0.66	0.16	0.67
Sohag 3		0.62	0.9	0.56	0.15	0.54
Beni Sueif		0.85	1.8	0.75	0.12	0.83
Mean	0.33	0.63	1.13	0.57	0.15	0.61
LSD (0.05)		0.22	0.65	0.13	0.05	0.22
Precipitation (mm)	198	200	221	221	138	

Impact of Durum Wheat Breeding

Durum is popularly known as *dakar*, and has been grown in Egypt for a long time. Early research work concentrated on selection from local materials, from which Dakar 49 and Dakar 52 were developed. In spite of their good adaptability they lack resistance to lodging and diseases, are low yielders, and have poor quality. Consequently, their cultivation has generally dwindled.

Durum improvement has made progress since the 1960s, thanks to ongoing help and cooperation from CIMMYT, FAO, ALAD, the Ford Foundation and, more recently, ICARDA. A number of screening nurseries, yield nurseries (regional and international) and segregating materials have been supplied for testing under local conditions. People have been trained, particularly in durum improvement, in international and regional training centers at CIMMYT, ICARDA, and in Italy.

At the same time, the results of joint FAO/IAEA Uniform Durum Mutant Yield Trials and the International Durum Yield Nurseries have contributed several outstanding genotypes. The varieties Brant "S," Anhinga "S," Garza, Cocorit 71, and Jori C-69 were among the highest-yielding group over all locations. However, release of these superior varieties has been surtailed due to lack of durum milling factories. At the same time, wheat breeders continue to improve high-yielding durum varieties with stable, high grain quality. Extensive efforts have been directed at improving durum through collaborations with CIMMYT, ICARDA, FAO, USDA, and the ICARDA/ EEC Nile Valley Regional Program (NVRP) with the result that several high-yielding durum varieties have been released. Four improved durum varieties (Sohag 1, Sohag 2, Sohag 3, and Bani Sweif 1) were released by 1991 (Appendix A). Since the 1970s, 306 primary and advanced yield trials have been carried out to evaluate about 1,700 durum genotypes under various Egyptian conditions (Table 5). The durum checks exhibited significantly higher grain yield than the bread wheat checks in trials planted since 1988 (Table 6). Two other superior durum lines are in the process of registration and seed increase:

- Beni Sueif 2: Ente/Mexi CD 8153-12M-3Y-4M-1Y-OM.
- Beni Sueif 3: Conr/Rufo CD 48693-10Y-1M-Y-OM.

Table 5. Performance of durum and bread wheat checks (t/ha) tested in durum yield trials (1977/78–1987/88).

Trial	DW Check	Means	DW Check	Means	D-B (t/ha)	D-B (%)	LSD (0.05)
D 1977/78	Strok "S"	4.78	Chenab 70	4.57	0.21	0.05	0.69
A 1978/79	Strok "S"	5.90	Giza 157	5.22	0.68	0.13	0.95
D 1978 79	Strok "S"	5.07	Giza 157	4.29	0.78	0.18	0.69
A 1979/80	Strok "S"	8.41	Giza 157	6.85	1.56	-0.23	0.80
B 1979/80	Strok "S"	6.42	Giza 157	4.91	1.51	0.31	0.80
D 1979 80	Strok "S"	5.59	Giza 157	4.72	0.87	0.18	0.75
A 1980/81	Strok "S"	8.55	Giza 157	7.11	1.44	0.2	1.34
B 1980/81	Strok "S"	6.50	Giza 157	5.88	0.62	0.11	0.63
D 1980/81	Strok "S"	5.59	Giza 157	4.92	0.67	0.14	0.70
A 1981/82	Strok "S"	5.70	Sakha 69	6.0	-0.3	0.05	1.10
B 1981/82	Strok "S"	5.87	Sakha 69	5.75	0.12	0.02	
D 1981/82	Strok "S"	6.98	Sakha 69	7.12	-0.14	0.02	0.52
A 1982/83	Strok "S"	8.20	Sakha 69	7.54	0.66	0.09	0.84
B 1982/83	Strok "S"	7.59	Sakha 69	7.59			0.45
D 1982/83	Strok "S"	7.63	Sakha 69	7.74	-0.11	0.01	0.23
A 1983/84	Bittern	6.31	Sakha 69	5.64	0.67	0.12	0.75
B 1983/84	Bittern	6.17	Sakha 69	5.69	0.48	0.08	0.43
D 1983/84	Bittern	6.88	Sakha 69	7.00	-0.12	0.02	0.26
A 1984/85	Bittern	6.27	Sakha 69	5.56	0.71	0.13	0.66

(continued next page)

(Table 5 continued)

Trial	DW Check	Means	DW Check	Means	D-B (t/ha)	D-B (%)	LSD (0.05)
B 1984/85	Bittern	4.69	Sakha 69	4.78	-0.09	0.02	0.51
D 1984/85	Bittern	5.72	Sakha 69	5.83	-0.11	0.02	0.35
A 1985/86	Bittern	6.98	Sakha 69	6.3	0.68	0.11	0.93
B 1985/86	Bittern	6.42	Sakha 69	6.18	0.24	0.04	0.60
D 1985/86	Bittern	6.08	Sakha 69	5.98	0.10	0.02	0.09
A 1986/87	Bittern	8.62	Sakha 69	5.70	2.92	0.16	0.93
B 1986/87	Bittern	7.40	Sakha 69	6.18	1.22	0.20	0.60
D 1986/87	Bittern	6.24	Sakha 69	5.83	0.41	0.07	0.34
A(L) 1987/88	Bittern	9.06	Giza 164	7.13	1.93	0.27	0.90
A(1) 1987/88	Bittern	6.80	Giza 164	6.51	0.29	0.05	0.95
B 1987/88	Bittern	6.60	Giza 164	6.00	0.60	0.10	0.58
D 1987/88	Bittern	5.24	Giza 164	5.03	0.21	0.04	0.40
A 1988/1989	Sohag 2	8.04	Giza 164	7.19	0.85	0.12	0.79
B 1988/1989	Sohag 2	7.53	Giza 164	7.27	0.26	0.04	1.11
D 1988/1989	Sohag 2	6.33	Giza 164	7.73	-1.4	-0.18	0.34
A 1989/1990	Beni Sueif 1	8.53	Giza 164	7.69	0.84	0	
A(1) 1989/1990	Beni Sueif 1	8.68	Giza 164	6.98	1.7	0.24	
B 1989/1990	Sohag 2	6.76	Giza 164	7.39	-0.63	-0.09	0.51
D 1989/1990	Beni Sueif 1	8.55	Giza 164	7.40	1.15	0.16	0.76
A 1990/1991	Sohag 3	6.94	Giza 164	6.31	0.63	0.1	0.66
B 1990/1991	Sohag 1	728	Giza 164	6.29	0.99	0.16	0.85
D 1990/1991	Beni Sueif 1	7.24	Giza 164	6.72	0.52	0.08	0.59
A 1991/1992	Sohag 2	8.10	Giza 164	7.58	0.52	0.07	0.63
B 1991/1992	Sohag 3	9.12	Giza 164	8.58	0.54	0.06	1.07
D 1991/1992	Beni Sueif	9.34	Giza 164	8.01	1.33	0.17	0.23
A 1992/1993	Sohag 2	8.01	Giza 164				0.2
B 1992/1993	Sohag 2	8.44	Giza 164				0.32
D 1992/1993	Beni Sueif	8.32	Giza 164				
A 1993/1994	Beni Sueif	8.47	Giza 164	6.85	1.62	0.24	0.29
B 1993/1994	Beni Sueif	6.60	Giza 164	7.07	-0.47	-0.07	0.23
D 1993/1994	Beni Sueif	7.56	Giza 164	7.04	0.52	0.07	0.28

Table 6. Performance (t/ha) of Egyptian durum wheat varieties in yield trials conducted under different environments (1988/89–1993/94).

	Sohag 1	Sohag 2	Sohag 3	Beni Sueif 1	Beni Sueif 2	Beni Sueif 3	LSD
A 1988/1989	7.85	8.04	7.28	6.03			
B 1988/1989	6.37	7.53	7.27	6.95			
D 1988/1989	6.17	6.33	6.40	6.14			
E 1988/1989	6.91	6.65	6.40	6.77			
A 1989/1990	7.19	7.21	7.23	8.53			
B 1989/1990	6.19	6.76	6.88	6.73	6.80		
D 1989/1990	8.05	8.23	8.09	8.55	8.03	6.75	
A 1990/1991	5.77	6.85	6.83	7.05			
B 1990/1991	7.28	6.33	7.03	7.10	7.28		
D 1990/1991	6.38	6.99	7.15	7.24		7.46	
A 1991/1992	7.61	8.10	7.94	7.90			
B 1991/1992	7.61	7.83	8.26	8.08			
D 1991/1992	8.13	8.91	8.90	9.34	8.67	9.90	
A 1992/1993	8.01	8.17	7.94	7.59			
B 1992/1993	7.90	8.08	8.43	7.99			
D 1992/1993	6.51	6.99	6.60	6.71	6.06	6.03	
D 1993/1994	4.86	5.23	5.94	6.60	5.90	5.96	0.31

Production Constraints Resolvable through Plant Breeding

In Egypt, most of the constraints of durum production are a consequence of cultivation under marginal conditions (Upper and Middle Egypt, the irrigated desert, and rainfed areas) characterized by drought and heat stresses, unbalance in soil fertility, and salinity. Terminal heat stress and water scarcity during grain filling are the most common abiotic stresses and have substantial effects on both yield and quality stability. Low tillering capacity is another constraint to maximum grain yield. Durum is frequently attacked by the three common rusts, powdery mildew, aphids, and BYDV. These constraints require a research plan which includes the following:

1. A variety adapted especially for drought and heat stress.
2. Plant types with appropriate rates of maturity.
3. More efficient fertilizer and water use.
4. High grain quality.
5. Increased biomass, harvest index, and related yield components.
6. Disease (rusts and BYDV) and insect (aphids) resistance.

Physical and Human Resources

Physical Resources

The wheat program is implemented through the wheat research section, Field Crops Research Institute, ARC. The headquarters are in Giza, where the rust greenhouse, aphid lab, and cytogenetic lab are located. This program is involved in interdisciplinary work with other institutes such as: Plant Pathology, Soil and Water, Plant Protection, and Food Technology. Collaboration with extension and seed multiplication agencies is also ongoing.

The program has five research stations: the Giza headquarters, Sakha and Gemmeiza for the Delta, and Sids and Shandweel for Middle and Upper Egypt, respectively. Supplementary research activities are carried out in Kom Ombo, Mattana, and the New Valley in Upper Egypt; Mallawy and Fayoum in Middle Egypt; Nubaria, El Kasr, and Sinai for the rainfed areas; and Ismailia and El Serw for the New Lands. About 30 on-farm sites are an integral part of the program.

Each of the four main stations consists of about 40 hectares. About 2,000 ha are used for basic seed increase. Durum research is concentrated at the Sids, Shandweel, and Mattana Research Stations. Rainfed research for durum is located at Nubaria, Matrouh, and Sinai.

Human Resources

The Wheat Research Section includes about 80 scientists and technical assistants, among whom about 25 are PhD holders. Among the PhD holders, only five concentrate on durum improvement.

Network Activities

The program collaborates with CIMMYT and ICARDA for the exchange of materials, information, and staff education and training. However, collaboration needs to be extended to other North African countries, Turkey, and Syria. The Nile Valley Research Program has organized an excellent network involving Nile Valley countries in several activities such as rust, aphid, BYDV, WUE, heat stress, and socioeconomic studies. This could be expanded to include a durum network managed through ICARDA in collaboration with CIMMYT. This is an effective and attractive means of smooth collaboration with equity among participating countries. It would also help the information flow and interaction among countries.

Current Breeding Efforts

- Breeding for water-use efficiency (Nubaria, Ismailia, and Shandweel).
- Breeding for heat tolerance (Shandweel, Mattana, Kom Ombo, New Valley).
- Breeding for high yield and quality (Sids, Shandweel, Nubaria).
- Breeding for disease and aphids (Giza, Shandweel, Sakha).
- Breeding for biomass and yield components (Sids, Gemmeiza, Nubaria).
- Breeding for adaptability under rainfed conditions (Nubaria, Matrouh, Sinai).

Because of the tetraploid nature and narrow available genetic variability in improved cultivars, improving durum is not an easy task. However, tremendous effort has been exerted to develop the crop. In general, research focuses on maintaining yield stability. The yield stability of the improved varieties must be increased by incorporating resistance to biotic and abiotic stresses.

Yield Stability through Disease and Insect Resistance

The most common diseases in Egypt are the three fungi which cause stem, leaf, and stripe rusts. Aphids cause severe economic losses, especially in terms of the transmission of barley yellow dwarf virus. Varietal resistance to these biotic stresses is essential for yield stability. Efforts have been made to develop germplasm with better tolerance to disease. Donors with the requisite level of resistance have been identified and used in resistance breeding. Data about physiological race identification, virulence analysis, epidemiology, and yield losses due to rust have been compiled. A series of surveys was carried out in Egypt, Sudan, Ethiopia, and Yemen to pool activities and to facilitate collaboration with international centers such as CIMMYT, ICARDA, USDA, and FAO. The surveys identified four stem rust resistance genes (Sr5, Sr9d, Sr26 and Sr36 [Tt-1]) and two leaf rust resistant genes (Lr23 and Lr24), which are common to the region.

Studies on population dynamics of aphids and their natural enemies in Upper and Middle Egypt found that the highest population of aphids and other parasites occurs in March, while that of predators occurs in April. Screening of wheat lines against an aphid (*Rhopalosiphum padi*)infestation found one resistant and three moderately-resistant lines with high yield potential under aphid infestation. Screening for resistance against *Schizaphis graminum* in some wheat lines and *Aegilops* species revealed 13 resistant wheat lines and four *Aegilops* accessions. Screening for BYDV resistance using the ELISA technique combined with yield loss estimates found twelve tolerant wheat lines.

Breeding for Water and Nitrogen-Use Efficiency

Increasing productivity per unit of both water and nitrogen is vital under marginal conditions. Research has been oriented toward identifying germplasm which is an efficient user of water and fertilizer. Research to increase productivity per unit of nitrogen will give parallel improvement in productivity per unit of water.

Modification of Plant Type

Since the green revolution, the modification of plant types has resulted in quantum jumps in the yield potential of field crops. Yield is a function of total dry matter and harvest index. Therefore, yield can be increased by increasing either the total biomass and/or the harvest index and related yield components. The low tillering capacity of durum and the pleiotropic effects associated with low tillering in cereal could substantially increase grain. Moreover, in low-tillering genotypes, the development of unproductive tillers is suppressed.

Developing improved varieties with shorter growth duration is essential. Such genotypes could be useful in avoiding major stresses during grain filling, and are good for crop intensification. Therefore, genotypes that are not photoperiod sensitive should be selected.

Conclusion

The area planted to wheat increased from about 500,000 ha in the mid 1980s to 1 million ha in 1994. Average grain yield increased from 3.7 to 5.3 t/ha in 1992. Wheat production rose from 1.8 to4.6 million t.

In the rainfed areas, durum has proved its superiority, as has bread wheat. In 1994, about 65,000 ha were planted to wheat under rainfed conditions. This can be increased to 100,000 ha. Several studies show that durum yield could reach 1–2 t/ha under rainfed conditions.

In conclusion, despite current progress in durum improvement, further efforts are needed to make use of the full potential of durums in Egypt, especially under rainfed conditions. In this connection, collaboration needs to be extended to include other North African countries, Turkey, and Syria for the enhancement of germplasm and information exchange.

Appendix 1. List of the wheat cultivars released by the Wheat Research Department during the last 70 years.

Cultivar	Crosses and pedigrees	Year	Yield (t/ha)
	Bread wheat		
Hindi D	Selected variety	1920–1950	1.0
Hindi 62	Selected variety	1920–1950	1.2
Mabrouk	Selected variety	1920–1950	1.2
Mokhtar	Selected variety	1920–1950	1.3
Giza 135	Selected variety	1920–1950	1.5
Tosson	Selected variety	1920–1950	1.7
Giza 139	Hindi 90/Kenya B 256	1947	2.2
Giza 144	Regent/2* Giza 139	1958	2.6
Giza 145	Hindi 62/Mokhtar	1958	2.4
Giza 146	Hindi 62/Mokhtar	1958	2.4
Giza 147	Hindi D/New Thatcher	1958	3.0
Giza 148	Regent/2* Mokhtar	1959	3.2
Giza 150	Mida-Cadet/2* Giza 139	1960	3.3
Giza 155	Regent/2* Giza 139// Mida-Cedat/2* Hindi 62	1968	4.0
Giza 156	RioNegro/2*Menatane//Kenya/3/2* Giza 135/Line 950	1972	4.2
Super x	Penjamo "S"/GB 55	1972	6.1
Mexipak 69	Penjamo "S"/GB 55	1972	5.8
Chenab 70	C.271//Willet dwarf/Sonora 64	1973	5.0
Giza 157	Giza 155//Pit 62 LR64/3/LR64/3/Tzpp/Knott	1977	5.1
Giza 158	Giza 156/7C	1977	4.4
Sakha 8	Indus/Norteno "S" PK 3418	1977	4.7
Sakha 61	Inia/RL 4220//7C/Yr "S"	1980	5.6
Sakha 69	Inia/RL 4220//7C/Yr "S"	1980	6.0
Giza 160	Chenab 70/G155	1982	5.3
Sakha 92	Napo 63/Inia66//Wern "S"	1987	5.7
Giza 162	Vcm//Cno67 "S"/7C/3/Kal/Bb	1987	6.0
Giza 163	T.aestivum/Bon//Cno/7C	1987	6.3
Giza 164	Kvz/Buha "S"//Kal/Bb	1987	5.3
Giza 165	B.W Cno/Mfd//Man "S"	1991	6.3
Gemmeiza 1	Maya 74 "S"/On//1160-147/3/ Bb/Gall/4/Chat "S"	1991	6.5
	Durum wheat		
Baladi 116 (*T.pyramidale*)	Selected local variety	1921–1950	2.3
Dakar 49	Selected local variety	1921–1950	2.4
Dakar 52	Selected local variety	1921–1950	2.5
Sohag 1	Gdo vz469/Jo "S"//61.130-Lds	1977	6.7
Sohag 2	Cr "S"/Pelicano//Cr "S"/G "S"	1987	6.6
Beni Sueif 1	Jo "S"/AA "S"//Fg"S"	1987	5.9
Sohag 3	D.W Mexi "S"/Mgh/51792//Durum 6	1987	6.0

Durum Breeding in France

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Abstract

High prices and increasing durum pasta consumption in Europe have had a positive effect on durum area, production, grain quality, processing industries, and export in France. However, the drastic reduction of both prices and financial subsidies in 1992, as a result of the European Agricultural Policy (EAP), has limited durum areas to the southern part of the country, where 64% of the durum is harvested. This area is characterized by moderate drought stress. The grain yield of durum in France is about 20% lower than that of bread wheat. Consumers now prefer easy-to-prepare and easy-to-store pasta, precooked couscous products, and the healthy, slow-to-metabolize carbohydrates of durum products. Average consumption of pasta and couscous products is about 7 kg/person per year. Quality research deals mainly with: (1) reducing brown endosperm coloration; (2) stabilizing kernel protein content at about 13.5% and eliminating low molecular weight glutenin subunit 1 genotypes, related to weak gluten; (3) reducing blackpoint affecting pericarp leading to black specks in pasta products; (4) reducing kernels affected by yellow berry, related to low semolina yield; and (5) maintaining the high level of yellow pigment obtained in French cultivars. The semolina and pasta industries are demanding: (1) durum grain with high durum protein content—a desirable trait when high temperature drying processing is used; and (2) durum varieties with alleles coding for durum storage protein units (45 gliadin and LMW 2 glutenin), related to cooked-pasta quality. In addition to the relation between LMW 2 glutenin and cooked-pasta quality, Glu A1 HMW 1 has now been incorporated into the breeding material. The potential positive effects of other alleles are under investigation. The SDS sedimentation test is a simple and rapid technique. However, it depends on protein content, vitreousness, and milling apparatus. Milling kernels into smaller particles induces higher SDS sedimentation ratings. Farinograph evaluation parameters are under study to obtain a better prediction of cooked-pasta quality. The viscoelastograph apparatus is also used on dried and cooked pasta, while a method derived from the alveograph is used on cooked fresh pasta. The French varieties Minodur and Armet have high values for yellow color parameters. Most of the landraces and varieties from Northern Africa, Italy, and the Iberian Peninsula are low in carotenoid content (2–5 ppm). The varieties and landraces from the former USSR, USA, and Canada are much better (about 8 ppm). Recent, promising breeding lines are nearing 15 ppm. Studies on carotenoids and brown endosperm components (peroxydases and polyphenoloxydase) are given high priority. Blackpoint of pericarp can be obtained under water-soaked, abiotic conditions in relation with cell wall or membrane damage and decompartimentation. Leaf rust, fusarium, powdery mildew, septoria, soil-borne wheat mosaic virus, and *Heterodera avenae* nematode are the main diseases. Fusarium affects the plant from seedling stage to maturity, and there is no reliable source of tolerance. Special seed dressings are necessary for better control of plant populations. About half of the French varieties are susceptible to the various leaf rust pathotypes. In the southeastern, typically Mediterranean, region, pathotypes are more specialized on durum than on bread wheat. Resistance bred in southeastern France (Ardente, Ixos, and Duriac varieties) are relatively satisfactory throughout the country. The foreign material is generally susceptible to local pathotypes. Tolerance to water stress during heading was found to be lower for durum than for bread wheat; in contrast, good tolerance during grain filling was observed, due to the ability to yield large vitreous seeds (related to large awns) under such conditions. Recent results suggest that osmotic adjustment during the cycle is related to yield stability. There is much more variability for tillering ability in durum landraces and related tetraploid species than in commercial varieties. Durum frost resistance is low despite the recent advances of French varieties (Télédur, Durelle). Populations segregating for genetic male sterility, developed from the original durum populations of western plant breeders, are under study for aegilops and tetraploid wheat introgression. The introgression of broad-based interspecific and intergeneric variability necessitates the use of genetic and molecular markers to recover the desired traits in the durum background.

Introduction

Durum production in France started more than 30 years ago. The relatively attractive minimum prices of the European Community and increasing durum pasta consumption in Northern Europe stimulated durum crop development, which peaked at 480,000 ha and 2.4 million t in 1991. This development was made easier by the improvement of French durum quality, which played a role in increased transformation and export of durum and semolina towards Northern Europe and Italy. The radical change of the European Agricultural Policy in 1992 has completely changed the amount and regional distribution of French durum, causing a drastic reduction of wheat prices and financial subsidies involving a maximum of 180,000 ha, limited to the south of the country. The consequences on French durum acreage and production were immediate: in 1994, 228,000 ha were sown and 1 million t harvested, compared with 4.3 million ha and 29 million t for bread wheat. Europe became an overall importer of durum, particularly from Canada. In 1994, 64% of French durum production was under Mediterranean rainfed conditions with moderate drought stress (more than 600 mm of precipitation). Some 46,700 ha were grown in the central region under rainfed but more intensive conditions. The genetic production potential of durum in France is considered to be about 20% lower than for bread wheat (Kaan 1993). This gap will not be easily lessened, due to genetic progress in bread wheat. Various genetic factors relate to this handicap, since—in addition to its tetraploid nature—the narrow basis of current durum genetic variability will not be easily overcome.

Durum consumption is slowly growing, due to consumer changes in favor of easy-to-prepare pasta and precooked couscous products. The importance of slowly metabolized sugars in durum products for the modern diet is now taken into account by consumers. Moreover, pasta products are easy-to-store and relatively cheap. Average consumption of pasta and couscous products is about 7 kg/person/year. More than 500,000 t of durum wheat are milled each year in France, producing 450,000 t of semolina. Some semolina is exported. The French pasta industry produces about 280,000 t/year, which are completed by 100,000 t of principally Italian pasta products. France is an important durum and semolina exporter, although the country does, however, import pasta products.

Durum grain transformation is mainly for semolina production and pasta making. Two leading groups (Danone-BSN and Skalli-Rivoire et Carret) have for many years controlled the industry, handling more than 60% of the semolina and pasta. The CRECERPAL laboratory of Marseilles (Danone-BSN) performs quality control and research analysis for the breeding programs. The technical institute of French cereal growers conducts trials on agronomic practices and varieties, with emphasis on the relation between plant production and environment.

The public research institution, Institut National de la Recherche Agronomique, works on genetic and breeding populations of durum in a cereal research group. Work on the physiological traits of adaptation to stress, and on the technology and biotechnology of durum quality, is carried out mainly in the Montpellier center. Six breeding companies (Benoist, Desprez, GAE, RAGT, Semences de Provence, and Verneuil) have working on durum for more than 10 years. These institutions have been grouped since 1983 in the GIE Blé dur association. With the help of the Ministry of Agriculture, they conduct research on germplasm, advanced lines, agrophysiology, and quality criteria methodology. The target is the development of quality durum varieties adapted to French environmental conditions.

Breeding Objectives

A general agreement between the seed industry, the growers, and the processing industry has set the following breeding priorities.

Quality

- Reduce brown endosperm coloration.
- Stabilize kernel protein content around 13.5% and eliminate low molecular weight glutenin subunit 1 genotypes with weak gluten.
- Reduce blackpoint affecting pericarp leading to black specks in pasta products.
- Reduce yellow berry, related to low semolina yield.
- Maintain the high level of yellow pigments obtained in French cultivars.

Disease Resistance

- Leaf rust.
- Fusarium, powdery mildew, and septoria.
- Soil-borne wheat mosaic virus and *Heterodera avenae* nematode.

Yield Stability Factors

- Water stress tolerance and related yield components.
- Cold stress tolerance.

State of Durum Breeding in France

Breeding for Quality

Quality is a prerequisite for durum production. If quality is not present the lower production cost and higher yield of bread wheat could lead to the substitution of bread wheat and a preference for better-quality durum imports. This actually happened after the introduction of the lower-quality variety in the 1970s, which was rejected by the industry after a rapid increase in acreage. The semolina and pasta industries are clearly asking that the quality standards for pure durum pasta products, defined legally in France in 1934, be followed.

Protein Content

Protein content is directly related to pasta quality. Moreover, a stable phenotypic and possibly genotypic negative correlation between protein content and yellow berry (soft, starchy endosperm) related to low semolina yield has been observed. However, this trait is difficult to breed due to various factors. Research shows that high durum wheat protein content is not a stable characteristic. Many studies indicate, conversely, that protein content is a complex genetic trait governed by polygenic quantitative factors. There is no complete agreement between industry, breeders, and growers about protein content priority. Due to the development of high-temperature drying processes, the pasta industry is presently asking that protein content receive more emphasis than protein quality. Some breeders and growers consider that increasing protein content is expensive because of the negative correlations with yield, fertilizer cost, and pollution. Genetic advances in protein quality could be easy to obtain and compensate for relatively low protein content. Much depends on the comparative rates of genetic advances.

Protein Quality

Investigation and use of alleles coding for storage protein in durum germplasm is carried out to improve cooked-pasta quality. The first correlation between a storage protein unit (γ-gliadin 45) and wheat quality was discovered at INRA (Damidaux et al. 1978) in durum (INRA and GIE Blé dur). All varieties presently grown in France have the 45 gliadin LMW 2 glutenin subunit type. Other rare alleles are also related to pasta quality. Convergent results indicate that the SDS sedimentation test can be improved using these newly introduced, rare alleles (Branlard et al. 1989) from genetic resources. An example is Glu A1 HMW 1 (Kaan et al. 1993; Ciaffi et al. 1991; Blanco and de Giovanni 1995), which is now incorporated into the breeding material. Potential positive effects of other alleles are under investigation. The SDS sedimentation test is a simple and rapid technique (Quick and Donnelly 1980; Dexter and Matsuo 1980). It is somewhat dependent on protein content, and possibly on the vitreousness of the kernels and the milling apparatus. Milling kernels into smaller particles could induce higher SDS sedimentation rates. However, good reproducibility of SDS rates compared with protein was found. Farinograph evaluation parameters are under study to improve prediction of cooked-pasta quality for the breeder. The present French registration system uses the viscoelastograph apparatus on dried- and cooked-pasta samples. This excellent method is not usable in breeding laboratories, and the French pasta industry laboratory is using a method derived from the alveograph on cooked-fresh-pasta samples.

Product Color

Research has been very fruitful in terms of scientific advance and varietal improvement. Some recent French varieties (Minodur, Armet) are outstanding for yellow color parameters, and French high yellow endosperm is grown in other European countries. Landraces and other varieties from Northern Africa, Italy, and the Iberian Peninsula are typically low in carotenoid (2–5 ppm, AACC). The varieties and landraces from the former USSR, the USA, and Canada are much better (about 8 ppm). Recent, promising breeding lines are nearing 15 ppm. Carotenoids, brown endosperm components (peroxydases and polyphenoloxydase), are under study in public and private sector breeding programs. Blackpoint of pericarp can be obtained under water-soaked, abiotic conditions in relation with cell wall or membrane damage and decompartimentation. (Kaan et al. 1993, 1995; Régnier 1994).

Breeding for Disease Resistance

Fusarium affects plants from seedling to maturity. Special seed dressings are necessary for better control of plant populations. We have no reliable sources of tolerance. Powdery mildew and *Septoria tritici* are also important. The worst epidemics are related to brown rust disease in all regions. About half the French varieties are susceptible to the known pathotypes. From the breeder's point of view, there are three main regions of pathotype reaction in France, based on multilocal and pluriannual evaluation networks. In the central region, the prevalent pathotypes are virulent on some bread wheat genotypes and less so on durum. In the southeast, some pathotypes are more specific to durum and less aggressive on bread wheat. Other pathotypes may occur in the southwest. Resistance bred in southeastern France (Ardente, Ixos, and Duriac varieties) has been relatively satisfactory throughout the country for some years. Foreign material is generally susceptible to local pathotypes.

Yield Component Breeding

Tillering of French and Mediterranean varieties is inferior to standard bread-wheat cultivars. This trait is partially compensated by superior kernel weight. Spike fertility is comparable (Kaan 1993; ITCF technical institute results). Tillering ability among genetic resources for durum and neighbor tetraploid species varies.

Breeding for Abiotic-Stress Tolerance

Despite recent advances in French durum varieties (Télédur and Durelle), frost resistance is inferior to standard bread wheats. This problem is, however, limited to the central region. Water stress tolerance during heading in durum is inferior to bread wheat (INRA and ITCF studies). Durum displays better resistance to water stress during grain filling than other cereals, with the characteristic ability to yield large vitreous seeds under such conditions. This may be related to awn features. Recently, extensive physiological and genetic work has been carried out in collaboration with Mediterranean institutes. Studies on morphological, physiological, and biochemical stress-adaptation factors have been led by INRA, Montpellier. Recent results suggest that osmotic adjustment during the growing cycle could be related to attaining the desired trait, i.e. yield stability.

New Breeding Tools and Prospects of Genetic Advances

Interspecific and Intergeneric Crosses and Broad-based Populations

The development of *in vitro* culture methods offers new possibilities for wide crosses. Our unit is starting to build up introgressed populations with new disease, stress tolerance, and other quality factors. Populations segregating for genetic male sterility are being developed using original durum populations from western plant breeders. They are presently being studied for ægilops and tetraploid wheat introgression. After a recombination period, the introgression of broad-based interspecific and intergeneric variability requires the use of genetic and molecular markers in order to recover the desired traits (marker-assisted breeding).

Intergeneric and Wide Crosses for Gynogenesis and Haplodiploidization

Much progress has recently been made in various laboratories and in France. This progress is reviewed by Coumans and Fernandez (this volume) and Coumans et al. (this volume).

Drought Stress

The analysis of physiological–genetical traits related to stress adaptation is carried out in cooperation with other Mediterranean institutes and scientists (Monneveux et al. 1995; Rekika and El Hakimi et al. 1995).

Conclusion

More than ever, the development of biotechnological tools such as cell biology and *in vitro* culture techniques, molecular markers, and transformation applied to durum breeding, requires an efficient network. French researchers and the seed and pasta industry want to increase cooperation for various reasons, some of which are trivial:

- It is presently difficult to raise support for cereal research from the European Union due to past overproduction.
- Private breeder activity is dependent on certified seed.

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Spanish Durum Breeding Program

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Abstract

Durum is cultivated on about 0.6 million ha in Spain. This is a drastic increase from the 1990 area, and is due to European Community subsidies for traditional growing areas. Durum is cultivated in Spain in two main zones: the southern (Andalucía and Extremadura) and northern (Aragón). The varietal structure of this species has changed over time. Mexa, which was cultivated on more than 80% of the area during the mid-1980s, has been replaced by Vitrón and Jabato in the south, and by Antón in the north. More than 700 local landraces of *Triticum turgidum* subsp. *durum* and subsp. *turgidum*, Spanish forms of *T. turgidum* subsp. *dicoccon* and even some *T. turgidum* subsp. *polonicum* were evaluated for morphological, agronomical, and grain quality. The biochemical characterization included eletrophoretic analysis of different isoenzymatic systems and the study of the composition of seed storage proteins. The association between γ-gliadin and high- and low molecular weight glutenin subunits (HMW and LMW), and gluten strength was analyzed. Linkage relationships and genetic distances between the different prolamin genes were determined. Moreover, the effect on gluten strength of gliadin genes and low relative molecular mass (Mr) glutenin subunit genes were studied. Breeding programs are run in Spain by public institutions and private companies. As the country is divided into autonomous regions, separate durum breeding programs are currently conducted by groups at Lleida, Madrid, Badajoz, Cadiz, and Zaragoza. All have incorporated wheat from ICARDA/CIMMYT in their programs, and since this germplasm generally shows very good adaptation, some groups use these materials exclusively. Spanish durum landraces were included in crossing blocks to increase genetic diversity. A small durum breeding program focusing on quality is being carried out at the School of Agriculture at the Polytechnic University in Madrid. Further, there are more than 14 private seed companies producing durum varieties in Spain. The aim of most of these breeding programs is to obtain high-yielding varieties adapted to the common climatic stresses of the growing areas. The southern zone of Spain has a Mediterranean climate, with an average annual precipitation of 450 mm, usually distributed evenly throughout the growing season. Germplasm from ICARDA/CIMMYT is well adapted to this area, where terminal drought stress is the main climatic constraint. The northern zone (Ebro Valley) has a more continental climate, with lower

temperatures during winter and spring, and very high temperatures and water stress during grain filling. Rainfall is lower (200–300 mm) and less evenly distributed. Late-heading varieties are better adapted to northern growing conditions. Resistance to frost, high tillering capacity, and short grain-filling period are suitable traits for incorporation into breeding material to avoid major drought stress. Breeding for resistance to the most important diseases (septoria, stripe rust, and powdery mildew in the south, and BYDV in the north), is considered in selection schemes. Environmental conditions greatly affect yield, test weight, and protein content. On the other hand, the genetic component is important for the SDS sedimentation test and yellow pigment. Vitreousness showed an important variation due to site × zone interactions. The low magnitude of interaction over years, and the different response of some sites in the same northern or southern zone indicates that vitreousness may be improved by the use of specific agronomical techniques (fertilization, irrigation, etc.). Using independent mean and standard deviations of the control varieties Roqueño and Mexa every year showed that most lines yielded equal to or less than the controls.

Introduction

Durum is cultivated on about 0.6 million ha in Spain. The area devoted to this crop has drastically increased since 1990, due to European Community subsidies for traditional growing areas. Durum is cultivated in Spain in two main zones: southern (Andalucía and Extremadura), and northern (Aragón).

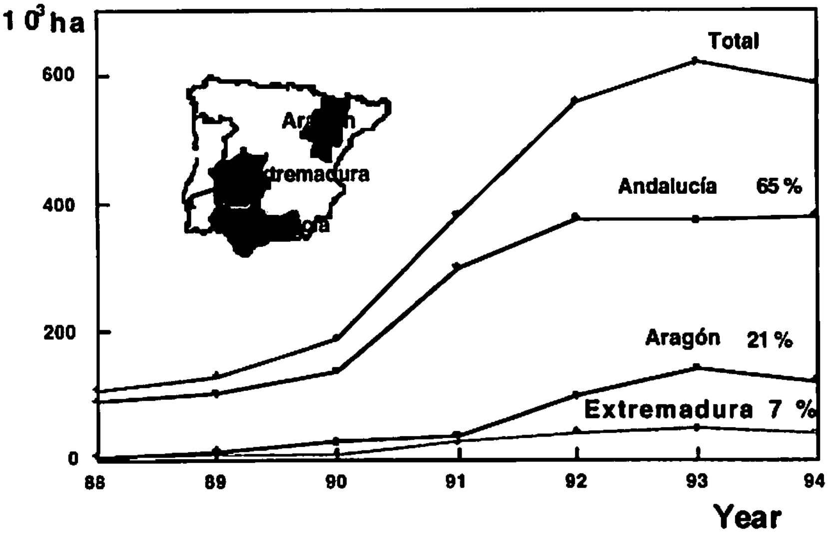


Figure 1. Evolution of the durum area in Spain (source: MAPA).

Table 1 shows the area, production, and yield of durum wheat in 1994. The varietal structure of this species has changed over time. Mexa, which was cultivated on more than 80% of the area during the mid-1980s, has been replaced by Vitrón and Jabato in the south, and by Antón in the north (Juan-Aracil and Michelena 1993). Figure 2 shows the evolution of durum wheat varieties in Spain.

Table 1. Area, production, and average durum yield in Spain in 1994.

	Rainfed	Percent	Irrigated	Total
Area (ha)	560,172	95	28,093	588,265
Production (t)	830,409	86	130,986	961,395
Yield (kg/ha)	1,482		4,662	1,634

Source: MAPA.

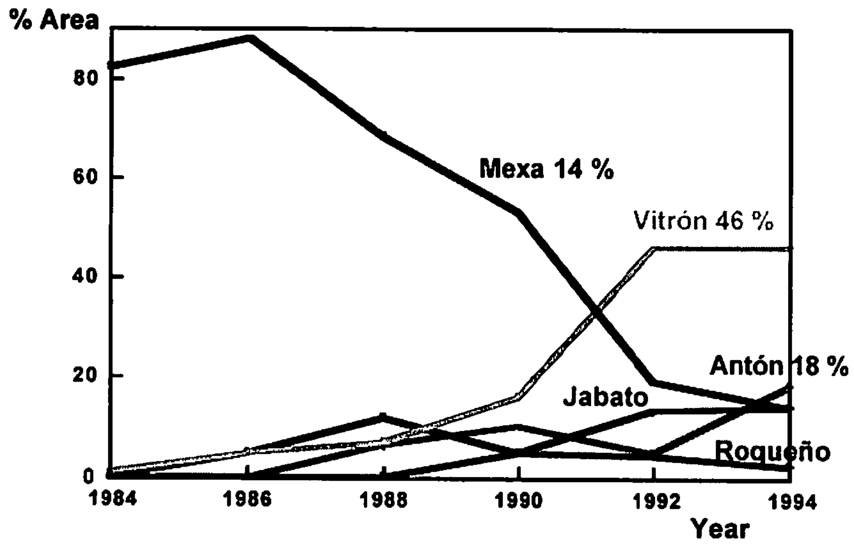


Figure 2. Evolution of durum wheat varieties in Spain.

Local Spanish Germplasm Evaluation

A research project coordinated by C. Soler has characterized the tetraploid wheats of the Spanish National Bank of Germplasm. More than 700 samples, including local landraces of *Triticum turgidum* subsp. *durum* and subsp. *turgidum*, Spanish forms of *T. turgidum* subsp. *dicoccon*, and even some *T. turgidum* subsp. *polonicum* were evaluated (taxonomic classification, Croston and Williams 1981).

Morphology, agronomy, and grain-quality were determined, and biochemical studies were carried out to assess variability within the collection and homogeneity of individual samples. The biochemical characterization included eletrophoretic analysis of isoenzymatic systems and the study of the composition of seed storage proteins: glutenins by SDS-PAGE, and gliadins by A-PAGE (Soler 1994).

Breeding Efforts

Breeding programs are run in Spain by public institutions and private companies. As the country is divided into autonomous regions, separate breeding programs are conducted in different regions. Groups at Lleida, Madrid, Badajoz, Cadiz, and Zaragoza are now working on durum. All have incorporated wheat from ICARDA/CIMMYT in their programs, and since this germplasm generally shows very good adaptation, some groups operate exclusively on these materials. Spanish durum landraces have been included in crossing blocks to increase genetic diversity. A small durum breeding program focusing on quality is being carried out by the School of Agriculture at the Polytechnic University in Madrid. There are more than 14 private seed companies producing durum varieties in Spain.

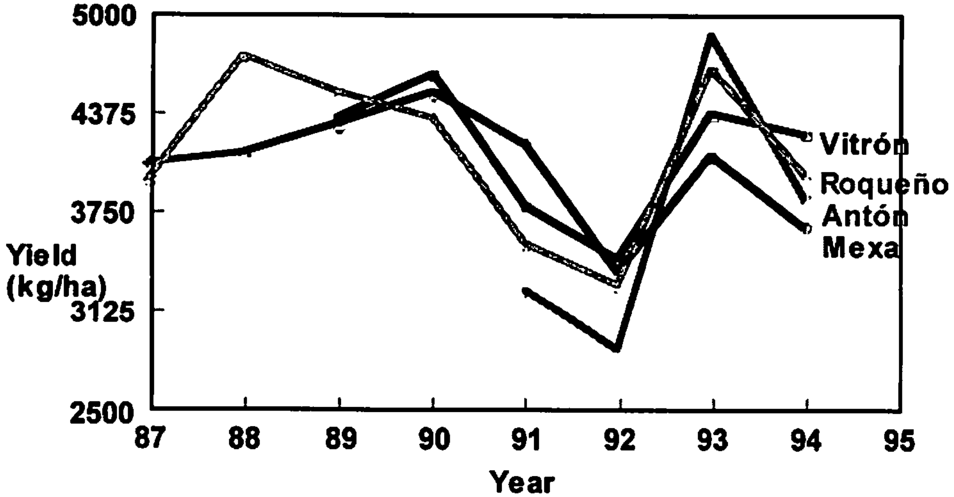
Breeding Goals

The aim of most of these breeding programs is to obtain high-yielding varieties adapted to the common climatic stresses of the growing areas, which are generally rainfed. The southern zone has a Mediterranean climate, with an average annual precipitation of 450 mm, usually evenly distributed throughout the growing season. ICARDA/CIMMYT germplasm is well adapted to this area, where terminal drought stress is the main climatic constraint. The northern zone (Ebro Valley) has a more continental climate, with lower temperatures during winter and spring, and very high temperatures and water stress during grain filling. Rainfall is lower (200–300 mm) and less evenly distributed. Late-heading varieties are better adapted to northern growing conditions. Resistance to frost, high tillering capacity, and short grain-filling period to avoid major drought stress are traits suitable for incorporation into breeding material.

Breeding for resistance to the most important diseases (septoria, stripe rust, and powdery mildew in the south, and BYDV in the north), is considered in selection schemes. Grain quality is discussed below.

Environmental Constraints

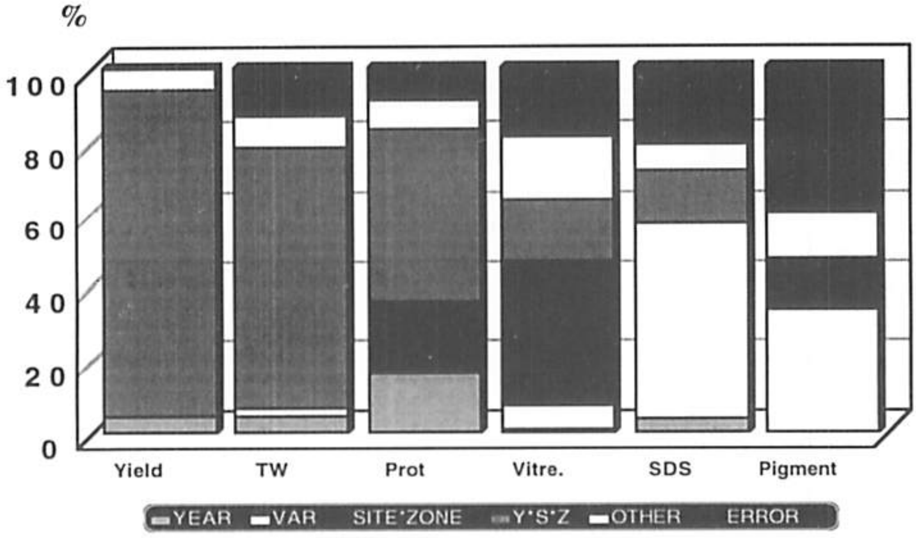
Drastic yield and grain-quality fluctuation is a consequence of seasonal climatic variations, i.e. low and uneven distribution of rainfall during the growing season. Figure 3 shows the average yield of control varieties from the National Variety Registration Trials, carried out from 1987 to 1994. Severe drought during the growing season and late rains in 1992 resulted in low yield, but also in low test weight, vitreousness, and falling number (Juan-Aracil and Michelena 1993). The development of rapid techniques for screening for drought resistance in a breeding program favors the development of varieties with more stable yields.



Source: INSPV

Figure 3. Average yield in official registration trials of the most cultivated varieties in Spain.

To analyze the causes of variation in durum yield and quality, official NVRT controls were used (Juan-Aracil and Michelena 1993). Ten sites were analyzed in 1991 and seven in 1992. REML variance components were estimated according to a mixed model in which zone was considered a fixed factor and year, site, and variety were considered random factors. Test weight, protein content, vitreousness, yellow pigments, and SDS sedimentation were determined by the Quality Laboratory of INSPV. Figure 4 shows the REML estimates of variance components for random factors. Yield, test weight, and protein content show an uncontrollable variation, because the main component of variance interactions was year. On the other hand, the genetic component was important for SDS sedimentation and yellow pigment. Vitreousness showed an important variation due to site x zone interaction. The low magnitude of the interaction involving year, and the different response of some sites belonging to the same northern or southern zone indicate that vitreousness may be improved by the use of specific agronomical techniques, such as fertilization, irrigation, etc. (Juan-Aracil and Michelena 1993).



Source: Juan-Aracil and Michelena, 1993

Figure 4. REML variance component estimates of yield and quality in durum wheat.

Quality

Quality is one of the main goals of durum breeding programs. Individual plants of early segregating generations are selected on the basis of seed size, vitreousness, and absence of grain disease. Grain test weight, 1,000 kernel weight, vitreousness, protein content, SDS sedimentation, and yellow pigment are common determinations in advanced materials. General surveys on quality (Table 2) of durum production have been available since 1990. Farmers, breeders, industry, and government are involved in this survey system. In 1994, a total of 169 samples (one sample for each 3,250 ha) were analyzed, covering 93% of the total area (AETC 1994).

The relationship between endosperm protein composition and pasta quality has been extensively investigated by the group from the School of Agriculture in Madrid. Associations between γ-gliadin and high and low molecular weight glutenin subunits (HMW and LMW, respectively), and gluten strength have been analyzed in various varieties and Spanish landraces (Carrillo et al. 1990, 1991). Linkage and genetic distance between the different prolamin genes have been determined (Ruiz and Carrillo 1993). The effect on gluten strength of gliadin genes and low relative molecular mass (Mr) glutenin subunit genes has also been studied (Ruiz and Carrillo 1995).

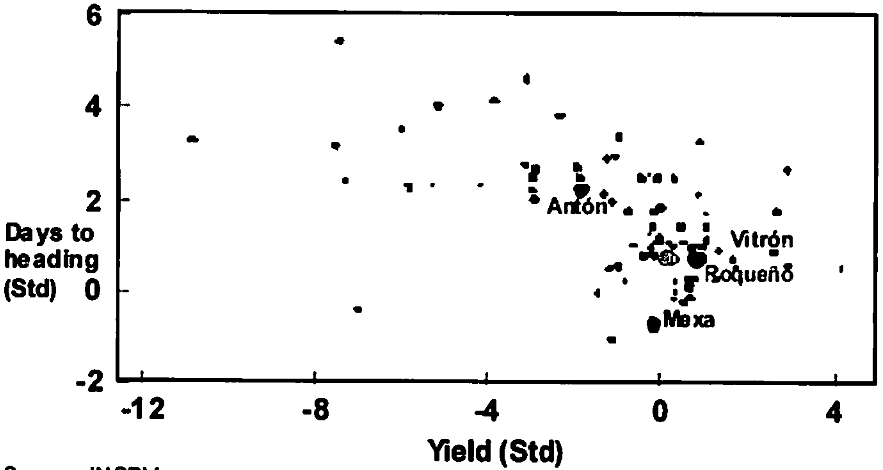
Table 2. Average quality of the most cultivated varieties in 1994.

	Variety		
	Vitrón	Antón	Mexa
Number of samples	57	32	27
Moisture (%)	9.1	9.2	8.9
Test weight (kg/hl)	80.7	78.2	78.4
TKW (g)	40.8	40.5	39.7
Protein (%)	14.8	14	15
Ash(%)	1.6	1.9	1.9
Vitreousness (%)	90	78	95
Yellow pigments (ppm)	7.5	8.5	8.4
SDS	26	24	30
Gluten index	30	7	55
Hagberg	397	372	369

Source: AETC.

Yield and Cycle Variation

More than 20 varieties are included every year in Spanish registration trials. In order to compare the candidates for release with the official controls, data from the multilocational trials of the NVRT are standardized by using the mean and standard deviation of the control varieties Roqueño and Mexa. Figure 5 shows the standardized values for yield and days-to-heading. High-yielding varieties are released, but most lines submitted yield equal to or less than the controls.



Source: INSPV

Figure 5. Standardized data of yield and days-to-heading in 55 official registration trials (1987–1994).

Only one variety had fewer days-to-heading than Mexa, the earliest check (118 days from sowing to heading). Many had a longer cycle than Antón (132 days-to-heading), the most frequently cultivated variety in the northern zone. Vitrón and Roqueño have an intermediate cycle length (about 122 days-to-heading).

Efforts have been undertaken by local governments to coordinate some of the activities developed by the different regions. Coordination at the scientific level must be improved to maximize the benefits of research.

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Impact of Modern Technologies on Durum Wheat Production in Syria

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Introduction

Wheat is the most important commodity food in Syria. Thanks to ongoing expansion of the cultivated area, the country was a wheat exporter until the 1950s. Since that time, increasing domestic demand unaccompanied by increases in area planted, and increased production of vegetables, fruits, and industrial crops at the expense of the wheat area, has meant that Syria no longer produces a surplus. Wheat and flour became the most important agricultural imports. The self-sufficiency rate for wheat was about 72% during 1985–1989.

Syria's agricultural policy pays special attention to wheat production, with the twin objectives of improving the living standard of producers and achieving wheat self-sufficiency. Planning concentrates on improving the productivity of the existing wheat land. Research is directed towards adapting modern high-yielding wheat varieties, chemical fertilizers, herbicides, and pest control measures suitable to Syrian conditions. Extension and credit institutions have been organized. Farmers are encouraged to mechanize production. The government has initiated various infrastructure projects to provide irrigation facilities. Wells are being dug by the government and the private sector.

Objectives of the Study

The Socioeconomic Department of the Directorate of Scientific Agricultural Research, Ministry of Agriculture and Agrarian Reform, in collaboration with the Farm Resource Management Program (FRMP) at ICARDA, conducted a multi-year study on the adoption and impact of improved wheat production technology. The study aimed to:

- Develop baseline measures for use in the adoption of improved wheat production technology among farmers in different agro-ecological zones.
- Describe adoption and impact levels of wheat technology according to the socioeconomic characteristics of the farmers and the farming systems.
- Assess reasons for adoption or non-adoption of particular technologies and factors contributing to performance gaps between research trial and on-farm

Primary data were collected through a field survey including nine provinces representing about 91% of the national wheat area.

There are many reasons why the national agricultural institutions, along with international and regional organizations concerned with agricultural research, focus their attention on adoption and impact studies. The efficiency and performance of new technology must be improved, links between institutions developing the technology and agricultural policy-makers must be improved, and the returns from investment in research and extension must be measured.

Most of the academic studies on technology adoption assume that the developed technology is appropriate and suitable to farmers. Such studies tend to focus on defining the characteristics of the farmers wishing to adopt such technology. However, a more complex method is desirable. In addition to descriptions of the adoption pattern, consideration should be given to whether or not the technology is suitable to the needs of the farmers. This type of study not only aims to make a certain technology successful, but to increase the effectiveness of research and extension by meeting the actual needs of the farmers.

Wheat Trends

National wheat area and production from 1973 to 1990 show the following trends:

- A general decline in the total area grown to wheat.
- Increased use of high-yielding varieties.
- Expansion in the area of irrigated wheat.
- A large fluctuation in total production from year to year.

Although there has been a clear improvement in irrigated wheat yield, rainfed yield is less easily interpreted. Adjustment for rainfall variation according to water-use efficiency indices shows no significant trend either upward or downward. However, high-yielding varieties performed better than local varieties under both rainfed and irrigated conditions.

Between 1991 and 1994 there was a clear increase in both wheat area and production in Syria. The total area cultivated to wheat increased by 16%, from 1,268,000 ha in 1991 to 1,474,000 ha in 1994. Fifty two percent of the area was irrigated, while only 2% was rainfed. Total wheat production increased from 2,350,000 to 3,664,000 t (56%). Average yield also increased during this period, from 1.7 t/ha in 1991 to 2.5 t/ha in 1994 (47%).

The growth of irrigated wheat in Syria was remarkable. The irrigated wheat area made up 9% of the total wheat area in 1973. It reached 20% by the late 1980s. Production of irrigated high-yielding varieties was about half of total production.

The irrigated wheat area increased from 369,000 ha in 1991 to 560,000 ha in 1994 (52%). It made up 38% of the total wheat area in 1994, while production represented 63% of the total (3.7 million t). There is no difference in Ministry of Agriculture statistics between full and supplemental irrigation.

High-yielding varieties accounted for about 8% of the total wheat area in 1973. Florence Aurore and Senator Capelli were among the first varieties introduced to Syria. They were latter joined by Jouri 69 and Mexipak, released prior to 1973. Florence Aurore and Senator Capelli have since been officially reclassified as local varieties. Between 1973 and 1987, nine new varieties were released, of which six were durum and three bread wheats. To a very limited degree, the varieties were targeted to different environments: zone 1, zone 2, full irrigation in any zone, or a combination (Table 1). Associated technologies, such as fertilizer, are based on zone rather than variety. Seed is multiplied by the General Organization for Seed Multiplication (GOSM), under contract to producers, and made available to farmers through governorate and local outlets. The seed is usually treated for seed-borne diseases and pests before sale.

Table 1. High-yielding durum varieties in Syria (1990).

Variety	Release date	Target environment	Experimental yield averages (various years)
Jori 69	1970	Irr. & zone 1	-
Gezira 17	1974	Irr. & zone 1	4.5 t/ha (irr.) 3.0 t/ha (rainfed)
Bohouth 1	1980	Irr. & zone 1	5.0 t/ha (irr.)
Cham 1	1983	Irr. & zone 1	4.5 t/ha (irr.)
ACSAD 65	1986	Zone 1 & 2	
Cham 3	1987	Zone 2	
Bohouth 5	1987	Irr. & zone 1	7.5 t/ha (irr.)

Source: FRMP Annual Report 1991.

Policy-makers and planners achieved their objective of having modern wheat technologies adopted by farmers. Mechanical tillage, planting, harvesting, use of high-yielding varieties, fertilizer application, and chemical weed and pest control have become common to most farmers. This is reflected in the rise of yield per unit area since 1991. Total wheat production now exceeds domestic need.

Survey Sample and General Characteristics

The survey sample was based on published wheat production statistics. Farmers were randomly selected using a multi-staged procedure according to stability zone and contribution to national wheat production. For the purpose of characterization and classification, the sample was grouped into five regions: (1) Western Region; (2) Al-Ghab Region; (3) Al-Jazirah Region; (4) Al-Furat Region; and (5) Hauran Region. Farmers were located in stability zones 1 and 2 (except for Al-Furat farmers, which use full irrigation from the Euphrates River). Data were obtained from the largest wheat plot, as experience shows this is the most accurate method.

The study lasted over three seasons, 1990/91 to 1992/93. A questionnaire was developed and 230 farmers were interviewed in each season. About 688 farmers were sampled over three seasons. Table 2 shows the distribution of the sample by region and farm size. In general, the Western Region demonstrates the expected distribution between small and medium-sized farms. Hauran farms tend to be neither very small nor very large, and Al-Jazirah farms are medium to large. In Al-Ghab and Al-Furat most farms are small, due to the recent history of agrarian reform, irrigation development, and settlement in the area.

Table 2. Sample distribution by region and farm size.

Farm Size	West Reg.	Al-Ghab	Al-Jazirah	Al-Furat	Hauran	Total
0–5 ha	46	31	19	21	4	21
	17%	75.6%	7.1%	50.0%	6.2%	17.6%
5–10 ha	64	3	42	17	13	139
	23.6%	7.3%	15.6%	40.5%	20.0%	20.2%
10–20 ha	87	3	60	2	21	173
	32.1%	7.3%	22.3%	4.8%	32.3%	25.1%
20–50 ha	58	3	78	2	23	164
	21.4%	7.3%	29.0%	4.8%	35.4%	23.6%
> 50 ha	16	1	70	0	4	91
	5.9%	2.4%	26.0%	6.2%	13.2%	
Total	271	41	269	42	65	688
	39.4%	6.0%	39.1%	6.1%	9.4%	100%

Some interesting comparisons emerge in land use and cropping system, according to region and farm size (Table 3). Hauran, for example, represents an area where wheat is grown traditionally, with large fallow areas and a wide range of crops. El-Jazirah is a main wheat-producing area, with some 81% of the arable land planted to wheat and barley. In addition to wheat, Al-Ghab and Al-Furat grow industrial crops such as cotton and sugar beet. The Western Region is largely similar to Hauran, but without much fallow. Other crops, mainly market vegetables, have been introduced to replace the fallow. The introduction of supplemental irrigation into the Western Region makes possible a more intensive land use than in Hauran.

Fifty five percent of the sample farmers have access to at least one source of irrigation, such as a canal, river, or well water. This figure is high compared with Syria as a whole. It is impossible to quantify any sample bias regarding access to irrigation, because there is no data at the national level on the number of farmers who have access to irrigation.

Three trends are noted. First, the irrigated wheat area is increasing, while there is a general decline in the total wheat area. Second, supplemental irrigation is more widespread than full irrigation. Third, most farmers do not choose the varieties they grow according to irrigation method. In other words, farmers in the irrigated area see no difference in varieties in terms of their response to irrigation.

Table 3. Land use (% of arable land averaged over three years).

	Average farm size (ha)	Wheat			Barley	Legume	Forage	Food	Tree	Other
		Durum	Bread	Total						
Region										
Western Region	19.2	20	5	25	27	11	1	9	16	12
Al-Ghab	7.8	54	2	56	1	1	1	0	37	4
Al-Jazirah	49.0	42	13	55	25	3	ns	ns	9	7
Al-Furat	7.2	36	25	61	1	ns	1	ns	36	1
Hauran	21.0	34	ns	34	5	21	4	5	11	20
Farm size										
0–5 ha	3.2	54	6	60	6	4	ns	2	25	2
5–10 ha	8.0	38	9	47	14	8	1	5	22	4
10–20 ha	15.4	31	6	37	23	9	2	6	17	6
20–50 ha	33.0	36	7	43	18	8	1	4	14	11
> 50 ha	118.6	37	13	50	29	4	ns	1	8	9
Mean of total	29.6	36	10	46	24	6	1	3	12	9

Table 4. Distribution of wheat by varieties (% of arable land).

	HYV durum varieties	HYV bread varieties	Local durum	Local bread
Region				
Western Region	63	18	18	1
Al-Ghab	96	4	0	0
Al-Jazirah	72	22	5	1
Al-Furat	58	42	0	0
Hauran	20	ns	80	0
Water Regime				
Full Irrigation	82	18	0	0
Supp. Irr.	88	10	2	0
Rainfed zone 1	58	32	11	0
Rainfed zone 2	66	15	16	2
Farm size				
0–5 ha	82	9	9	0
5–10 ha	70	19	11	0
10–20 ha	67	17	15	ns
20–50 ha	73	17	10	ns
> 50 ha	66	24	9	1
Total	68	21	10	1

HYV=High-yielding variety.

Since the statistics of the Ministry of Agriculture do not distinguish between durum wheat and bread wheat, the results of the survey have been relied upon to estimate the cultivated area for each type of wheat (Table 4). The area grown to high-yielding varieties of durum wheat was about 68%, high-yielding varieties of bread wheat about 21%, local varieties of durum wheat about 10%, and local varieties of bread wheat about 1%. There were clear differences in the distribution of each species according to geographical region, irrigation system, and farm size.

Given the importance of durum wheat in Syria and the relative advantage of its production, analysis is restricted to farmers who grow durum wheat. They make up about 85% of the total sample.

Recommended Package for Durum

Recommendations are made according to variety, and whether the cultivar is high-yielding or local. Such recommendations tend to increase inputs for high-yielding varieties. The package recommends six irrigations in Al-Furat and Hasakeh, one or two supplementary irrigations in the Western Region, two or three irrigations in zone 1, and four irrigations in zone 2 (the latter two in Dara province). Each irrigation equals about 750 m^3/ha.

Modern Technologies for Durum

Irrigation

Irrigation has seen a remarkable increase in Syria, doubling since 1973. The fully-irrigated wheat area expanded from 9% of total area in 1973 to 20% in the late 1980s.

This study provides clear evidence that, when given the opportunity and the means, farmers will adopt irrigation technologies because of the benefits they receive in terms of higher yield. The survey shows that about 85% of farmers with a water source irrigate their wheat crop or part of it. The rest, mostly from Al-Ghab, Hauran, and the Western Region, do not irrigate the wheat crop. Rather, they use their available water to increase yield of more valuable crops such as cotton and vegetables. Of the farmers who irrigate their wheat, 39% use full irrigation, 58% use supplemental irrigation, and 3% use both methods.

Traditional flooding was the dominant method with most farmers when irrigating their wheat. Less than 10%, mostly live in the Western Region, use sprinkler and supplemental irrigation. Most farmers increase more than the number of irrigations recommended.

Mechanization

Wheat producers use mechanization intensively: 98% of surveyed farmers use a tractor for pre-seeding tillage; 69% use some sort of machinery for seeding, and the rest rely on hand broadcasting (mostly small holders). About 88% use a combine to harvest wheat; hand harvesting was significant in Hauran as well as in small plots in Al-Furat, where mechanization is difficult. Table 5 shows the distribution of surveyed farmers by number of cultivations and harvesting method.

Table 5. Farmer practice: number of tillages and harvesting method (% of farmers).

	No. of tillages			Harvesting method	
	1	2	3	Combine	Manual
Region					
Western Region	15	56	29	88	12
Al-Ghab	0	61	39	100	0
Al-Jazirah	10	71	19	100	0
Al-Furat	18	65	17	68	32
Hauran	50	42	8	51	48
Irrigation Regime					
Full Irr.	10	69	21	91	9
Supp. Irr.	5	55	40	94	6
Rainfed zone 1	23	62	15	87	13
Rainfed zone 2	22	61	17	83	17
Total	16	61	23	88	12

Table 6. Farmer practice: fertilizer application (% of farmers).

	Phosphate	Nitrogen			
		No. of fertilizations	Only at planting	Only top dressing	Both
Region					
Western Region	87	5	11	25	59
Al-Ghab	45	0	16	7	77
Al-Jazirah	88	9	9	19	64
Furat	96	0	11	7	82
Hauran	87	18	54	10	19
Irrigation Regime					
Full Irr.	98	0	8	7	84
Supp. Irr.	91	4	10	14	72
Rainfed zone 1	80	5	11	31	53
Rainfed zone 2	80	17	29	17	38
Total	86	7	15	19	59

Fertilizer

About 86% of the farmers surveyed apply phosphorous on durum wheat. This rate is higher than 90% when irrigation is used, and 80% for rainfed agricultural. The regional distribution of non-phosphate users is fairly even, except in Al-Ghab, where 55% of the farmers do not use phosphate. This may be because available phosphorous in the soil was relatively high and the response to phosphorous slight.

Farmers use nitrogen fertilizer more than phosphorous. Only 7% of farmers, mostly from Hauran and zone 2, do not use nitrogen (Table 6). Fifty nine percent of the surveyed farmers use two applications, one at planting and one at tillering (most are farmers of irrigated wheat). Some of the farmers in zone 2 apply nitrogen in one lot, either at seeding or tillering.

Phosphate fertilizer rates are close to those recommended for irrigated areas and zone 2, and less than the recommended rate in zone 1. Farmer use of nitrogen fertilizer is slightly higher than recommended (Table 7). Al-Ghab farmers tend to increase nitrogen fertilizer above the recommended rates.

Table 7. Farmer practice: fertilizer rate (kg/ha).

	Average N	Average P_2O_5
Region		
Western Region	107 ± 71	75 ± 57
Al-Ghab	203 ± 61	53 ± 65
Al-Jazirah	100 ± 75	72 ± 47
Furat	180 ± 76	116 ± 52
Hauran	28 ± 18	35 ± 21
Irrigation regime		
Full Irr.	165 ± 78	105 ± 46
Supp. Irr.	152 ± 74	104 ± 60
Rainfed zone 1	89 ± 63	55 ± 42
Rainfed zone 2	45 ± 36	38 ± 28
Total	105 ± 79	70 ± 53

Herbicides

Herbicides have the lowest adoption rate of the major external inputs. Only about 42% of the farmers have used it at least once. The majority of non-users are in Al-Jazirah (84%), Al-Furat (68%), and Hauran (57%). Herbicides are most popular in Al-Ghab (87% use) and the Western Region (62% use).

Seed Rate

Table 8 summarizes the seed rates used by farmers and their seed sources according to region and method of irrigation. It is clear that farmers in all regions and under all irrigation methods use higher rates than those recommended.

Durum producers in Syria tend to rely on the General Organization of Seed Multiplication for seed. About two thirds of the sample report that GOSM is their only source of pure and dressed seed. This rate is higher under irrigation than under rainfed agriculture. The farmers in Al-Ghab, Jazirah, Al-Furat, and Hauran rely on GOSM more than those in the Western Region.

Table 8. Farmer practice: seed rate and sources.

	Seed rate (kg/ha)	Seed source			
		Own	Market	GVT	More than one source
Region					
Western Region	212 ± 69	22	13	52	14
Al-Ghab	298 ± 46	0	3	87	10
Jazirah	240 ± 82	14	4	73	9
Furat	277 ± 65	4	7	82	7
Hauran	162 ± 31	22	3	71	3
Irrigation regime					
Full Irr.	306 ± 73	5	2	83	10
Supp. Irr.	266 ± 67	6	8	72	13
Rainfed zone 1	206 ± 55	28	9	58	6
Rainfed zone 2	164 ± 32	20	9	60	11
Total	225 ± 77	17	7	66	10

Preceding Crop

The farmers were asked about the crop preceding wheat. The largest plot grown to wheat was used to define this crop. Table 9 shows the distribution in terms of the previous crop, region, and irrigation system.

Table 9. Farmer practice: previous crop (% of farmers).

	Summer crop	Legumes	Fallow	Cereals	Other	More than one crop
Region						
Western Region	47	20	8	12	8	5
Al-Ghab	55	3	0	19	10	13
Al-Jazirah	35	5	7	45	1	7
Al-Furat	79	0	0	4	11	7
Hauran	21	27	44	2	6	0
Irrigation Regime						
Full Irr.	75	1	0	14	5	5
Supp. Irr.	66	2	3	15	3	11
Rainfed zone 1	33	28	6	21	6	6
Rainfed zone 2	10	14	29	39	6	3
Total	41	13	11	23	5	6

Forty one percent of farmers grow durum wheat after a summer crop, 13% after a legume crop, 16% after fallow, 23% after a cereal crop, 5% after other crops, and 6% after more than one crop. There is a significant difference according to region and irrigation system. Most of the farmers in Al-Ghab and Al-Furat grow wheat after summer crops. In the Western Region, wheat is grown after a summer crop or legume crop. At Hauran, most of the farmers grow wheat after fallow, followed by a legume crop and then a summer crop. Forty five percent of Jazirah farmers report that they grow wheat continuously on the same land.

More than two thirds of the surveyed farmers grow wheat after summer crops if full or supplemental irrigation is available. For rainfed farming in zone 1, wheat is grow after a summer crop (33%), or after legumes (28%); 21% of the farmers in this zone report that they grow wheat continuously. In zone 2, 39% of the sample report that they grow wheat continuously, and 29% that the land is fallow prior to wheat. About a quarter of the sample in zone 2 grows wheat after a summer or legume crop.

Distribution of Varieties

Adoption of new varieties by wheat producers is more complicated than the adoption of other components of the technological package. There has been a significant increase in the total area grown to improved varieties over the past 20 years. In our survey, adoption of high-yielding varieties conforms to published agricultural statistics.

The survey enabled us to look at adoption patterns of HYVs in several ways. First we looked at the general distribution of varieties. Table 10 shows the percentage of areas growing each variety of durum and the percentage of farmers using each variety. Cham 1 and Cham 3 rank first in terms of durum cultivated area. They cover about 63% of the total durum wheat area. About 56% of wheat producers grow these two varieties. These varieties are the result of scientific collaboration between the Directorate of Agricultural Scientific Research and ICARDA.

Table 10. Distribution of durum varieties in Syria (average 1990/91-1992/93).

	Area (%)	% Farmers
Gezira 17	3	9
Cham 1	33	24
Cham 3	30	22
Bohouth 1	4	12
Bohouth 5	1	3
ACSAD 65	13	16
Jori 69	3	9
Other HYV Durum	ns	2
Local varieties	13	7
Total	100	100

Second, we looked at variety distribution in terms of water regime: rainfed, supplemental irrigation, and full irrigation (Table 11). This invites comparison with the target environments listed in Table 1. For example, Cham 3, which is targeted for zone 2, is in fact grown extensively in zone 1 and under irrigated conditions (either supplemental or full).

Table 11. Distribution of durum varieties by water regime in Syria (% of area, average 1990/91-1992/93).

	Rainfed		Supp. irr.	Full irr.	Total
	Zone 1	Zone 2			
Gezira 17	1	3	4	12	4
Cham 1	28	34	34	35	32
Cham 3	26	33	26	33	30
Bohouth 1	4	1	7	7	4
Bohouth 5	ns	ns	1	5	1
ACSAD 65	23	5	19	7	13
Jori 69	2	2	7	ns	3
Other HYV Durum	1	0	2	ns	ns
Local varieties	16	23	1	0	13
Total	100	100	100	100	100
Varieties (%) developed by ICARDA and Syrian National Program to total HYVs	65	86	62	69	71

Third, we looked at the distribution of cultivars among durum wheat producers. Twenty four percent of the surveyed farmers grow Cham 1, and 22% grow Cham 3. Local durum varieties are grown by 27% of farmers. Some farmers grow more than one variety. The average number of varieties per farmer is 1.4.

Fourth, we looked at weighted average age of high-yielding variety areas. This average was developed by Brennen and Byerlee in 1991. It is useful for a comparison of high-yielding variety changes and rate of spread across regions and over time. The weighted average age of the sample was 6.8 years. It is rather high in the Western Region and with small farmers. This means that when small farmers or the Western Region farmers adopt one of the high-yielding varieties, they tend to continue growing this variety rather than switching to a new one. Table 12 shows the distribution of farmers using high-yielding durum wheat varieties according to the date of adoption.

Table 12. Distribution of farmers using high-yielding durum varieties by date of adoption (%)

	1-5 years	6-10 years	11-15 years	>15 years
Region				
Western Region	30.6	47.8	18.5	3.2
Al-Ghab	2.9	51.4	25.7	20.0
Jazirah	57.4	31.4	4.4	6.9
Al-Furat	20.7	31.0	24.1	24.1
Hauran	100.0	0	0	0
Farm size				
0-5 ha	22.6	46.2	21.5	9.7
5-10 ha	34.1	46.6	12.5	6.8
10-20 ha	52.1	26.6	10.6	10.6
20-50 ha	51.0	34.0	9.0	6.0
> 50 ha	46.3	42.6	7.4	3.7
Water regime				
Full Irr.	37.5	35.4	8.3	18.8
Supp. Irr.	32.0	45.3	17.2	5.5
Rainfed zone 1	41.9	39.5	12.9	5.6
Rainfed zone 2	58.0	30.9	9.9	1.2
Total	41.0	38.7	12.6	7.7
n= (429)	(176)	(166)	(54)	(33)

Technologies Associated with High-yielding Varieties

Adoption of new varieties is usually accompanied by changes in crop management. Most often cited are: chemical fertilizers, herbicides, and new agronomic practices. High-yielding variety adopters were asked if they had changed their production practices when they adopted the new varieties. A majority report that they made at least one change, and others that they made more than one change (Table 13). An increased seed rate is the most frequent change associated with adoption of high-yielding varieties. Increased fertilizer use is common when adopting new varieties.

Table 13. Change in practice associated with high-yielding variety durum adoption (%).

Change	Date of adoption			Positive reply	
	1-5 years	6-10 years	11-15 years	>15 years	For total
Increase seed rate	73	64	63	63	67
Increase use phosphate fert.	60	55	59	52	57
Increase use nitrogen fert.	60	62	67	64	62
Increase number of tillages	42	38	33	46	40
Increase herbicide use/rate	18	32	40	36	28
Shift HYV to more fertile soil	8	16	26	24	15
Estimate yield increase by farmer	44%	60%	76%	49%	54%

Impact of Technology on Durum Production

Based on the data collected from the survey over three seasons, a production function has been developed using multiple regression analysis, linking the productivity of each hectare with multiple variables such as rainfall, high-yielding variety, number of irrigations, quantity of applied nitrogen fertilizer, previous crop, and application of herbicides. This equation is significant. Table 14 shows the coefficient values of the production function. This function explains about 53% of the changes of durum wheat yield in Syria.

Table 14. Estimation of durum-wheat-production function in Syria based on wheat survey data (1991–1993).

Label	Coefficient	SE	Variable
N	6.32	2.3	N added (kg/ha)
N^2	-0.004	0.007	Sq. of N added
Rain	1.68	0.5	Rainfall rate (mm)
Seed	6.82	4.0	Seed rate (kg/ha)
S^2	-0.0106	0.007	Sq. of seed rate
Variety	879.8	154.0	Variety (1=HYV) (0=local)
NOIR	232.9	32.1	No. of irrigations
Herbicide	330.3	116.2	Herbicide Application (1-applied) (0-No)
Rot	675.4	126.2	Previous crop (0-cereals) (1-other)
Constant	-944.4	477.6	

Adj R^2 = 0.51; F(9,526)= 63***.

Multiple regression analysis indicates that the application of herbicides may increase average yield by 302 kg/ha. Improved varieties may increase the yield by 847 kg/ha compared with local varieties. Growing durum wheat after legumes or a summer crop increases yield by about 659 kg/ha compared with growing durum wheat continuously in the same field. Each 1 mm of rainfall increases durum yield by 1.91 kg. The use of 1 kg of net nitrogen unit increases yield by about 6.5 kg. This increase slows with the application of N fertilizer until the optimal limit is reached, then production decreases.

Farmers were divided into three groups in terms of number of irrigations. The first group includes those who irrigate their fields 1–3 times annually (supplemental irrigation). The second includes those who irrigate their fields 4–6 times (full irrigation). The third group includes those farmers who irrigate wheat more than 7 times a year (more than the recommended rate). Multiple regression analysis shows that with supplemental irrigation, the expected yield is 1,049 kg/ha. For full irrigation, the will be about 1,755 kg/ha. If the number of irrigations is more than recommended, the expected yield will be 1,430 kg/ha.

Based on the above production function, and after replenishing the average values of the variables with the data collected, the impact of the most important technological components of durum wheat cultivation was estimated, namely: irrigation, cultivar, fertilizer, and factors relating to crop and land management.

The impact of irrigation on productivity was significant at Jazirah and Al-Furat, compared with the Western Region and Al-Ghab. The impact of fertilizers in Al-Ghab was higher than in other regions. The impact of improved varieties was noted in all regions except Hauran, due to the delay in introducing them.

The effect of the various components of the package on total productivity was identical in the irrigated areas, the supplemental irrigation areas, and zone 1. In zone 2, the total impact was less than in the other areas.

Agricultural technology affected all groups of farmers: small, medium, and large holders. This is a more positive result than experienced in other countries, where technology aids large holders and small farmers do not benefit at all.

The average increase in net revenue at the farm level resulting from the technological package was estimated by deducting the cost of each component of the package from the total yield increase. Due to the difficulty of estimating costs relating to crop management, it was assumed that the cost of crop management equals 40% of the total increase resulting from the component. Table 15 shows such estimates. Increases were higher in the irrigated and supplemental irrigation areas than in the non-irrigated areas. The net revenue/cost ratio in the rainfed areas was higher than the irrigated areas; this is an indication of the economic profitability of investment in rainfed agriculture.

A preliminary estimate of the impact of modern agricultural technology on durum wheat in Syria was made, and the increase in national income as a result of such technologies calculated. The following formula was used:

$$\text{Total increase} = Ar_j \times A_i \times X_i$$

where: X_i=the increase obtained from technological component i in the package; A_i=the adoption rate of technological component i; and Ar_j=the average area grown to durum wheat in area j.

The preliminary estimate is an increase of 1,661,000 t of durum wheat, and about 17.4 billion Syrian Lira annually (407 million US$) in national income (Table 15). About 23% of this increase is due to the impact of irrigation, 34% to the use of improved varieties, 24% to fertilizer, and 19% to land and crop management (Table 16). About 32% of the impact comes from fully irrigated areas, 31% from supplemental irrigation areas, and 37% from rainfed areas.

In spite of this remarkable increase, there is still a gap in productivity between potential and actual yields. This requires further effort, either in agricultural research or extension.

Table 15. Estimated annual increase in national income due to modern technologies used on durum wheat in Syria (000 tonnes).

	Area	Increase				Total
	(000 ha)	Variety	Nitrogen	Irr.	Manag.	increase
Full Irr.	197	136	115	211	64	526
Supp. Irr.	216	145	113	169	78	505
Zone 1	324	162	107		103	372
Zone 2	353	111	57		60	228
Total	1,090	554 (34%)	392 (24%)	380 (23%)	305 (19%)	1,631 (100%)

Table 16. Estimated average increase in net revenue at the farm level due to technological packages (kg/ha).

	Due to variety	Due to nitrogen	Due to irrigation	Due to management	Total increase
Water regime					
Full Irrigation	688	582	1073	380	2,723
Supp. Irr.	687	547	782	423	2,439
Rainfed zone 1	595	347		404	1,346
Rainfed zone 2	390	193		278	861
Region					
Western Region	556	405	305	454	1,720
Al-Ghab	692	693	161	452	1,998
Jazirah	702	380	489	232	1,803
Al-Furat	703	625	1,076	426	2,830
Hauran	97	123	40	467	727
Total	610	390	377	368	1,745

Breeding for Resistance to Drought and Salinity in Durum

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Abstract

In Morocco, the durum area is about 1.3 million ha, and per capita consumption is more than 100 kg/year. Durum yield is comparable to bread wheat yield. Four landraces and 21 new cultivars have been released by INRA. Among these, 60% were selected from germplasm introduced from ICARDA and CIMMYT. After 20 years of work, grain yield in Morocco has risen from less than 5,000 kg/ha to more than 7,500 kg/ha, with genetic gains in farmer fields of almost 40%. In the absence of a multidisciplinary approach, very little attention has been paid to resistance to abiotic stresses. Many new research activities have been recently initiated. The specificity of environmental constraints calls for separate breeding projects for low rainfall, high rainfall, and high elevation. In the low rainfall areas, drought and heat stresses, Hessian fly, and root rots are the main constraints. Drought research assesses early and late drought resistance in existing cultivars. Salt tolerance trials are conducted in the laboratory using NaCl and saline water. Results show that NaCl slightly lowers germination and greatly reduces germination speed and coleoptile length. Genotypic differences are apparent among cultivars. Massa and Sarif are the most salt-tolerant varieties. Under field conditions, durum is, on average, more salt sensitive than barley, bread wheat, and triticale.

Introduction

Durum wheat is an important cereal species in Morocco, with 1.3 million ha sown annually and an average per capita consumption of more than 100 kg/year. At the farmer level, durum is preferred over bread wheat due to its specific uses. Morocco ranks third in the Mediterranean region and first in the North Africa and Middle East region in term of durum acreage. Although average national grain yield is low, due mostly to drought and pest stress, substantial genetic gains have been achieved by the Moroccan Institut National de la Recherche Agronomique breeding program in collaboration with the international centers and other breeding programs in the region. Yield potential is comparable to bread wheat, and this has stimulated a boost in acreage. Four landraces and 21 INRA cultivars are registered in the cereal catalogue. Among these, almost 60% were selected from germplasm introduced from ICARDA and CIMMYT. Seventeen percent of the durum acreage is sown with certified seed of newly released varieties. Karim, Marzak, Sebou, and Omrabi are the most widely grown cultivars.

In 1934, INRA started the only durum wheat breeding program in Morocco, with selection within local populations and the introduction of cultivars such as Keyprounda. During 1968–1972, the joint INRA/CIMMYT effort led to the release of cultivars Cocorit and Jori. Collaboration with CIMMYT continued, and after 1981 a strong collaboration developed with the durum program at ICARDA. These international efforts with have led to the release of 13 new cultivars since 1985. ICARDA has contributed extensively to the build-up of an effective durum wheat network, with INRA Morocco playing a major role, thanks to the expertise of the institution and the diversity of the environment.

This paper discusses what the INRA durum wheat improvement program is doing and what it can do to enhance the productivity of durum wheat in the North African and Mediterranean regions. INRA is eager to contribute to these joint efforts. Breeding for abiotic stresses will be emphasized. Other aspects will be developed further by colleagues at INRA and the Institut Agronomique et Vétérinaire Hassan II (IAV HII).

Ongoing Research and Long-term Projects

Until recently, the main objective of the durum wheat breeding program was to develop new genotypes which will outyield the widely spread landraces. In the last 20 years, grain yield potential in Morocco has gone from less than 5,000 kg/ha to more than 7,500 kg/ha, with genetic gains in farmer fields of almost 40%. These gains have been achieved through the introduction and development of semi-dwarf and shorter-cycle cultivars. Most of the released cultivars have shown low stability in grain quality and some of them are even sold at the same price as bread wheat.

In the absence of a multidisciplinary approach, very little attention has been given to pest resistance, quality improvement, or the evaluation of tolerance to abiotic stresses. The availability of a qualified staff, and the development of close collaboration with ICARDA/CIMMYT and institutions within the country and the region, has stimulated the development of a more comprehensive and efficient durum wheat improvement program. New research has been recently initiated and is supported by ICARDA. The process of programming by objectives, adopted at INRA, has facilitated the setting up of research priorities.

The durum breeding strategy is based on the improvement of both grain yield and grain quality through the use of landraces with good grain, and the incorporation of resistance to major pests. Data show that most of the recent releases are highly susceptible to yellow berry and to pests such as Hessian fly, septoria, tan spot, and rusts. The specificity of environmental constraints makes it necessary to develop separate breeding projects for low rainfall, high rainfall, and high elevation environments. Drought and heat stresses, Hessian fly, root rots, rusts, and BYDV are mostly encountered in semi-arid and arid regions. Tan spot, BYDV, septoria, and rusts are prevalent in high rainfall and irrigated environments. At high elevation, BYDV, tan spot, Russian wheat aphid, frost, and heat stresses are the most limiting factors. INRA has developed expertise in Morocco and within the Maghreb region in the areas of:

- Septoria screening, germplasm development, and virulence studies.
- Tan-spot screening, germplasm development, and virulence studies.
- Root-rot screening, germplasm development, and virulence studies.
- BYDV screening, germplasm development, and virulence studies.
- Hessian fly and RWA screening, germplasm development, and virulence studies.
- Evaluation of durum wheat quality for pasta and bread making.
- Physiology of heat and drought stresses, and germplasm development.
- Germplasm development through the use of interspecific crosses, irradiation, and dihaploid techniques.
- Some research is carried out in close collaboration with scientists from IAV Hassan II (leaf rust) and ENA-Meknes (tan spot).

Most of these projects, supported by ICARDA and the UNDP, have already helped the region. Detailed contributions are presented at annual coordination meetings.

Breeding for Resistance to Abiotic Stresses

Breeding for Drought Resistance

In Morocco, most of the durum wheat acreage is located in the high rainfall and semi-arid zones. Compared to bread wheat, very little durum is sown in the arid zones. This can be explained by the type of germplasm developed. Earliness is limited in durum wheat, and all breeding material introduced or developed locally heads at about the same time as ACSAD 65 and Karim (about 10 days). The development of early-heading genetic material could help to extend durum wheat into drier areas and improve water-use efficiency. In the arid and semi-arid zones, Hessian fly resistance is a prerequisite for further improvement. Attempts are underway to incorporate effective genes from resistant bread wheats and wild relatives. BYDV, leaf rust, and root rots are important limiting pests for which effective sources of resistance have already been identified.

Trials at Jemaa Shaim have shown that winter types are not suited for semi-arid and arid environments. Evaluation of early and late drought resistance of existing cultivars is ongoing.

Salt Tolerance

Salinization of soil and water limits the productivity of most annual crops on 37% of the irrigated land in Morocco. Cereals, especially barley, are among the glycophyte species with good tolerance to salt. Trials have been conducted at the germination stage in the laboratory using 200 mM of NaCl, and under irrigation with saline water at Ain El Atti (water EC=11,67 dS/m) and Dar Bouazza (water EC=3.75 dS/m) to determine the effects of salt on grain yield. In the laboratory, NaCl slightly reduced germination percentage and greatly reduced germination speed and coleoptile length. Genotypic differences were apparent, and cultivars Sarif and Tessaout were the most salt sensitive at germination. Under field conditions, durum was on average more salt sensitive than the barleys, the bread wheats, and the triticales. These differences were only apparent at Ain El Atti under severe salt stress, although significant differences were apparent between cultivars, and Massa and Sarif were the most salt-tolerance varieties.

Conclusion

In light of ongoing research, the genetic gain achieved, and the qualified staff, the INRA durum wheat breeding program is ready to play a major role in the region.

Studies on Drought Tolerance in Durum and Genotype–Environment Interaction

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Abstract

Hydric status, energetic status (chlorophyll fluorescence), and osmoregulation (proline and soluble sugars) were studied. The analysis of yield stability showed three major varietal groups: wide adaptation, intermediate adaptation, and narrow adaptation. Varieties Bidi 17, GG Rkhem, Gloire de Montgolfier, Montpellier, and Oued Zenati had low yield and low response to favorable conditions. Mexicali, Vitron, Waha, and ZB/Flg showed high yield and linear response to favorable conditions. Capeiti, INRAT 69, Polonicum, and Sahel showed stable yield. Yield was found to be negatively correlated with the sensitivity index to environment constraints (ISCE). Vitron was identified for favorable areas, Bidi 17 for unfavorable areas, and Polonicum, INRAT 69, and Capeiti for moderate water-stress areas. Stress physiological traits, water status, energetic status, and osmoregulation contribute to varietal adaptation. The use of contrasting models was proposed: (1) Hedba 3 (low values for proline and fluorescence) and Stork (high values for proline and fluorescence); (2) Hedba 3 (low value for proline) and Waha (high value for proline); (3) Bidi 17 (high values for proline and psi, low yield) and Waha (high value for proline, low value for psi, high yield); 4) Polonicum (low values for proline and psi, low yield) and Stork (high values for proline and psi, high yield). Results showed a consistent relationship between groups in terms of physiological traits and expressed yield stability. Chlorophyll fluorescence was found to have transgressive inheritance. The other physiological parameters have intermediate heritability. Analytical and synthetic approaches were proposed to identify drought-resistant varieties.

Introduction

In areas where environmental constraints are less pronounced, the preference for yield potential is justified because the conditions allow for the expression of the genetic ability (Monneveux 1991). When constraints are more severe, yield alone does not constitute suitable criteria. The analysis of spatio-temporal variability of major environmental components and interaction with genotypes remains the most reliable way to study the mechanisms involved in yield. Different models have been proposed to analyze genotype–environment interaction. All of them require follow-up of the behavior of a group of varieties. Regression coefficient, mean yield by site, and standard deviation values (Finlay and Wilkinson 1963) were used by several researchers to study varietal adaptation. However, these linear models have their critics (Boggy et al. 1973). Other multivariate models have recently been proposed (Kempton 1984; Gauch 1988). The additive main effect and multiplicative interaction (AMMI) model might be used for durum screening in the Mediterranean region (Nachit et al. 1992).

Although yield is a main objective (Frey 1964), results reported by Ceccarelli (1989), Monneveux (1991), and Nachit et al. (1992) suggest that selection for drought resistance would be useful in areas where constraints exists and where selected genotypes are supposed to be grown. Analytical and, later, synthetic approaches have been extremely convincing as they deal with identification, hierarchization, and introgression of tolerance traits in a breeding program. Durum breeding of necessity involves germplasm use, including landraces and wild relatives. According to Nachit et al. (1988), selection is more efficient when it is done in the region where varieties are to be cultivated. Rasmusson (1987) introduces the concept of ideotype in order to overcome the great delay caused by trait-by-trait selection. Some correlation between physiological and morphological traits have been reported; e.g. between hydric potential and height of plant (Kirkham and Smith 1978).

Improvement of genetic diversity (Nachit et al. 1988) should utilize material adapted to semi-arid and arid regions. The results reported by Nachit and Ouassou (1988) on yield variations under water stress have contributed to a better understanding. Varieties with high yield under drought combining drought tolerance, yield potential, and appropriate phenology should be developed. The physio-genetic breeding approach, as it is applied in the CIMMYT/ICARDA durum project in the Middle East and North Africa using severe stress, moderate stress, and favorable environment testing, has resulted in the development of durum cultivars that aggregate yield potential, yield stability, and stress tolerance.

Results

Testing has dealt with plant hydric status (RWC, RWL, IS, HI), energetic status (chlorophyll fluorescence), and osmoregulation (proline and soluble sugars accumulation). The results can be summarized as follows:

- The analysis of yield stability shows three major groups: (1) varieties with wide adaptation; (2) varieties with intermediate adaptation; and (3) varieties with narrow adaptation.
- Physiological adjustment responses are a function of the genotype. Some genotypes may express a fair tolerance to drought without having the same mechanisms. Generally, there is an origin effect of cultivars on tolerance. Some traits seem to be reliable indicators of tolerance to water stress (osmoticums, fluorescence, and leaf hydric potential).

- Chlorophyll fluorescence was found to have transgressive inheritance. The other physiological parameters have only intermediate heritability compared to the parents. Finally, physiological models have been proposed and may serve as selection tools.

Given the primary results and supplementary biometrical tests, yield stability analysis shows a diversity in response to environment constraints. The genetic expression of varieties depends upon the intensity of the imposed constraints. Analysis of stability parameters and curves of regression allow us to group varieties according to their behavior:

- Bidi 17, GG Rkhem, Gloire de Montgolfier, Montpellier, and Oued Zenati have low yield which does not increase with favorable conditions.

- Mexicali, Vitron, Waha, and ZB/Flg have higher yield than the mean of the site. Yield increases linearly when conditions become more favorable. These types are to be recommended for favorable sites.

- Capeiti, INRAT 69, Polonicum, and Sahel represent the group of genotypes that are stable They are, as a matter of fact, old introductions that may have been adapted to existing durum regions.

In all cases, yield was found to be negatively correlated with the sensitivity index to environment constraints. Further, from the correlation found between morphological parameters and yield, significant traits can be identified that explain yield expression under the various types of stress in terms of severity, occurrence, and time of occurrence. Plasticity of varieties has also been checked in terms of spatio-temporal conditions.

- For favorable zones, Vitron should be a good ideotype.

- For unfavorable conditions, Bidi 17 is an appropriate ideotype.

- For moderate water stress, ideotypes such as Polonicum, INRAT 69, and Capeiti are recommended.

Among the physiological traits which favor a water deficit, fluorescence, hydric leaf potential, RWC, RWL, IS, and accumulation of osmolytes such as proline and soluble sugars seem to contribute to the general adaptation behavior. The combination of several traits (good accumulation of proline, high RWC, and low hydric potential) may justify the choice of physiological models, but only when yield is high. Cultivars may be chosen for inclusion and used in a germplasm program according to various approaches (analytical, trait-by-trait, or synthetic).

The use of contrasting models allows correction of failures observed in some varieties (Table 2):

- Hedba 3 (low values for proline and fluorescence).

  Stork (high values for proline and fluorescence).

- Hedba 3 (low value for proline).

  Waha (high value for proline).

- Bidi 17 (high values for proline and psi, low yield).

  Waha (high value for proline, low value for psi, high yield).

- Polonicum (low values for proline and psi, low yield).
- Stork (high values for proline and psi, high yield).

Table 1. Relation between grain yield, sensitivity index to environmental constraints (SIEC), and morpho-phenological traits.

Site	Year	Correlation between traits	
Khroub	1991	Yield / Height	-0.70
	1992	Yield / Height	-0.70
	1993	Yield / Fertile tillers	+0.43
		SIEC /Fertile tillers	-0.42
Tiaret	1991	SIEC / Maturing date	-0.57
	1992	Yield / Height	-0.81
		Yield / Heading date	-0.84
		SIEC / Height	-0.63
	1993	SIEC / Maturing date	-0.55
		SIEC / Heading date	-0.67
		SIEC / Height	-0.70
		SIEC / Yield	-0.99
		Yield /Maturing date	-0.55
		Yield / Heading date	+0.69
Tessala	1991	SIEC / Height	-0.70
		SIEC / Awns length	-0.62
	1992	Yield / Height	-0.67
		Yield / Heading date	-0.69
	1993	SIEC / Yield	-0.99
		Height / Awns length	+0.70
Zidane	1991	SIEC / Height	-0.76
		SIEC / Heading date	-0.64
	1992	Yield / SIEC	-0.65
		SIEC / Height	-0.74

There is a consistent relation between groups in terms of physiological traits and expressed yield stability (Table 1). Varieties that belong to either one of the three groups in the yield-stability analysis express similar physiological responses. Does that mean that physiological traits construct yield? This hypothesis is justified in several cases, with the exception of those varieties that are resistant to drought but also resistant to yield.

Table 2. Expression of physiological traits in some durum varieties.

Traits	Varieties				
	Hedba 3	Stork	Waha	Bidi 17	Polonicum
Proline +/-	-	+	+	+	-
Fluorescence +/-	-	+			
Psi +/-		+	+	+	-
Grain yield +/-		+	+	-	-

The preliminary genetic study, even though incomplete, deals with F_1 populations to detect any possible transgressive effects. Fluorescence does in fact generally display this tendency, and thus may be used as an important trait indicator for drought tolerance in durum.

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Abiotic Stress Tolerance in *Aegilops* Species

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Abstract

Broadening the genetic base of durum by introducing genes from wild progenitors and primitive forms is a well-recognized technique. This type of germplasm has been used by breeders as a source of genes for disease resistance, and, to a lesser extend, for improving abiotic-stress tolerance. For more efficient use of *Aegilops* spp. germplasm, a better understanding of its potential usefulness in the improvement of tolerance to abiotic stresses is needed. Four different techniques can accomplish this: ecogeographical surveys, field evaluation, evaluation in controlled conditions, and identification and study of morphophysiological traits related to abiotic-stress tolerance. Experimental data concerning salt, cold, and drought tolerance in *Aegilops* spp. were summarized. Ecogeographical surveys in Syria showed that *Ae. crassa* was salt tolerant. *Ae. tauschii* (D genome) had a lower Na accumulation in aerial parts. High salt tolerance was found in *Ae. tauschii* and *Ae. cylindrica* (CD genome). Enhanced K/Na discrimination was also observed in *Ae. cylindrica*). This trait was found to be absent in the species of the *Sitopsis* spp. section. *Aegilops* spp. with C genome (*Ae. umbellulata*, *Ae. kotschyi*, *Ae. triuncialis*, and *Ae. ovata*) tend to accumulate more Na in aerial parts than *Ae. tauschii* and *Ae. cylindrica*, but considerable variability for total salt load was observed in *Ae. ovata*. Other salt-tolerance-related traits (vegetative growth, proline accumulation, and fluorescence inhibition under salt stress) were examined: accessions with low inhibition of vegetative growth and high fluorescence belong to the species *Ae. columnaris*, *Ae. kotschyi*, *Ae. ventricosa*, *Ae. crassa*, and *Ae. sharonensis*. Salt tolerance can be related to constitutive genomes (D genome) and geographical origin. High intraspecific variability and variability within a population exist for all traits. Cold resistance covers two phenomena (chilling and freezing): chilling resistance corresponds to resistance to low temperatures (above-freezing), and frost resistance to freezing temperatures. Screening of B genome donors showed low cold resistance, while D genome donors showed high frost resistance. The G genome (originated from *Ae. speltoides*) has good frost tolerance. Field observations showed that *Ae. sharonensis*, *Ae. kotschyi*, *Ae. umbellulata*, *Ae. triaristata*, and *Ae. ovata* may be frost resistant. Screening in controlled conditions at -13 °C and -16 °C showed that *Ae. tauschii* and *Ae. speltoides* were more frost resistant than *Ae. sharonensis* and *Ae. bicornis*. High frost resistance was found in *Ae. cylindrica*, low in *Ae. caudata*, *Ae. comosa* (M), and *Ae. speltoides (S)*, and intermediate in *Ae. triuncialis*, *Ae. triaristata*, *Ae. ovata*, and *Ae. biuncialis*. This suggests the positive effect of the U genome on frost resistance.

The capacity to maintain leaf water potential (LWP) is an important mechanism of dehydration avoidance. *Aegilops kotschyi* (the most xerophytic species) maintained its LWP until 40% of soil moisture had been depleted; it showed a higher degree of relative stomatal opening at all LWPs and a higher rate of CO_2 fixation. In *Aegilops kotschyi*, the enzymes involved in photosynthesis and electron transport are less affected by stress, and total soluble plant proteins and leaf chlorophyll remained constant. Relative water content (RWC), recognized as a criteria of drought tolerance related to osmotic adjustment, was determined on 82 *Aegilops* spp. accessions from different origins. Few accessions have RWC values inferior to Kabir 1 (the drought-susceptible check); most ICARDA drought-selected accessions had RWC values superior to Cham 1 (the drought-tolerant check). Important intraspecific variability was noted for RWC (particularly in *Ae. ovata).* Evaluation for 13C/12C discrimination was also made. Leaf water loss (LWL) of 12 *Aegilops* spp. accessions was followed under controlled conditions in growth chambers. Along with RWC data previously obtained, three groups were distinguished according to residual transpiration rate (RTR) values. *Aegilops speltoides* accessions were characterized by low stomatal transpiration. A strategy was developed to reduce the number of accessions and to extract a subsample representing a large part of the variability of the total collection. Information was taken from morphophysiological traits, biochemical and molecular polymorphism, and neutral markers. The homeologous pairing in wheat is controlled by a major locus (Ph1) on chromosome arm 5BL; the isolation of an induced mutant at the Ph1 locus has made it possible to transfer the alien gene to wheat. In addition, homeologous transfers can be obtained using certain accessions of *Ae. speltoides*, *Ae. caudata*, and *Ae. mutica*, which suppress Ph1 activity in appropriated hybrids. An assessment of the amount of alien chromatin introgressed into wheat is the key step in this analytical approach. This information is actually limited by the number of markers that are available, and a development of biochemical and molecular markers is required for the characterization and monitoring of transfers. Information on biotic and abiotic stress must be developed for use in the constitution of *Aegilops* spp. core collections, according to the methodologies of introgression (analytic vs holistic) and the objectives of selection (specific stress vs multi-stress resistance).

Introduction

The necessity of broadening the genetic base of durum by introducing genes from non-conventional genetic resources (wild progenitors, primitive forms) is now understood. This type of germplasm has been widely used by wheat breeders as a source of genes for disease resistance (Dhaliwal et al. 1986). Its utilization for improving abiotic-stress tolerance is considerably less developed. For more efficient use of *Aegilops* spp. germplasm, a better understanding of its potential usefulness in the improvement of abiotic stress tolerance is needed. Four different ways are available for this purpose, as discussed below.

Ecogeographical Surveys

Ecogeographical surveys are particularly convenient to examine the relationship of wild relatives with environmental factors through distribution patterns. Such surveys have been carried out for different *Aegilops* spp. by Sankary (1990) in Syria, Zaharieva (1993) in Bulgaria, Vojdani and Meybodi in Iran (1993), and Della and Bari (1993) in Cyprus.

Field Evaluation

Screening of wild germplasm for salt and drought tolerance has been attempted on the shores of Lake Jabboul in northern Syria (Damania et al. 1988) and in the Pendjab desert (Damania et al. 1992), respectively. Results are, however, highly dependent on seasonal climate.

Evaluation in Controlled Conditions

This procedure, widely used to screen cultivated wheat genotypes for salt (Timm et al. 1991) and frost tolerance, has been also applied to wild relatives (Farooq et al. 1989; Barashkova and Vavilov 1991; Damania et al. 1993).

Morphophysiological Traits Related to Abiotic Stress Tolerance

This approach aims at a better understanding of stress tolerance mechanisms as well as a definition of the morphophysiological criteria permitting analysis of intra- and interspecific variations and a rapid screening of potentially useful accessions or recombinant lines.

The objective of this paper is to summarize experimental data concerning salt, cold, and drought tolerance in *Aegilops* spp. and to define a strategy of management and utilization of this germplasm in durum breeding.

Salt Tolerance in *Aegilops* spp.

Ecogeographical surveys carried out in Syria by Sankary (1990) show that some species such as *Ae. crassa* are able to survive in salt-affected environments. In controlled conditions, Shah et al. (1987) observed lower Na accumulation in aerial parts in *Ae. tauschii* (D genome) than in other species. Farooq et al. (1989) found high salt tolerance in *Ae. tauschii* and *Ae. cylindrica* (CD genome). Enhanced K/Na discrimination was also observed in *Ae. cylindrica* by Farooq et al. (1989). Gorham (1990) shows that this trait is absent in the species of the *Sitopsis* section. *Aegilops* spp. with C genome (*Ae. umbellulata*, *Ae. kotschyi*, *Ae. triuncialis*, and *Ae. ovata*) tend to accumulate more Na in aerial parts than *Ae. tauschii* and *Ae. cylindrica*, but considerable variability for total salt load was observed in *Ae. ovata* (Farooq et al. 1989).

Other salt-tolerance-related traits (vegetative growth, proline accumulation, and fluorescence inhibition under salt stress) were examined on 45 accessions belonging to 17 species (Xu et al. 1993; Fig. 1). In the PCA carried out among *Aegilops* spp., 62.3% of the variation is explained by the two first PCs; the X axis is closely related with the vegetative growth inhibition of roots and aerial parts (r=0.83** and 0.71**, respectively), and the Y axis with the fluorescence emission (r=0.56*). Most accessions with low inhibition of vegetative growth and high fluorescence emission belong to species *Ae. columnaris* (No. 7), *Ae. kotschyi* (No. 9), *Ae. ventricosa* (No. 11), *Ae. crassa* (No. 12), and *Ae. sharonensis* (No. 17).

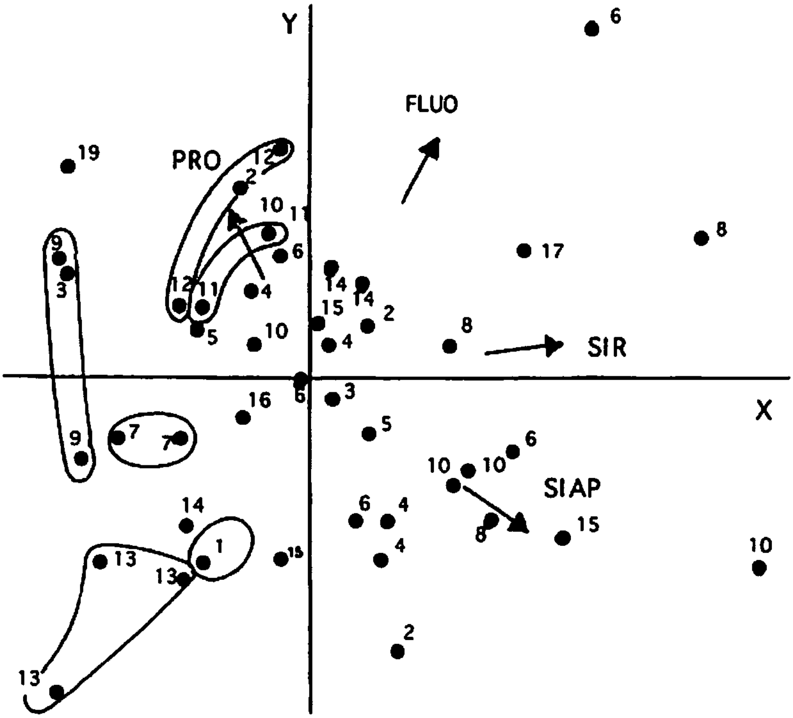

Figure 1. Multivariate analysis (PCA) of four salt-tolerance-related traits in *Aegilops* accessions plotted against the first two principal components. SIAP=susceptibility index of aerial part; SIR=susceptibility index of roots; PRO=proline content; FLUO=fluroescence inhibition. From Xu et al. (1993).

The following can be concluded:

- Salt tolerance in wild relatives may be related to constitutive genomes (e.g. high tolerance associated with D genome) and with geographical origin (most tolerant accessions originate from salt-affected areas).
- Very high intraspecific variability and variability within a population exist for all morphophysiological traits. Screening and breeding procedures specifically adapted to this type of germplasm need to be defined.

Cold Resistance in *Aegilops* spp.

Cold resistance covers two different phenomena (Levitt 1980):

- Chilling resistance, which corresponds to resistance to low (above-freezing) temperatures.
- Frost resistance, which corresponds to resistance to freezing temperatures.

Chilling and freezing stresses and their respective resistance mechanisms have different meanings in theory, practice, and implications (Blum 1988).

Data on chilling and frost resistance in bread wheat and durum and in wild relatives are rather limited. Extensive screening of ancestral wheat species was carried out by Barashkova (1981) and Limin (1981) to identify potential sources of frost resistance. Both authors concluded that B genome donors, originating from the Mediterranean region, have poor resistance, while D genome donors are valuable sources of frost resistance. In addition, Barashkova (1981) determined that the G genome (originating from *Ae. speltoides*) has good frost tolerance. Study of *Aegilops* spp. populations for improvement of frost resistance in wheat (and particularly in durum) has been carried out. Preliminary information obtained by Damania (1990) from observations in the field (Aleppo, 1991/92) indicate that *Ae. sharonensis*, *Ae. kotschyi*, *Ae. umbellulata*, *Ae. triaristata*, and *Ae. ovata* are frost resistant. Screening in controlled conditions at -13 °C and -16 °C, attempted by Barashkova and Vavilov (1991), shows that *Ae. tauschii* and *Ae. speltoides* are more frost resistant than *Ae. sharonensis* and *Ae. bicornis*. These authors confirm that D genome is responsible for the high frost resistance of *Ae. tauschii*, and postulate than the D genome may neutralize the depressive effects of other genomes associated with it: *Ae. cylindrica* (CD) is more resistant than *Ae. caudata* (C), and the hexaploid wheats (ABD) more resistant than durum (AB) or *Aegilops* spp. of the *Sitopsis* spp. section.

Data obtained by Stankova and Zaharieva (unpublished), under controlled conditions at -18 °C, also confirm the high frost resistance of *Ae. cylindrica* and the low resistance of *Ae. caudata* (Fig. 2). Low resistance was observed in *Ae. comosa* (M) and *Ae. speltoides (S)*. Intermediate resistance was found in *Ae. triuncialis*, *Ae. triaristata*, *Ae. ovata*, and *Ae. biuncialis*, which suggests a positive effect of the U genome on frost resistance. The most resistant species have the highest plasticity. In a preliminary experiment on *Aegilops* spp. of Bulgarian origin, an important intraspecific variability was noted, particularly in *Ae. ovata* (Table 1). Accessions originating from mountainous regions (Strandja and Rhodopes mountains) generally have higher resistance than accessions from plains and coastal regions. High frost-resistance was found in populations 89E134, 89E140, and 89E162. Experiments are presently being carried out to confirm the role of various genomes in frost tolerance, to more deeply analyze the intraspecific variability and its possible causes (particularly in *Ae. ovata*), and to screen accessions with potential usefulness to enhance frost resistance in durum and even in bread wheat.

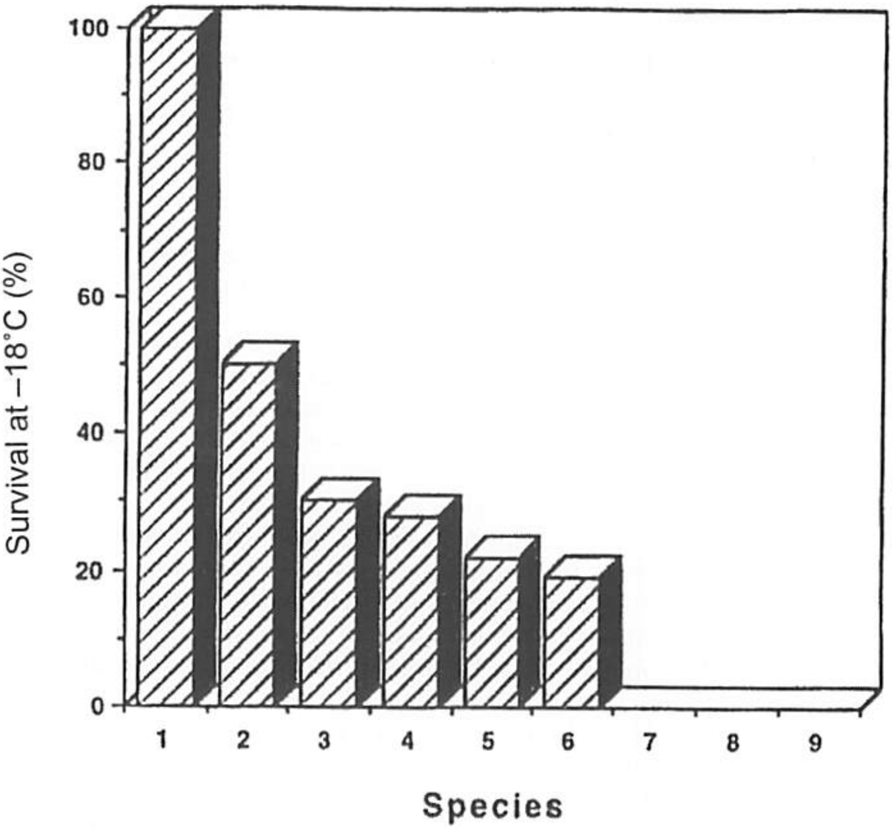

Figure 2. Average frost tolerance of different *Aegilops* species (percent of survival at -18 °C). 1=*Aegilops umbellulata* (U); 2=*Aegilops cylindrica* (CD); 3=*Aegilops triuncialis* (UC); 4=*Aegilops triaristata* (Umt); 5=*Aegilops ovata* (Umo); *Aegilops biuncialis* (Umb); 7=*Aegilops caudata* (C); 8=*Aegilops speltoides* (S); and 9=*Aegilops comosa* (M).

No information is currently available concerning the effects of low (no-freezing) temperatures on growth in *Aegilops* spp. (chilling resistance). Analysis of the effect of low temperatures on photosynthesis (based on CO_2 assimilation and chlorophyll fluorescence measurements) is, however, projected, in collaboration with the Faculty of Agronomical Sciences of Gembloux, Belgium. Assessing these traits on the Bulgarian collection will also clarify the genetic relationship between frost resistance and reaction to low temperatures.

Drought Tolerance in *Aegilops* spp.

Since the capacity for maintaining leaf water potential has been described as an important mechanism of dehydration avoidance (Levitt 1980), this trait was used by Shimshi et al. (1982) to compare *Aegilops kotschyi* (the most xerophytic of the wild wheat species that inhabit the Negev desert) with bread wheat cultivars (cvs Chinese Spring and Seeon). *Aegilops kotschyi* was found to maintain its LWP until 40% of soil moisture had been depleted. Concomitant with higher LWP, *Ae. kotschyi* maintained a higher degree of relative stomatal opening and a higher rate of CO_2 fixation. Using the same germplasm, Mayoral et al. (1981) found that the enzymes involved in photosynthesis and electron transport are less affected in *Ae. kotschyi*. At the whole plant level, he found that total soluble plant proteins and leaf chlorophyll remain constant under stress in wild species.

Relative water content, recognized as a criteria of drought tolerance related to osmotic adjustment, was studied on 82 *Aegilops* accessions from three gene banks (ICARDA, Syria; IIPGR, Bulgaria; and INRA Rennes, France) following 12 days of water stress (Fig. 3). *Aegilops* accessions from ICARDA had been previously screened based for survival under the conditions of the Pendjab desert (Damania et al. 1992). Experiments were carried out under controlled conditions (12 hour photoperiod, air temperature 21 °C during the day and 18 °C at night, RH 60%, photosynthetic photon flux density 0.45 µmol.m-2/s-1). The *Aegilops* accessions (six plants per accession) were compared to two durum checks: Cham 1 (drought tolerant) and Kabir 1 (drought susceptible). After the stress, leaf water potential reached -2.0 MPa in Cham 1 and -2.5 MPa in Kabir 1, with relative water content 78 and 64%, respectively. Few accessions showed RWC values inferior to Kabir 1; most of the preselected accessions from the ICARDA gene bank showed RWC values superior to Cham 1. Average RWC values for some species are given in Figure 4. Since important intraspecific variability was found for RWC (particularly in *Ae. ovata)*, each plant was individually replanted, and families originating from promising plants were re-tested for RWC and also evaluated for 13C/12C discrimination.

Leaf water loss for 12 *Aegilops* accessions was studied under controlled conditions (according to Clarke and Romagosa 1989) in growth chambers at Barcelona University. Results on epidermal and stomatal water loss after 2 hours of foliar desiccation are presented on Table 1. Three groups were identified according to residual transpiration rate. High RTR values were observed in accessions preselected in the Pendjab desert. Both *Ae. speltoides* accessions are characterized by low stomatal transpiration. *Aegilops ovata* accession 88E206 from the Tundja valley of Bulgaria, previously identified as unable to maintain its RWC under water stress, has high stomatal and residual transpiration. Detailed curves of water loss vs time are presented for two contrasting accessions (91/24 and 88E206 from Bulgaria) in Figure 5.

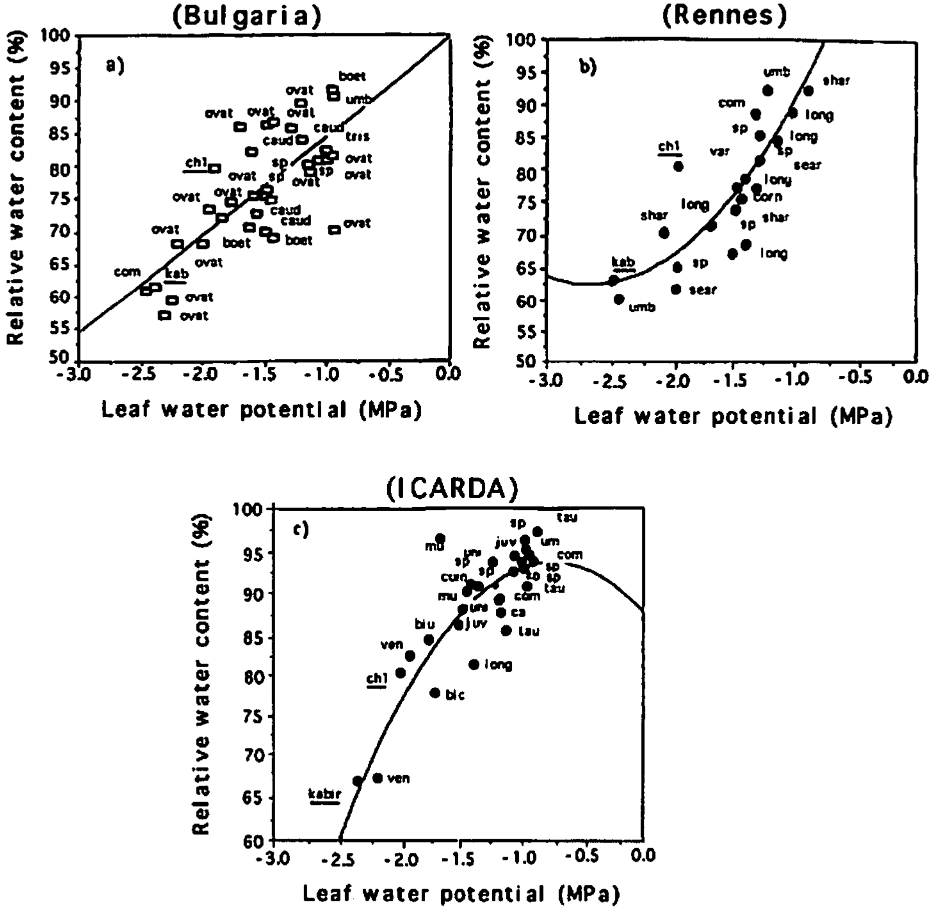

Figure 3. Relationship between relative water content (%) and leaf water potential (Mpa): a) subset of the *Aegilops* collection from IIPGR Sadovo, Bulgaria; b) subset of the *Aegilops* collection from INRA Rennes, France.; and c) subset of the *Aegilops* collection from ICARDA. For experimental conditions, see text. ch1=Cham 1; kab=Kabir; bic=*Aegilops bicornis*; biu=*Aegilops biuncialis*; ca=*Aegilops caudata*; com=*Aegilops comosa*; juv=*Aegilops juvenalis*; ko=*Aegilops kotschyi*; long=*Aegilops longissima*; mu=*Aegilops mutica*; ovat=*Aegilops ovata*; sear=*Aegilops searsii*; shar=*Aegilops sharonensis*; sp=*Aegilops speltoides*; tau=*Aegilops tauschii*; tris=*Aegilops triuncialis*; umb=*Aegilops umbellulata*; uni=*Aegilops uniaristat*; var=*Aegilops variabilis*; ven=*Aegilops ventricosa*.

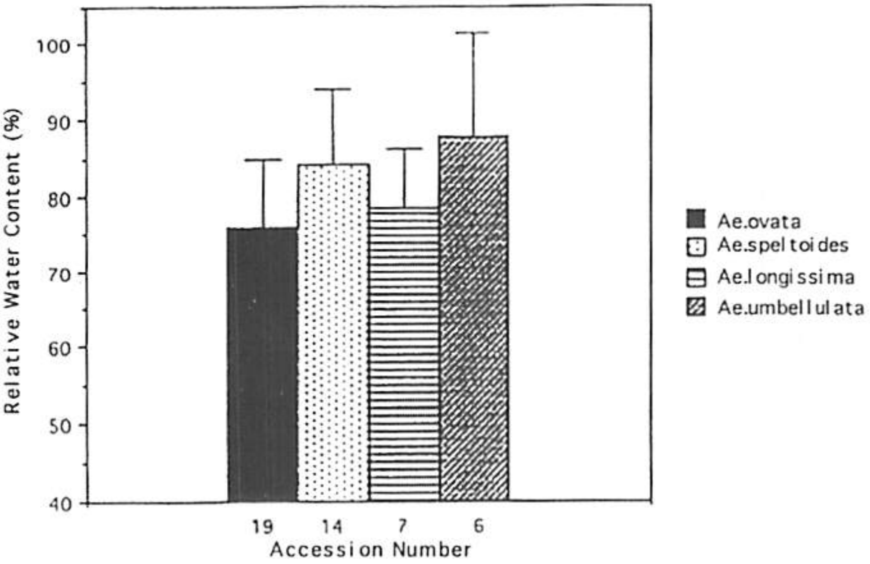

Figure 4. Average RWC value for different *Aegilops* species under water stress.

Table 1. Water loss in *Aegilops* accessions.

Species	Accession	Origin	RTR	ST	TWL 2h	TRE (%)
			Low RTR			
A. speltoides	91/24	Bulgaria	0.0075	0.34	1.240	79.06
A. ovata	88E211	Bulgaria	0.0076	1.20	2.110	70.10
A. caudata	91/63	Bulgaria	0.0078	0.96	1.896	72.69
			Intermediate RTR			
A. ovata	88E228	Bulgaria	0.0095	1.41	2.550	61.35
A. ovata	89E186	Bulgaria	0.0100	0.36	1.560	68.39
A. ovata	89E124	Bulgaria	0.0108	0.43	1.726	80.95
A. ovata	M94/1	France	0.0112	0.24	1.584	86.45
A. ovata	88E164	Bulgaria	0.0120	0.90	2.340	89.20
			High RTR			
A. ovata	88E149	Bulgaria	0.0135	0.54	2.160	80.32
A. ovata	88E206	Bulgaria	0.0147	1.53	3.294	69.20
A. speltoides	90E762	Bulgaria	0.0148	0.22	1.996	76.35
A. ovata	89E162	Bulgaria	0.0160	0.50	2.420	82.55

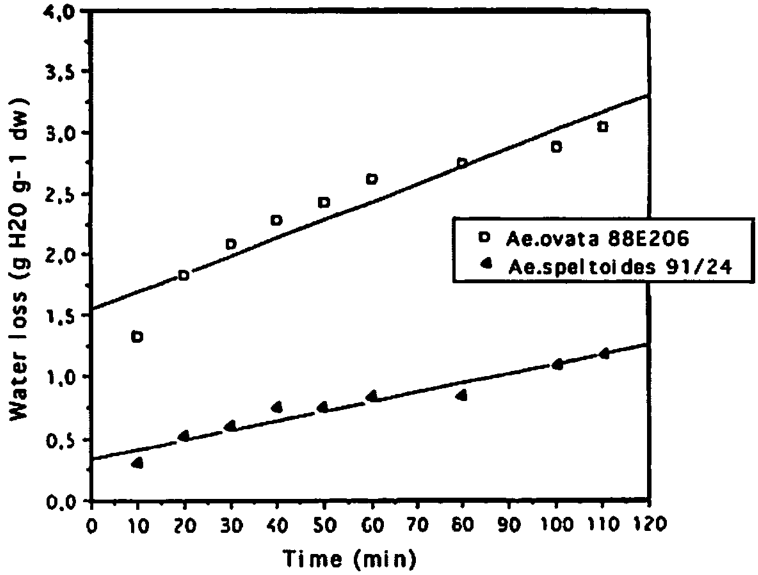

Figure 5. Water-loss curves determined by weighing at frequent intervals after leaf excision.

Management of *Aegilops* Germplasm

Core Collections

Collection, conservation, and management of wild genetic resources are now recognized by cereal breeders as an important strategy. Two problems remain to be solved:

- How to characterize and evaluate wide quantities of accessions already present in the national or international gene banks.
- How to efficiently manage utilization of this material in breeding programs.

Many authors (Frankel 1984; Erskine and Muehlbauer 1991; Spagnoletti, Zeuli and Qualset 1993; and Brown 1994) have insisted on the importance and the feasibility of core collections. The principle of this strategy is to reduce the number of accessions studied by extracting "a subsample representing a large part of the variability of the total collection" from the bank. Experimental work is being carried out to define the principles of stratification in groups and of sampling within these groups.

Stratification of the collection must take into account the type of material, the type of information available, and the objectives of the breeder. Successful stratification must:

- Involve biogeographical information.
- Use the tools of hierarchical classification.

In *Aegilops* species, where diffusion is probably strongly dependent on environmental factors, it is convenient to stratify by species, then by biogeographical data. Information needed for this procedure includes:

- The location of the collection, and morphological descriptors.
- Agronomical or morphophysiological traits potentially useful to the breeder (disease resistance, abiotic-stress tolerance).
- Information provided by highly polymorphic neutral molecular markers (e.g. RAPD markers)

The choice of accessions within the groups can be based on:

- Classical random-sampling methods (Lande and Barrowclough 1987; Brown 1989; Chakraborty and Neel 1989; and Schoen and Brown 1994).
- Determinist sampling, based on the maximization of allele number (Schoen and Brown 1994) or of phenotypic diversity (Noirot et al. 1994) within the core collection.

An important problem in the constitution of core collections is to know which "type of diversity" to maximize or, in other words, which criteria to use to analyze the genetic diversity. These criteria can be:

- Morphophysiological traits, which show a continuous distribution easily expressed by the use of quantitative genetics. This variability presents a direct interest for breeders; the expression of these traits, however, is highly dependent on environmental factors (Mitchell-Olds and Rutledge 1986) and could be expensive in a large collection.
- Biochemical or molecular polymorphism, in which the determination of the number of alleles allows the calculation of Neil or Shannon diversity indices. Little information is available concerning the role of major genes in adaptation to environmental factors and the characterization of selected polymorphisms is rather difficult. Progress with this type of approach is highly dependent on access to advances in cloning major genes of agronomical interest.
- Neutral markers, which give information on the evolutionary history of a group of populations. They allow redundancy to be eliminated by calculating genetic distance between accessions. It is possible that, in the near future, rapid screening of a whole collection will be feasible through the use of markers such as intermicrosatellite amplification.

The constitution of a core collection of Bulgarian *Ae. ovata* is complete, based on 13 quantitative morphological characters and nine qualitative characters (morphological traits, frost and sodium resistance, and ecogeographical origin). Traits related to drought tolerance, and isoenzymatic and RAPD markers are actually used as additional indicators to improve the definition of the core collection. Three different groups have been identified, corresponding to three ecogeographical origins (Zaharieva, personal communication). This research can be further extended to other species present in Bulgaria (*Aegilops cylindrica*, *Aegilops triaristata*) and other Mediterranean countries.

The groundwork has already been laid for the creation of a core collection for *Aegilops* spp. for the SEWANA project:

- Extensive collections have been made in West Asia and North Africa by ICARDA (Damania et al. 1992), in Bulgaria by Zaharieva (1993) and in Morocco (Benlhabib and Zaharieva, unpublished). Collections have also been initiated in the south of France.
- Various biotic and abiotic stresses have been evaluated.
- Isoenzymatic and molecular tools are available in many countries for this purpose.

Aegilops in Durum Breeding: Analytical and Holistic Approaches

The analytical approach to transferring desirable genes from wild relatives to wheat conventionally involves producing an amphiploid between durum and wild genitors. However, these plants, which combine the D genomes of wheat with alien species, are usually unstable, with low yield and quality, and are thus inadequate for direct use by the breeder. Homeologous pairing in wheat is controlled by a major locus (Ph1) on chromosome arm 5BL (Riley and Chapman 1958). The isolation of an induced mutant at the Ph1 locus (Sears 1977) has made it possible to transfer the alien gene to wheat (Liang et al. 1979). In addition, homeologous transfers can be obtained using certain accessions of *Ae. speltoides*, *Ae. caudata*, and *Ae. mutica*, which suppress the activity of Ph1. An assessment of the amount of alien chromatin introgressed into wheat is the key step in this analytical approach. This information is actually limited by the number of markers available, and a development of biochemical and molecular markers is required for the characterization and monitoring of transfers. For this purpose, several complementary methods must be developed, such as isolation, cloning, and characterization of tandem repeated species-specific sequences of wild relatives. In the SEWANA program, this analytical research has been developed by the ETSIA Universidad Politecnica in Madrid, with the aim of introgressing desirable genes from *Aegilops* into durum.

The holistic approach, developed by ENSA-INRA Montpellier, France, is a complementary approach to using *Aegilops* germplasm in wheat breeding. It consists of a massive and non-specific widening of the genetic basis by means of interspecific crosses, followed by the "dynamic management" of the interspecific pools (Henry et al. 1991; Le Boulch et al. 1994). This approach requires:

- A method of interspecific hybridization that obtains a great number of interspecific hybrids with the least amount of time and money. The method consists of a durum previously doubled by colchicin as a female progenitor. The hybrid obtained by crossing with an *Aegilops* accession has the same genomic composition as obtained in the classical way, but two steps are eliminated: doubling of each embryo by colchicin treatment, and manual crossing of the recurrent parent.
- A limitation in the number of crosses carried out. This goal can be achieved by identifying individual plants or families representing the widest variability, which requires the previous constitution of a core collection.
- An experimental network using contrasting and well-known selection pressures and consequent selection for different environmental constraints.

This approach is supported by the results of a dynamic management of genetic resources of bread wheat (David, personal communication), which shows that natural selection leads rapidly to adaptation in populations. It is also supported by the observation that ecogeographical differentiation among *Aegilops* accessions can be attributed to adaptation to environmental stresses.

Conclusion

Information is still needed concerning the potential for salt, cold, and drought resistance of the various *Aegilops* species. Since abiotic-stress resistance depends on several mechanisms and morphological traits, basic investigation is still needed to decide which traits have priority for introgression into durum without affecting productivity. Several laboratories have been asked to carry out this type of research within the SEWANA project. Information concerning biotic and abiotic stresses must be organized and managed to help create core collections and to identify accessions for crossing programs according to introgression methodology (analytic vs holistic) and selection objectives (specific stress vs multi-stress resistance).

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The Use of Tetraploid Wheats to Improve Drought Tolerance in Durum

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Abstract

Some taxa, such as *Triticum turgidum* subspp. *dicoccoides*, *dicoccon*, *polonicum*, and *carthlicum*, represent a promising source of disease resistance, high protein content, and drought resistance. *Triticum dicoccon* shows many interesting agronomical characters, such as biomass production, number of tillers and spikes per plant, number and deepness of roots, and maintenance of high relative water content (RWC) under water stress. *Triticum polonicum* has a long peduncle, long awns, good spike fertility, high 1,000 kernel weight, and a good superficial rooting pattern. *Triticum carthlicum* generally has good tillering ability and good spike fertility. *Triticum dicoccoides* has good tillering capacity. The osmotic adjustment capacity of *T. dicoccoides*, the genetics of RWC, and quality traits were studied. The capacity for maintaining high RWC, or increasing leaf osmotic potential (LOP) when leaf water potential (LWP) is decreasing, were also studied. RWC, LOP, and LWP were assessed in five durum genotypes (Cham 1, Jennah Khetifa, Korifla, Oued Zenati, and Kabir 1) and *T. dicoccoides* 600-808. Results show that the durum cv Cham 1 and *T. dicoccoides* 600-808 maintain their RWC under stress, while Kabir 1, Korifla, Oued Zenati, and Jennah Khetifa rapidly decrease RWC with increasing LWP and Ln(LOP). Leaf RWCs for *T. polonicum*, *T. dicoccoides*, and *T. carthlicum* were higher than for *T. durum* and *T. dicoccon*. Accessions (*T. polonicum*, *T. dicoccon*, and *T. carthlicum*) with the highest RWC were crossed with Cham 1 (drought tolerant durum), and their F_2 populations were assessed. Similarly, *T. dicoccoides* were crossed with the durum variety Korifla and assessed for RWC. The distribution of RWC in the four populations suggested a polygenic determinism for this trait. Broad- and narrow-sense heritabilities were determined. The high mean value of the F_2 over the mean of the parents suggested overdominance effects and transgressive inheritance. A divergent selection was initiated for the crosses of *T. polonicum*, *T. dicoccon*, and *T. carthlicum* to determine narrow-sense heritability and response to selection, and to validate the possible use of this trait in breeding programs. In the *T. polonicum* × *T. durum* F_2 population, RWC showed significant correlation with the harvest index ($r=0.66^{**}$). One single cycle of divergent selection appears to increase the average RWC value. Realized heritabilities for RWC showed the highest value for the *T. polonicum* cross and the lowest value for *T. carthlicum*. Although backcrossing has decreased, the transgressive positive effects of RWC, approximately 10% of the BC_1 plants maintained higher values than the *T. dicoccoides* parent, and 75% higher values than Korifla. The effect of using *T. polonicum*, *T. dicoccon*, and *T. carthlicum* on durum quality was evaluated. Protein content was higher in all tested interspecific lines than in the durum check (Omrabi 5), and some lines also showed higher grain yield and 1,000 kernel weight than the check.. Evaluation of AB tetraploid wheats for drought-tolerance-related traits indicates that many accessions are potentially useful for the improvement of drought tolerance in durum.

Introduction

Since the variability of drought-tolerance-related traits is rather limited in durum, interspecific crosses would appear to be a good tool to introduce such traits into durum germplasm. While the utilization of wild species of the *Aegilops* genus presents many difficulties (see Rekika et al., this proceedings), interspecific crosses between durum and the other tetraploid species of the *Triticum* genus (particularly those with AB genome) could represent a promising and more rapid tool. Arguments for a more extensive use of AB tetraploid wheats in durum breeding have been previously presented (Al Hakimi and Monneveux 1993a). Crosses between *T. durum*, *T. polonicum*, and *T. dicoccon* produce high proportions of fertile F_1 seed (Grignac 1965). Some species, such as *T. dicoccoides*, represent a promising source for disease resistance (Joppa and Williams 1988; Nachit 1990) and high protein content (Nachit 1987; Damania et *al.* 1988). High levels of drought tolerance have been reported in *T. dicoccon* (Grignac 1965), *T. polonicum*, and *T. carthlicum* (Van Slageren et al. 1991).

Yield components and some morphophysiological drought-tolerance traits have been described in the AB tetraploid species (Al Hakimi and Monneveux 1993a). *Triticum dicoccon* shows many interesting agronomical characters, such as biomass production, number of tillers and spikes per plant, and number and deepness of roots. Many accessions of this species also exhibit a capacity to maintain high RWC under water stress. *Triticum polonicum* has a long peduncle and awn length. Both attributes are desirable under terminal water stress (Nachit 1984; Ali Dib and Monneveux 1992). This species is also characterized by good spike fertility, a high 1,000 kernel weight, and a good superficial rooting pattern. *Triticum carthlicum* generally has good tillering ability and good spike fertility. *Triticum dicoccoides* presents a good tillering capacity (Nachit 1990; Damania et al. 1990). Other studies in this proceedings dealing with the potential interest of these four tetraploid species include:

- Osmotic adjustment capacity of *T. dicoccoides*.
- Genetics of RWC in crosses between *T. durum* and some tetraploid species.
- Quality traits of some interspecific hybrids.

Osmotic Adjustment Capacity in *Triticum dicoccoides*

The capacity of maintaining high RWC, or increasing LOP when LWP is decreasing, appears to be a good criterion of drought tolerance in cereals (Acevedo 1987; Schonfeld et al. 1988). RWC, LOP, and LWP were assessed in controlled conditions (20 °C day/18 °C night; relative humidity 60% day/70% night; PPFD 450 μmol.m-2.s-1) for 12 days of water stress on 5 durum genotypes (Cham 1, Jennah Khetifa, Korifla, Oued Zenati, and Kabir 1) and on *T. dicoccoides* 600-808. Results of this experiment, presented in Figure 1, show that the drought tolerant durum Cham 1 and the *T. dicoccoides* 600-808 accession were able to maintain their RWC during the stress, while the RWC values for Kabir 1 and Korifla, and for the landraces Oued Zenati and Jennah Khetifa, decreased rapidly with increasing LWP and Ln(LOP). These results let us postulate a good osmotic adjustment capacity for the wild genotype, which could be transferred into durum.

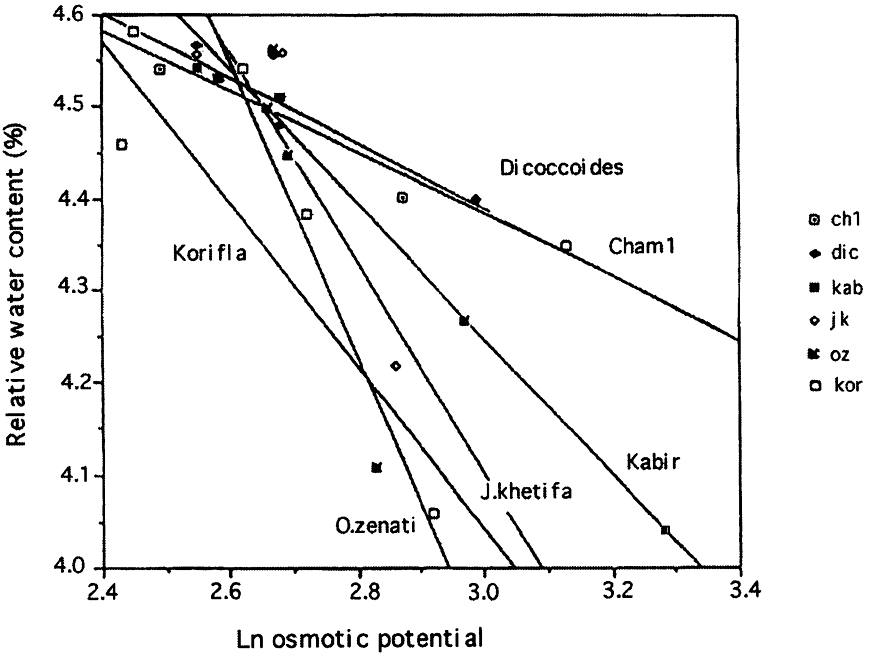

Figure 1. Osmotic adjustment of durum varieties and one accession of *T. dicoccoides* 600808.

Genetics of Relative Water Content

Segregation of RWCs in F_2 Interspecific Populations

Measuring leaf RWC (greenhouse conditions, at sixth leaf stage, on the sixth full expanded leaf) under moisture stress conditions shows that *T. polonicum* and

T. carthlicum had higher values than *T. durum* and *T. dicoccon* (Al Hakimi and Monneveux 1993b). High RWC values were also observed in *T. dicoccoides* (Fig. 1). The accession with the highest RWC value was chosen from each of the domesticated species (*T. polonicum*, *T. dicoccon*, and *T. carthlicum*) and crossed with Cham 1 (drought-tolerant durum). RWC was assessed under the same experimental conditions in the interspecific F_2 populations (Figs. 2a, b, and c). *Triticum dicoccoides* was crossed with the durum variety Korifla (with *T. dicoccoides* as the male parent) and RWC was measured in controlled conditions (Fig. 3). The distribution of RWC in the four interspecific populations suggests a polygenic determinism for this trait. Broad-sense heritability (h2), evaluated using data obtained from the F_2 segregative populations, according to the formula

$$h2 = [\text{var } F_2 - \text{var } (P1 + P2)/2] \text{ var } F_2$$

was found to be high, particularly in *T. durum* Korifla × *T. dicoccoides* 600-808 and in *T. polonicum* × *T. durum* Cham 1 (Table 1). In the crosses with *T. polonicum*, *T. carthlicum*, and *T. dicoccoides*, the mean value of the F_2 was higher than the mean of the parents (Figs. 2a and b), which suggests the effects of overdominance. The high heritability of this trait, as well as its transgressive inheritance shown in all F_2 populations, is encouraging for the manipulation of this attribute in a breeding program. Consequently, a divergent selection was initiated for *T. polonicum*, *T. dicoccon*, and *T. carthlicum* to determine the narrow-sense heritability and response to selection, and to validate the possible use of this trait in breeding programs. In addition to RWC measurements, some morphological traits of the F_2 plants were assessed; a significant correlation was noted in the *T. polonicum* × *T. durum* F_2 population between RWC and harvest index (r=0.66**).

Table 1. Heritability for relative water content in interspecific crosses.

F_2 population	h2 Broad sense	h2 Narrow sense	h2 Realized heritability
T. polonicum 9 × Cham 1	0.73	0.59	0.91
T. dicoccon 1 × Cham 1	0.30	0.74	0.79
T. carthlicum 12 × Cham 1	0.58	0.26	0.51
Korifla × *T. dicoccoides* 600808	0.97		

For abbreviations, see text.

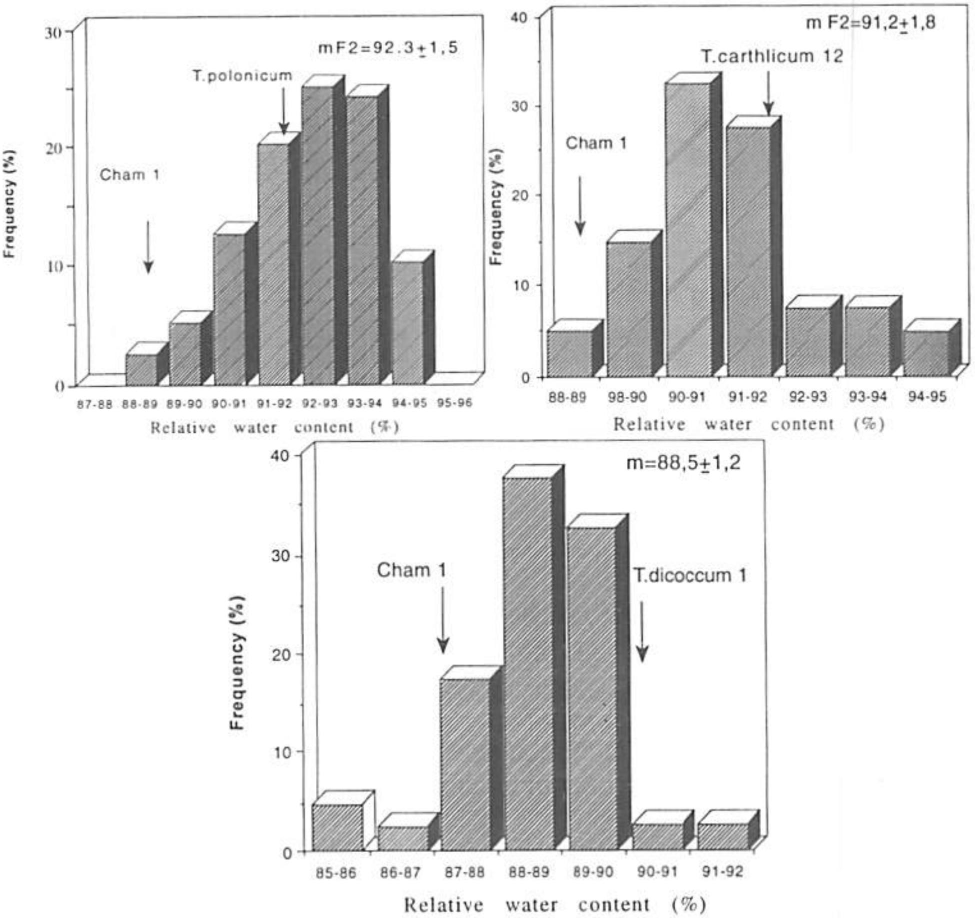

Figure 2. Distribution of relative water content in three F_2 populations: a) *T. polonicum* × Cham 1; b) *T. carthlicum* × Cham 1; and c) *T. dicoccon* × Cham 1.

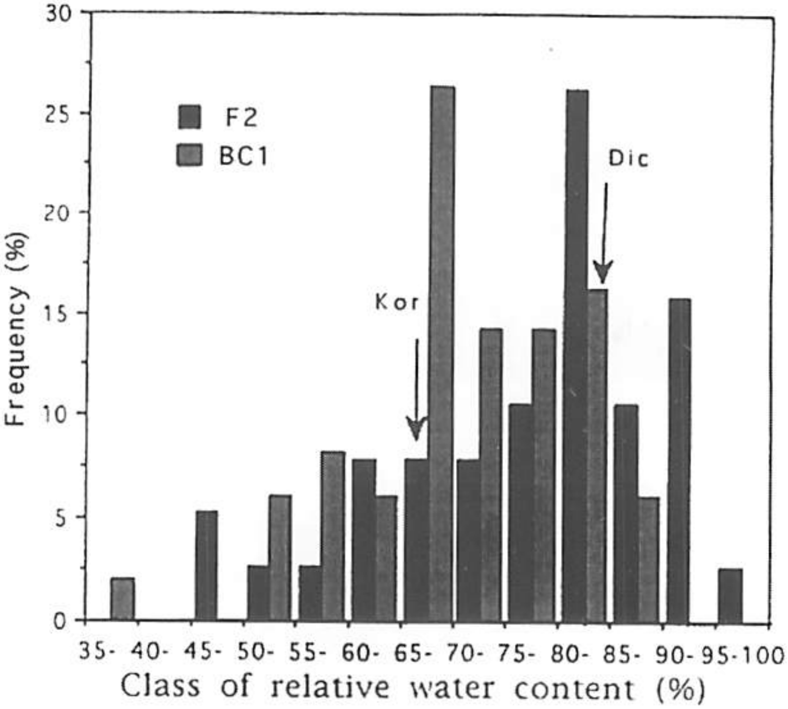

Figure 3. Frequency histogram of relative water content in *T. durum* (Korifla) × *T. dicoccoides* 600808 F_2 and BC_1 populations.

Divergent Selection for RWC

The effects of divergent selection based on the RWC of the F_2 population on the RWC of F_3 population are presented in Figures 4a and b. One single cycle of divergent selection appeared to increase the average RWC value. The F_3/F_2 regressions (narrow-sense heritabilities) are given in Table 1. The F_3 plants were submitted to a second cycle of selection for RWC, and the F_4 seed from these plants was sown at Montpellier for agronomical study. Although the climatic conditions (moderate drought) did not permit an optimal expression of the role of RWC in tolerance, differences were observed for several agronomical traits between the RWC- and RWC+ groups for the crosses *T. polonicum* × *T. durum*, and *T. dicoccon* × *T. durum* (Figs. 5a and b). Realized heritabilities (which are a good indicator of selection efficiency) were calculated for the three crosses (Table 1). The highest value was obtained when *T. polonicum* was used in the cross, and the lowest when *T. carthlicum* was used.

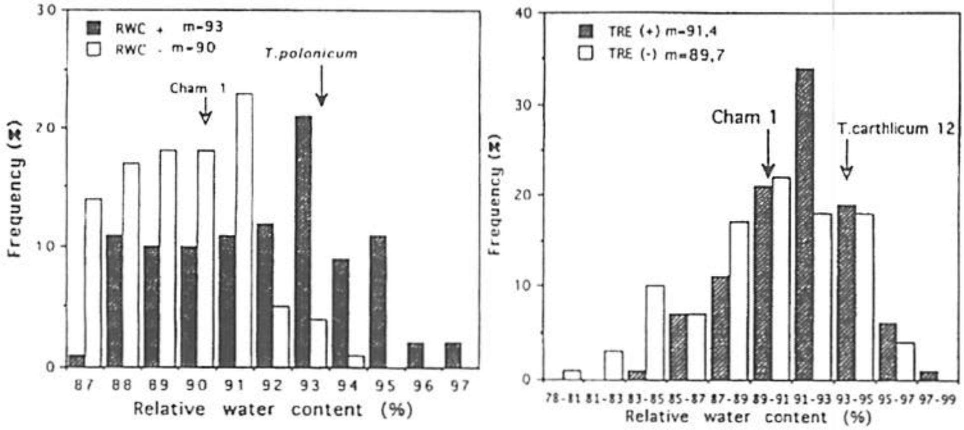

Figure 4. Effect of divergent selection in F_2 based on RWC and the distribution of RWC in F_3: a) *T. polonicum* × Cham 1; b) *T. carthlicum* × Cham 1.

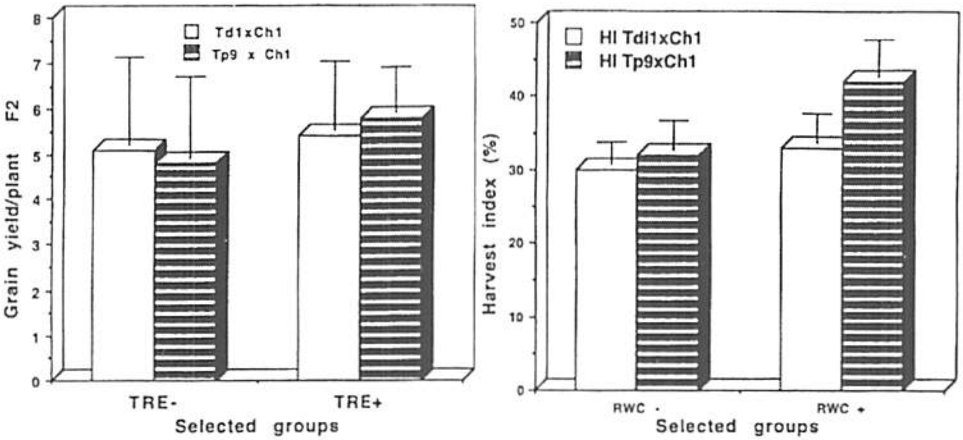

Figure 5. Yield (a) and harvest index (b) under water stress in F_2 RWC+ and RWC- populations.

Effects of Backcrossing

If progenies of single interspecific crosses often present undesirable traits inherited from wild or exotic progenitors, backcrosses (with the improved variety as a recurrent parent) are needed to conserve the favorable traits. The possibility of conserving the introgressed trait (RWC in our case) through successive backcrosses must be evaluated. Figure 3 presents the distribution of RWC values in F_2 and BC_1 populations from *T. durum* × *T. dicoccoides.* Although backcross by the improved parent decreased the transgressive positive effects on RWC, 10% of the BC_1 plants maintained higher RWC values than the *T. dicoccoides* parent, and 75% had higher values than Korifla. Preliminary results are encouraging for the future of introgressing this trait . However, crossing durum with related species is reported to strongly affect the quality of the cultivated species. Thus, the effect on durum quality of using *T. polonicum*, *T. dicoccon*, and *T. carthlicum* in was evaluated.

Quality Traits in Interspecific Hybrids

Thousand kernel weight, protein content, sedimentation value, yellow berry, and semolina color were assessed in F_3 and F_4 interspecific populations selected within an F_2 progeny described by Al Hakimi et al. (1994). Multivariate analysis of the F_3 populations was carried out by the use of principal component analysis (PCA). The first two PCs accounted for 70.5% of the variation (Fig. 6). The *x* axis, which is the component extracted first, was positively correlated with SDS (r=0.63) and protein content (r=0.62), and negatively correlated with 1,000 kernel weight (r=0.58), yield (r=0.79), and yellow berry (r=0.60). Highest protein content and SDS sedimentation values were obtained in a cross between a landrace from Iraq and a *T. carthlicum* accession, and in a cross between *T. dicoccon* and the durum variety Omrabi 5. These F_3 populations were sown at two locations (Montpellier and Aleppo) and individual plants selected on the basis of agronomical traits. The corresponding F_4 seed was sown at Montpellier. Protein was assessed on the bulked lines. Protein content was higher in all tested interspecific lines than in the durum check (Omrabi 5), and some lines also had higher grain yield and 1,000 kernel weight than the check. Higher protein content was registered in the lines derived from F_3 plants selected in the favorable environment (Montpellier).

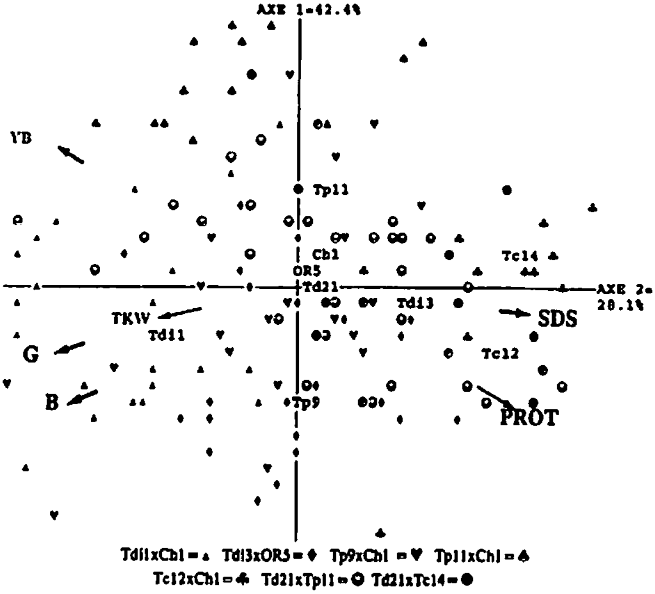

Figure 6. Principal component analysis for several quality traits conducted for 140 F_3 lines belonging to seven crosses.

Conclusion

Evaluation of AB tetraploid wheats for drought-tolerance-related traits indicates that many accessions are potentially useful for the improvement of drought tolerance in durum. The ability to maintain RWC under water stress, a criterion closely related to osmotic adjustment capacity, appears to be polygenic and highly heritable. Important positive transgressive effects were, moreover, observed in some interpecific crosses. All this, as well as the possibility of introducing grain quality characteristics, such as protein content, into durum by way of interspecific crosses, is encouraging for the use of AB tetraploid wheats in durum improvement.

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Some Insights into Morphophysiological Traits Associated with Cereal Yield Increases in Mediterranean Environments

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Abstract

The lack of identification of appropriate morphophysiological traits has been a major factor preventing progress in improving yield in water-limited environments, as well as the reason why plant breeders have not adopted more analytical approaches for selection. Based on the single framework established by Passioura's identity, the critical traits that currently show substantial potential to improve yield under Mediterranean drought conditions are discussed. Particular emphasis is given to single traits which integrate the function of the crop either at a higher level of organization (i.e. canopy), or according to a part of the plant cycle (e.g. grain filling) or both. The potential uses of carbon isotope discrimination and several substitutive approaches, as well as remote sensing techniques, are discussed. Finally, attention is devoted to the photosynthetic performance of the ear. For cereals under Mediterranean conditions, where terminal drought stresses are common, the ear becomes the main photosynthetic organ during grain filling.

General Framework

Cereal yield (per unit growth area) under optimal conditions has increased considerably over the last 50 years (Austin et al. 1989; Romagosa and Araus 1991a). However, yield under stress conditions has remained much more steady (Perry and D'Antuono 1989; Siddique et al. 1989).

Between 60 and 80% of yield variability from year to year in the suboptimal areas may be explained exclusively by environmental factors (Jones and Qualset 1984). Among these, water availability is the most important (Boyer 1982). Mediterranean regions are characterized by yield fluctuations, normally due to the unpredictable occurrence, duration, frequency, and intensity of abiotic (drought, cold, and heat) stresses (Nachit et al. 1992). In addition, if climatic changes occur as predicted due to the accumulation of greenhouse gases, the effect of abiotic stresses such as drought will be of even greater importance in some areas. In fact, the water status of the main cereal crops (durum and barley) in the Mediterranean basin, which may have been stable during the past seven millennia, seems to have undergone drastic changes in recent years (Araus and Buxó 1993). This highlights the need to work on the breeding of cultivars that lead to higher and more stable yields under Mediterranean rainfed conditions (Srivastava 1991).

The main option for stabilizing production is the development of drought-resistant varieties. Genetic improvement of drought resistance faces conceptual and methodological problems. Ample knowledge about the physiological components of drought resistance has been collected. However, the number of factors which affect drought resistance, as well as their interactions and different levels of organization (subcell, cell, organ, plant, and canopy), make it difficult to predict which traits confer the greatest improvement in a particular environment.

In recent years, many different selection criteria, based on morphological, physiological, and biochemical traits associated with cereal yield increases in Mediterranean environments, have been suggested (for durum: Ali Dib et al. 1991, 1994; Nachit and Ketata 1991; Nachit et al. 1992; Loss and Siddique 1994). Despite the volume of information on morphophysiological traits, there are few examples (if any) in which the physiological approach to the breeding of drought-adapted cultivars has been successful. Blum (1983) suggests that this lack of success has two main reasons. First, there is a serious lack of information on the relationships between particular drought-adaptative traits and economic yield in arid and non-arid environments. Second, there have been few attempts to determine the extent of cultivar variation in, and inheritance of, particular drought-adaptative traits. Where genetic variation has been identified, the method is often slow, difficult, or expensive to measure, and inappropriate for routine screening work in a breeding program. Ceccarelli et al. (1991) argues that selection for a single trait is often unsuccessful, particularly in unpredictable environments where the frequency, timing, and severity of stresses are unknown. In these situations, different combinations of many traits may produce the same grain yield. In durum, for instance, the large differences in environmental conditions across the Mediterranean basin may explain the presence of morphologically and physiologically diversified plant types (Ali Dib et al. 1992).

Loss and Siddique (1994) conclude that there are several reasons for the lack of adoption of an analytical approach by breeders. First, empirical breeding programs have been very successful at producing consistent yield increases, especially in high-yielding environments (Slafer et al. 1993). Breeders are not convinced that a physiological approach will give better results, and they believe that improvements in field experimentation and computerization will ensure the continued success of the empirical approach (Loss and Siddique 1994; Ceccarelli and Grando 1995). In fact, breeders do not generally select for specific traits to improve yield under drought, principally because drought adaptative traits are usually poorly defined, and any advantages have not been adequately demonstrated (Richards 1995). Moreover, when selection traits are proposed, there is frequently confusion between traits related to productivity under drought, which are important for agricultural plants, and traits related to survival mechanisms, which characterize xerophytes. Yet many adaptations favoring survival tend to reduce economic yield, and may make sense only when the target environment is very unfavorable.

Given the complex physiological process that determines yield, and its large genotype–environmental interaction, it is often easier to show that a trait improves a short-term plant function or characteristic under stress, rather than improving yield itself. Often, physiologists being their studies at too low a level of organization (e.g. molecular or cellular) and in controlled environments that are of little relevance to the breeder, who is interested in the grain yield of crops in the field. Moreover, those traits that provide "instantaneous" pictures of a given process of the plant are poorly associated with yield response, and can provide contradictory information. An example of the latter is the accumulation of metabolites such as proline (see references in Bergareche et al. 1993). Furthermore, many traits are measured with complex, time-consuming techniques that are unsuitable for screening large numbers of progeny in breeding programs. Given the equivocal evidence associated with some of these traits and the difficulty of selection, Loss and Siddique (1994) conclude that these resources are probably most efficiently used in a parental identification subprogram. Using "equivocal" traits at this level or at any other of a breeding program is an easy way to waste effort. The association of morphophysiological traits with RFLP markers will perhaps improve the usefulness of some of these traits in future breeding programs (Nachit et al. 1993; Backes et al. 1995), but at present, the application of molecular markers in this field is still under development and unavailable to most breeders.

Identifying Proper Morphophysiological Criteria

There is no single index by which to directly predict yield potential and genotype stability, particularly in unpredictable environments where the frequency, timing, and severity of stresses are unknown. Therefore, it is necessary to develop a set of single and quickly-measured criteria. In principle, morphophysiological traits affecting crop productivity could be identified by comparing genotypes grown under contrasting environments—or even within the same environment—by comparing modern and old cultivars or wild relatives. Characterization of morphophysiological traits altered in the process of selecting for higher yield could make it possible to identify the nature of the selection pressure causing the change, and the relationship between the altered trait and the yield increase. Desirable physiological traits could then be specifically targeted for selection. These traits would have to comply with (Acevedo and Fereres 1993) and feature:

- Greater heritability (or cheaper to measure) than yield.
- Significant genetic correlation with yield and/or stability of yield.
- Causal relationship with yield.
- The ability to be tested by means of physiological assays that are easy to use.

- The possibility of screening early generations when yield *per se* cannot be assessed.

One approach to search for "potential" traits to be used in breeding programs is to define ideotypes suited to the agro-ecological zones in the Mediterranean basin (Nachit 1990; Ali Dib et al. 1991, 1992). Under Mediterranean conditions, the framework most widely used was proposed by Passioura (1977) and allows the study of those indices which maximize yield per unit of rainfall:

$$\text{Economic yield} = T \times WUE \times HI$$

where T is the water transpired by the crop; WUE is the water-use efficiency; and HI is the harvest index.

In theory, these components should be independent (Passioura 1977). Therefore, any increase in one of them should not affect the others. In practice, however, these components have been shown not to be totally independent. Thus, Blum (1993) concludes that there may be a negative association between WUE and transpiration, such that relatively drought-resistant genotypes that sustain transpiration and maintain plant water status may show relatively low WUE compared with susceptible ones. This may complicate the feasibility of selecting for any of these components, although Passioura's identity still remains as a pedagogical framework to search for more critically important traits to improve yield under drought.

Several principles (advanced above) can help refine the identification of the critical traits that are most likely to improve yield under drought. First, the degree of influence of a trait on yield depends on the time scale over which it is effective (Passioura 1982). For example, a trait that influences the development of leaf area is more important than a trait that influences stomatal response to the onset of drought. Another principle is that, as a general rule of thumb, the closer a trait is to the level of organization of the crop the more influence it will have on productivity. Thus, a trait such as plant height will have a large effect on yield because it is expressed at the crop level, and the doubling in activity of a key enzyme may have little effect because its expression may be greatly modified by other steps along the path, by other products, by the growth of other organs, and by environmental factors (Richards 1995). If this is generally correct, then it is difficult to perceive how altering the level of a biochemical trait will impact on crop performance.

Some physiological techniques have been modified and, although not as accurate, provide a reasonable (and thus useful) method for plant screening. Several traits are combined in a single measurement. In addition, the traits measured can provide integrated information (either through time or at the canopy level, or both) on the performance of the crop. The end product is selection for yield. In fact, selection for yield automatically integrates all the unknown factors that are important for improving drought resistance. The limitations of a purely empirical breeding approach have already been discussed.

In this regard, current promising physiological criteria, such as discrimination against the stable isotope ^{13}C (Δ) or canopy temperature, are to a greater or lesser extent, integrative in time, level of organization, or both. For example, a high Δ or low canopy temperature can be used to screen for increased stomatal conductance or maintenance of leaf hydration under drought stress and therefore general drought avoidance, whether due to factors associated with root growth, phenology, osmotic adjustment, or non-stomatal transpiration, etc. (Romagosa and Araus 1991a; Blum 1993).

In addition, most known morphophysiological criteria concern the study of vegetative plant parts (usually leaves), ignoring the fact that, for cereals under Mediterranean rainfed conditions, the main photosynthetic organ contributing to the growth of grains is not the leaf but the ear. Under Mediterranean rainfed conditions, terminal stresses (e.g. drought and high temperature at grain filling) are the main factors limiting cereal yield. In this situation, the role of the ear as a photosynthetic organ supporting kernel growth is very important. New insights in this area will allow us to develop alternative criteria, or improve existing ones, in the future.

The final general rule, important for identifying the most likely traits to improve yield, is to know the nature of the target environment (Richards 1995). Thus, it is generally accepted that under conditions with unpredictable or mild water deficit, selecting for high grain-yield potential may still be the best option (Slafer et al. 1993). Under frequent and more severe water stress, physiological attributes aimed at avoiding or tolerating such stresses should be considered for selection, even though these attributes could produce some reduction in grain-yield potential. For cereals under Mediterranean conditions, Ceccarelli (1989) concludes that in environments where the average grain yield is greater than 2–3 t/ha, selection for traits is successful under optimum conditions (see also Ceccarelli and Grando 1995). But where yield is less than 2 t/ha, direct selection in the target environment is the most efficient strategy. In these harsh environments, yield stability is often a high priority.

Traits that fall within the framework established by the components of the Passioura identity are discussed below. Particular emphasis is given to single integrative measurements.

Integrative Traits and Available Methodologies

Water-use Efficiency

The term water-use efficiency is used to express the amount of above-ground dry matter produced per unit of transpired water. This yield component is the most difficult to manipulate. Generations of breeders have failed to improve the water-use efficiency of dryland crops by empirical means (Richards 1995). In water-limited environments, total biomass of current cultivars is about the same as cultivars grown over a century ago (Siddique et al. 1989). However, there are some traits that show substantial potential as fast and single indicators of WUE. These traits are helping breeders and crop physiologists to better understand the sometimes contradictory relationship between WUE and yield, and the factors that influence WUE. Providing that there is enough genetic variability for this character, these traits may became useful tools to improve yield under drought. Among the traits worth mentioning are carbon isotope discrimination and its surrogates. Remote-sensing techniques, which allow, for example, estimation of water status at the canopy level, are included in the next point (water transpired by the crop), although they could be also placed here. Estimation of photosynthetic efficiency by remote sensing is also discussed below.

Discrimination against ^{13}C

In C_3 plants, such as small-grain cereals, Δ provides an integrated measurement of WUE during the previous period of growth (Farquhar and Richards 1984; Hubick and Farquhar 1989; Araus et al. 1993a). Thus, the value of Δ is positively related to the ratio between the atmospheric and the intercellular partial pressures of CO_2 (p_i/p_a). Therefore it is negatively related to WUE (measured either as net photosynthesis/transpiration or plant biomass produced/water transpired). In this regard, Δ has been proposed as a criterion of genetic variability in WUE among genotypes of wheat (Farquhar and Richards 1984; Condon et al. 1990; Ehdaie et al. 1991) and barley (Hubick and Farquhar 1989). Considerable genotypic variation for Δ has been demonstrated in wheat (Condon an Richards 1992), barley (Romagosa and Araus 1991b; Acevedo 1993) and durum (Araus et al. 1993a), but environmental factors may cause even larger changes in the value of Δ measured in dry matter. This could compromise the effective use of Δ in breeding programs. Condon and Richards (1992) for wheat and Romagosa and Araus (1991b) for barley conclude that assessment of genotypic variation in Δ is most effective under well-watered conditions. An alternative for rainfed crops is sampling at the early stages of the crop (Condon and Richards 1992). Richards and Condon (1993) conclude that, provided growing conditions are adequate, the advantages of selecting for Δ are numerous: it is highly heritable, there is substantial genetic variation, genotype × environment interactions are small, and its measurement is non-destructive and must be carried out early in the plant's life.

However, for crops grown under rainfed Mediterranean conditions, even if variation for Δ in mature kernels (as well as other upper parts of the plant) reflects environmental effects rather than true genotypic differences, analyzing Δ may be still of value. For example, Δ of mature kernels can provide an overall view of the water status during grain filling, a critical period for cereals under Mediterranean conditions when drought develops. Within a given environment, a higher Δ in mature kernels may be attained by phenological differences among genotypes. Thus, the early flowering lines are likely to have a higher Δ than later flowering lines, due to a lesser transpirative demand which allows higher stomatal conductance (Ehdaie et al. 1991; Acevedo 1993). At other times, higher Δ in mature kernels is related with a higher transpiration during grain filling, in which case Δ remains positively correlated with canopy temperature (Araus et al. 1993c), an integrative (at the canopy level) criterion of transpiration.

In water-limited environments, the low Δ genotypes should have greater biomass and hence the potential for higher yield, providing all genotypes use the same amount of water for transpiration (Richards 1995). However, a review of the current literature reveals that this is frequently not the case. For instance, Δ values in mature kernels seem to be positively correlated with grain yield and total biomass in wheat (Condon et al. 1987; Morgan et al. 1993; Araus et al. 1993c; Sayre et al. 1995) and barley (Romagosa and Araus 1991a, b) under well-irrigated or rainfed conditions. However, under rainfed conditions, this correlation, although positive, decreases (Romagosa and Araus 1991). The fact that the correlation is positive seems to indicate that plants with lower WUE (because of higher transpiration rates), and thus higher Δ, are more productive. Some of the possible explanations to this were developed in the last paragraph (see also Araus et al. 1993c and Richards 1995). For example, early flowering lines were related to higher yield under Mediterranean conditions (Nachit et al. 1992) and showed a higher Δ than late ones. On the other hand, mechanisms which prevent the loss of water, such as a constitutive lower stomatal conductance, may limit potential yield because p_i (and thus photosynthesis) decreases. These genotypes show consistently lower Δ values (Morgan et al. 1993). In fact, stomata which only close in response to severe water stress may be more adequate, in terms of yield, than stomata which show a permanent lower stomatal conductance (Jones 1987). Moreover, selection for low Δ (i.e. high WUE) may favor genotypes of low production under drought stress (drought susceptible). In fact, the measurement of Δ in different experiments repeatedly confirms that WUE for biomass tends to increase under conditions of drought stress. This indicates that drought-stressed plants tend to have relatively higher WUE than non-stressed plants (Blum 1993). Thus, if two genotypes are compared under drought stress, the one maintaining a relatively better plant water status may show a lower WUE (and higher Δ). For example, depending on the conditions of drought stress, the ability of certain genotypes to maintain a better plant water status results from their capacity to extract deep soil moisture. Therefore, WUE may be a questionable selection criterion for improving yield in water deficit environments. Plant production under drought stress depends not only on WUE, but largely on the genotype's capacity to sustain transpiration (Blum 1993).

Lower values of p_i (and thus lower Δ and higher WUE) may also be attained by means of an increase of internal photosynthetic activity, without any concomitant decrease in stomatal conductance (Araus et al. 1989). In peanut, a strong negative correlation between the specific leaf weight (ratio between dry weight and leaf area) and Δ has been reported using an extensive set of cultivars and varieties

(Wright et al. 1988, 1993). This suggest that cultivars with thicker leaves present higher photosynthetic activity per unit leaf area. Alternatively, higher photosynthetic rate per unit leaf area and lower p_i could be associated with greater leaf nitrogen content (Araus and Tapia 1987; Araus et al. 1989), which in turn would increase water-use efficiency and thus carbon isotope discrimination (see Condon et al. 1992; Amaro et al. 1995). However, selecting for higher leaf thickness and/or nitrogen content can indirectly favor genotypes with lower leaf growth and early vigor (see above).

Alternative Criteria to Δ

Several surrogates for the measurement of Δ have emerged, such as specific leaf weight in grain legumes (Wright et al. 1993, 1994), the amount of minerals such as K or Si, ash content in leaves of cereals and forages (Walker and Lance 1991; Masle et al. 1992; Mayland et al. 1993), and near infrared reflectance spectroscopy (NIRS) in forages (Clark et al. 1995). Indeed, NIRS is a very promising technique because it could allow a very fast and inexpensive estimation of Δ. This technique could be also used to determine, in a faster and non-destructive way, the total ash content of samples (Windham et al. 1991). Indeed, in laboratory settings, NIRS is currently the basis for accurate, highly repeatable assays of many biological traits, including digestibility, nitrogen, energy content, moisture, ash, crude fats, total reducing sugars, alkaloids, and a number of other compounds and classes of compounds in plant matter (Clark 1989).

As pointed out by Mayland et al. (1993) and Clark et al. (1995), further research is needed to evaluate the potential of these alternatives to Δ as a criteria in selecting for WUE. This is particularly true during the early phases of a breeding program when large populations are involved. Later selection could be based on the more precise and accurate—but costly—Δ analysis. Total ash content in mature kernels has been proposed as a criterion complementary to Δ to asses genotype differences under rainfed, poor-yielding environments (Febrero et al. 1994; Voltas et al. 1995). The relationship between Δ and other alternative criteria, such as nitrogen content, needs more conclusive evidence (Condon et al. 1992; Amaro et al. 1995).

Photosynthetic performance at the canopy level

Photosynthetic capacity per unit of leaf area is one of the factors determining in a broad sense the water-use efficiency of a crop. Photosynthetic capacity is strongly determined by organic nitrogen content (Araus and Tapia 1987), and indirectly by chlorophyll content, which generally follows changes in nitrogen. Chlorophyll content per unit leaf area can be fairly well estimated using portable chlorophyll meters. Although measurement is reliable and fast, they measure only isolated leaves. Remote sensing techniques based on canopy reflectance allow measurement of nitrogen content in a fast, non-destructive, and integrative way. Nitrogen content in bread wheat has been related to a linear combination of green (545 nm) and red (660 nm) reflectances in the range of visible light (Fernández et al. 1995). As pointed out before, environmental factors, such as the occurrence of abiotic stresses, strongly affect WUE and Δ, partly because they operate at the photosynthetic level. In this context, indices such as the photochemical reflectance index (PRI), based on reflectance at the 531 nm band, may provide a useful non-destructive assessment of photosynthetic radiation-use efficiency (defined as net CO_2 assimilation rate/incident radiation) either at the leaf or canopy level (Filella et al. 1995). This is similar to techniques for measuring laser-induced fluorescence at the canopy level currently under development (Lichtenthaler 1988).

Water Transpired by the Crop

Early vigor and rapid attainment of ground cover

These traits have been demonstrated to be valuable in true Mediterranean environments (Fischer 1980), providing good yield and biomass (Nachit et al. 1992; López Castañeda and Richards 1994). They enable the wet soil surface to be covered rapidly by the crop, thus minimizing direct evaporation from the soil. Moreover, water-use efficiency is higher in winter because of the lower vapor pressure deficit. There may also be fewer weeds, because a more vigorous crop should prove more competitive. Faster growth is beneficial for improving cereal yield in Mediterranean environments (Blum 1993; Richards 1995).

Current methods for measuring biomass production in cereal plots involve destructive sampling which is not suitable for routine use by plant breeders where large numbers of samples are needed. The measurement of spectral reflectance using ground-base remote sensing techniques has the potential to provide a fast, nondestructive, and accurate estimate of plant biomass production. Fast evaluation of early vigor may be carried out by measuring radiometric characteristics. This implies a comparative assessment of canopy reflectance in the red-near infrared contrast by using, for example, the normalized difference vegetation index (NDVI; Ashcroft et al. 1990) or by direct spectral reflectance in the visible and mid infrared regions (Elliott and Regan 1993). Nevertheless, further work is required to study the effect of environmental factors and genotypic differences in morphological characters such as tiller number and growth habit on reflectance measurements (Loss and Siddique 1994).

The plant characteristics that are responsible for differences in early vigor among and within cereal species are still elusive. Some simple characteristics may be important. For example, the higher early vigor of barley compared with wheat seems to be related to earlier germination and a higher specific leaf area (leaf area to leaf weight ratio) during the early stages in the former species (López Castañeda et al. 1995). For barley, early vigor seems to be positively related to embryo size. For wheat, additional factors and traits associated with early vigor are the development of coleoptile tillers (Liang and Richards 1994), the absence of the major dwarfing genes (Richards 1992), and the width of the first leaf (Richard

1995). A negative relationship between large leaves and good frost tolerance has been observed in durum (Pecetti et al. 1993), which may suggest that early vigor might be associated with sensitivity to winter frost episodes.

In addition, increased early growth and leaf area development may be inherently linked with decreased water-use efficiency (Turner 1993). Therefore, selecting for increased WUE (and therefore lower Δ) may be counterproductive. It may be preferable to select for early vigor and low specific leaf weight in order to improve early vigor (Turner 1993). Thus, under well-watered and fertilized conditions, the Δ of the water soluble fraction of seedlings is positively related to growth (Febrero et al. 1992). Therefore, high Δ in seedlings could be used as a selection criterion for early vigor.

Plant biomass at heading/anthesis

Apart from estimating early vigor, non-destructive remote sensing techniques based on reflectance measurements allow evaluation of the effect of environmental factors such as water and nitrogen stress on total biomass, total leaf area, and plant yield later in the crop cycle (Flagella et al. 1992; Fernández et al. 1994). Thus, NDVI is widely accepted as a radiometric indicator of these crop parameters (see references in Fernández et al. 1994).

Plant water status

Selection for the ability of plants to maintain leaf hydration under drought stress, whether due to factors associated with root growth, low non-stomatal transpiration, or osmotic adjustment, can be evaluated visually or by remote sensing techniques. Simple yet effective visual criteria for water status and green area (leaf) duration are various leaf symptoms such as leaf rolling and leaf desiccation or "firing" (Nachit et al. 1992; Blum 1993). For example, leaf rolling is an expression of turgor loss in cereals. It serves to protect the leaf against an overload of solar radiation which cannot be dissipated by transpiration. Moreover, it produces severe photoinhibition and early senescence. Therefore, leaf rolling upon wilting is important, but delayed rolling is taken, in a positive sense, as an indicator of retained turgor (Blum 1988; Nachit 1992).

Several plant responses to drought stress are amenable to detection by remote sensing techniques. For instance, canopy infrared thermometry as a screening technique for drought resistance has been used with wheat (Blum et al. 1982). This technique allows, for example, screening for deep roots, increased stomatal conductance, and general drought avoidance (Blum et al. 1982, 1988, 1989). Of course, the maintenance of transpiration may be either an advantage or disadvantage, depending on the stage of growth and the total seasonal distribution of water use. For example, lower canopy temperature was associated with better yield stability when measured just before heading under a Mediterranean climate (Nachit 1992; Blum 1993). Although infrared thermometry has been widely used in breeding programs because of the low price of instrumentation and the apparent simplicity of utilization, results have been much less promising than many expected. The lack of success may be because the plants were not properly stressed (Blum et al. 1982) or it may be due to disturbances in measurements because of time of day, weather, and ground cover (Turner 1986).

Water status can also be monitored at the canopy level using reflectance techniques. For example, the ratio between reflectance at 870 nm (one of the water absorption bands) and at 900 nm (reference wavelength) closely follows the changes in relative water content, leaf water potential, stomatal conductance, and canopy temperature when the plant water stress is well developed (Peñuelas 1993). For cereals exposed to a soil salinity gradient the water index measured during grain filling was correlated with carbon isotope discrimination of mature kernels and canopy temperature (Peñuelas et al. 1995).

Harvest Index

It is generally accepted that the main physiological change produced by the genetic improvement of wheat grain yield in recent decades has been an increased harvest index (HI) associated with an increased number of grains per unit land area (see references in Slafer and Andrade 1993). However, even when current cultivars are able to develop large HIs when cultivated under high input conditions, under Mediterranean rainfed conditions HI is modest. This arises from the setting and filling of kernels. Therefore, the period from anthesis to maturity is crucial in determining HI under our conditions, although sometimes total grain yield may be associated with previous stages, which determine, for instance, spike density (Nachit et al. 1992).

Grain Filling

Genotypes maintaining better water status during this period should show a higher HI. Evaluation of traits associated with the two other components of Passioura's identity after heading and during grain filling would indeed help to improve HI. For example, final kernel weight depends mostly on duration of grain filling (see references in Hay and Walker 1989), which is associated with green area duration. For green area duration, evaluation with a portable chlorophyll meter may be feasible. Under drought conditions, early senescence may be in turn associated with the water status of the plant. Evaluation of water status during grain filling, either by means of visual scores, remote sensing techniques, or Δ in mature kernels can help improve the harvest index under Mediterranean conditions.

Preanthesis Reserves

The capacity to support grain filling from mobilized stem reserves is difficult to assess under natural drought stress conditions (Blum 1993). The application of chemical desiccants on leaves and stems has been used to simulate postanthesis water stress and to screen genotypes for their ability to retranslocate assimilates to the grain (Blum 1988). Although chemical selection seems to have been successfully used in mass selection to improve grain filling under stress (Blum et al. 1991), this method is best conducted in the absence of leaf diseases under irrigated conditions or in wetter environments (Nicolas and Turner 1993). There are other restrictions to the use of the technique; it is convenient to use with genotypes of similar maturity (Loss and Siddique 1994). The results may vary in relation to the desiccant used; for instance, magnesium chlorate can show a much stronger effect than potassium iodide, sometimes killing the plants (Romagosa and Voltas, personal communication). Although the desiccant should be applied properly on leaves and stems (Blum 1988; Nicolas and Turner 1993) it has been applied to the whole canopy, affecting the spike, which is not only a photosynthetic organ but also the sink of assimilates.

Importance of the Ear

The contribution of ear photosynthesis to final grain weight ranges between 10 and 76% (Biscoe et al. 1975; Evans et al. 1975; Duffus et al. 1985), depending not only on the genotype, but also on growing conditions and the method of measurement. Hence, the photosynthesis of the ear may contribute more to grain yield than that of the flag leaf. This is particularly evident in awned genotypes under drought conditions (Johnson and Moss 1976; Blum 1985), although it has also been reported under irrigation (Araus et al. 1993a). Differences in WUE between the ear and the vegetative parts of the plant may be involved in the photosynthetic role of the spike. Even though the ear (due to its position) is the warmest organ of the plant, the WUE of the spike is higher than that of the vegetative parts of the plant (Araus et al. 1993a). The presence of awns seems to confer to the ear an additional increase in WUE (Bort et al. 1994), although this point is now controversial (Weyhrich et al. 1995). The ear has a much more xeromorphic anatomy than the lower parts of the plant (Araus et al. 1993a). This may be an adaptation to environmental conditions becoming more arid during the cycle of the plant (Araus et al. 1986, 1991).

The adaptative response of the spike may be related to its photosynthetic function in several ways (Blanke and Lenz 1989). First, there is the possibility of a high degree of refixation by ear bracts of CO_2 released by growing grains. Second, anatomy may be related with the presence of an intermediate C_3—C_4 photosynthetic metabolism in ears. Third, a crassulacean acid metabolism (CAM) may be present. Each of these possibilities could confer ecological advantages to the ear under conditions of high temperature and water shortage (Schuster and Monson 1990) by diminishing, for example, photorespiratory losses during grain filling (Ziegler-Jöns 1989). Several papers on immature grain in wheat and barley (Duffus and Rosie 1973; Meyer et al. 1978; Watson and Duffus 1988; Aoyagi and Bassham 1984) report substantial activity of PEP carboxylase (PEPcase) in immature grains, as well as other enzymes of the C_4 cycle. On the other hand, there is also considerable PEPcase activity and high levels of C_4 products in bracts (glumes and lemmas) of C_3 cereals (Wirth et al. 1977). This and other observations about enzymatic activity and the presence of C_4 metabolites (Blanke and Lenz 1989), as well as anatomy, chloroplast ultrastructure and gas-exchange, suggest that in bracts and immature grains of C_3 cereals there is an intermediate C_3—C_4 metabolism (Ziegler-Jöns 1989). However, recent results have provided conclusive direct (Bort et al. 1995) and indirect (Araus et al. 1993b) evidence of the lack of C_4 metabolism in ears of durum and barley. The possibility of some degree of inducible CAM metabolism, as well as the effect of abiotic stresses, has been less studied and still needs to be elucidated.

Various studies support the refixation of CO_2 released from the respiration of the ear (Kriedemann 1966; Watson and Duffus 1988; Araus et al. 1992, 1993a). Because of the importance of the CO_2 released by respiration, the photosynthetic rate of the ear has usually been underestimated, and the role of the ear as a photosynthetic organ during grain filling may be more important than previously supposed (Araus et al. 1993a). Evaluating genotypic variation for CO_2 refixation by ears by developing fast and simple criteria to asses the degree of refixation may be of interest. One of the simplest criteria may be the difference in CO_2 evolution in light and dark using CO_2-free air.

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Calibration of CropSyst Simulation Model and Assessment of Durum Wheat Production Risk Across Agro-climatic Zones of Northwest Syria using Simulation/GIS Technology

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Introduction

The rainfed farming systems of West Asia and North Africa (WANA) have developed over the centuries in areas receiving annual rainfall of only 200–600 mm, varying from year to year and place to place. This variable and often chronic rainfall deficiency is coupled with widespread nutrient deficiencies and improper soil and crop management. Cereals are the principal crops grown in the region, with wheat (*Triticum aestivum* subsp. *aestivum*, *T. turgidum* subsp. *durum*) covering about 50% and barley (*Hordeum vulgare* subsp. *vulgare*) about 20% of the cropped land (Pala 1991). A cereal/fallow rotation integrated with livestock is the traditional system in the region. This increases the amount of water available for the subsequent crop, but "fallowing efficiency is low and variable" (Harris et al. 1991). In addition, the increased need for food and feed associated with population growth is causing the abandonment of this practice for continuous cropping throughout the region (Acevedo et al. 1991). In Syria, wheat is grown from the wettest to the driest areas. In dry areas, where barley predominates, it is produced for subsistence, even up to the edge of the steppe (Thomson et al. 1985). In wetter areas (over 325 mm mean annual rainfall), wheat is the dominant crop and is in rotation with food legumes and summer crops such as water melon, sesame, cotton, cucurbit, etc. Farming systems in the area have to cope with problems of highly variable and frequently deficient rainfall (Cooper et al. 1987). As this factor cannot be controlled by farmers, soil and crop management practices combined with adapted improved cultivars geared for improved water-use efficiency (WUE) provide the best chance for farmers to increase and sustain crop production. Crop establishment is the most important step in crop production, and sowing date is one of the key factors in achieving this, provided there is a properly tilled seed bed.

Under continuous cropping systems, almost all the extractable water is used by the crops every year, and further evaporation during the summer decreases soil water far below the wilting point. Therefore crops depend on precipitation in autumn for crop emergence and canopy development.

For dryland crop production in winter rainfall areas, the sowing date may have a substantial effect on WUE, and thus yield, by ensuring that growth is adjusted to the availability of soil moisture. There are many indications in the region and in similar areas of the world that early crop establishment and early canopy development are associated with higher wheat and barley yield (Bolton 1981; Cooper et al. 1983). The possibility of extending the growth period, resulting in higher yield, is possible only by earlier sowing (Keatinge et al. 1986; Photiades and Hadjichristodoulous 1984; French and Shultz 1984). However, early sowing will only be an advantage if emergence is also early and if the crop can survive potential drought conditions at the seedling stage (Pala 1991). Currently, farmers in the Mediterranean basin tend to sow wheat later than the optimum time (mid November), as defined by the above-mentioned and other researchers, because of unreliable initial rains (Dennet et al. 1984), the popularity of weed control through pre-sowing tillage operations, and the risk of frost damage.

The effect of sowing date on crop yield, given the long-term spatial and temporal variations in a given region, must therefore be studied. Field experiments are the best tools to assess this effect. However, they are usually conducted over short periods. To attain reliable results for analysis of the production risk, a minimum of 30 years of data are generally required. In this respect, crop simulation models are possible alternative tools for such study. Geographic information systems can be used to map soil and crop characteristics over a wide region to direct research and development projects for the welfare of the farmers.

The impact on water productivity and N availability, two critical resources for growing wheat and other crops in the region, cannot be analyzed independently from weather data, soil characteristics, field hydrology, crop characteristics, and rotation system, among other factors. Therefore, in addition to studying the optimum sowing date for the improved durum wheat cultivar, Cham 1, our second objective was to determine potential yield levels with sufficient water and nitrogen, using CropSyst whenever needed to assess regional productivity. Supplementary irrigation, i.e., supplementation of deficits with minimum quantities of water, is one way to increase productivity and meet the food requirements of the rapidly increasing populations of WANA (Perrier and Salkini 1991). The third objective was to identify the nitrogen levels for different soil and climatic zones under rainfed conditions to increase nitrogen-use efficiency.

The Model

CropSyst is a multi-year and multi-crop daily time step simulation model. It was developed to serve as an analytical tool to study the effect of cropping systems management on productivity and the environment. The model simulates soil water budget, soil–plant nitrogen budget, crop canopy and root growth, dry matter production, grain yield, residue production and decomposition, and erosion. Management options include cultivar selection, crop rotation (including fallow years), irrigation, nitrogen fertilization, tillage operations, and residue management.

The water budget in the model includes rainfall, irrigation, runoff, interception, water infiltration and redistribution in the soil profile, crop transpiration, and evaporation. The nitrogen budget in CropSyst includes nitrogen application, nitrogen transport, nitrogen transformations, ammonium sorption, and nitrogen uptake. Daily crop growth, expressed by biomass increase per unit area, is calculated on the basis of a minimum of four limiting factors; light, temperature, water, and nitrogen. Details on the technical aspects and use of the CropSyst model are reported elsewhere (Stockle et al. 1994; Stockle and Nelson 1994). The model and documentation are available from the second author of this paper upon request.

Soil characteristics, initial conditions of available moisture N and organic matter, and daily weather data were input into CropSyst. The crop input parameters used for the cultivars were either typical for the crop species, determined from experimental data, or determined by calibration as mentioned below.

The field data used for model validation were obtained from a line-source sprinkler experiment (Perrier and Salkini 1991) conducted at the International Center for Agricultural Research in the Dry Areas (ICARDA) headquarters, Tel Hadya, near Aleppo, Syria, during 1989/90, 1990/91, and 1991/92 using a strip block design with three replications. Cham 1, an improved durum wheat cultivar, was tested under three water levels (W0, W60, and W100) and two levels of N fertilizer, in a wheat/chickpea rotation. To calibrate crop parameters, two plots (W100/N100 and W0/N0) in 1991/1992 (the best season during the experimental period) of the cultivar were used. These plots were not used in the validation, as explained below. Calibration consisted in adjustments of these parameters within their usual range of fluctuation to produce a reasonable tracking of green area index (GAI), aboveground biomass, evapotranspiration (ET), and N uptake throughout the season.

Validation of the model for Tel Hadya soil conditions was carried out using 16 out of 18 combinations of three growing seasons, three water treatments, and two nitrogen treatments as described above, each including the fluctuation of GAI, aboveground biomass, cumulative ET and cumulative N uptake throughout the season. In addition, above-ground biomass, grain yield, cumulative ET, and cumulative crop N uptake at harvest were also available.

The Model's Performance

To evaluate the ability of CropSyst to track the GAI, aboveground biomass, ET, and N uptake progression throughout each growing season, the data points were plotted against the daily simulated data for each treatment. An example is presented in Figure 1 for 1990/91. Most results were similar to this example, with a few cases better or slightly worse. In general, the model was well able to track changes in GAI, biomass, ET, and N uptake. This is important, because it provides a base to support the reliability of the model to predict these quantities at harvest time, the information usually utilized in the long-term analysis of management practices.

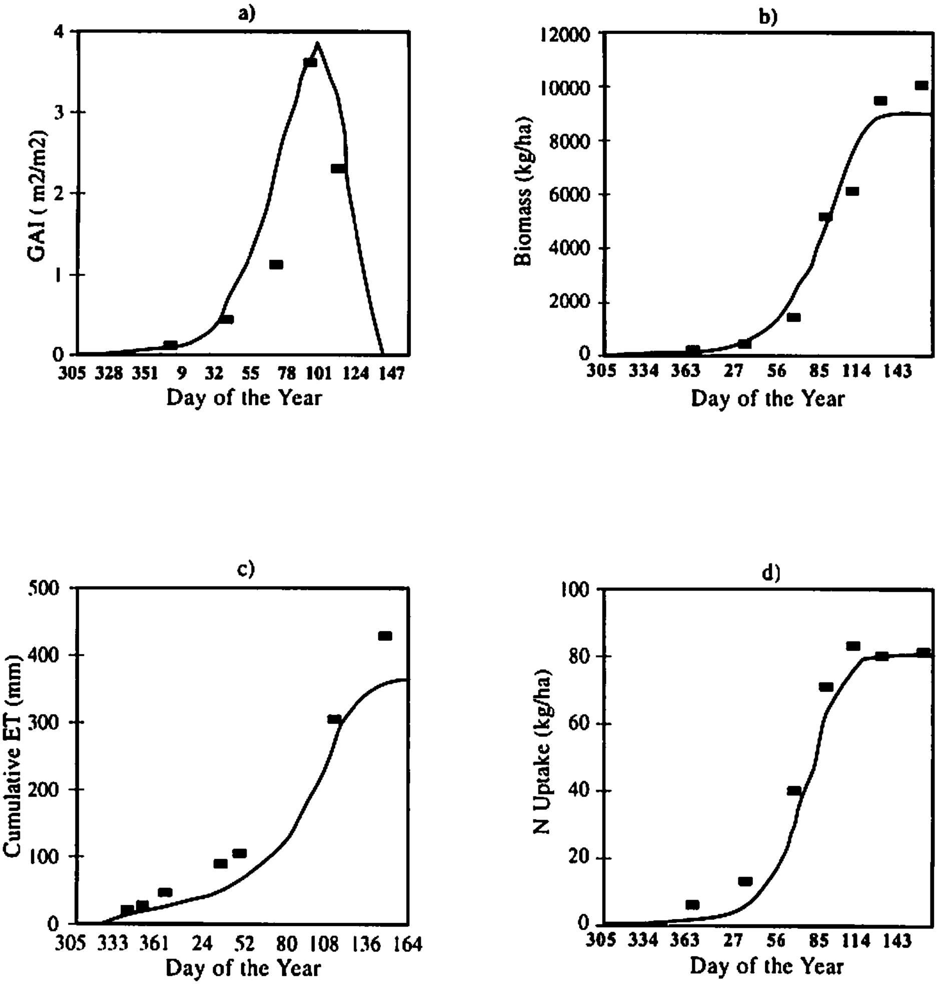

Figure 1. Distribution of daily simulated data compared with the onserved data obtained at different times during the 1990/91 growing season of Cham 1 durum cultivar, treatment W/100/NO: a) GAI; b) above ground biomass; c) cumulative ET; and d) cumulative N uptake. The rectangles represent observed data points, and the lines represent simulated daily data.

Observed and simulated above-ground biomass, grain yield, cumulative ET, and cumulative N uptake at harvest are compared in Figure 2. Statistical analysis of this information is presented in Table 1. The simulated outputs for Cham 1 closely follow the 1:1 line when plotted against the experimental data (Fig. 2). Statistical analysis reveals that CropSyst predicted the outputs reasonably well, with a high index of agreement (d), and root mean square errors (RMSEs) of 9 (Cumulative ET) to 25% (grain yield) of the observed mean values. The observed and simulated mean values for the 16 data points of each cultivar were very close (Table 1).

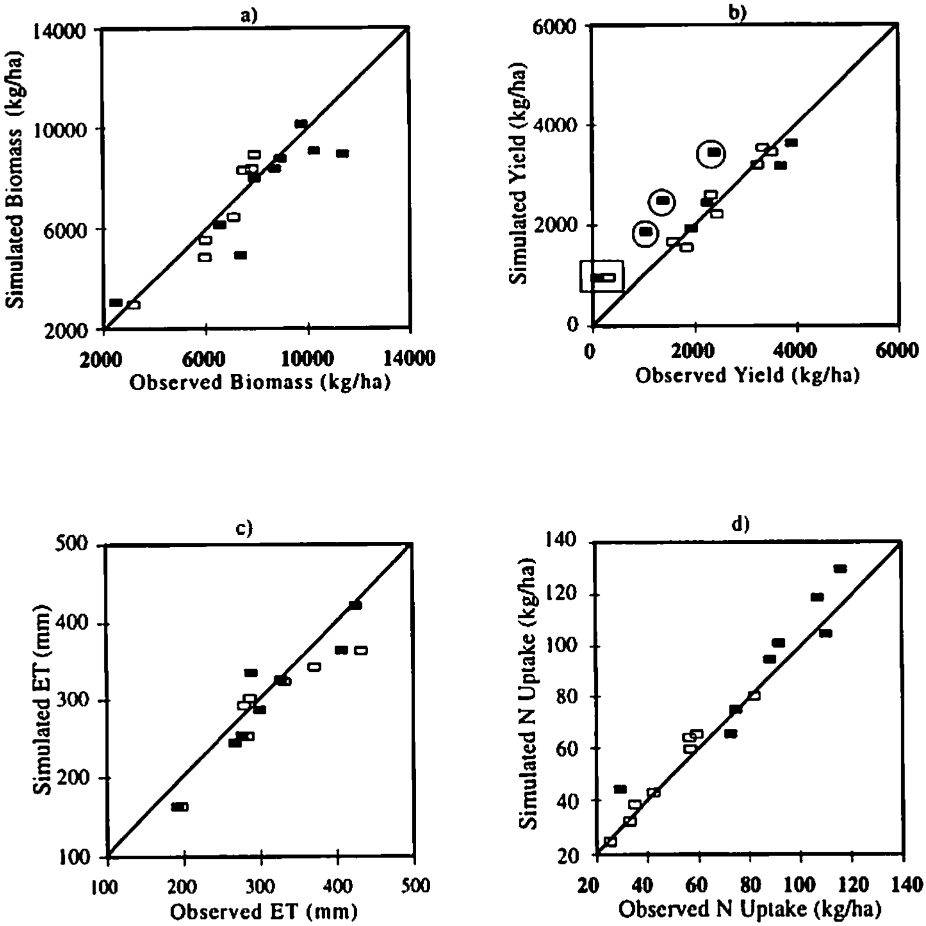

Figure 2. Simulated data plotted against observed data for the Cham 1 wheat cultivar during the three seasons of the experiment: a) above ground biomass; b) grain yield; c) cumulative ET; and d) above-ground crop N uptake.

The reason for the over-predicted grain yield data (marked with squares) was the severe drought associated with frost and strong hot winds on rainfed plots in 1989/90, which the model was unable to detect. The reason for the overestimated points (marked with circles) was the unusual negative response of grain yield to N fertilization during 1990/91. In spite of these problems, the statistical analysis shows a reasonable agreement between observed and predicted grain yield data, with an index of agreement of 0.92 for Cham 1 (Table 1).

Soils of the Study Area

The soils of the study area are diverse, with four major soil groups recognized on the FAO/UNESCO soil map of the world found in northwest Syria (Map 1).

Calcareous soils, formed from limestone residuum, predominate in the area, with highly variable texture, depth, slope, and stoniness. Organic matter levels are generally low, and structural stability is poor in some soils. Sabet and Harris (1986) report surface capping by rainfall as a serious constraint to production on the soils of calcic Xerosols in Syria.

Agro-ecological Zones

Syria has been divided into six agricultural stability zones based on mean annual rainfall (Watson 1979). Five of these stability zones transect the study area. However, closer intervals of mean annual rainfall isohyets were used in the simulation to highlight the changes across rainfall zones (Pala et al. 1992).

Data from 24 meteorological stations were used to generate the weather data for pre-defined rainfall isohyets at 20 mm intervals (21 rainfall zones): 11 stations had daily rainfall, maximum and minimum temperature data ranging from 12 to 29 years; 6 had daily rainfall data ranging from 13 to 28 years; and 7 had only monthly rainfall data, ranging from 15 to 24 years. Radiation data, available from the ICARDA Tel Hadya station for only 12 years, was used as a standard value for generating radiation values for all stations (summer B parameter was 0.302818 and winter B parameter was 0.00575).

Production Systems

About one third of the dryland area lies in northwest Syria. A two year crop rotation is almost universally practiced with wheat as the main crop, generally preceded by chickpea or lentil, depending on locality.

Chickpea and lentil crops dry the soil profile to about the same extent as wheat, therefore the water available to these crops comes from the current season's rainfall only. However, continuous wheat, in contrast to chickpea and lentil, performs poorly due to factors other than water availability (Harris 1990). Therefore, continuous wheat was assumed to behave as a wheat/legume sequence in the simulation with respect to water use. Since the modeling of legumes has not been tested in the study area, the use of continuous wheat was thought to be useful for the study of long-term wheat production risk. Biotic factors, related to the poor performance of continuous wheat, could not be traced by a simulation model.

Cham 1, which is an improved durum wheat cultivar highly adopted by farmers in the study area (Pala and Rodriguez 1992) was used in the study.

Effect of Sowing Date on Production and Risk

Production

An example is given in Map 2 using a model/GIS combination for yield output based on mid-November sowing. Half of the study area (about 40–60% of cropped land) yields only 0.5–1.5 t/ha of wheat, which is about the mean wheat yield in Syria. However, it becomes clear that wheat sown later than mid November provides less than 1 t/ha yield in about 25–50% of the area. Yield over 2 t/ha is obtained on about 30% of the area, generally from early sowing (Fig. 3).

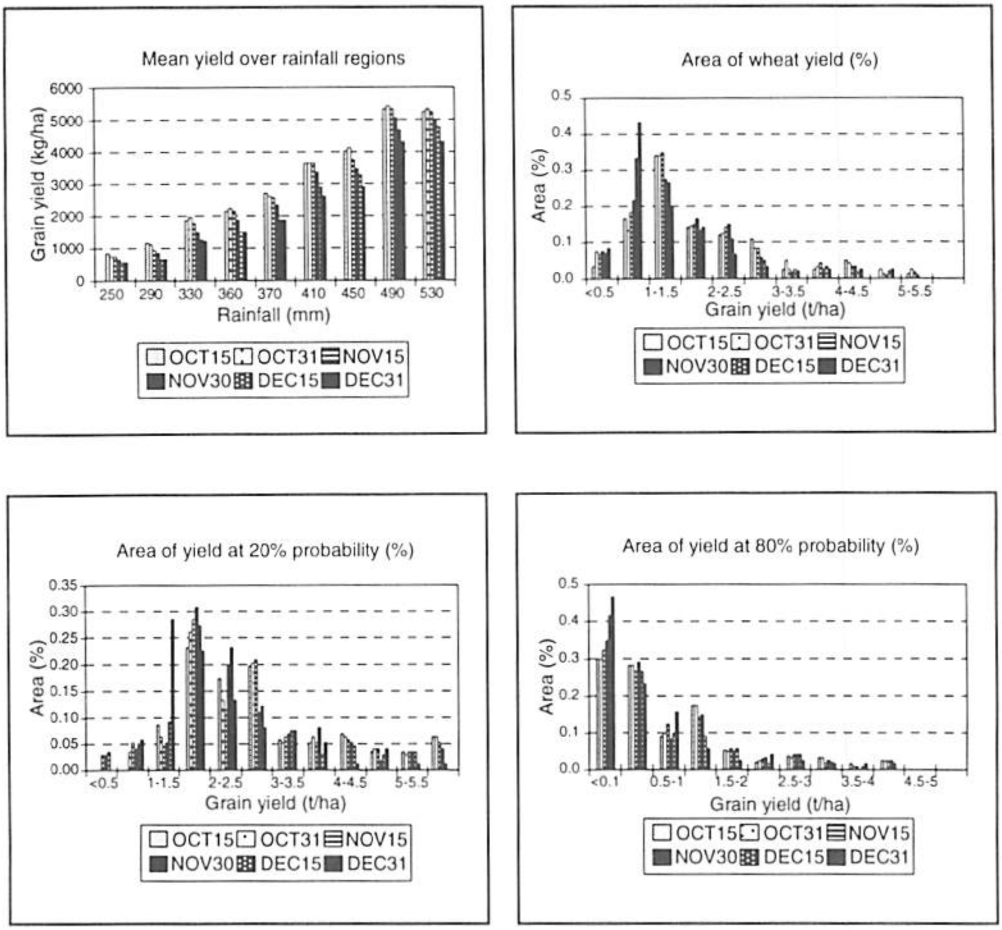

Figure 3. Mean durum wheat yield with different sowing dates across rainfall regions, area percentage of yield ranges, and their probabilities at 20 and 80%.

In general, yield increases along with increased rainfall up to about 500 mm. In the higher rainfall areas, delaying the sowing date has a substantial negative effect on yield (5 t/ha at earlier sowing decreases to about 4 t/ha in late December sowing).

However, this effect lessens as conditions dry. In general, sowing wheat between mid October and mid November does not have a remarkable effect on yield, while delaying it to later dates decreases yield (Fig. 3).

Risk

If the farmers like the risk and want higher yield 20% of the time, they should adopt earlier sowing. About 70% of the cropped land yields 3 t/ha or less. At higher yields, earlier sowing is generally the norm (Fig. 3). However, most of the farmers are against risk and would like to be on the safe side at least 80% of the time. In this case, yield more than 1.5 t/ha will be obtained on only 15% of the area, and once again earlier sowings generally provide more production. More important is the increasing area of yield less than 0.1 t/ha, which is practically zero with delayed sowing.

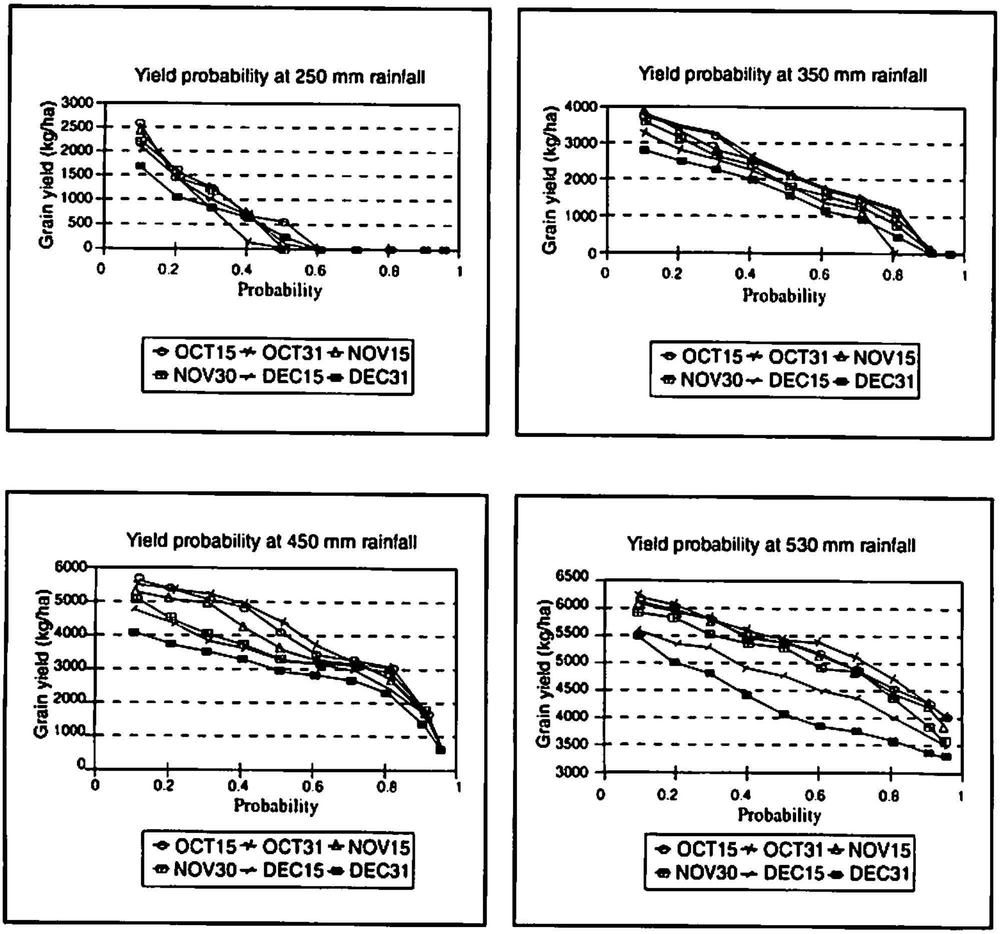

Figure 4. Yield probabilities with different sowing dates at different rainfall areas.

The probability of yield distribution from drier to wetter areas is given in Figure 4. However, an explanation is needed for the driest and wettest areas. In drier areas (250 mm rainfall), the probability of yield between 1 and 1.5 t/ ha is just 20%, decreasing substantially when wheat is sown in late December. The probability of no yield is 40% for all sowing dates, revealing a great risk for wheat in dry areas. However, farmers grow barley in these areas to alleviate the risk of crop failure.

The situation markedly improves for wheat production in northwest Syria when it gets wetter. In areas with more than 500 mm mean rainfall, early sowing until mid November has a great advantage over later sowing, with no risk at all for any date of sowing. Twenty percent of the cases yield about 5.8 t/ha through late November sowing, although this falls to about 5 t/ha when sowing is further delayed. In eighty percent of cases, yield is about 4.4 t/ha for the first four sowing dates, but only about 3.5 t/ha when sowing is delayed to December (Fig. 4).

Effect of Supplementary Irrigation and N Application on Production and Risk

Production

Rainfall does not have much effect on wheat yield since automatic irrigation has solved the water deficit problem in drier areas (Fig. 5).

About 42% of the cropped area needs 250–300 mm/year irrigation water, about 27% needs 300–400 mm, another 25% needs 200–250 mm, and the rest (about 6%) needs less than 200 mm. This shows that the majority of the area needs more than 250 mm irrigation water/year to achieve optimum yield (Fig. 5). Supplementary irrigation requirements decrease from about 400 mm in the 250 mm rainfall zone to 100 mm in the 500 mm zone (Fig. 5). Scarcity of water resources, however, allows a small percentage of the area to be supplemented by irrigation, thus water should be used efficiently.

The N requirement is not affected by rainfall either, since irrigation solves the problem of the water deficit (Fig. 6). About 10, 30, 25, and 17% of the study area requires 90–95, 95–100, 100–105, and 105–110 kg N/ha, respectively. The rest of the area (about 22%) requires more than 110 kg N/ha as fertilizer (Fig. 6). These N requirement values are in agreement with earlier studies (Perrier and Salkini 1991; Pala et al. 1992.

Risk

In 20% of cases, about 500, 400, 300, and 100 mm of irrigation water would be required for areas receiving 250, 350, 450, and 530 mm of rainfall, respectively (Fig. 5). In 80% of cases, about 350, 250, 200, and 100 mm of irrigation water would be required for zones receiving 250, 350, 450, and 570 mm rainfall, respectively (Fig. 5). This shows that to achieve potential yield, drier areas would deplete water resources. Therefore farmers rightly decide to grow barley in dry areas and supplement wheat with lesser amounts of water to increase wheat production. These results are in agreement with farmer application. However, farmers in the region apply more than the necessary amount of water to increase wheat production (Pala and Rodriguez 1993).

In 20% of cases, the N requirement is about 100, 110, 130, and 140 kg N/ha for zones receiving 250, 350, 450, and 570 mm of rainfall, respectively (Fig. 6).

In 80% of cases, the N requirement is about 75, 90, 105, and 115 kg N/ha for areas receiving 250, 350, 450, and 570 mm of rainfall, respectively (Fig. 6).

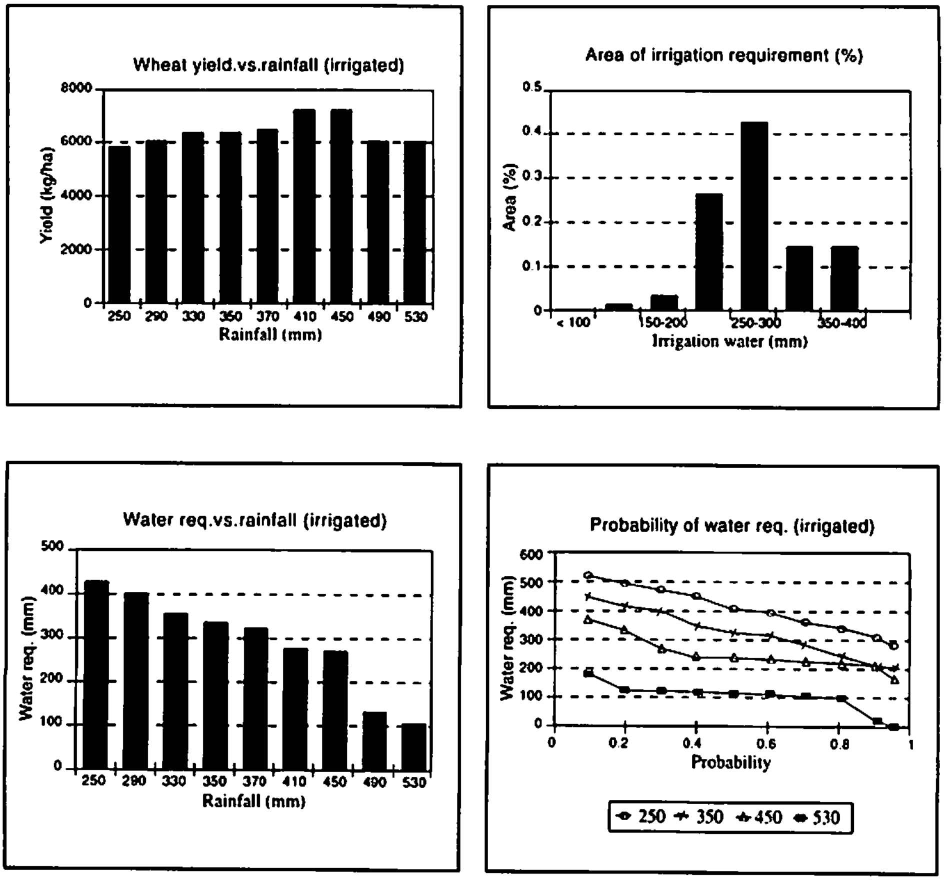

Figure 5. Mean durum wheat yield across rainfall regions, area percentage of ranges for irrigation requirement, and their probability distributions under irrigated conditions.

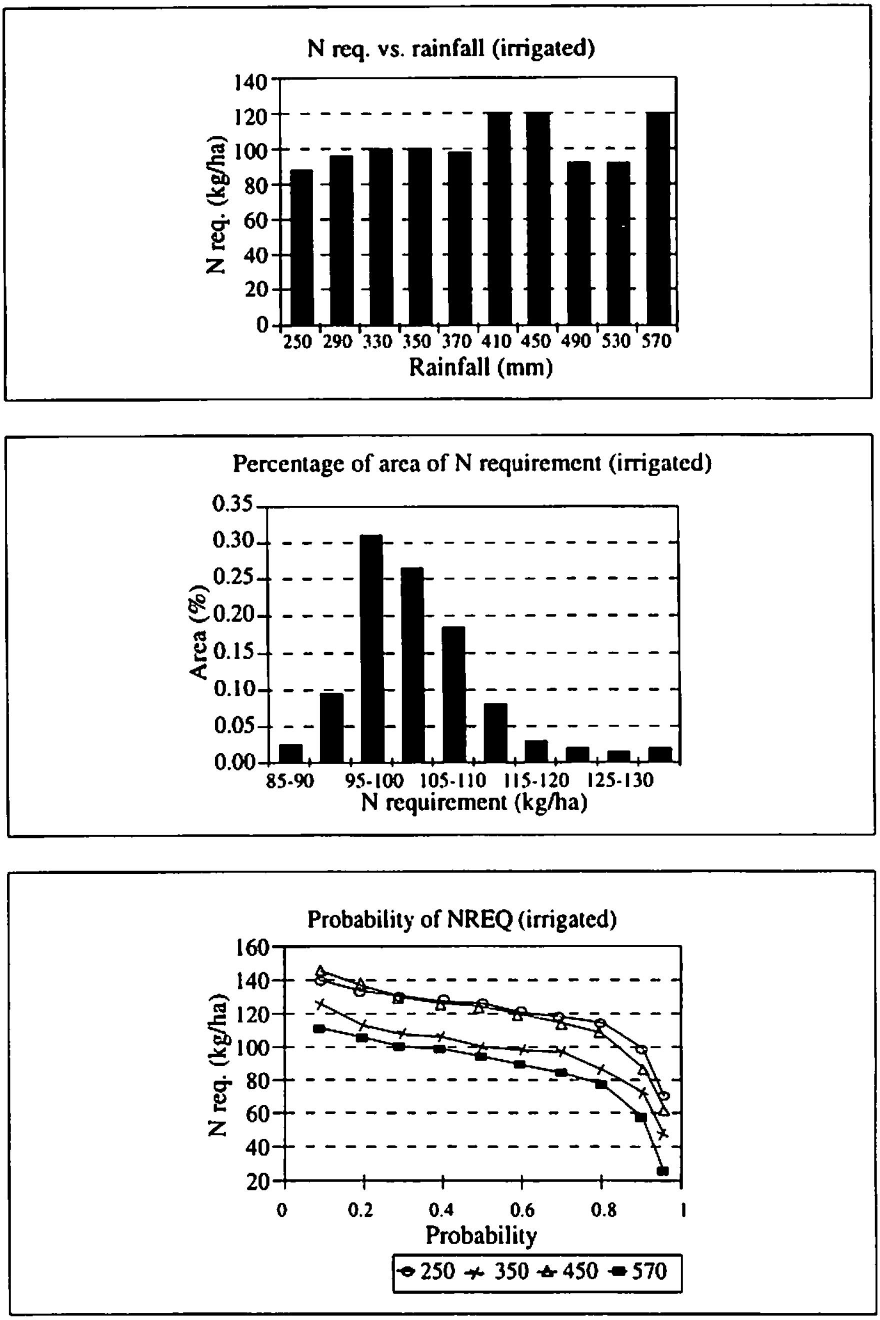

Figure 6. Nitrogen requirement of durum wheat under different rainfall area, percentage of area for range of N requirement, and probability distribution under irrigated conditions.

Effect of N Fertilizer on Production and Risk in Rainfed Areas

Production

Yield increases linearly from about 0.5 t/ha at 250 mm rainfall to about 5.5 t/ha at 530 mm rainfall, and then levels off as rainfall increases (Fig. 3). This is in agreement with a study on the assessment of environmental factors on wheat response to N fertilizer (Pala et al. 1992). Wheat's N requirement ranges from 25 kg N/ha at 250 mm rainfall to 110 kg N/ha at more than 500 mm rainfall.

About 65% of the area needs 50–80 kg N/ha, about 17% needs 20–50 kg N/ha, and about 18% requires 80–120 kg N/ha for efficient fertilizer use for wheat production (Fig. 7).

Risk

In 20% of cases, yield is about 1.5, 3, 5, and 6 t/ha at 250, 350, 450, and 570 mm rainfall, respectively (Fig. 7).

In 80% of cases, yield is about 0, 1, 2.5, and 3.5 t/ha at 250, 350, 450, and 570 mm rainfall, respectively (Fig. 7). This shows that production of wheat is risky at less than 300 mm rainfall. This is well understood by farmers of the region, who have a long experience growing barley, which is relatively more drought resistant than wheat.

In 20% of cases, the N requirement is about 50, 80, 120, and 120 kg N/ha at 250, 350, 450 and 570 mm rainfall, respectively (Fig. 7).

In 80% of cases, the N requirement is around 5, 50, 85, and 100 kg N/ha in the respective rainfall zones (Fig. 7). This is in agreement with earlier on-farm studies (Pala et al. 1992).

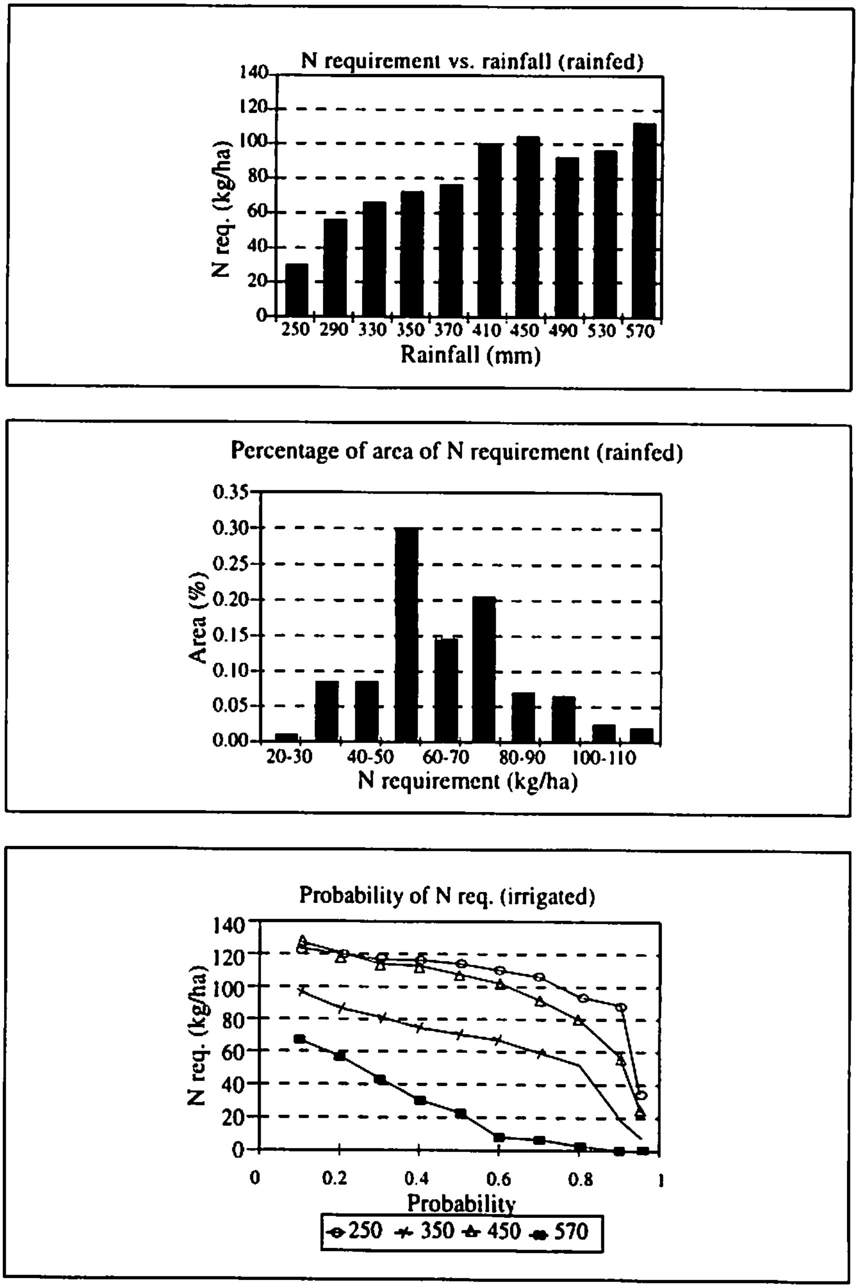

Figure 7. Nitrogen requirement of durum wheat under different rainfall area, percentage of area for range of N requirement, and probability distribution under rainfed conditions.

Acknowledgments

This study is based on data concerning supplementary irrigation and nitrogen needs of wheat during 1989–1992, as well as soil and climatic data, from northwest Syria. The author is grateful to Hazel Harris, Haitham Halimah, Sabih Dehni, and Samir Barbar for their efforts in soil moisture and crop measurements, to Wolfgang Gobel and Galia Martini for providing long-term weather data, and to Zuhair Masri for providing a soil map of northwestern Syria. The author is also thankful to Claudio Stockle and the Department of Biological Systems Engineering of Washington State University for sharing the CropSyst model and for hosting the author while on sabbatical.

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Boron-toxicity Tolerance in Durum Wheat

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Abstract

Boron (B) toxicity occurs mainly in arid and semi-arid regions, especially in alkaline soils. It has been observed in the semi-arid areas of many countries in West Asia and North Africa (WANA). Selecting or breeding crop cultivars with high tolerance or resistance to B toxicity is the only effective approach to increasing yield on soils high in B. In view of the importance of durum wheat in WANA, especially in semi-arid areas, work to identify B toxicity tolerant durum wheats was initiated at the International Center for Agricultural Research in the Dry Areas (ICARDA) in 1993. Experiments were conducted in a plastic house under controlled temperatures and natural sunlight. Boric acid was added to create soil media with different B levels. Durum wheat had foliar B toxicity symptoms similar to bread wheat. When subjected to high soil B levels, grain yield was reduced, and heading delayed. There was significant variation in response to high soil B levels among durum wheat genotypes. Screening of seedlings detected some germplasm accessions with high levels of B toxicity tolerance.

Introduction

When we talk about boron, most of us think of B deficiency. Boron is one of the seven essential micronutrients, and B deficiency is one of the most widespread nutritional problems affecting high-yielding crops in the more humid parts of the world (Gupta 1979). But this does not mean that B toxicity does not exist.

Boron is phytotoxic if present in excess amounts in the soil. In fact, the range of B concentrations between deficiency and toxicity is narrower than all other nutrients. Generally speaking, deficiency may occur when hot-water soluble soil B is less than 1 ppm, and definitely occur when greater than 5 ppm (Reisenauer et al. 1973). The first account of B toxicity in a crop was probably reported as early as 1934 in the USA (Jenkin 1993). But it was not until 1983 that the problem was unveiled in South Australia (Cartwright et al. 1984), which has a Mediterranean type climate and alkaline soils, as do most of the WANA countries. Serious efforts to tackle the problem in cereal crops began only after this discovery.

There are three main causes of high B levels in soil: (1) soils inherently high in B, such as those formed from sediment of inland seas; (2) use of irrigation water high in B, often from deep wells; and (3) excessive application of fertilizers or other sources high in B, especially industrial or commercial wastes (Jenkin 1993). In WANA, the third cause is probably of no practical importance.

In contrast to B deficiency, B toxicity occurs mainly in arid and semi-arid regions, especially in alkaline soils (Leyshon and Jame 1993; Marschner 1986). This is probably because precipitation is insufficient to leach the bulk of the B down below the root zone. Alkaline soils are able to adsorb more B than acid soils (maximum adsorption of B by soil particles occurs around pH 9), so the amount of free B available for movement down the soil profile is reduced.

One of the reasons why B toxicity has not been widely understood is that high soil B concentrations usually occur in subsoils (Cartwright et al. 1984; ICARDA 1994), while soil surveys usually sample the topsoil. Figure 1 summarizes the distribution of water-soluble B at various depths at a dry site (annual rainfall of 233 mm) in northern Syria where B toxicity symptoms were observed on barley (*Hordeum vulgare* subsp. *vulgare*). Eight out of the ten profiles show that B concentration increases substantially at or below a depth of 30 cm.

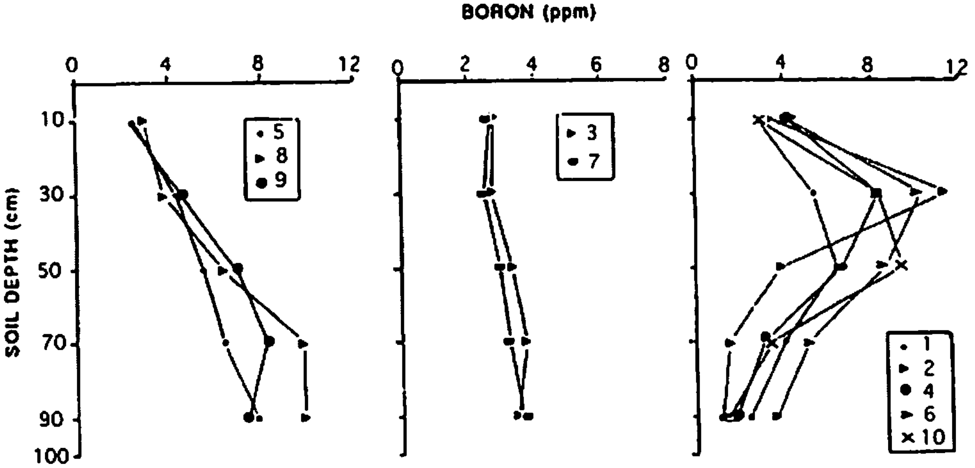

Figure 1. Distribution patterns of water-soluble boron in various profiles at Boulder, northern Syria (ICARDA 1994).

Boron toxicity (based on published reports and observations of symptoms supported by plant and/or soil analysis) in crops occurs in the semi-arid areas of many WANA countries. It occurs in all five countries in North Africa: Algeria, Egypt (northwest coast), Libya, Morocco, and Tunisia. In West Asia, it has so far been known to occur in Iraq, Jordan, Syria, and Turkey. Many soil samples collected in Iraq, Syria, and Turkey (Anatolian Plateau) have high B levels (Sillanpaa 1982). In addition, B toxicity has been reported in India (Chauhan and Asthana 1981), Spain (Salinas et al. 1981), Australia, and the USA.

It is neither practical nor easy to detoxify high B soil by agronomic means (Leyshon and Jame 1993). Soil can be leached extensively by water, using much more than needed to remove soluble salt, but the availability of water in semi-arid and arid areas is always a constraint. Chemicals, such as triisopropanolamine, are expensive and may not work under field conditions. Lime applied to increase pH and adsorption by soil particles is not suitable for alkaline soils.

Selecting or breeding crop cultivars with high tolerance or resistance to B toxicity is the only effective approach to increasing or maintaining yields on high B soils. Extensive work on screening for B toxicity tolerance and on understanding the underlying mechanisms has been carried out for bread wheat (*Triticum aestivum* subsp. *aestivum*) and barley in Australia (Nable 1988; Moody et al. 1988; Paull et al. 1988, 1991; Jenkin and Lance 1991), but little has been done on durum wheat (*T. turgidum* subsp. *durum*).

In view of the importance of durum wheat in WANA, especially in semi-arid and high-altitude areas, work to identify and develop B toxicity tolerant durum wheats was initiated at ICARDA in 1993. In this paper, we report the results obtained so far on the following topics: (1) effect on yield and other characters; (2) genotypic variation; and (3) sources of higher tolerance.

Materials and Methods

All experiments were conducted in a plastic house under controlled temperatures of 4–25 °C and natural sunlight. In the experiment studying the effects of soil B level on yield, plants were grown in pots. To create soil media with three different B levels, boric acid was added at rates of 0, 25, and 50 mg B/kg soil mixture (designated B0, B25, and B50, respectively). The boric acid was mixed uniformly throughout the soil mixture, giving hot water extractable B concentrations of 2.3, 9.1, and 19.4 ppm, respectively. The soil mixture was made up of one part soil (fine clay, montmorillonitic, thermic, Calcixerollic Xerochrept) and two parts sand. Nine durum wheat cultivars, advanced lines, and landraces, selected to represent the range of symptom scores in an earlier screening experiment, were tested. Shoot B concentrations were measured colorimetrically by the Azomethine-H method (Bingham 1982) after dry-ashing.

For tolerance screening, seedlings were grown in 200×75×25 cm trays filled with soil, to which was added 50 or 100 mg B/kg soil mixture. Symptom severity on leaves and plant growth were scored 4–6 weeks after sowing. CIMMYT/ICARDA regional observation nurseries and germplasm accessions were screened.

Results and Discussion

Foliar Symptoms of Boron Toxicity

Symptoms first develop on the tips of the oldest leaves, with successively younger leaves being affected to a lesser extent. Durum wheat has similar symptoms as those described for bread wheat by Snowball and Robson (1991). Mottled chlorosis (beige color) develops at the leaf tip, followed by necrosis of the leaf tip and development of chlorotic spots or blotches at leaf edges. These symptoms gradually spread down to the leaf base.

Based on the authors' experience, foliar symptoms of B toxicity on wheat are less easy to recognize than those on barley. In order to find out whether B toxicity occurs in fields where wheat is grown, it may be help to include sensitive barley checks.

Effect on Yield and Other Characters

Mean grain yield was significantly reduced at B25 and B50 when compared with B0 (Table 1). The B treatments also had a significant effect on B toxicity symptom score, seedling dry weight, shoot B concentration, days-to-heading, and plant height, but not on straw yield or harvest index (Table 1). The delay in heading is expected to have a large deleterious effect on grain yield under field conditions in semi-arid areas.

In another experiment conducted by the authors (unpublished data), durum yield reduction was similar to barley yield reduction, and both experienced a similar delay in heading when grown at B50. Thus, one should not assume that although there are fewer symptoms than in barley, the effect of B toxicity on durum wheat yield is negligible.

Table 1. Effect of boron application rate on yield and other characters.

Character	Boron level			
	B0	B25	B50	LSD(5%)
Symptom score (0–5)	0	1.1	1.7	0.12
Seedling dry weight (mg/pl)	99	86	75	5.9
Shoot B concentration (ppm)	9	186	459	31.4
Days-to-heading	90	93	96	1.0
Plant height (cm)	71	75	81	1.9
Grain yield (g/pl)	1.6	1.4	1.3	0.12
Straw yield (g/pl)	3.3	3.0	3.0	ns
Harvest index (%)	33	32	30	ns

Genotypic Variation

Oued Zenati (Algerian landrace), Gezira 17 (old Syrian cultivar), and Omrabi 5 (CIMMYT/ICARDA advanced line) appear to have higher tolerance to B toxicity than the other six entries. These three had lower symptom scores and shoot B concentrations, and showed less delay in heading and reduction in grain yield (Table 2). In terms of shoot B concentration, delay in heading, and reduction in grain yield, Omrabi 5 (sister line of Cham 5, the most recently released cultivar in Syria) had higher B toxicity tolerance than Cham 3 (released in 1987 in Syria), which in turn had higher tolerance than Cham 1 (released in 1984 in Syria).

Table 2. Mean boron-toxicity-symptom score and shoot boron concentration at B25 and B50, and delay in heading and reduction/increase in grain and straw yields.

Name/Cross	Toxicity symptom score	Shoot concentration (ppm)	Delay in heading (days)	Percent yield difference Straw	Percent yield difference Grain
Gezira 17	0.7	282	3.7	+34.6	- 2.8
Omrabi 5	0.8	240	6.0	-15.6	-10.4
Oued Zenati	1.1	245	2.0	- 6.7	- 1.4
Haurani	1.3	334	7.0	-11.6	-31.7
Jordan 21	1.1	356	8.0	-16.6	-25.6
Deraa	1.3	380	8.0	-13.1	-11.9
Cham 1	1.3	391	11.3	+ 5.0	-29.0
Cham 3	2.3	329	7.7	-11.8	-14.7
Cakmak	2.8	347	4.3	-19.3	-30.0
Mean:	1.4	323	6.4	- 7.9	-16.3
LSD (P=0.05)	0.3	77	4.2	20.8	ns

0–5 scale: 0=no symptoms, 5=severe symptoms.

Sources of Tolerance

Screening of the 1992/93 and 1993/94 CIMMYT/ICARDA regional observation nurseries at B50 showed a small but significant variation in symptom development among entries. Lines with the lowest symptom scores and shoot B concentrations were selected for further testing. Awalbor was a promising lines found as a result of this screening.

To find higher tolerance to B toxicity, germplasm accessions were screened at a higher B level (B100). In the Durum Core Collection of 125 entries, which includes many landraces from WANA and southern Europe, the following were found to have a high level of tolerance based on growth (entry number in parenthesis): Chahba 88 (46) and Siliana (73) had the highest tolerance, followed by Kishk (6), Jordan Coll. 86 No. 80 (26), Tensift-1 (43), Jordan (68), Awali-1 (74), HO-FAO 25918 (77), Aric 31708.70/3/Bo//C.de Chile/Br/4/Cit/Gta (78), N. Dakota 86 line No. 10 (79), Wakooma (80), Entre largo de Montijo No. 7621 (116), and Candeal de Grao Escuro No. 7746 (119).

In a preliminary screening of 25 durum wheat accessions from six WANA countries (Afghanistan, Iran, Iraq, Jordan, Syria, and Turkey), a few accessions from Afghanistan looked outstanding. Accession ICDW 7675 grew as vigorously and had a lower symptom score than Greek G61450, a very tolerant bread wheat.

The identification of tolerant durum wheat accessions from Afghanistan was in agreement bread wheat results (Moody et al. 1988). In a screening of 1,576 accessions of spring bread wheat from the Australian Winter Cereals Collection, Moody found that Asia and Asia Minor, and, within Asia Minor, Afghanistan, have the greatest number of tolerant lines. It appears that many soils in Afghanistan have high levels of B. Tolerant accessions will be used to introduce higher B toxicity tolerance into the CIMMYT/ICARDA durum wheat breeding program. Genetic studies using traditional means and new biotechnology techniques are planned.

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Resistance to Fungal and Viral Diseases in Durum Wheat

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Durum Wheat Diseases

Major wheat diseases in West Asia and North Africa (WANA) are loose smut (*Ustilago tritici*), common bunt (*Tilletia laevis* and *T. tritici*), and flag smut (*Urocystis agropyri*). All three rusts are present in the majority of WANA countries. However, yellow rust (*Puccinia striiformis*) is prevalent in West Asia and Yemen. Leaf rust (*P. recondita*) and stem rust (*P. graminis*) are more prevalent in Egypt and Sudan. Leaf rust predominate in North Africa. Among the *Helminthosporia*, tan spot (*Pyrenophora trichostoma*) is restricted to Iran, Turkey, Libya, Tunisia and Morocco. Septoria tritici blotch (*Mycosphaerella graminicola*) occurs in endemic form in Tunisia, Algeria, and Morocco. Septoria nodorum blotch (*Leptosphaeria nodorum*), scattered across only four countries in the region, is becoming more prevalent in Morocco. Powdery mildew (*Erysiphe graminis*) is prevalent in the wetter areas of WANA, mainly in North Africa. The foot and root rots of wheat are attracting more and more attention in WANA. The causal agents, *Fusarium* spp., in some cases associated with *Cochliobolous* sp., form a major disease problem in Morocco and Tunisia, especially in years of drought. Head scab (*Fusarium* spp.) is reported in Pakistan and Iran, but information on the presence of the disease in other countries is lacking. The bacterial leaf streak on wheat (*Xanthomonas campestris* pv *translucens*) is the main bacterial disease in WANA. Among the viruses, the seed-borne barley stripe mosaic virus has been reported in Lebanon, Syria, and Jordan, but seems to be present in most countries of WANA. Barley yellow dwarf virus is spread across the region. This virus re-occurred widely in Egypt in 1998/90 and 1990/91. The seed gall nematode (*Anguina tritici*) has been reported in several countries in the region. Cereal cyst nematode (*Heterodera* spp.), root knot nematode (*Meloidogyne* spp.), and root lesion nematode (*Pratylenchus* sp.) have also been reported in several countries in the region, but their importance as wheat pathogens has not yet been quantified in most countries. Durum wheat diseases are more or less the same as bread wheat diseases, except for peculiarities related to agro-climatic area and specific interactions between host and pathogen, i.e., host preference. In common bunt, *T. laevis* mainly attacks bread wheat, whereas *T. tritici* attacks bread wheat and durum wheat without preference. In field and greenhouse trials, pycnidial formation is greatest in both durum and bread wheat cultivars when the source of inoculum is from the same species. During the last decade, yellow rust epiphytotics hit badly and devastated all widely grown bread wheat cultivars in West Asia. Despite the differences in genetic background, it is assumed that the yellow rust population developed and multiplied on widely-grown bread wheats in areas the epiphytotics are more adapted to, i.e., that they displayed a preference for bread wheats. There are good indications that the pathogen population of leaf rust epiphytotics in WANA is more adapted to durum wheats.

Using Resistance to Control Durum Wheat Disease

Considering the availability of disease control measures to farmers in WANA, the use of resistance in plants remains the best control of wheat diseases. It is unlikely that fungicides will be widely used in the short-term to control diseases such as rusts, septoria blotch, and powdery mildew. Only chemical seed treatments for the control of seed-borne disease are used, and farmers are encouraged to sow certified and treated seed. However, many farmers in WANA still use their own seed, which is in most cases untreated.

Screening is the tool used to upgrade resistance in the germplasm developed by the breeders at the International Center for Agricultural Research in the Dry Areas (ICARDA) and the National Agricultural Research Systems (NARSs).All breeding material at ICARDA undergoes at least two screenings for the major diseases: leaf, stem, and yellow rusts, septoria blotch, common bunt, and barley yellow dwarf virus. The screening is done at the Center's principal station, Tel Hadya, at the substation at Lattakia in Syria, and at the sub-station at Terbol in Lebanon. Data on powdery mildew and tan spot are obtained from the multilocational screening system.

Material in the advanced stages (advanced yield trials) is also exposed to different diseases and biotypes of pathogens when it is tested through the multilocational screening system. Several "hot spot" locations in WANA constitute the multilocational screening system. The principal hot spots for durum wheat diseases are: Tel Hadya and Terbol for yellow rust; Tel Hadya, Terbol (summer cycle), Safi and Marchouch in Morocco, and Sakha and Sids in Egypt for leaf rust; Tel Hadya, Terbol (summer cycle), Marchouch and Tessout in Morocco, and Sakha and Sids in Egypt for stem rust; Tel Hadya and Lattakia in Syria, and Marchouch and Meknes in Morocco for septoria tritici blotch; and Tel Hadya for both common bunt and barley yellow dwarf virus.

The disease inoculum used at each screening site represents the prevailing biotypes of the pathogen in the respective country. In Syria and Lebanon, disease inoculum is renewed every year. Inoculum of septoria blotch is adjusted to a ratio of 1:1 for bread and durum wheat. The common bunt inoculum is adjusted for the two pathogens, mixed to 1:1.

Screening for common-bunt resistance follows a different scheme. In the first year, material is screened (Common Bunt Nursery I), using a bulk of isolates collected in Syria. Lines that perform well are re-tested for a second year and screened in the third year (Common Bunt Nursery II) against 8–10 different isolates from WANA.

Germplasm resistant to a disease, selected through screening and multilocational testing, is included in a Special Purpose Disease Nursery, and is re-tested for 2–3 years at different locations. During this re-testing, the material is selected for acceptable agronomic traits and for reasonable resistance to non-targeted diseases. In the third phase, lines with resistance to a disease are pooled together in the so-called Germplasm Pools for Sources of Resistance, increased, and distributed to the NARSs and other collaborators. These pools are the end-product of the screening work carried out at ICARDA.

Current Level of Disease Resistance in Durum Germplasm

The performance of durum germplasm against yellow rust, leaf rust, stem rust, and septoria blotch is summarized in Figure 1. Germplasm tested in the 1994/95 season is represented in the Durum Key Location Disease Nursery (DKL-95), the Durum Preliminary Disease Nursery (DPD-95), the Durum Aleppo Crossing Block (DAC-95), and in the special purpose disease nurseries, Durum Yellow Rust Nursery (DYR-95), Durum Leaf Rust Nursery DLR-95), Durum Stem Rust Nursery (DSR-95), and Durum Septoria Nursery (DST-95). Selection criteria were: <2 CI for yellow rust, <5 ACI for leaf and stem rust, <3–5 average score for septoria, and <15% head infection for common bunt.

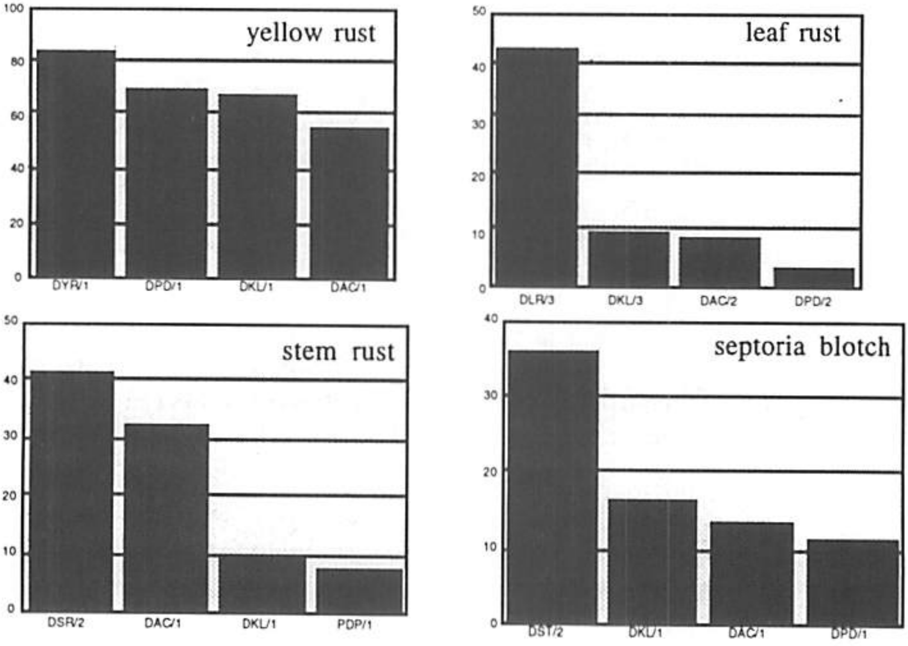

Figure 1. Performance of durum wheat germplasm against yellow, leaf, and stem rusts, and septoria blotch (1994/95).

The special purpose disease nurseries have the most resistant lines for the corresponding diseases. For yellow rust, 83% of the screened lines are resistant, but a relatively high percentage of resistant lines is found in the other germplasm screened: DPD (68%), DKL (66%), and DAC (55%). For leaf rust, all germplasm (except DLR) shows a low percentage (<10%) of resistant lines. For stem rust, DKL and PDP have a low percentage of resistant lines. For septoria, 16% of DKL lines were resistant, followed by DAC (13%) and PDP (11%).

Germplasm Pools for Sources of Resistance

Several germplasm pools for sources of resistance to various diseases precipitated from our screening system (Table 1). These were pools for yellow rust (DYRGP), septoria tritici blotch (DSTGP), stem rust (DSRGP), leaf rust (DLRGP), common bunt and yellow rust (DCBYRGP), and common bunt (DCBGP). Each of these pools was distributed in several sets to the WANA NARSs and other collaborating institutions beyond WANA. These lines, with relevant information on each line in each pool, are preserved at the ICARDA gene bank.

Table 1: Durum wheat germplasm pools for sources of resistance to disease (1988–1996).

GPPL	Disease	No. of lines	No. of sets distributed	First release
DYRGP-87	Yellow rust	1-26	29	1988/89
DYRGP-92	Yellow rust	1-22	30	1992/93
DYRGP-95	Yellow rust	1-12	40	1995/96
DSTGP-87	Septoria blotch	1-19	30	1989/90
DSTGP-95	Septoria blotch	1-15	35	1995/96
DSRGP-92	Stem rust	1-17	29	1992/93
DSRGP-95	Stem rust	1-12	35	1995/96
DLRGP-95	Leaf rust	1-18	40	1995/96
DCBYRGP-88	Common bunt/yellow rust	1-19	29	1989/90
DCBGP-92	Common bunt	1-16	29	1992/93

One particular line, DYRGP-87, Waha=Cham 1, released in 1984 and planted on a large scale in several countries of WANA, still maintains its excellent resistance to yellow rust. Currently, this cultivar occupies a quarter (roughly 300,000 ha) of the wheat acreage in Syria. All lines in the DSRGP, DLRGP, DSTGP, and DCBGP pools have good resistance to the non-targeted disease, yellow rust, which did not exceed 20 MR.

Some lines in DSTGP-87 combine earliness, septoria resistance, and high yield potential. Lahn, for example , a cross between Schearwater and Bittern made in Mexico and selected from F_2 in Syria, had 16% higher yield than Gezira 17, the local check, and 4% higher than Cham 1. Lahn is the favorite cultivar for the irrigated areas in Syria.

A recent pool for sources of resistance to common bunt included 26 resistant lines. Lines in this pool were resistant to nine common bunt isolates from different countries and sites in WANA. Correlation, principal components, and cluster analysis grouped the resistant lines into three clusters. Cluster one comprises genotypes with genetic interrelationship to Jennah Khetifa, a Maghrebian landrace. Cluster two comprises genotypes close to Senatore Cappelli and Haurani (the latter a landrace from Syria). Cluster three comprises advanced genotypes containing resistance genes from Mindum (a Turkish landrace). Donor sources of resistance for lines in this pool appear to be related to the three major sources mentioned. Cultivar Senatore Cappelli is considered resistant, since it has been grown by farmers on a large scale for many years and has remained resistant to common bunt throughout seven years of testing. Since 1986, Senatore Cappelli, an old cultivar which used to be planted in Syria on a large scale, has, been introduced in all common-bunt nurseries as a resistance check. Except in 1988, when this cultivar had 20% head infection, its performance never exceeded 10% head infection over the five years of testing. The consistency of resistance of this cultivar suggests durable resistance.

Situation of Durum Diseases in the Maghreb

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Abstract

In the Maghreb countries Durum cultivars are subject to attack by various fungal diseases. The severity and resulting yield losses vary from year to year. The purpose of this study was to ascertain the status of durum diseases. Disease surveys were conducted in Morocco, Algeria, and Tunisia. Data included severity level, type of host cultivar (local, semi-dwarf), and growth stage at the time of assessment. In Morocco, the most prevalent diseases were tan spot, septoria blotch, and leaf rust. The latter occurred every year. Tan spot and septoria diseases varied in prevalence. Powdery mildew was present in significant amounts. In Algeria, the most prevalent diseases were tan spot, septoria blotch, and leaf rust. Tan spot was significant every year. Leaf rust attained epidemic proportions in 1993. Among other diseases, yellow rust, previously reported as an important disease in Algeria, was present in two years out of four; powdery mildew was present every year, and particularly abundant in 1990. In Tunisia, septoria blotch, tan spot, and leaf rust were present every year. Powdery mildew was present every year on about 25% of the surveyed wheat fields. The pathogens studied were *Septoria* species, *Pyrenophora tritici repentis*, and *Puccinia recondita* f. sp. *tritici*. Septoria blotch is the most important foliar disease of wheat in Tunisia. *Septoria tritici* is the major pathogen. In Morocco, *S. nodorum* was more frequent on durums than *S. tritici*, especially in the northern part of the country. *S. nodorum* was twice as frequent (69%) than *S. tritici* (31%) on durums. The frequency of occurrence of *S. tritici* was higher than that of *S. nodorum* on bread wheats. In Morocco, tan spot caused by *P. tritici repentis* was found on both durum and bread wheats, although the disease was more prevalent on the former. However, the pathogenic specialization of *P. tritici repentis* was only moderate in the Maghreb countries. Isolates from durums infected bread wheats and vice versa. In an attempt to determine pathotype and virulence variation in the pathogen, 18 isolates of *P. tritici repentis*, collected from Morocco, Algeria, and Tunisia, were characterized. All 18 isolates were of the necrosis type, varying only in the extent of chlorosis. The isolates interacted significantly with the cultivars of the same host species, therefore, the search for resistance to tan spot considered the pathogenic variation encountered in *P. tritici repentis*. The isolates were utilized to screen durum for resistance in the greenhouse. The cultivars of durum with resistance in greenhouse testing, as well as in the field, were Sarif, Karim, INRA 1769, and INRA 1749. Systematic race surveys of *P. recondita* f. sp. *tritici* have been conducted in the region, particularly in Morocco. The characterization of pathogen races in Maghreb countries indicated diversity is generated each year from the alternate host, *Anchusa italica*. Among the races identified, the race UN1, avirulent on genes Lr1, 2a, 2c, and 3, was frequently isolated from *T. durum* cultivars. This race, present in the three Maghreb countries, is much more virulent on durum than on bread wheats. Analysis of the virulence data in relation to the geographical area showed that the central plains south of Casablanca and the northwest area (Morocco) had highly diverse races due to the presence of the alternate host and to favorable conditions for rust development. A site where the alternate host is functional is now used to screen for resistance to leaf rust. Among the entries found highly resistant to leaf rust at this site were Altar, Sarif, Mahmoudi, Kohini, Azizi Ap1, Derbani Ac1, and Bidi Ap44.

Introduction

Durum is widely grown in the Maghreb countries. Both local and improved high-yielding cultivars are planted, in different proportions, in each country. These cultivars are subject to attack by various fungal diseases. Severity and resulting yield losses vary from year to year depending mainly on prevailing climatic conditions. The absence of a reliable database on disease distribution and intensity, and on yield loss from disease, has permitted certain misconceptions to be perpetuated regarding the relative importance of cereal diseases in general.

It is only in the last few years that there has been a constant effort in the Maghreb countries to determine the annual distribution of wheat diseases through systematic surveys. A significant amount of information is now available on the physiological specialization of the most important fungal diseases of wheat in the Maghreb. The purpose of this paper is to ascertain the status of durum diseases in the Maghreb countries by summarizing the results of disease and pathogen surveys.

Disease Surveys

Surveys of diseases in commercial durum fields were conducted in three Maghreb countries (Morocco, Algeria, and Tunisia) from 1990 to 1994. The surveys determined the prevalence of fungal diseases in durum. Additional data were sometimes collected: severity level, type of host cultivar (local, semi-dwarf), and the growth stage at time of assessment. Data summaries for each country follow.

Morocco

Surveys were conducted in the main cereal growing areas of Morocco from 1991 to 1994 (Table 1). The most prevalent diseases were tan spot, septoria blotch, and leaf rust. The latter occurred every year due to successful local oversummering and the occurrence of the alternate host (Ezzahiri et al. 1992). Tan spot and septoria diseases varied in their prevalence. Tan spot was particularly important in 1991 and 1994. These two years saw above-average rainfall. Powdery mildew was present in significant amounts during the relatively dry years of 1992 and 1993.

Table 1. Prevalence (% of fields infected) of fungal diseases on durums in Morocco (1991–1994).

Disease	Year				Average annual prevalence
	1991	1992	1993	1994	
Tan spot	92	36	29	57	53
Septoria blotch	39	56	27	22	39
Leaf rust	70	76	62	68	74
Yellow rust	12			01	
Stem rust	02	11	11	06	8
Powdery mildew	02	29	22	12	12
Flag smut	13				
Total fields	132	46	79	174	

Algeria

The results of the surveys conducted in commercial fields on durum in Algeria between 1990 and 1993 are presented in Table 2. The most prevalent diseases were tan spot, septoria blotch, and leaf rust. Tan spot was significant every year. Leaf rust attained epidemic proportions in 1993 on durum. Among other diseases, yellow rust (reported previously as an important disease in Algeria), was present in two years out of four. Powdery mildew, present every year, was particularly abundant in 1990.

Table 2. Prevalence (% of fields infected) of fungal diseases on durums in Algeria (1990–1993).

Disease	Year				Average annual prevalence
	1991	1992	1993	1994	
Tan spot	50	73	45	51	55
Septoria blotch	11	05	26	20	15
Leaf rust	55	05	31	72	41
Yellow rust	11	10			
Stem rust	02	11	11	06	08
Powdery mildew	33	05	11	05	13
Total fields	132	46	79	174	

Data from Sayoud (1993).

Tunisia

Among the many diseases found on durum in Tunisia between 1990 and 1994, septoria blotch, tan spot, and leaf rust were present every year (Table 3). The relative importance of each disease varied according to climate in the given year. Powdery mildew was found every year in about 25% of the surveyed wheat fields.

Table 3: Prevalence (% fields infected) of fungal diseases on durums in Tunisia (1990-1993).

Disease	Year				Average annual prevalence
	1991	1992	1993	1994	
Tan spot	06	27	27	16	19
Septoria blotch	13	10	34	81	34
Leaf rust	56	17	07	10	22
Yellow rust					
Stem rust	26	02			
Powdery mildew	35	29	19	09	23
Flag smut	18	15	10	07	12

Data from Chaghlaf et al. (1993).

Pathogen Characterization

A significant amount of research is conducted in the Maghreb countries an attempt to characterize the variation of the most important pathogens in durum. The pathogens studied in detail were *Septoria* species, *Pyrenophora tritici repentis*, and *Puccinia recondita* f. sp. *tritici*.

Septoria Species

The septoria diseases are economically more important in bread wheat than in durum. In fact, septoria blotch is reported to be the most important foliar disease of wheat in Tunisia. Among the two species (*S. tritici* and *S. nodorum*), the former is reported to be the major pathogen in the area. The symptoms produced by *S. tritici* and *S. nodorum* and even tan spot can be confusing. For this reason, researchers attempted to complete pathogen identification in the laboratory.

Maazouz (1992) studied a number of samples collected from durums in Morocco. He reported that *S. nodorum* was more frequent inn durum than *S. tritici*, especially in the northern part of the country. In another survey, Farih (1993) confirmed that *S. nodorum* was twice as frequent (69%) in durum as *S. tritici* (31%). The frequency of occurrence of *S. tritici* was higher than that of *S. nodorum* in bread wheats (Table 4). Within pathogen species, researchers have characterized the isolates utilized in screening bread wheats for resistance to septoria blotch (Maazouz 1992).

Table 4. Frequency (%) of *Septoria* species occurrence on durum and bread wheats in Morocco (1993).

Septoria species	Durum	Bread Wheat
S. tritici	31	58
S. nodorum	69	42
Total fields	32	31

Data from Farih (1993).

Pyrenophora tritici repentis

Tan spot caused by *P. tritici repentis* is found on both durum and bread wheats, and is more prevalent on the former. However, Boulif et al. (1994) found that the pathogenic specialization of *P. tritici repentis* is only moderate in the Maghreb countries; isolates from durums infected bread wheats, and vice versa. In an attempt to determine pathotype and virulence variation in the pathogen, Boulif and Saoud (1994) characterized 18 isolates of *P. tritici repentis* collected from Morocco, Algeria, and Tunisia. They found that all 18 isolates were of the necrosis type, varying only in the extent of chlorosis. The isolates interacted significantly with the cultivars of the same host species. Therefore, the search for resistance to tan spot should consider the pathogenic variation encountered in *P. tritici repentis*. The isolates were used to screen durum for resistance in the greenhouse. Some cultivars developed in the Maghreb were found to be resistant in greenhouse testing as well as in the field. These cultivars are Sarif, Karim, INRA 1769, and INRA 1749.

Puccinia recondita f. sp. *tritici*

Among the three wheat rusts, leaf rust is by far the most prevalent in the Maghreb countries (Ezzahiri et al. 1994b). Systematic race surveys of *P. recondita* f. sp. *tritici* have been conducted in the area, especially in Morocco (Ezzahiri et al. 1994a). The study of pathogenic variation by means of race surveys is an effort to support epidemiological studies and to enhance the usefulness of resistance to be incorporated in commercial cultivars. The characterization of pathogen races in the Maghreb indicates a wide array of virulence combinations on the Lr genes utilized as differentials (Tables 5, 6). This diversity is generated each year from the alternate host, *A. italica* (Ezzahiri 1992).

Table 5. Percent distribution of unified numeration races of *Puccinia recondita* f. sp. *tritici* identified in Morocco (1992 and 1994).

Host	UN race											
	1	2	3	5	6	9	10	11	12	13	14	17
					1992							
Bread wheat	4	2	2	19	27	2	2	4		37		2
Durum	30	7		10	17		3	10		13	7	3
					1994							
Bread wheat	59	1	3	3	5			4	4	20		1
Durum	88				2		6	2		2		

No. of isolates tested:135 (105 from BW and 30 from DW) in 1992; 158 (92 from BW and 66 from DW) in 1994.

Table 6. Unified numeration races of *Puccinia recondita* f. sp. *tritici* in Algeria (1992 and 1993) and in Tunisia (1993).

Country	UN race											
	1	2	3	5	6	9	10	11	12	13	14	17
					1992							
Algeria	3	3	1	4	1	1	1	1	1	2		1
					1993							
Algeria	8											
Tunisia	4	1										

No. of isolates tested: Algeria 21 (1992); 8 (1993); Tunisia 5 (1993).

Among the races identified, race UN1, avirulent on genes Lr1, 2a, 2c, and 3, was frequently isolated from *T. durum* cultivars. It is present in the three Maghreb countries, and is much more virulent on durum than on bread wheat (Table 7).

Table 7. Seedling reactions of selected durum and bread wheat cultivars to isolates of the race UN1.

	Algerian isolate (A17)	Tunisian isolate (TU1)	Moroccan isolate (C20)
	Durum		
Kyperounda	2	2+	12c
Acsad 65	2	2+	3
Jori	3	3	4
Cocorit	2+	23	3
Karim	3	3	3
Marzak	3	4	3
Belbachir	3	2+	3
O. Zenati	3c	23c	4
Zeramek	4	23c	
Selbara	4	4	3-
Omrabi	23	23	3
Sebou	3	4	3
Sarif	1c	1c	0
Tensift	3	4	1c
Massa	23	4	3
Isly	23c	4	4
Tassaout	4	4	4
Waha	3c	3	3
Sahel	3+	23	3

(continued next page)

(Table 7 continued)

	Algerian isolate (A17)	Tunisian isolate (TU1)	Moroccan isolate (C20)
		Bread wheat	
Nesma	0	0	0
Siete Cerros	12c	12	1c
Saiss	0	0	1N
Jouda	0	0	1c
Potam	c	1N	1c
Merchouche	3c	23-	2+c
Tilila	0	0	0
Baraka	0	1+c	3
Khair		1	1c
Saba	0	0	-
Kanz	0	1c	1+N
Arz		1	
Hork		1	
Zidane		0	0
Ziad	0	0	0

The isolates A17, TU1, and C20 are avirulent on Lr1, 2a, 2c and 3.

Table 8. Shannon diversity for unified numeration races of *Puccinia recondita* f. sp. *tritici* in relation to crop species and areas of Morocco (1990 and 1992).

	Bread wheat			Durum		
	No. of isolates	No. of races	Shannon index	No. of isolates	No. of races	Shannon index
			1990			
Souss	21	6	1.63	10	5	1.47
Central plains	39	7	1.31	33	10	2.17
Tadla	5	3	1.05	7	4	1.15
Gharb	7	3	0.79	4	3	1.03
Sais	3	2	0.63	1	1	
Tangerois	9	5	1.56	11	5	1.52
			1992			
Souss	26	7	1.46	18	5	1.60
Central plains	51	9	1.60	7	4	1.15
Tadla						
Gharb	16	5	1.4	5	2	0.7
Sais	9	4	1.2	2	2	0.7
Tangerois	5	3	0.9	3	3	1.0

Shannon Index=piln(pi); pi=frequency of the *i-th* phenotype.

Analysis of virulence data in relation to geographical area (Table 8) in Morocco shows that the central plains south of Casablanca and the northwest area (Fig. 1) have diverse races, due to the presence of the alternate host and to favorable conditions for rust development. One site where the alternate host is present is now used to screen durums for resistance to leaf rust. Among the entries highly resistant to leaf rust at this site are Altar, Sarif, Mahmoudi, Kohini, Azizi Ap1, Derbani Ac1, and Bidi Ap44.

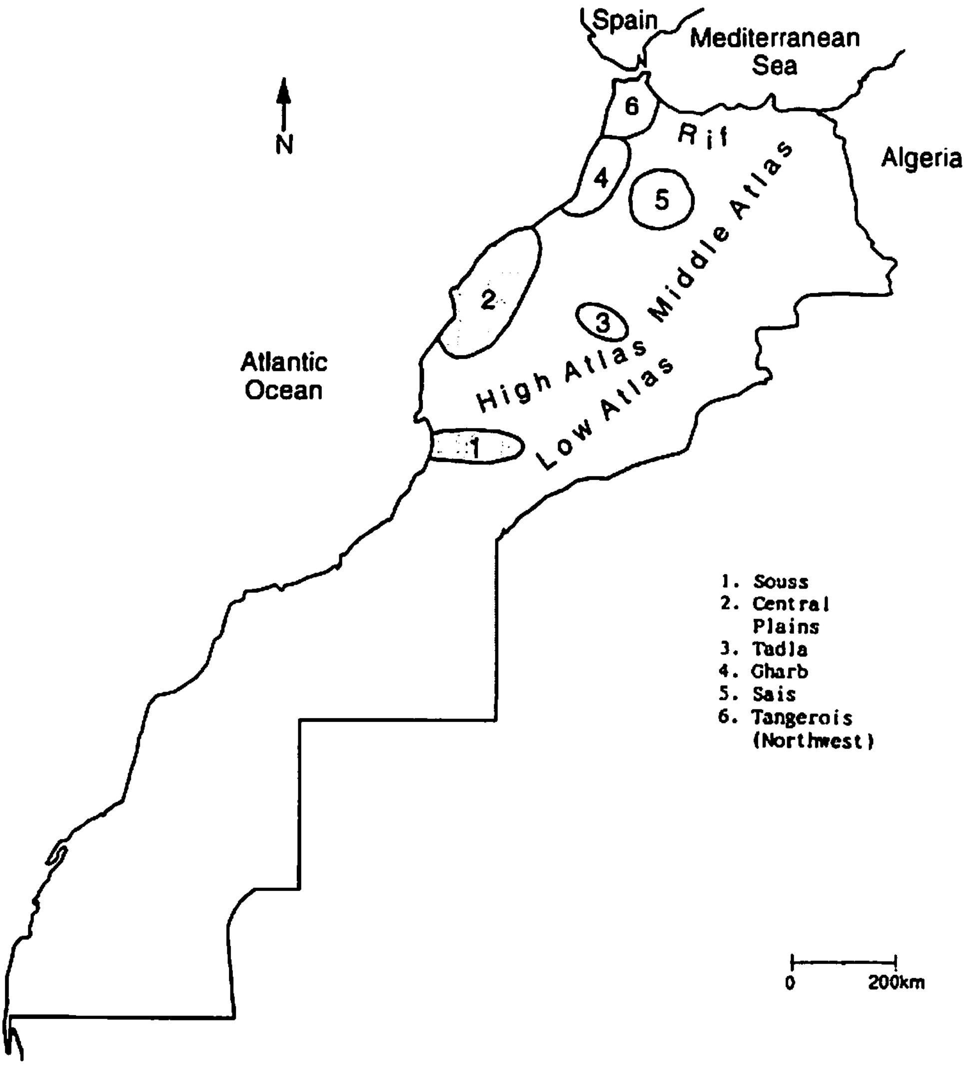

Figure 1. Surveyed wheat-growing areas of Morocco.

Conclusion

Among the many diseases that attack durums, tan spot, septoria blotch and leaf rust are the most prevalent in the Maghreb. The fact that these diseases occur widely in the region is sufficient reason to justify epidemiological studies.

The discovery that *S. nodorum* is more frequently found on durum than *S. tritici* in Morocco needs to be pursued at the regional level. There is a need to assess the geographical distribution of *Septoria* species and the impact on durums in the Maghreb in order to more effectively direct the use of resistant cultivars.

Some of the research activities on tan spot have focused on the characterization of pathogen variation. Specific isolates of *Pyrenophora tritici repentis* were utilized to screen durums for resistance to this pathogen.

For leaf rust, the particularities of the life cycle of the causal agent are well known. Areas of high pathogenic variation have been identified in Morocco. Results of the race surveys indicate a common pattern of racial distribution of *P. recondita* f. sp. *tritici* on durum in the Maghreb.

Acknowledgments

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Durum Resistance to Insect Pests in Morocco

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Abstract

In Morocco, durum production is severely affected by Hessian fly, wheat stem saw fly, and the Russian wheat aphid. Hessian fly losses are estimated at 32%. Nine major wheat resistance genes were identified against Hessian fly in Morocco. The mechanism of resistance in these wheat genes is antibiosis. The first durum line resistant to Hessian fly has been identified. To diversify sources of resistance for Hessian fly, wild relatives of wheat, such as *Aegilops ovata* (UM), *Ae. squarrosa* (D), and *Ae. triuncialis* (UC) were studied. Since few sources of resistance to Hessian fly in durum are available, we transferred the H5 gene located on the A genome from bread wheat to durum. Field surveys showed that saw fly attacks all cereal species in Morocco, with the level of infestation similar on the cereals (40%). Russian wheat aphid is found in all cereal-producing regions of the country. Two hundred and sixty four lines of the main cereals (barley, bread wheat, durum and triticale) were evaluated in the greenhouse for resistance; six barley lines, three bread wheat, and two triticales showed good resistance; however, no resistance was found in durum.

Introduction

Insect surveys conducted in the cereal production areas of Morocco since 1982 have shown that durum (*Triticum turgidum* subsp. *durum*) production is severely affected by damage caused by a group of insect pests. The most important of these are Hessian fly, *Mayetiola destructor* (Say), wheat stem saw fly, *Cephus* spp., and Russian wheat aphid, *Diuraphis noxia* (Mordvilko). Our research effort concentrates mostly on Hessian fly, an insect that is the most damaging to durum. Our strategy of insect control is oriented toward genetic resistance, due to the following reasons:

- It does not pollute the environment.
- It does not cost the farmer; everything is incorporated in the seed.
- It has given satisfactory results in other parts of the world.

Hessian Fly (Diptera: Cecidomyiidae)

Importance of the Insect

In Morocco, damage caused by Hessian fly can result in total loss of the crop if high infestations occur at the early stages of crop development. Lhaloui (1992), using the insecticide Furadan 5G, estimated Hessian fly losses on durum at 32%.

Our five year survey (1986–90) in the west central semi-arid region of Morocco showed that 85% of the 916 fields surveyed were infested, and 55% had economic infestations, considered as >20 percent tillers infested (Lhaloui et al. 1992).

Sources of Resistance

Table 1 summarizes the nine major wheat resistance genes effective against Hessian fly in Morocco. The resistance reaction of these genes has been confirmed both in the greenhouse and in the field. The mechanism of resistance in these wheat genes is antibiosis, i.e., the first instars of avirulent biotypes die after feeding on resistant plants (El Bouhssini et al. 1995). Two other genes, H7H8 and H9, are only moderately resistant against Hessian fly in Morocco. H9 genes exhibit antibiosis against Hessian fly larvae, whereas H7H8 genes allow for avirulent larvae to survive on resistant plants (El Bouhssini 1988; El Bouhssini 1992).

Table 1. Major wheat genes for resistance to Hessian fly in Morocco.

Gene designation	Source	Genome or chromosome location
H5	*T. aestivum*	1A
H7, H8	*T. aestivum*	5D,?
H11	*T. turgidum*	1A
H13	*A. squarrosa*	6DL
H14, H15	*T. turgidum*	5A
H21	*S. cereale*	2BS.2RL
H22	*A. squarrosa*	1D
H23	*A. squarrosa*	6D
H25	*S. cereale*	4AS.4AL-6RL.4AL
H26	*A. squarrosa*	4D

Table 2 summarizes four other sources of resistance effective against Hessian fly in Morocco; all of them are bread wheats. The two CIMMYT lines, L222 and L254, and the Algerian line, ADC14, express antibiosis against Hessian fly in Morocco. L222 and L254 have high resistance (about 100%), similar to that provided by the major resistance genes, whereas ADC14 is just moderately resistant. We are in the process of studying the inheritance of resistance in these lines. The cultivar Massira and the line BT92P1.20, which originated from the ICARDA wheat breeding program, exhibit tolerance against Hessian fly. We are in the process of determining at which wheat growing stage this tolerance is expressed.

Table 2. Other sources of resistance to Hessian fly in Morocco.

Line	Source	Mechanism of resistance
Massira	*T. aestivum*	Tolerance
BT92P1.20 (NS 732/HERM)	*T. aestivum*	Tolerance
L222	*T. aestivum*	Antibiosis
L254	*T. aestivum*	Antibiosis
ADC14	*T. aestivum*	Antibiosis

Table 3 summarizes the first durum sources of resistance to Hessian fly in Morocco. Their resistance reaction was confirmed both in the field and in the greenhouse. The lines CM829 (from the international collection) and BD MS3 (from the Moroccan breeding program) are interesting because they combine two mechanisms of resistance: antibiosis and tolerance. There is a mixture of live and dead larvae on resistant plants. The lines CI 112, CI 113, and CI 115 (from the ICARDA collection) have a high level of resistance and express antibiosis against Hessian fly larvae. We are in the process of studying the inheritance of their resistance to see if they carry new resistance genes.

Table 3. Resistance of ICARDA and international durum collection to Hessian fly (Settat, Morocco).

Line	Percentage of resistant plants	Mechanism of resistance
CM 829	78.5	Antibiosis/tolerance
CI 112	75.0	Antibiosis
CI 113	100.0	Antibiosis
CI 115	100.0	Antibiosis
BD MS3	41.0	Antibiosis/tolerance
NASMA (susc. check)	0.0	
SAADA(resis. check)	100.0	Antibiosis

In order to diversify sources of resistance for Hessian fly, we have explored wild relatives of wheat. Tables 4 and 5 show that eight species of these wild relatives have resistance to Hessian fly in Morocco. *Ae. ovata* (UM), *Ae. squarrosa* (D), and *Ae. triuncialis* (UC) showed the highest number of resistant accessions. Most of the resistant accessions of these wild species were collected in Morocco (Bentikka 1994; Houari, unpublished data).

Table. 4. Reaction of a Moroccan collection of *Aegilops* for resistance to Hessian fly (Settat, Morocco 1995).

Species and genome	Origin	No. of accessions tested	No. of resistant accessions
A. ovata (UM)	Mekens, Fes, Nador, Chefchaouan, Tetouan	36	5
A. triuncialis (UC)	"	15	0
A. triaristata (UM)	Ksar El Kebir	3	1
A. ventricosa (DUn)	Azrou, Sefrou	4	2

Table 5. Resistance of an ICARDA *Aegilops* collection to Hessian fly (Settat, Morocco, 1950.

Species and genome	Origin	No. of accessions tested	No. of resistant accessions
A. ovata (UM)	Morocco (14), Portugal (5), Syria (1)	88	20
A. triuncialis (UC)	Morocco (2), Portugal (4)	41	6
A. triaristata (UM)	Morocco	8	1
A. cylindrica (CD)	Turkey	4	1
A. squarrosa (D)	?	7	5

Gene Deployment Strategies

All major resistance genes identified have been transferred to Moroccan wheats through a backcross breeding method. In addition to its presence in a winter bread wheat, Arthur 71, the H5 gene is also present in a spring wheat germplasm, SD8036 (Cholic et al. 1987). SD8036 was recently released in Morocco as the cultivar Saada, targeted for production in the southern part of the country where Hessian fly is the most damaging. This cultivar is in very limited use, however, because its red kernels are not liked by farmers. The bread wheat cv Massira was released to farmers in 1994. Two bread wheat lines carrying H13 and H23, as well as L222, are in registration yield trials. If they perform well during two years of yield testing in several locations, they will be released to farmers in 1996. Since we have identified few sources of resistance for Hessian fly in durum, we have transferred the H5 gene located on the A genome from bread wheat to durum. Several promising durum lines have been selected out of this backcross program.

Although the optimum strategy for gene deployment is open to debate, we adopted a single-gene strategy. Cox and Hatchett (1986) favor sequential gene deployment or simultaneous deployment of different genes, whereas Gould (1986) proposes pyramiding genes for more durable resistance. In both cases, the authors based their conclusions on simulation models in which certain assumptions related to the genotypic interaction between resistance genes and virulence genes and to other genetic parameters had not been verified.

Knowledge of genetic parameters such as the survival of homozygous avirulent/heterozygous Hessian fly genotypes, and wheat carrying specific resistance genes is essential to understanding host insect interaction. Resistance genes, such as H7H8, against Hessian fly in Morocco allow a high survival of avirulent larvae on resistant plants (El Bouhssini 1992), and thus should be deployed to reduce selection for biotype development. It would be nice to deploy wheat varieties that express tolerance against Hessian fly larvae. This should dilute virulence genes in the fly populations and thus slow down biotype development.

Wheat Stem Saw Fly (Hymenoptera: Cephidae)

Importance of the Insect

Surveys and field trials conducted in 1987/88 show that saw fly attacks the four cereal species grown in Morocco: bread wheat, durum, barley, and triticale. It is found in all cereal-growing areas of the country, with similar levels of infestation for all cereals (40%). The highest percent weight loss per 1,000 kernels (17%) and reduction in number of kernels per spike (45%) were found on durum (El Bouhssini et al. (1987).

Sources of Resistance

No major effort has been mounted to find solid-stem durum. Nor does the entomology laboratory in Settat have any source of resistance for saw fly in durum. ICARDA saw fly nurseries should be evaluated in Morocco. In bread wheat, Settat does have a line that combines resistance to saw fly and Hessian fly. It originates from a cross between the variety Potam, which has solid stems, and a line carrying the H13 gene. It would be helpful to develop durum lines with combined resistance to both pests, Hessian fly and saw fly.

Russian Wheat Aphid (Homoptera: Aphididae)

Importance of the Insect

Russian wheat aphid, *Diuraphis noxia* (Mordvilko), is a new insect pest of cereals in Morocco. In the last few years this aphid has been limited to high elevations, but it is now found in all cereal producing regions of the country.

Sources of Resistance

Two hundred sixty four lines of the main cereals (barley, bread wheat, durum, and triticale) were evaluated in the greenhouse for resistance to this pest. Based on the degree of damage and plant height in comparison to non-infested checks, six barley lines, three bread wheat, and two triticales showed good resistance to Russian wheat aphid (Boulmane 1994). These sources of resistance have been given to breeders to use in their breeding programs. From this first screening, it appears that special efforts will need be made to find sources of resistance for durum.

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Towards Efficient Doubled Haploid Methods in Durum

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Abstract

Haploidization is one important plant biotechnology for the improvement of durum. Doubled haploid (DH) methods not only enable the plant breeder to quickly obtain pure lines but also provide the ideal material for genetic mapping with molecular markers. The use of such doubled haploid homozygous lines in breeding programs will save time and improve the accuracy of evaluation of qualitative and quantitative traits. The main methods for haploid production are crosses with inducer lines, interspecific and intergeneric crosses followed by *in vitro* embryo rescue, and *in vitro* gynogenesis and androgenesis. Within the network, each of the available techniques will be investigated to enable the development of a method to produce DH lines in durum in high numbers. Currently, isolated microspore culture and intergeneric crosses with maize are the most promising methods.

Importance of Haplodiploidization for Durum

Drought is the major stress limiting durum yield in the low-input environments of West Asia and North Africa. In 1995, drought resulted in 70% losses at harvest. Southern Europe is exposed to the same stress. Other abiotic stresses are also important, such as cold, salinity, and heat. The selection for new stress-tolerant varieties is difficult, time consuming, and requires more accurate tools to overcome existing bottlenecks. Among these tools, *in vitro* and molecular biotechnologies are promising.

Given this context, the general objective of biotechnological research applied to durum wheat is the development of efficient tools for abiotic (drought, salinity, heat, and cold) stress-tolerance improvement of durum wheat in the Mediterranean basin. These tools are not yet well developed in this species, despite previous research efforts. In fact, durum represents a challenge for all scientists concerned with biotechnology. The aim of researchers concerned with *in vitro* biotechnologies in the SEWANA durum research network is to make a comprehensive attempt to eliminate the recalcitrance of durum wheat to *in vitro* approaches.

Haploidization is likely to become one of the most important plant biotechnologies, mainly to improve small-grain cereals such as durum. Doubled haploid (DH) methods enable the plant breeder to quickly obtain pure lines, with better accuracy due to the lack of heterozygosity and a better genetic gain when additivity and additive epistasis are predominant. The use of such doubled haploid homozygous lines in breeding programs will save time (2–3 years) and can enhance the genetic gain per cycle. Homozygous lines improve the accuracy of evaluation of qualitative and quantitative traits.

Monocellular haploid systems such as isolated microspores would be very helpful in the area of *in vitro* selection for stress tolerance (Lashermes 1991; Mihamou-Ziyyat et al. 1994). In addition, DH methods allow a simplification of genetic quantitative analysis as well as molecular mapping. The most important international genetic mapping programs in cereal involve studies on targeted crosses and their DH line populations, if DH methods are available for the crop.

The number of DH lines registered around the world as new varieties is increasing for species such as barley, rice, bread wheat, and rape seed. For barley in Europe, for example, the number of DH cultivars is more than 20. For bread wheat, four new DH-derived varieties have been released since 1990.

The continuous improvement of DH methods is, therefore, strategically critical for a species such as durum where this biotechnology is so far not yet available for population development. *In vitro* production of DH lines could hasten the production of new varieties of durum in the Mediterranean basin, providing breeders of durum with powerful techniques of pure (homozygous) line production and saving time in the evaluation of progenies and in the release of new varieties. The consequences of successful DH methods in this important crop are very important. So far, the difficulty in obtaining DH lines in durum, irrespective of the method, is a bottleneck (Picard et al. 1995).

Methods of Producing Haploids in Durum

The main methods for haploid production are: crosses with inducer lines, interspecific and intergeneric crosses followed by *in vitro* embryo rescue, and *in vitro* gynogenesis, and androgenesis. The most important methods are described below.

Intergeneric Crosses

In durum, an intergeneric cross with corn (*Zea mays*) is currently the most successful method of DH line production. Utilization of maize or other species such as teosinte in intergeneric crosses has provided the geneticist with an alternative way to produce haploid lines in durum (Cattaneo et al. 1991; Mihamou-Ziyyat, 1992). Intergeneric crosses between *T. turgidum* subsp. *durum* and *Z. mays* offer a good solution to the problem of albinism, as almost all plants derived from this technique are chlorophyllian (Riera-Lizarazu et al. 1992; Comeau et al. 1988; Amrani et al. 1993; Coumans et al. 1993). Nevertheless, strong genotypic effects have been observed in durum, when durum is used as the female. This technique is used in private companies to produce DH lines for their breeding programs.

In Vitro Androgenesis or Gynogenesis

In vitro anther cultures (also called *in vitro* androgenesis), discovered 31 years ago on solid as well as liquid media, have evolved very quickly during the last seven years. However, durum appears to be one of the more recalcitrant species for *in vitro* androgenesis. Aissa (1974) was the first to obtain green plantlets during wheat anther culture. This recalcitrance is characterized by a strong genotypic effect leading to a low frequency of genotypes giving chlorophyllian plantlets or low embryo rates (Ghaemi et al. 1993; Mihamou-Ziyyat 1992; Cattaneo et al. 1991). Mihamou-Ziyyat and Picard (unpublished results) obtained *in vitro* androgenetic embryos on 95% of 101 homozygous lines of durum wheat in France (with a percentage of embryos ranging from 0.1% to 38.2%). But the regeneration of 52 chlorophyllian plants was obtained with only 17 lines (16% of the total). The best green plant regeneration rate was obtained with the variety Mondur (average 1.2 green plants per 100 cultivated anthers).

An understanding of the *in vitro* behavior (culturability: aptitude to produce haploid chlorophyllian plants) of the durum landraces of North Africa is a very important objective which will facilitate the future use of genetic diversity to improve this cereal in the SEWANA region.

Isolated Microspore Systems

In order to eliminate the recalcitrance of this species, we have chosen to adapt and develop all the available methodologies of other cereals on a restricted core of durum lines and a common target cross. Recently, the culture of isolated microspores has been considered the most efficient method because of its simplicity, the absence of maternal tissue, and the lower competition between microspores (Mejza et al. 1995). This method not only allows a better application of the DH method in crop breeding as exemplified in rape seed or barley, but also opens new possibilities for genetic manipulation and *in vitro* selection. In our laboratory (Picard et al. 1995) we have obtained results with isolated microspore culture for bread wheat. With one bread wheat genotype (DH line 112) we were able, on a modified Chu medium containing 0.26 M glucose, to produce hundreds of green plants at a maximum rate of 0.2% of microspores developing a green plant. Currently, de Buyser and Picard are trying to apply this method to durum genotypes such as Cham 1, Jennah Khetifa, Korifla, and Kabir.

Finally, it is anticipated that isolated microspore cultures in durum are likely to overcome the genotypic effects caused by maternal tissue in intergeneric crosses, as well as in anther culture. Such a method is also very attractive for *in vitro* selection.

Haplodiploidization Research in the SEWANA durum Network

The SEWANA durum network has integrated within its program research on DH methods in order to obtain DH populations for the development of molecular marker maps for durum and to ease selection for breeders. Within the network, the partners will try to eliminate the recalcitrance of durum to haplodiploidization. All available methods (intergeneric cross of durum x maize followed by haploid embryo cultures, anther culture, ovary culture, and isolated microspore culture) will be studied in parallel with the same genotypes. Genetic studies will be undertaken to further our understanding of the origin of albinism. Genetic diversity of landraces for their culturability *in vitro* will be evaluated.

Induced Gynogenesis through Intergeneric Crosses

Induced gynogenesis after crossing durum wheat with maize must be improved in order to obtain higher embryo frequency and related higher frequencies of green plant recovery. These experiments will be carried out on the targeted cross (Cham 1 x Jennah Khetifa) and on durum genotypes of the durum core collection (Coumans, Dusautoir). Intergeneric crosses with *Hordeum bulbosum* strains selected for their aptitude to produce a high rate of embryos will also be investigated (El Haddoury).

In Vitro Anther Culture or Androgenesis and Analysis of Genetic Effects

A sufficient number of DH lines (about 200) are planned to be produced from the current targeted cross (Cham x 1 Jennah Khetifa), with Jennah Khetifa as a responsive line in anther culture (Amara, Benlhabib, de Buyser, Lepoivre, Picard). A complete study of the genetic variability of androgenetic responses of durum landraces from Maghreb countries will be undertaken. Screening of this genetic pool of durum for anther culture response and selection of highly responsive DH lines will be carried out (Becheikh, Daaloul, Amara, Mihamou-Ziyyat, Benlhabib).

Improvement of the existing culture technique will be attempted with a limited number of genotypes. The parameters to be analyzed are: pretreatment conditions, influence of culture medium and modification of gelling agent, osmotic pressure, sugar supply, and environmental conditions. Crosses and selfed progenies, will be analyzed for genetic effects by diallel analysis (Lepoivre, Anceau, Amara).

Backcrosses of durum on bread wheat are now available and will be studied for cytoplasmatic effects on androgenetic responses. The role of the bread wheat cytoplasm with respect to the durum nuclear genome will be evaluated in order to detect unknown specific effects of the plastid genome (Chlyah and Chlyah).

Molecular studies could be undertaken to map deletions in the plastid genome of microspore-derived albino plants and to trace their origin during androgenesis (Coumans).

In Vitro Gynogenesis

Experiments will be carried out to develop *in vitro* gynogenesis through the transfer of knowledge from barley to durum.

Isolated Microspore Culture

The objective is to transfer methods recently developed in other cereals such as barley, maize, and bread wheat to durum. Besides the expected increase in embryo production, isolated microspore culture should allow a closer control of the induction phase and possibly a decrease of albinism. A few selected genotypes among the core collection (Cham 1, Jennah Khetifa, Azizi) will be tested with different experimental designs along the following parameters: culture conditions of donor plants, stress pretreatment, extraction method, purification of embryogenic microspores, co-culture, and timing of embryo transfer (Picard, deBuyser, Coumans).

Conclusion

The efforts of the SEWANA durum network on the various aspects of haplodiploidization are very important. This technique may become widespread in the near future. It is particularly important to provide the members of the durum network involved in molecular mapping with a population of 200–250 doubled haploid lines of a common targeted cross (Cham 1 × Jennah Khetifa). Currently, a population of single-seed descent derived lines is used. Theoretically, haplodiploidization is a very helpful method for molecular mapping because DH lines are completely homozygous, and therefore correlations between markers and agronomical traits are relatively easy to control. Thus, this project will allow comparison of single-seed descent lines with the DH method for molecular mapping.

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In Vitro Techniques, an Overview

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Abstract

During the sabbatical year of A. Comeau at ICARDA, the potential of *in vitro* methods was evaluated. Haploid production in barley was carried out by means of anther culture, and the same genotypes were also studied at Laval University. Barley germplasm developed from Syrian landraces belongs to the most recalcitrant barley genotypes. The use of interspecific crosses with *Hordeum bulbosum* may be an alternative for haploid production in barley. Similarly, androgenesis in durum might be tried with interspecific crosses with maize or related species. Interspecific crosses between durum and *Aegilops* species were also investigated. Synchronization of flowering between *Aegilops* species and durum requires attention. A tiller culture method was used to carry out pollination and allow embryo formation under controlled environmental conditions. Sucrose solution supplemented with 2,4-D auxins were sufficient to allow pollination and embryo formation until embryo rescue techniques could be applied. For systematic introgression of *Aegilops* germplasm into durum, new genotypes have to be identified and used. Otherwise, crosses during the summer, when temperatures exceed 30 °C, may deactivate necrosis genes.

Overview of *In Vitro* Research for Key Cereal Species

In vitro technology related to durum has received attention only quite recently. In Canada, as well as at ICARDA, more efforts had been invested in bread wheat (Picard et al. 1990; Lashermes et al. 1991; Trottier et al. 1993). In this species, interspecific crosses, androgenesis, and haploid production by wheat × maize, wheat × teosinte, and wheat × *Tripsacum* crosses are rather easy to obtain (Laurie and Bennett 1988). Two areas of *in vitro* research have also been rather successful: haploid production through anther culture or crosses with *H. bulbosum* (Kaha and Kao 1970; Pickering and Deveaux 1990) and, recently, microspore culture (Olsen et al. 1991, Jähne et al. 1991), especially at Guelph University (Ziauddin et al. 1991). A method intermediate between microspore and anther culture, called shed pollen culture, is being used at Laval University. As for durum, only interspecific crosses are now being pursued in Canada, although the Montpellier results may stir interest for durum × maize crossing to create haploids.

In durum androgenesis, the main problem is the lack of regeneration. This has been noted in research carried out in France, Morocco, and Tunisia (Coumans et al.

1997; Amara et al. 1997, this volume). If this species does regenerate, it yields albinos.

Recent results allow us to postulate that cereals adapted to drought are more recalcitrant to androgenesis, and this holds true for barley, rye, and durum, to name a few. All these species tend to have similar regeneration problems, and albinism. Recent promising results with barley indicate that their study might give clues about how to improve the androgenesis method for durum. Until the androgenetic pathway works better, the durum x maize method of producing haploids is of more immediate usefulness (Coumans et al. 1997, this volume).

Controlling Regeneration and Albinism by Improved Media

The androgenesis work done at ICARDA in 1993/94 was paralleled by studies at Laval University by graduate student J. Ouedraogo, using similar media and methods. The media were modifications of Hunter's Ficoll liquid medium (Hunter 1987), to which amino acids and hormones were added. The ICARDA work concentrated on androgenesis of Tadmor barley and related drought-adapted material; the Laval student studied more diversified germplasm. The results obtained at ICARDA were positive, but not optimum when compared to the possibilities of the *bulbosum* method, discussed below. However, the Laval data shed new light on the difficulties encountered at ICARDA. The first part of the Laval research was to confirm that an amino acid mixture developed by Comeau (Trottier et al. 1993) was also useful in barley. Trials with three cultivars showed that amino acid doubled the success of green plant production. Research on various hormones gave negative results. None of these hormonal modifications, tried in factorial design with the amino acid supplement, was any better than the Hunter medium with U2.5 amino acids; in fact these media were almost always worse.

Research was extended to various barley lines and F_1 hybrids of various complexity. The best medium (Hunter plus BA and amino acids) was used. Recalcitrance varied strongly among genotypes. In this germplasm, embryos per 100 anthers and green plants per 100 anthers were strongly correlated. Green plants per 100 anthers and albino plants per 100 anthers were not correlated. However, the number of green plants per 100 anthers and the percentage of albinism were strongly and negatively correlated.

It was obvious that Tadmor was the very worst of all lines for embryo production. In the correlation between green plants with percent albinism, Tadmor was again one of the worst performers. Using totally novel media, we had obtained some promising results with Tadmor at ICARDA, but we did not realize the significance of these result until we compared our results with those of Ouedraogo. These novel media need further research, planned for the spring of 1995 at Laval. This part of a project is sponsored by private industry (Coop Fédérée and Semico Inc.), with support from the Industry and Trade Ministry of the Quebec government.

Some authors claim that albinism and embryo induction are controlled by completely different genes. The Laval results are contrary to this conclusion. It is clear that the best media improved the embryo formation and, at the same time, reduced albinism. It is also clear that genotypes that were recalcitrant suffered from two correlated problems: reduced embryo formation and a high rate of albinism. It thus becomes clear that improving the speed and rate of embryo formation is the way to reduce albinism, although the difficulties inherent to regeneration are not to be underestimated.

Haploid chromosome number must be doubled to restore fertility. Chromosome doubling technology was improved at ICARDA by using Laval University methods using locally-made equipment to handle the plants without killing them (Tawkaz et al. 1994). This system worked satisfactorily at Laval, and has been improved by the use of a novel hydroponic nutrient solution, a formula developed by Comeau and Van Leur at ICARDA. Green, fast-growing plantlets are essential to doubling success.

Haploidy from Alien Crosses

Comeau's original U2.5 amino acid medium (Trottier et al. 1992) was designed for embryo rescue, and was known to be adapted to the rescue of haploid embryos from cultivated barley x *H. bulbosum* and durum x maize. At ICARDA, we were hoping to find some *H. bulbosum* in an old nursery planted by Inagaki and Tahir about six years ago. As exact pedigrees were not available, we crossed a random series of *H. bulbosum* with various winter and spring barleys.

This was useful to confirm that a lot of embryos can be rescued from such crosses; the disappointment was that the material turned out to be derived from tetraploid *bulbosum*, which is not appropriate for haploid production.

Fresh pollen of *bulbosum* is shed as soon as the rays of the sun touch the spikes, before 6 AM. Later in the day, fresh pollen cannot be found. A novel idea was to cut the *bulbosum* heads one day in advance, in the afternoon, and store these heads in wet towels, in a cooler at about 0° C (a cooler that fits inside a car is ideal). The next day, one can conveniently take out a few spikes at a time, let the pollen shed at any time of day, and do the crossing with fresh pollen using a "pollen funnel" to direct the pollen onto the flowers.

Recalcitrance does not seem to be a problem with this method. The only aspects that need attention are pollen quality (it must be fresh), and special media for very tiny embryos. The *bulbosum* method should be very cost-effective for barley at ICARDA.

The durum x maize method is really not very different from the *bulbosum* method used for barley. Durum embryos are, however smaller. Yet, with proper attention, the problem can be solved with a tiller culture system. This idea could not be tried at ICARDA with durum × maize, as the two species are difficult to synchronize. However, the technique can be tried out using fast-growing *Tripsacum* species. Ideally, both male and female should be grown in cabinets, although this is impractical where space is limited. In practice, *Tripsacum* can be grown indoors, although its weediness potential must be considered before releasing it outdoors. The females could be field-grown, but planted very early so that they flower when the weather is cool.

The Tiller Culture System

Female spikes can be cut 1–3 days before flowering and then grown in a nutrient solution, perhaps simply a sucrose syrup. Trials with *F. jaiti* from Hassan II University confirm that an 8–9% sucrose solution is best for young durum spikes, and that 4.5–6% solutions seem better for more advanced grain filling. In CIMMYT, Mexico, and at Montpellier (Coumans et al. 1997 this proceedings), this method was combined with the use of 75 mg/L of auxin 2,4-D after pollination, to delay embryo mortality and facilitate embryo rescue. The auxin can be added to the sucrose solution.

Application to Durum Interspecific Crosses

When durum females are attainable for crossing, the *Aegilops* are generally not fully headed. When the *Aegilops* are flowering, the durum are generally in the middle of grain fill—too late for crossing. So, synchronization of the species is a major problem. Lack of growth-cabinet space is a limiting factor. Also, excess heat in the field at flowering often causes serious damage to durum flowers, especially to pollen, which reduces seed set when durum is used as the pollinator.

A tiller of durum harvested 2–3 days before flowering can be kept in a test containing an 8% sucrose solution (renewed every 2–3 days). As these test tubes are a very compact form of storage, durum tillers can be prepared in numbers in advance, and kept in a growth cabinet at 2–4° C, with light 2–16 hours per day to maintain some photosynthesis. These durum tillers will remain receptive for as long as two weeks at this temperature. We experimented with this idea for crosses of *T. durum* × *Aegilops* and *T. durum* × *T. monococcum* made at ICARDA in the spring of 1994.

The pollen donors were harvested about one day before flowering and also kept in tubes containing a sucrose solution. Crosses were done in the laboratory. Anther extrusion was stimulated by placing the male spike about 10 cm away from a 60 W incandescent light bulb. Pollinated females were then grown about 13–15 days at 16° C in a cabinet with 16 hours of light. The sucrose percentage was decreased from the initial 8% to 6 and 5% for early and late grain filling, respectively. The success rate of this method was satisfactory, as about 1 spike out of 3 pollinated gave embryos that could regenerate plantlets.

Hybrid Necrosis in Durum Interspecific Crosses

Some interspecific crosses are erroneously considered to be easy. For example, the high failure rate of gene transfer from *T. aestivum* to *T. durum* is a fact of life. Almost all of this problem can be ascribed to necrosis gene effects. Most durum lines belong to the necrosis group Ne_1. A very large group of spring bread wheats are endowed with the necrosis Ne_2 allele. Crosses of an Ne_1 to an Ne_2 carrier give heterozygous Ne_1Ne_2 plants that always die before heading because of the complementary gene action of the necrosis genes. Crosses of wheat to *Aegilops* or rye can die for the same reason. In fact, species that cross easily to bread wheat can often be impossible to cross to durum, and conversely, those that cross to durum will fail with bread wheat. Canadian attempts to transfer BYDV and fusarium resistance from bread wheat to *T. durum* were therefore largely unsuccessful.

Null alleles (Ne_0) have been found, mostly in bread wheat. Null alleles are more common in CIMMYT bread wheat; however, these CIMMYT bread wheats are not good sources of BYDV resistance. The search for bread wheat with null alleles is still going on in Canada. In the meantime, it might be possible to use a rare source of null necrosis alleles for durum, which is Vernal Emmer, as suggested by Tsunewaki. This is being tried at Agriculture and Agri-Food Canada.

In the meantime, ICARDA has a unique advantage in dealing with necrosis genes. These genes do not express at high temperature (30 °C or above). Therefore, necrosing Ne_1Ne_2 hybrids can grow and set F_2 seed in June or July if planted late (April) in the fields near Aleppo. Additional water may be needed. This is a unique natural advantage of being at ICARDA; the same cannot be done in Canada. Perhaps this could be the basis for joint projects.

Conclusion

In vitro techniques are being developed with different goals in mind. Accelerated cultivar production is being done through haploid production, which can come from androgenesis or from certain alien crosses aided by embryo rescue. Expansion of the biodiversity of the durum gene pool to improve resistance to diseases and stresses is another goal that can be served by embryo rescue technology. These methods need further research, and are promising for the near future.

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Interspecific/Intergeneric Crosses Program for Wheat Improvement at IAV Hassan II, Morocco

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Abstract

Aegilops spp. were collected in Morocco and identified as mainly *Aegilops ovata* and *Ae. triuncialis*, as well as some *Ae. ventricosa* and *Ae. triaristata*. These *Aegilops* spp., along with other collections, were screened for resistance to Hessian fly. Some *Ae. ovata* accessions were highly resistant. Interspecific crosses with *Ae. ovata* and *Ae. triuncialis* and also with *Agropyron* spp. were performed. The result was genotypic and sometimes even plant specific. Hybrids and backcross progenies were cytologically examined. Anther culture, already successfully used for bread wheat and barley, and intergeneric crosses with maize, are being used to fix karyotypes.

Introduction

Wheat production fluctuates greatly under the Moroccan environment. Besides technical and climatic factors, this variation is largely due to biotic stresses (El Bouhsini et al. 1994). Sources of resistance are limited in currently cultivated varieties, which have narrow genetic diversity. The incorporation of disease resistance into cultivars remains a major objective of Morocco's national breeding program, many of which integrate alien species in their hybridization activity. Wild wheat relatives constitute a valuable genetic pool for many agronomical traits (Sharma et al. 1981). Program objectives are: (1) to search for sources of resistance to Hessian fly in *Aegilops* accessions collected in Morocco; and (2) to transfer selected resistance into durum wheat cultivars by interspecific hybridization.

Germplasm Collection

Wild wheat relatives were collected in northern Morocco in July 1994. The aim was to enlarge the genetic pool and search for resistance and adaptation to biotic and abiotic factors. More than 30 sites were visited and 56 samples were gathered. *Aegilops* spp. are widespread in the Atlas mountains and plains of Morocco, where the climate is relatively humid. Four different species were collected (Table 1). *Aegilops ovata* and *Aegilops triuncialis* were found at most sites. *Aegilops ventricosa* and *Aegilops triaristata* are uncommon and found only in specific environments. Other collection missions are scheduled to cover the remaining regions of Morocco.

Table 1. Moroccan *Aegilops* collection: screening results for Hessian fly resistance.

Code	Origin	PR	PS
	Aegilops ovata		
0003	Tiflet	0.0	6.0
0004a	Meknes	9.5	71.4
0004b	Meknes	85.0	10.0
0005	Meknes	60.7	0.0
0006	Fes	60.9	0.0
0007	Meknes	60.0	0.0
0009	Elhajeb	40.9	40.9
0011	Elhajeb	25.0	62.5
0013	Azrou	37.1	51.4
0017	Ifrane	45.7	48.6
0018	Sefrou	29.0	58.1
0022	Oujda	61.9	28.6
0023	Oujda	6.7	76.7
0024	Oujda	13.4	73.9
0025	Berkane	40.0	28.0
0026	Berkane	21.1	63.3
0027	Nador	77.8	5.6
0028	Nador	88.9	0.0
0029	Elhoceima	56.5	30.4
0031	Elhoceima	76.6	13.3
0033	Elhoceima	69.7	18.2
0035	Elhoceima	68.0	20.0
0037	Ketama	18.5	4.4
0039	Chefchaoune	64.6	8.8
0040	Chefchaoune	55.6	14.8
0042	Chefchaoune	11.1	85.2
0044	Chefchaoune	91.3	4.4
0046	Tetouan	73.0	9.0
0047	Tetouan	22.0	72.0
0048	Tetouan	100.0	0.0
0049	Larache	70.0	30.0
0051	Larache	57.0	43.0

(continued next page)

(Table 1 continued)

Code	Origin	PR	PS
	Aegilops triuncialis		
0008	Elhajeb	0.0	100.0
0010	Elhajeb	12.5	87.5
0030	Elhoceima	0.0	100.0
0032	Elhoceima	0.0	100.0
0034	Elhoceima	0.0	100.0
0036	Elhoceima	3.8	88.5
0038	Ketama	0.0	96.0
0041	Chefchaoune	0.0	100.0
0043	Chefchaoune	0.0	100.0
0045	Tetouan	3.7	96.5
	Aegilops ventricosa		
0012	Azrou	40.0	5.0
0020	Sefrou	80.0	0.0
	Aegilops triaristata		
0050	Larache	18.0	55.0
0052	Ksar kbir	92.0	8.0

PR=Percentage of resistance; PS=Percentage of susceptibility.

Screening for Resistance to Hessian Fly

Wild *Aegilops* from ICARDA and Morocco are being evaluated for major diseases found in the country, essentially for resistance to widespread biotypes of Hessian fly, which remains a high priority (Tables 1, 2). The 56 *Aegilops* accessions collected were screened under two environments, Jemaâ Shaim experimental station and greenhouse infestation. Many resistant accessions were identified in local *Aegilops* (Table 2).

Table 2. Resistant accessions from the SEWANA collection.

Code	Species	Origin	PR	PS
400027	*ovata*	Syria	50.0%	0%
401155	*cylindrica*	Turkey	60.0%	0%
401667	*ovata*	Morocco	87.9%	0%

PR=Percentage of resistance; PS=Percentage of susceptibility.

The distribution of resistance sources depends on the frequency of insect occurrence. The Meknes, Fes, Nador, Chefchaoune, and Tetouan regions had the highest rates of resistance (Table 1). Differences between species were significant. *Aegilops triuncialis* accessions were the most susceptible to Hessian fly attack, while many *Aegilops ovata* accessions were totally uninjured, with a 100% resistance rate.

Interspecific Cross and Embryo Rescue

Gene transfer from *Aegilops* spp. to durum is quite laborious. Cultivated durum wheat (AABB) has no common genome with Aegilops spp. However, bread wheat shares the D genome with some *Aegilops* spp., and gene introgression from *Ae. squarosa* into bread wheat is quite feasible. Hybridization of durum varieties and *Aegilops* spp. is restricted by the incompatibility barrier, and requires intergenomic translocation for alien DNA incorporation.

Durum wheat cultivars were crossed to resistant accessions of *Ae. ovata* and *Ae. triuncialis*. Immature embryos were rescued at 14 days on an appropriate medium (Sharma and Baenziger 1986). Hybrids at the 2–3 leaf stage were transferred to a plantlet regeneration medium to increase tiller number. When young tillers were vigorous, they were treated with colchicine to double their chromosome number.

The success of the cross depended on the genotype of the wild parent (Table 3). This result is similar to those reported by Sharma and Baenziger (1986) and Sharma and Ohm (1990). Crosses with *Ae. ovata* produced seed and regenerated hybrids at a rate of 3.88% and 1.66%, respectively. *Aegilops triuncialis* crosses produced no hybrid, although a maternal effect for the crossability was noticed. Seed set was significantly higher in reciprocal (6.25%) than direct (3.88%) crosses. However, plants regenerated from direct crosses more often had a normal morphology and a better survival rate after transferal to soil.

Table 3. Interspecific crosses (*Triticum durum* x *Aegilops* spp.).

FP	MP	NF	NS	NP
Ae. durum	ovata	640	3.88%	1.66%
Ae. ovata	durum	80	6.25%	2.50%
Ae. durum	triuncialis	252	0.39%	0.00%
Ae. triuncialis	durum	68	0.00%	0.00%

FM=Female parent; NF=Number of flowers; MP=Male parent; NS=Number of seeds; NP=Number of plants.

Chromosomic Analysis of Interspecific Hybrids

Chromosome number of hybrids and backcrosses was identified according to the procedure described by Sharma and Gill (1983). Root tips were collected from 1–2 day old seedlings, fixed, hydrolyzed, and stained with Feulgen solution.

BC1 plants from a *T. aestivum* x *A. elongatum* cross had between 42 and 65 chromosomes, of which 6–7 came from the elongatum parent (Table 4). Chromosome elimination was significant between BC2 and BC3 in the *T. aestivum* x *Ae. intermedium* cross, while chromosome number in hybrids with *Ae. trichophorum* remained the same in BC2 and BC3. *Agropyron trichophorum* chromosomes appear to be stable in *T. aestivum* cytoplasm. This stability may be explained by a gametocidal effect, which can maintain a whole alien genome (Kirbirige and Knott 1983). *Agropyron intermedium* is able to suppress ph gene activity and promote chromosome pairing between homeologous chromosomes (Sharma and Gill 1983).

Table 4. Chromosome analysis of BC progenies (*Triticum aestivum* × *Agropyron* spp).

Female	Male	BC	NP	NCh	CV	Range
T. aestivum	*A. elongatum*	BC1	35	48.40	0.09	42-65
T. aestivum	*A. intermedium*	BC2	23	45.67	0.08	42-56
T. aestivum	*A. trichophoru*	BC2	30	46.60	0.09	38-56
T. aestivum	*A. intermedium*	BC3	90	43.52	0.03	42-56
T. aestivum	*A. trichophoru*	BC3	141	47.65	0.10	38-58

BC=Number of backcross; NP=Number of plants; Nch=Number of chromosomes (average); CV=Coefficient of variation.

Triticum durum × *Aegilops* spp. crosses were performed under field condition at ICARDA, Syria. F_1 hybrid plants from this program were analyzed in our laboratory. Chromosome numbers varied from 28 to 65 and were influenced by the ploidy level of both parents (Table 5). Hybrids of Haurani and Omruf 1 fixed 22 and 18 chromosomes, respectively, when crossed to *Aegilops vavilovii*. The significant interaction between the two parents is probably under genetic control. The occurrence of a high percentage of unreduced gametes could explain the large number of alien chromosomes that remained and/or the inhibition of univalent chromosome elimination in the hybrids.

Table 5. Chromosome analysis of F_1 (*Triticum durum* × *Aegilops* spp.).

Female (*T. durum* cultivar)	Male	NP	NCe	NCh	CV
Omsnima	*Ae. squarosa*	8	132	29.14	0.88
Lahn	*Ae. umbullulata*	2	13	31.46	2.54
Omsnima	*Ae. umbullulata*	2	98	28.82	0.45
Brachoua	*Ae. kotchyi*	2	128	28.80	0.43
Haurani	*Ae. kotchyi*	2	16	38.42	1.96
Lahn	*Ae. kotchyi*	5	135	29.65	1.16
Brachoua	*Ae. kotchyi*	4	59	30.91	1.13
Gerumal	*Ae. syriacum*	5	91	28.07	0.99
Haurani	*Ae. vavilovii*	2	41	50.21	5.11
Lahn	*Ae. vavilovii*	2	20	29.75	1.00
Omruf1	*Ae. vavilovii*	2	20	46.52	4.60
Gerumal	*Ae. vavilovii*	2	27	28.86	0.47

NP=Number of plants; Nce=Number of cells; Nch=Number of chromosomes (average); CV=Coeffecient of variation.

F_1 plants showed a large morphological diversity in terms of size, tillering, and leaf characteristics. They also showed viability and fertility differences. The instability of the chromosome number in the early generations leads to embryo malformation, hybrid mortality, and low fertility.

Anther Culture

Once alien chromosomal segments are transferred into a durum cultivar, they require stabilization to allow undisturbed chromosome pairing. Conventional selfing methods are time consuming and risk the loss of alien chromosome segments by elimination. Diplohaploidization by anther culture and subsequent chromosome doubling through colchicine treatment may solve this problem by hastening homozygosity. Since 1988, we have been able to regenerate bread wheat and barley haploid plants by using anther culture. However, it is very difficult to induce androgenesis in durum wheat. Currently, we are screening for cultivars with androgenetic response, and testing intergeneric hybridization with maize to produce durum haploids.

Anther culture of *T. aestivum* × *Agropyron* backcrosses BC1, BC2, and BC3 was performed (Table 6). At the pollen uninucleate stage, anthers were plated on a modified MS medium (Foroughi-Wehr 1976). Anther response averaged a low induction rate of 3%. However, a significant variation was noticed among families and individuals from the same backcross. Response to anther culture was very low in BC1 (0.22%). BC3 plants presented the highest induction rate (3.4–4%), and some individuals even reached 25%. The increased androgenetic response in BC3 could be explained by better chromosome stability and therefore higher pollen viability.

Table 6. Anther culture response of BC progenies(Triticum *aestivum* × *Agropyron* spp).

	BC	NA	PI	CV	NP
A. elongatum	BC1	472	0.2%	3.13	0.00%
A. intermedium	BC2	466	1.3%	1.80	0.00%
A. trichophoru	BC2	636	1.2%	2.07	0.00%
A. intermedium	BC3	2,,312	3.4%	1.80	0.08%
A. trichophoru	BC3	4002	4.0%	1.23	0.12%
Total		7,888	3.0%	1.43	0.089%

BC=Backcross generation; NA=Number of anthers; PI=Percentage of calli induction; CV=Coefficient of variation; NP=Number of plants.

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Double Haploid Line Production in Durum Wheat using Anther Culture

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Abstract

Sixteen durum wheat and four bread wheat genotypes were studied for their androgenetic capacity. The percentage of calli/embryoid induction varied from 0.41 to 4.60%, with an average of 1.6%, for Protocol A, and from 0.20 to 5.80%, with an average of 1.40%, for Protocol B. The local durum genotypes Jenah Khotifa AP4 and Azizi AP9 had the highest percentages of induced embryoids under both protocols: 4.60–3.60% for Protocol A and 5.20–5.80% for Protocol B. The absolute highest regeneration of calli was obtained for Jenah Khotifa. From four genotypes, green plants were regenerated: Azizi, Bidi (*Triticum turgidum* subsp. *durum*), Sabine, and Florence Aurore (*T. aestivum* subsp. *aestivum*). A high variation for green plant regeneration was observed between the two protocols. Colchicine, added directly to the induction media, was effective in directly producing doubled haploids. Interspecific crosses with *Zea mays* are currently being investigated in our laboratory as an alternative to doubled haploid production in *Triticum durum*.

Introduction

Haploid plants, as a direct product of meiosis, are obtained by *in vitro* culture of male (androgenesis) or female (gynogenesis) gametophytes. The return to the diploid state, or haplodiploidization, consists of spontaneous or induced chromosome doubling, which obtains homozygous lines in which all the traits are fixed in a single operation and in the absence of any allelic interaction. This process allows considerably simplified genetic analysis (Snape and Simpson 1981; Snape 1989).

The convenient use of this process is dependent on success in obtaining haploids through anther culture or though intergeneric hybridization between agropyron × wheat (Li and Dong 1991), triticale × wheat (Wang et al. 1993), and maize × wheat (O'Donoughue and Bennett 1994). Therefore, haplodiploidization constitutes a useful tool, particularly in a breeding program. The technique is also useful in combination with the use of mutagenesis and direct gene transfer. Thus, research efforts in this field are well justified. In addition, the study of heritability of androgenetic traits is very important to the successful use of regeneration process.

Materials and Methods

Plant Material

Sixteen durum wheat and four bread wheat genotypes (Table 1) were studied for their androgenetic capacity.

Table 1. List of genotypes used.

Code	Genotype/ploidy	Origin
01	Azizi AC2	Tunisia
02	Azizi AP9	Tunisia
03	Jenah Khotifa AP4	Tunisia
04	Hedhba	Tunisia
05	Mahmoudi	Tunisia
06	Karim	Tunisia
07	Bidi AP9	Tunisia
08	D76-53-3b	Tunisia
09	Tanit	Tunisia
10	Florence Aurore (6x)	Tunisia
11	Byrsa	Tunisia
12	Khiar (BD 23-35)	Tunisia
13	Hybride 2	Tunisia
14	Biancullida ICM 27	Tunisia
15	Taganrock AC1	Tunisia
16	Sabine (6x)	FSA Gembloux
17	Omrabi	ICARDA
18	Cham 1	ICARDA
19	Oued Zenati	Algeria
20	Ardente	ENSA/INRA Montpellier

Methodology

Experimental design

A completely random design with one factor, the genotype, was used. The experimental plot was composed of five lines of two meters each, interspaced at 18 cm.

Planting

The genotypes were planted in a covered plastic house at four different seeding dates, in order to spread out the time of spike collection for anther culture (8–28/11/1993; 13/12/1993, and 3/01/1994).

Spike collection

Morphological test. Spikes with flexible and yellowish rachis were selected for anther culture. This rachis aspect corresponds to the distance between the top of the ear to the first internode, which is equal to two thirds of the distance to the base of the flag leaf.

Cytological test. Only spikes with pollen in the uninuclear stage were kept and used for the anther culture procedure.

Colchicine treatment

Colchicine is the most frequently used chromosome doubling agent. It stops mitosis at metaphase by deactivation of the spindle mechanism (Jensen 1974). In general, the treatment is carried out on whole plants by soaking the crown for 72 hours in a 0.04% colchicine solution (Henry and de Buyser 1980). In the present study, the 0.04% colchicine concentration was added directly to the induction media (Barnabàs et al. 1991

Table 2. Induction medium I2.

Composition	Quantity (mg/L)
KNO_3	1900
$CaC1_2, 3H_2O$	440
$MgSO_4, 7H_20$	370
KH_2PO_4	170
NH_4NO_3	165
$FeSO_4, 7H_2O$	27.9
Na_2EDTA	37.3
$MnSO_4, 4H_2O$	22.3
$AgNO_3$	10
$ZnSO_4, 7H_2O$	8.6
H_3BO_3	6.2
K1	0.83
$Na_2Mo_4, 2H_2O$	0.25
$CuSO_4, 5H_2O$	0.025
Glutamine	750
Caseine hydrolisat	250
Myo-inositol	100
Ascorbine acide	0.4
Biotine	0.4
Nicotin acide	0.4
Pyridoxine HCL	0.4
Saccharose	85.5
Agarose	6

pH=5.8.

Testing protocols

Anther culture was performed according to two different protocols, described in Figure 1.

Protocol A: I2/RW/RHIZ./MP. This protocol was obtained from ICARDA's Cereal Biotechnology Laboratory. The anthers are placed on induction medium I2 (Lashermes et al. 1991) (Table 2). The embryoids formed on the pollinic stalls of the anthers are transferred to regeneration medium RW (Table 4). This phase is followed by a transfer to a rooting medium for root initiation (Table 6), and a transfer to the plant regeneration medium MP (Table 7).

Table 3. Induction medium Z1,5.

Composition	Quantity (mg/L)
KNO_3	1000
$(NH_4)_2\ SO_4$	100
$MgSO_4\ 7H_2O$	125
KH_2PO_4	200
$Ca(NO_3)_2$, $4H_2O$	100
KCl	35
$F_2SO_4\ 7H_2O$	27.8
Na_2EDTA	37.3
Potato extract	400 mL
Saccharose 9%	450 mL
Glutamine	200
Thiramine HCl	1
2,4-D	1.5
Kinetine	0.5
Agraose	5.0

pH=5.8.

Table 4. Regeneration medium RW.

Composition	Quantity (mg/L)
Distilled water	800 mL
Macro-elements	100 mL
Micro-elements	10 mL
$AgNO_3$	5 mL
Casein hydrolisat	500 mg
Vitamins	10 mL
AJA	10 mL
Glucose	18 g
Agarose	6 g

pH=5.8.

Table 5. Regeneration medium R8.

Composition	Quantity (mg/L)
KH_2PO_4	300
KNO_3	1000
NH_4NO_3	1000
$Ca(NO_3)_2.4H_2O$	71.5
KCL	65.0
$MnSO_4\ 4H_2O$	4.9
$ZnSO_4\ 7H_2O$	2.7
H_3BO_3	1.6
KI	0.75
Na_2EDTA	37.0
$FeSO_4\ 7H_2O$	27.8
Nicotin acid	5
Pyridoxine HCL	5
Thiamine HCL	1
Inositol	100
Glycin	2
Saccharose	20
AIA	1
Agar	3
Gelrite	3

pH=5.8.

Protocol B: Z1,5/R8/RHIZ/MP. This protocol was obtained from FSA Gembloux, the Belgium Phytopathology Laboratory.

The medium Z1,5 (Piri 1991) is used for the embryoid induction phase (Table 3), while the medium R8 is used for the regeneration (Table 5). The development and the differentiation of a root system is obtained on a rooting medium (Table 6). Developing plants are transferred to a regeneration medium MP (Table 7).

Parameters measured

Percentage of induced embryoids. This was calculated as induced embryoids per 100 cultured anthers.

Percentage of regenerated calli. This was calculated as regenerated calli per 100 induced embryoids.

Percentage of regenerated plants. This was calculated as regenerated plants per 100 calli.

Protocol A	Protocol B
Harvest of spikes	Harvest of spikes
Morpho-cytological tests	Morpho-cytological tests
Cold pretreatment 2 days at 4° C	Cold pretreatment 2-3 days at 5° C
Sterilization of spikes	Sterilization of spikes
Plating on I2 induction medium	Plating on Z1,5 induction medium
Incubation in the dark	Incubation in the dark.
Transfer of embryos on RW regeneration medium	Transfer of embryos on R8 regeneration medium
Incubation in green house	Incubation in green house
Transfer on rooting medium	Transfer on rooting medium
Transfer to MP plant regeneration medium	Transfer to plant regeneration medium
Transfer of plants to soil	Transfer of plants to soil
Determine of chromosome number	Determine chromosome number
Chromosome doubling (in case of haploidy) by colchicine treatment	Chromosome doubling (in case of haploidy) by colchicine treatment
Acclimatization	Acclimatization

Figure 1. Schemes of *in vitro* anther culture.

Table 6. Rhizogenesis medium.

Composition	Quantity mg/L
Macro. MS	50 mL
Micro. MS	5 mL
Fe EDTA MS	5 mL
Myo-inositol	100 mg
Saccharose	30 g
Nicotin Acide	0.5 mg
Pyriodoxin HCL	0.5 mg
Thiamine HCL	0.1
Glycine	2 mg
ALA	5 mg
Kinetine	0.1 mg

pH=5.8.

Table 7. Regeneration medium MP.

Composition	Quantity mg/L
Distilled water	870
Macro elements	100
Micro elements	10
Vitamins	10
Fe EDTA	10
Saccharose 2%	20 g
Agar	7 g

pH=5.8.

Results

Percentage of Induced Embryoids

The percentage of induction varied from 0.41 to 4.60%, with an average of 1.6%, for Protocol A (Table 8; Fig. 2), and from 0.20 to 5.80%, with an average of 1.40%, for Protocol B (Table 9; Fig. 4). The local durum genotypes Jenah Khotifa AP4 and Azizi AP9 had the highest percentage of induced embryoids under both protocols: 4.60–3.60% for Protocol A and 5.20–5.80% for Protocol B. However, for the other genotypes, wide genetic variability was observed.

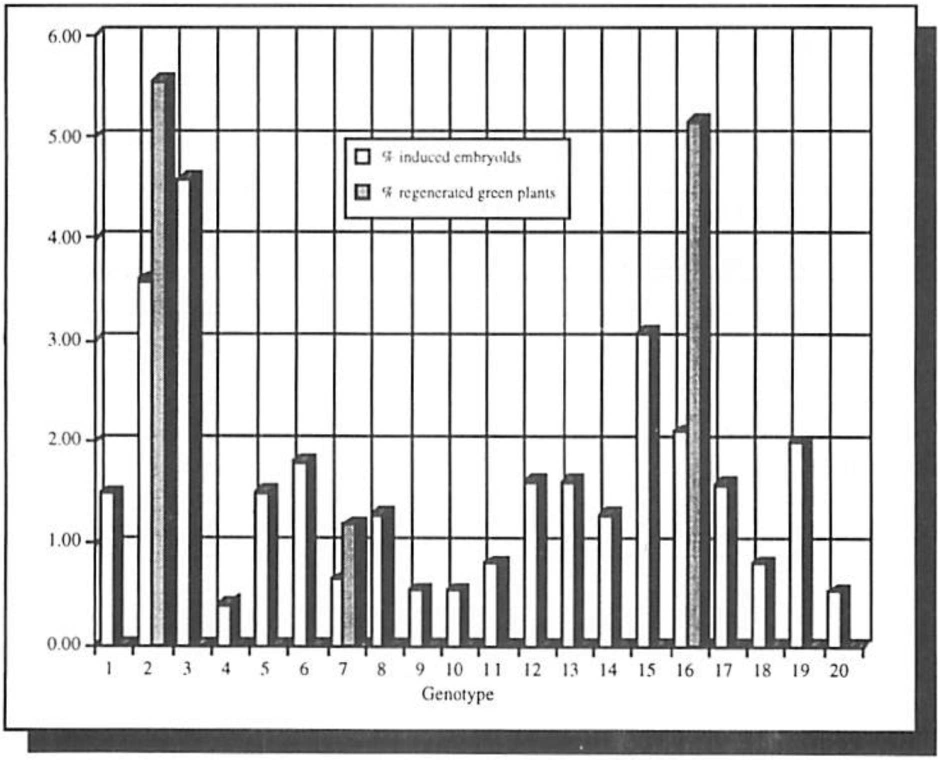

Figure 2. Percentage of enhanced embryoids and regenerated green plants (Protocol A).

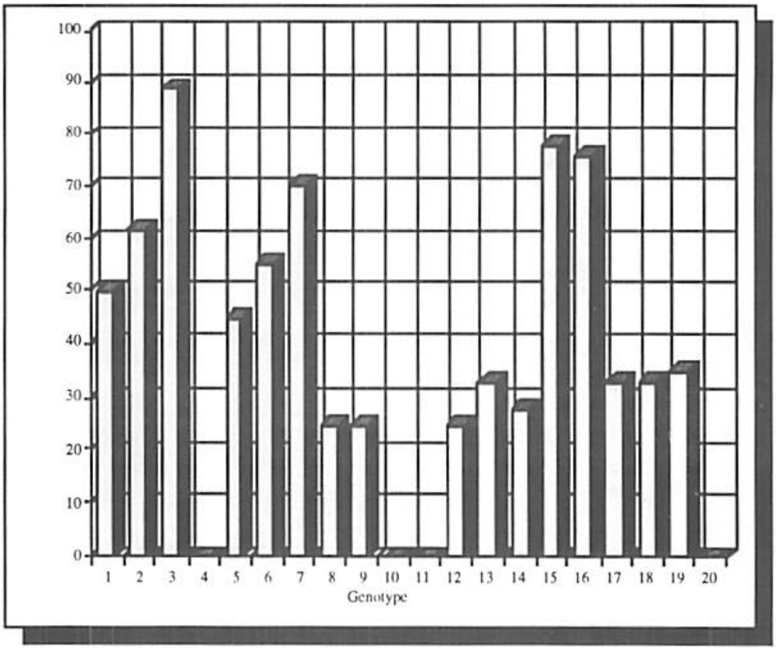

Figure 3. Percentage of regenerated calli (Protocol A).

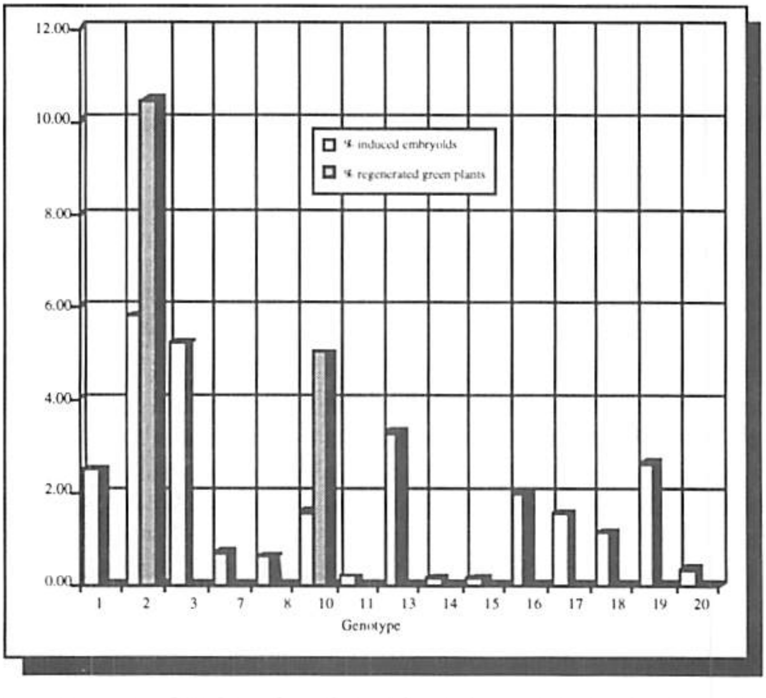

Figure 4. Percentage of induced embryoids and regenerated green plants (Protocol B).

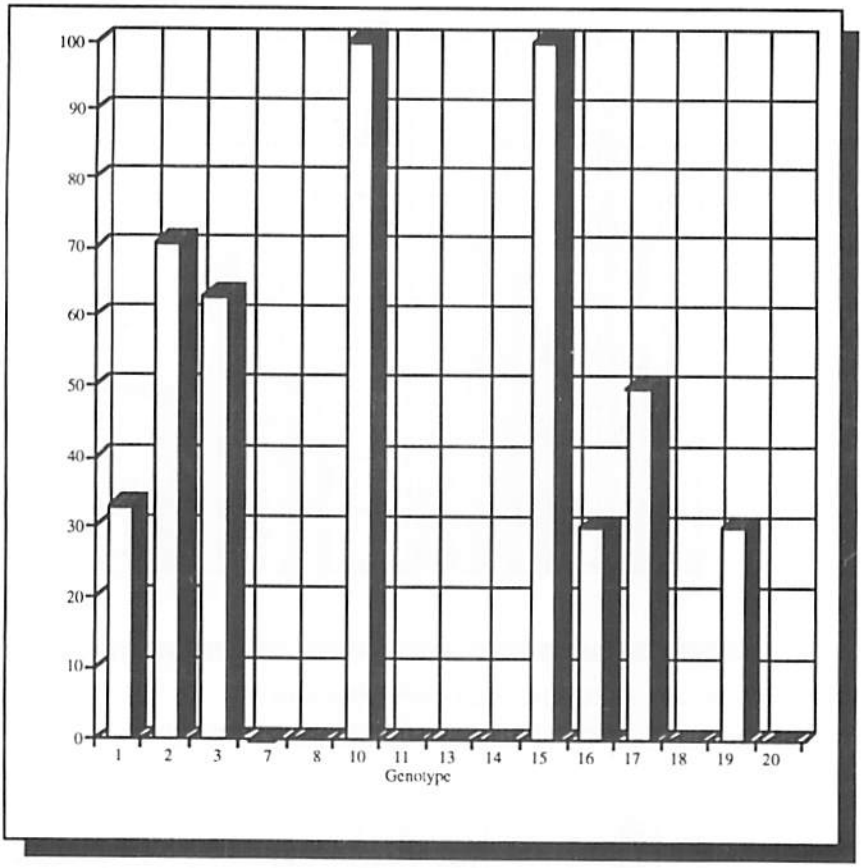

Figure 5. Percentage of regenerated calli (Protocol B).

Percentage of Regenerated Calli

Genotypes Jenah Khotifa AP4 and Azizi AP9, as well as Taganrock, Sabine, and Bidi AP9, showed the highest percentage of regenerated calli for Protocol A. The absolute highest regeneration of calli was obtained for Jenah Khotifa (Table 8; Fig. 3). As for protocol B, Taganrock, Jenah Khotifa, and Azizi had the best regeneration percentages (Table 9; Fig. 5). The percentage of regenerated calli varied from 25 to 89%, with an average of 38.5%, for Protocol A, and from 30 to 100%, with an average of 23.8%, for Protocol B. For both protocols, two genotypes (Azizi and Jenah Khotifa) were distinguished by a high rate of induction and of regeneration. These results confirm those found by Daâloul et al. 1993.

Percentage of Plant Regeneration

Four genotypes produced green plants: Azizi, Bidi (*T. durum*), Sabine, and Florence Aurore (*T. aestivum*). A high variation for green plant regeneration was observed between the two protocols, with the exception of Azizi AP9, which demonstrated a certain stability: 20 and 25 green plants for protocols A and B, respectively (Tables 8 and 9; Figs. 2 and 4).

Table 8: *In vitro* anther culture: Protocol A (12/RW/Rhiz./MP).

Genotype	Percentage Induced embryos	Percentage regenerated calli	Percentage regenerated green plants
1	1.50	50	0
2	3.60	61	5.55
3	4.80	89	0
4	0.41	0	0
5	1.50	45	0
6	1.90	55	0
7	0.66	70	1.20
8	1.30	25	0
9	0.55	25	0
10	0.55	100	0
11	0.83	0	0
12	1.60	25	0
13	1.60	33	0
14	1.30	28	0
15	3.10	78	0
16	2.10	76	5.17
17	1.60	33	0
18	0.83	33	0
19	2.00	35	0
20	0.55	0	0

Table 9: *In vitro* anther culture: Protocol B (Z1.5/R8/Rhiz./MP).

Genotype	Percentage induced embryos	Percentage regenerated calli	Percentage regenerated green plants
1	2.50	33	0
2	5.80	71	10.42
3	5.20	63	0
7	0.74	0	0
8	0.66	0	0
10	1.60	100	5.00
11	0.20	0	0
13	3.30	0	0
14	0.16	0	0
15	0.18	100	0
16	2.00	30	0
17	1.60	50	0
18	1.20	0	0
19	2.70	30	0
20	0.41	0	0

Colchicine Treatment

The colchicine treatment of haploid plants is rather sensitive and can cause high mortality due to the toxic effect of the agent. The developmental stage of the exposed plants may also play an important role in the effectiveness of the treatment. It has been reported that anthers cultured in the presence of colchicine produce a significantly higher percentage of embryos than calli (Barnabàs et al. 1991). In our experiments, the effect of colchicine, added directly to the induction media, did not negatively affect the anther response or the green plant regeneration capacity.

Conclusions

Protocol A seems to be more selective and better than protocol B for the induction of embryoids, the production of regenerable calli, and the regeneration of green plants. The use of protocol A allowed the regeneration of 69 plants, while only 37 were regenerated with protocol B.

It is important to note that we have rarely observed the phenomenon of albinism with anther culture in durum (2– 3%).

Chromosome doubling produced by colchicine added directly to the induction media was effective in our experiments. However, at this time, it is not known at which stage of *in vitro* culturing chromosome doubling occurs. It is also not clear whether spontaneously doubled genomes differ from chemically doubled genomes in degree of genetic stability. Therefore, effective methods to produce true homozygotes with high doubling rates, low risk of damage, and easy applicability should be developed.

Initial tests are currently being conducted in our laboratory on the effectiveness of intergeneric hybridization between *Zea mays* × *Triticum durum* (Comeau et al. 1988; Dusautoir, Station d'Amélioration des plantes de Melgueil/INRA Montpellier) for doubled haploid production in durum. The embryo rescue technique being used is described by Ushiyama et al. (1991).

For the study of heritability of androgenetic parameters, we developed a half diallele with 6 parents. The F_1 population, DH lines, and F_2 population will be characterized with agro-physiological and molecular markers.

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Durum Wheat Haplodiploid Plant Production

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Abstract

Three main haploidization techniques are discussed: anther culture, microspore culture, and induced gynogenesis with foreign pollen. Anther culture has recently shown some promise. It is now almost as efficient as bread wheat anther culture in regards to the number of haploid calli or embryos. However, the vast majority of plants produced were albino, rendering that technique useless. Microspore culture techniques have not been fruitful so far, which may be a lack of research rather than a lack of success. The development of such a technique in durum wheat would be useful for better control of androgenesis and to solve the albinism problem. Induced gynogenesis is the only technique currently able to produce green doubled haploid plants in workable numbers. The technique can still be improved by focusing efforts on: a more general technique, less dependence on the genotype, overall efficiency in haploid embryo production, and efficiency in embryo germination.

Anther Culture

Techniques of haploid and doubled haploid plant production are powerful tools, widely used in pure-line development of cereals (de Buyser et al. 1987) and in genetic analysis (Snape and Simpson 1981). In cereals, anther culture is by far the preferred haplodiploidization technique (Dunwell 1985), even if haploid bread wheat plants can be obtained using ovule and embryo sac culture (Zhu and Liu 1981, Yang and Zhou 1982) or intergeneric crosses with foreign pollen (Laurie and Bennett 1988). These latter methods are not very efficient. In spite of important genotypic variability observed for a majority of species (Fadel and Wenzel 1990) and relatively low efficiency—between 5 and 100 haploid structures per 100 plated anthers (Huang 1987)—bread wheat anther culture is now routinely producing inbred lines, which are being incorporated into breeding programs (Picard et al. 1990; Veilleux 1994).

Durum wheat anther culture, however, has so far proven more recalcitrant (Sharma et al. 1982), as it is for other biotechnological applications in general. Fewer structures are produced, with only some genotypes. Further, most if not all regenerated plants (>95%) are albino (Zhu et al. 1980; Hadwiger and Heberle-Bors 1986), although one report claimed 30% green plants (Daaloul et al. 1992). Mihamou-Ziyyat (1992) found that few durum wheat cultivars well-adapted to

Induction, Initiation, and Development

Anthers of durum wheat, cv Ardente, obtained by Semences de Provence, Beaucaire, France, which grows well in the fields of southern France, were collected when microspores were at the late uninucleated stage and plated onto PII medium with potato extract (Xing Zhi and Han 1984). Microspore development in anthers was not synchronous at collection (when spike tops, without awns, reach 2 cm over the penultimate leaf ligule). In most of the anthers, all developmental stages—from early uninucleated to mid or late binucleated—were found. A cold pre-treatment has not proven useful. However, spikes can be left for up to 14 days at 4° C without damage. Microspore development has been observed during culture after acetocarmine squash of the anthers (Fig. 1). The mean viable microspore number after 21 days was 59.8 per anther, and the number of microspore divisions was about 13 per anther, representing 0.28% of the total microspores. An average of 41.3 microspore-derived structures per 100 anthers were transferred on regeneration medium (R9 Picard and de Buyser 1973) after 42 days, and more than 95% of regenerated plants were albino.

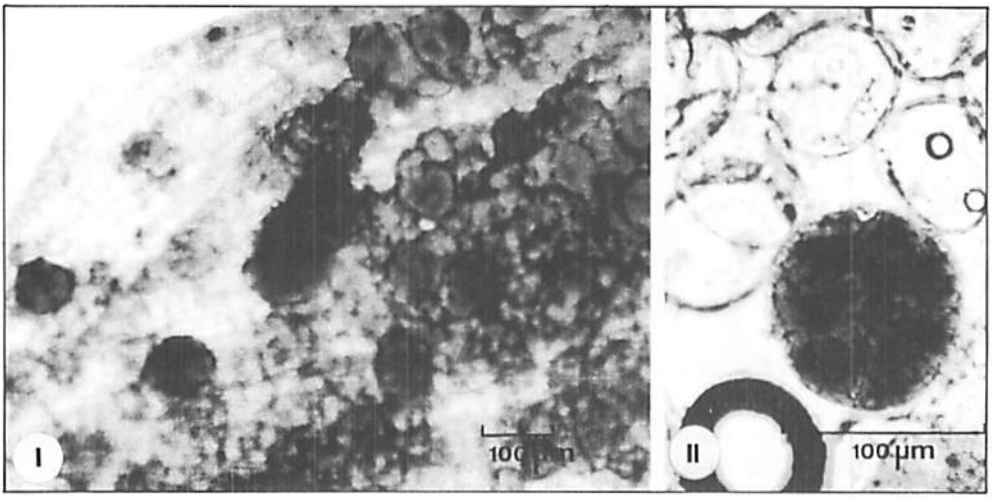

Figure 1. Squash in arcetocarmine of durum wheat anther after 15 days in culture. I) General view showing dividing microspores. II) Detail of one microspore-derived microcolony.

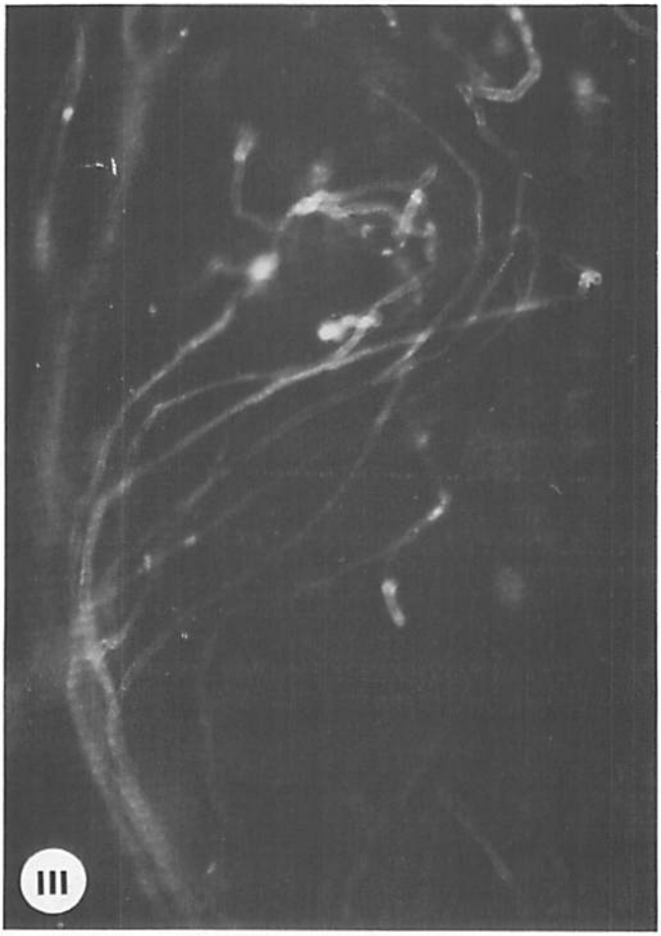

Figure 2. Germination of maize pollen and growth of pollen tubes through durum wheat silks and styles to the ovule. Fluorescent observation after staining with alinine blue.

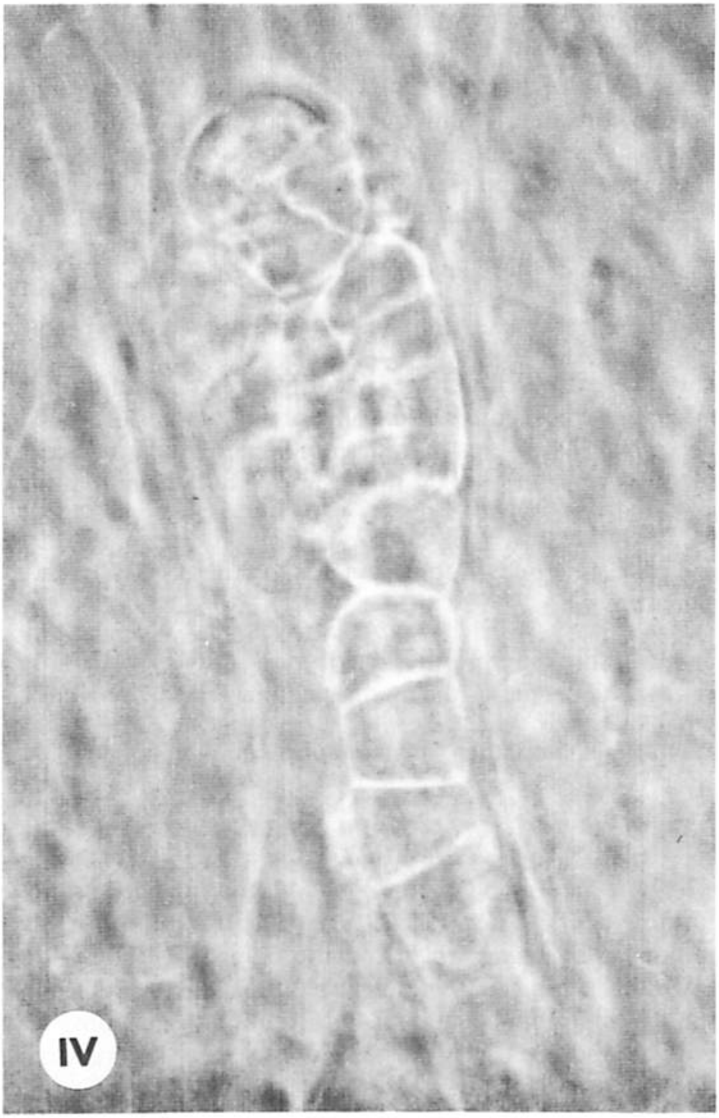

Figure 3. Visualization of a putative "haploid" embryo with suspensor after fertilization with maize pollen. Observation in phase contrast microscope after ovule clearing with Herr solution.

Ethylene and Polyamine Implication on Androgenetic Response

Several problems are encountered during durum wheat anther culture. Amongst them are an insufficient number of microspores able to divide and develop into structures (embryos or calli).

Because it is known that ethylene and polyamines are involved in many *in vitro* physiological processes (see Adkins 1992 for a review), these two families of growth regulators, as well as their metabolic pathways, were followed during culture. Effectors of these metabolisms were added to the culture medium. Their effects have been confirmed or inferred by endogenous quantification of ACC, M-ACC, ethylene, and polyamine, and their physiological responses recorded. The influence of one metabolism has also been tested on the other (see Sevenier 1995; and Sevenier et al. 1995 for detailed results).

The main conclusions of these investigations are:

- Ethylene and polyamine are implicated in the microspore response.
- The results were different for the two different phases of development studied: when microspore divisions are enhanced, the number of structures decreases, and vice versa. That means that the requirements with regard to ethylene and polyamine are opposite for the microspore induction-initiation phase and for structure development.
- Ethylene increases microspore division, while it is negative for structure development.
- A high putrescine/(spermidine+spermine) ratio is favorable for structure development and decreases the number of microspore divisions.

In the best conditions, more then 100 structures could be obtained per 100 anthers.

Albinism

Most of the regenerated plants were albino and no medium improvements of the anther culture have so far decreased this problem. Nothing is known about the causes of albinism. By using wheat-specific chloroplastic DNA probes we have recently shown that albinism in durum wheat is accompanied by deletions in the chloroplastic genome (unpublished results), as already demonstrated for rice and bread wheat (Day and Ellis 1984). The next subject to be investigated will be the time when these deletions occur.

Perspectives

The technique of durum wheat anther culture needs to be improved because efficiency is still too low, and genotypic problems have not been solved. Knowing how endogenous ethylene and polyamine production can modulate the physiological response of microspores, it should be possible to optimize each of the steps. The anthers must be transferred between the induction-initiation phase (characterized by division potential) and the growth phase (characterized by the number of structures that can, later on, develop on the regeneration medium).

However, anther culture can be considered an efficient technique for the production of doubled haploid lines if, and only if, the bottleneck of albinism can be solved. Slim-Amara (this proceedings) and Daaloul et al. (1992) studied anther culture of Tunisian genotypes and landraces. While the overall response was relatively low (a maximum of 5.8 structures per 100 anthers and 0.43 regenerated plants for the best genotype, Azizi A P 9), most of the plants were green. Lines with positive androgenic reaction (green plants) could be classically introduced in useful germplasm and used as starting material. A comparison of protocols may improve the final number of chlorophyllian doubled haploids that can be obtained with anther culture.

Using specific chlorophyllian DNA probes, we can also focus on learning when and how albinism is instigated. We can decide whether it occurs naturally during microsporogenesis as a result of the quasi-strict maternal cytoplasmic fertility, or whether it is caused by the shock received during culture, or at microcallus or proembryo stage.

Isolated Microspore Culture

The best isolated microspore-culture systems have been developed on tobacco (Kyo and Harada 1985) and rapeseed (Swanson et al. 1988). Isolated microspore culture has also shown some success in cereals such as bread wheat (Mejza et al. 1993; Picard et al. 1993), rice (Ogawa et al. 1992) and maize (Coumans et al. 1989). The potential of shed microspores to yield haploid calli and embryos has also been demonstrated with bread wheat (Datta and Wenzel 1987). No assay has been so far reported for durum wheat.

Perspectives

Expertise on corn (Coumans et al. 1989) and, more recently, bread wheat (Picard et al. 1990, 1993) microspore culture sheds light on the use of the same technique on durum wheat. With isolated microspore culture and the possibility of performing non-destructive observations, it should be easier to control the progress of androgenesis and to determine conditions that will decrease albinism and improve overall efficiency. If the technique can be made viable for durum wheat, haploid microspore will be the best technique to use for *in vitro* selection (haploid single cells carrying the gametoclonal variability) or genetic transformation, as developed on rapeseed (Swanson et al. 1988; Neuhaus et al. 1987).

Induced Gynogenesis

An alternative way to produce haploid plants relies on interspecific or intergeneric crosses. Gynogenesis induced by foreign pollen was first used in cereals for the production of haploid barley plants with *Hordeum bulbosum* pollen (Kasha and Kao 1970). This technique has since been adapted to bread wheat, but necessitates the absence of some Kr alleles (Koba et al. 1991; Sitch and Snape 1987). With crosses using bread wheat as the female parent and maize as the pollen parent, rapid elimination of the male genome under controlled conditions was observed by Laurie and Bennett (1988), allowing the development of haploid embryos. Using crosses between durum wheat and maize, fertilization potential was also assessed (O'Donoughue and Bennett 1988) and green plants recovered (Coumans et al. 1992; Amrani et al. 1993).

Background

Emasculation and pollination were carried out according to Laurie and Bennett (1986). To facilitate the castration process, spikelets of the basal third of the spike, as well as each central flower of the remaining spikelets, were removed about three days before anthesis. The apical third of the spike was cut out. The remaining flowers were emasculated after cutting out the apical part of the lemmas and paleas. After emasculation, spikes were protected by a light paper bag in order to maintain sufficient moisture (Suenaga and Nakajima 1989). The flowers were pollinated about three days later. Immediately after the pollination, the culms were placed in a growth chamber in a nutrient solution under continuous light at 25° C.

Observations of the pollen tubes showed that, whatever the wheat or *Zea* genotype, corn pollen germinated and entered the silk in less than 30 minutes in all observed pollinated flowers (Fig. 2). The first pollen tubes reached the style after two hours and the ovule after four hours. In wheat genotypes with good fruit setting, such as Acalou and Mondur, pollen tubes were visible at the ovule level in all pollinated flowers. However, the aniline blue technique does not allow a clear view of the fertilization event. This technique has been proposed for durum wheat (O'Donoughue and Bennett 1994).

Ten days after pollination, well-developed proembryos were easily recovered. All plants regenerated were chlorophyllian and haploid, as confirmed by flow cytometric analysis.

Genotypic Analysis

Eleven durum wheat genotypes were crossed with four *Zea mays* and one *Z. mexicana* genotypes. A genotypic effect was observed between durum wheat genotypes when all the pollen donors were combined (Table 1).

Lloyd, Ardente, and Primadur were significantly higher than the mean for the number of fruit-like structures, with more than 65% for Lloyd. For the number of developed embryos per hundred fruits, the best genotypes were Villemur, Aristan, and Primadur, while no embryos could be recovered for Lloyd.

Table 1. Pollinated flowers, fruit-like structures, and embryo number for fertile spikes.

Durum wheat genotypes	No. of flowers on fertile spikes	No. of fruit-like structures	Percentage of fruit-like structures†	No. of embryos	Embryos / 100 fruits‡
Acalou	409	194	47.4°	15	7.7
Ambral	577	306	53.0	22	7.2
Arcour	103	56	54.4	1	1.8°
Ardente	195	122	62.6°°	17	13.9
Aristan	209	90	43.1°°	18	20.0**
Armet	390	146	37.4°°	8	5.5**
Lloyd	458	302	65.3°°	0	0.0**
Mondur	789	392	49.7°	32	8.2
Néodur	151	73	48.3	0	0.0**
Primadur	1,385	814	58.8°°	124	15.2**
Villemur		279	52.7	57	20.4**
Mean			53.4		10.6

† Probability calculated by chi square test: °=P >95 and °°=P >99.

‡ Probability calculated by Fichers exact test (2-tail): *=P >95 and **=P >99.

The same analysis was performed on pollen donor genotypes. In that study, *Z. mexicana* cv teosinte was better than the four tested maize genotypes.

Two other genotypes are being tested with maize pollen. Cham 1 is giving outstanding results, with 3–5 green plants per spike. Jenah Khetifa is starting to produce a few embryos. A concentrated effort will be undertaken for the landrace Jenah Khetifa and for the hybrid Cham 1 × Jenah Khetifa.

Perspectives

No embryos were recovered from the durum wheat genotypes Lloyd and Neodur. Other results were genotype-dependent, either on the genotype of the pollen donor or on the one of the female receivers. There is still room for improvement. However, since all recovered plants were green, it is already possible to produce doubled haploid plants in sufficient number for some genotypes.

Two problems remain. First, why can so few viable embryos be rescued while the number of swollen ovaries is relatively high? In other words, is ovary swelling a sign of fertilization, and do most of the embryos abort at a very precocious stage? The second is that we have no final proof that induced gynogenesis by interspecific or intergeneric crosses does not implicate a possible introgression of maize DNA in the durum wheat genome. To solve the first problem, a clearing-up technique is being considered in the laboratory. It will allow the development of very young embryos without dissecting the ovaries (Fig. 3).

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Cell Suspension and Somatic Embryogenesis in Durum

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Abstract

Two biotechnologies need to be developed and improved for durum wheat: somatic embryogenesis and cell suspension culture. The first concerns the possibility of growing whole plants via uni- or oligocellular development, ideally through somatic embryogenesis. Work focused on one (immature zygotic embryos) of several starting materials (mature zygotic embryos, immature inflorescences, and anthers). Shoots as well as embryos can be neoformed. The main objective for durum wheat is the definition of a protocol that allows the efficient use of genetic transformation methods. The second objective deals with cellular reactions to abiotic stresses such as heat and drought. This may represent an additional parameter, based on intracellular behavior, for ranking genotypes according to their susceptibility or tolerance. If a difference can be detected at that level, *in vitro* selection of this specific parameter is possible.

Somatic Embryogenesis

An experimental model for durum wheat must be defined that allows plant regeneration originating from single cells. Future objectives include in vitro selection or genetic transformation. Despite abundant literature on cereals, very few reports are available on durum. The establishment of such a model presents numerous difficulties: choice of reactive explants, disposition on the culture medium, culture conditions, and the nature of growth regulators and genotype (Bhaskaran and Smith 1990).

Immature zygotic embryos are the explants most often used (Ozama and Komamine 1989 for rice; Shillito et al. 1989 for corn; He et al. 1986 and Breiman et al. 1987 for bread wheat; Yang et al. 1993 for durum wheat) with anthers (Armstrong et al. 1987 and Reynolds 1993 for bread wheat).

Because albinism in durum wheat is a critical problem encountered with the use of anther culture (with a second minor problem of chromosome doubling), immature zygotic embryo culture is the preferred technique (Bennici et al. 1988). However, in immature embryos, there is no clear-cut distinction between callogenesis and somatic embryogenesis: both types of regeneration sometimes coexist on the same explant (He et al. 1986). Only by use of a histological follow-up of culturing can the two processes be discriminated.

It is also likely that intra- and intergenotypic variations are somehow related with endogenous hormonal status. So, a hormonal supply is needed in bread wheat for the dedifferentiation and initiation of the embryogenic process (Mathias et al. 1986), or for aiding maturation of somatic embryos (Brown et al. 1989).

If genetic transformation is the major objective, the experimental model must have two major characteristics: responsive cells must be very close to the surface of the explants to be reached by foreign DNA, and the tissues must spend the least possible time in vitro to avoid the genetic variations frequent in cereals (Chowdhury et al. 1991; Larkin and Scowcroft 1983 for review).

Two Protocols Developed in Montpellier

Zygotic immature embryos

Ardente was the genotype of durum wheat used in the following experiments. Only immature grains of the lateral flowers of each spikelet from the medium third of the spike were collected. Immature embryos were dissected 12, 16, or 20 days after anthesis and immature embryos placed with the embryo axis in contact with the medium (see Fernandez 1994 for details).

Callus, shoot, and somatic embryo development was followed for eight weeks (Fig. 1). There was a clear interaction between medium composition, level of 2,4 D in the medium, and age of zygotic embryos. In these conditions, histological observations have found both types of regeneration. Among the additives tested in culture (2.4 g/L NaCl), numerous small proembryos developed at the callus surfaced after 28 days (Fig. 2), showing that the ratio of shoots to embryos can apparently be displaced (in which case they fail to develop further).

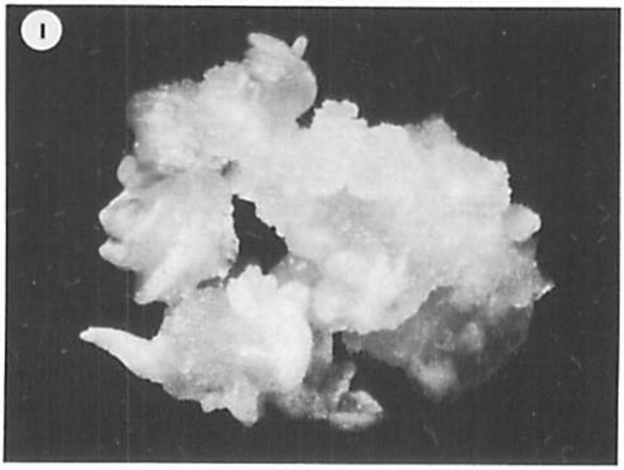

Figure 1. Shoot and embryo development on callus derived from immature zygotic embryos of durum wheat after five weeks in culture.

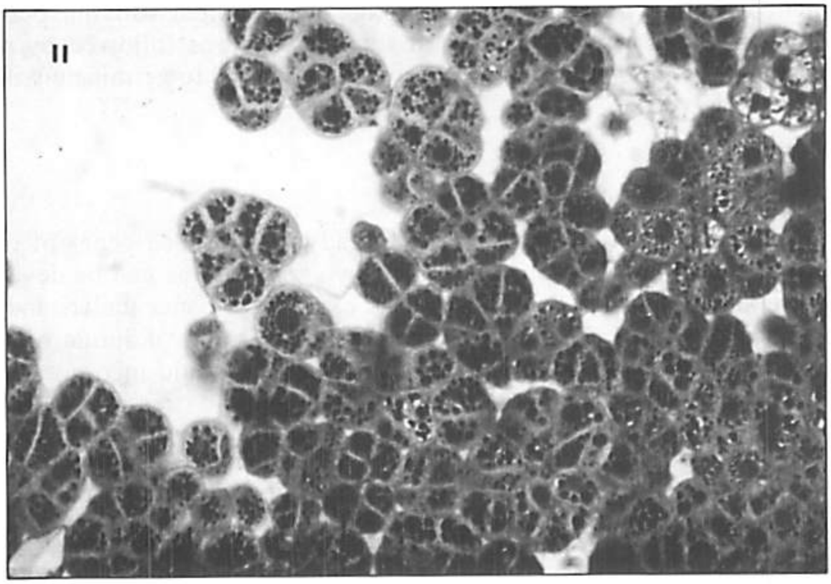

Figure 2. Small proembryos developed at the surface of calli derived from immature zygotic embryos of durum wheat after four weeks on culture medium supplemented with 2.4g NaCl.

Immature inflorescence culture

Immature inflorescences were tested, because most explants responsible for somatic embryogenesis are located at both ends of the developmental cycle. Possibly, they are somehow more juvenile—young tissue developed from the zygote (immature embryos, mature embryos, and, to a lesser extend, hypocotyls, epicotyls, and cotyledons), or tissues from the floral meristem—and in the neighborhood of the gametes such as anthers (Zhong et al. 1995), nucelle (Bacchi 1944), or integument (Carron and Enjalric 1985).

Immature inflorescences possess all the flower organs at a very juvenile stage. After four weeks, shoots as well as somatic embryos are observed on most of the explants. The complexity of the tissue present in immature inflorescences at a very juvenile stage makes it more difficult to recognize the type of shoot development (callogenic or embryogenic) and the position of the cells at the base of shoots or embryos.

Short-term Objectives

Besides increasing the percentage of responding embryos independent of the genotype, several improvements of the model are needed before oggenetic transformation can begin. The number of somatic embryos has to be enhanced, as they originate from cells that can combine embryogenic potential and transformation capacities. Medium composition has to be refined to allow development of most proembryonic structures. A comparison must also be performed between *in vitro* maturation of somatic embryos followed by normal germination, and the possibility of forcing somatic embryos to germinate without a maturation period.

Long-term Objectives

A model of somatic embryogenesis must be ready to introduce genes of interest into durum wheat by genetic transformation. Two applications can be developed. One fundamental application should allow, for example, a better understanding of the presence of proteins or metabolisms involved in the growth of durum wheat and the behavior of grains or zygotic embryos. The second should incorporate genes involved with general/specific resistance to biotic and abiotic stresses and quality.

We will focus on two transformation techniques: shot gun, biolistique, or particle bombardment (Sandford 1988) and tissue electroporation (D'Halluin et al. 1992; Songstad et al. 1993).

Cell Suspension

Embryogenic cell suspensions can be accomplished for most cereals (Vasil 1987). The main objective is to obtain protoplast that can regenerate plants. Cell suspensions can originate from microspore culture (anther and isolated microspore culture: Su et al. 1992 for rice; Jähne et al. 1994 for barley) and be used in genetic transformation. In durum wheat, however, the presence of a high rate of albinism renders this process inadequate if normal plants are to be recovered.

Embryogenic cell suspensions can also be obtained from immature zygotic embryo cultures (Hodges et al. 1986 for maize; Felföldi and Purnhauser 1992 for bread wheat; Bennici et al. 1988 for durum wheat). However, to decrease the length of time spent in vitro, direct somatic embryo production is often preferred to embryogenic cell suspensions.

Lines more tolerant or resistant to biotic or abiotic stresses were selected by classical means. Agro-physiological and biochemical criteria were developed by other teams (this proceedings) to help breeders (root development, stomatal density and conductance, content in ABA, proline and other osmoticum, chlorophyllian fluorescence, etc.). For example, as a result of these markers, several drought-tolerant genotypes were selected in diverse species such as sunflower, peanut, maize, and rice.

Two main difficulties complicate the search for plants resistant to culture conditions in dry areas:

- In the field, it is not easy to separate the two constituents of stress: hydric and thermic factors. It is possible that plants can overcome the two constituents by different mechanisms.

- As a consequence, specific reactions are not well understood. The literature concerning the use of parameters such as proline content and chlorophyllian fluorescence is not clear or easily reproduced, and it does not always correlate with field tolerance. It is more likely that tolerance can be reached, in distinct genotypes, by alternative cellular or tissue strategies.

Results

Plants of several wheat genotypes (11 bread and 16 durum wheats), were agro-physiologically tested by Galiba in the phytotron at Martonvasar, Hungary, in order to characterize lines as a function of their sensitivity to heat. Embryogenic cell suspensions are under development in Hungary and at Montpellier. Results will be published.

Short-term Objectives

Many stresses attack plants at the cellular level by common degradation effects such as those caused by free radicals. Stress conditions may then create oxidative disorders which disturb cellular development. The objective is then to recognize how cells, faced with such stress, react, and to search for discriminating reactions between susceptible and tolerant genotypes. These genotypes will be proposed as cellular parameters related with the tolerance, in complement to other criteria such as agro-physiological or molecular markers. These genotypes, physiologically tested in the Martonvasar phytotron, are generating our first in vitro cell suspensions corresponding to well-defined reactions of the plants to heat stress. They will be compared to heat and drought factors at the cellular level.

Long-term Objectives

If a discriminant reaction to thermic or other stresses is demonstrated between known susceptible and tolerant plants, an in vitro strategy can be used. Only tolerance characters at the cellular level are accessible this way. To be selectable, the degradation process must somehow be detected. For example, cell-membrane weakening starts with cell leaking that can be monitored by changes in medium conductance. With an increase in stress conditions, a more drastic membrane degradation will cause the death of susceptible cells. *In vitro* selection can be improved by increasing variability by using a mutagen and by passage through protoplasts that are more sensitive to cell membrane decay. The cell membrane would, in this case, indicate endogenous oxidative stress. If variation of this parameter is seen as logical for high temperatures, it may be used with other stresses that may have a similar effect on the cell membrane.

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Comparative Mapping of Gramineae Species: Implications for Durum Improvement

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Abstract

Comparative mapping using DNA markers offers a method of combining research efforts in different species. In the present study, we developed a consensus map for members of the Triticeae tribe (*Triticum aestivum* subsp. *aestivum*, *Aegilops tauschii*, and *Hordeum* spp.) and compared them to rice, maize, and oat. The aneuploid stocks available are invaluable for comparative mapping because almost every DNA fragment can be allocated to a chromosome arm, thus preventing erroneous conclusions about probes that could not be mapped due to a lack of polymorphism between mapping parents. The orders of the makers detected by probes mapped in rice, maize, and oat were conserved for 93, 92, and 94% of the length of triticeae consensus maps, respectively. Homeologous segments conserved across Triticeae species, rice, maize, and oat can be identified for each Triticeae chromosome. Putative orthologous loci for several simply-inherited and quantitatively-inherited traits in Gramineae species were identified.

Introduction

Geneticists have directed much effort towards the molecular genetics of wheat (*Triticum aestivum* L. subsp. *aestivum*) and its relatives. Restriction fragment length polymorphisms (RFLPs) have been assigned to wheat chromosome arms (Anderson et al. 1992; Devey and Hart 1993), and RFLP linkage maps exist for hexaploid wheat (Chao et al. 1989; Liu and Tsunewaki 1991; Devos et al. 1992; 1993a; Xie et al. 1993; Nelson et al. 1995a, b, c; Van Deynze et al. 1995a) and the related species, *Aegilops tauschii* Coss (Gill et al. 1991; Lagudah et al. 1991), *T. monococcum* subsp. *monococcum* (Van Deynze et al. 1995a, Dubcovsky et al. in preparation), barley, *Hordeum* species (Heun et al. 1991; Kleinhofs et al. 1993; Graner et al. 1991; 1994), rice, *Oryza sativa* L. (Causse et al. 1994; Kurata et al. 1994a), maize, *Zea mays* L. (Burr and Burr 1991; Gardiner et al. 1993), and oat, *Avena* spp. (O'Donoughue et al. 1992, 1995; Rayapati et al. 1995). To date, tetraploid *Triticum* species have received little attention, although one of the parents of the hexaploid mapping population used by Van Deynze et al. (1995a) and Nelson et al. (1995abc) was a synthetic wheat line consisting of the durum cultivar, Altar 84 and a *Ae. tauschii* accession.

Genetic linkage maps can be compared among crop plants using a common set of DNA probes (Bonierbale et al. 1988). Comparisons of molecular maps of wheat, barley, *Ae. tauschii*, and *T. monococcum* (Devos et al. 1993a; Nelson et al. 1995a, b; Van Deynze et al. 1995a) indicate that the order of molecular markers on the linkage maps of these species, detected with the same probes, are largely homosequential. Thus, consensus maps can be constructed to represent linkage maps for the chromosomes of these species (Nelson et al. 1995a, b; Van Deynze et al. 1995a).

This paper assesses the degree of genome conservation between Triticeae species and rice, maize, and oat, and the implications for durum wheat improvement.

To map this project, anchor probes were selected because: (1) they were previously mapped on rice and represent single-copy DNA sequences in rice; (2) they have also been previously mapped onto maize (Ahn and Tanksley 1993) and/or wheat (Ahn et al. 1993); and (3) they provide good genome coverage in rice. A set of 142 markers was chosen based on position in the rice RFLP map (Causse et al. 1994), because the small size of the rice genome suggested that it was the best model for comparative mapping among Gramineae species. Also, rice is a basic diploid with a large proportion of single copy DNA (approximately 85% of DNA behaves as single copy at high stringency, 0.5X SSC at 65 °C; McCouch et al. 1988), and no apparent pattern in the distribution of duplicated loci. It is important to develop an accurate model of the ancestral Gramineae genome for comparison, so that gene duplication and amplification in DNA which have happened since the divergence of these species from their last common ancestor do not obscure the underlying structural similarity among grass species genomes. Additional clones were chosen from the linkage maps of each species to make comparisons in areas not represented by those selected based on the above criteria. The probes, enzymes, and methods used are described in VanDeynze et al. (1995a).

Development of Consensus Maps

Consensus maps based on species-specific maps from wheat (Nelson et al. 1995abc; Van Deynze et al. 1995a), *Ae. tauschii* (Gill et al. 1993), and barley (Heun et al. 1991; Kleinhofs et al. 1993; Graner et al. 1991, 1994) were developed using wheat as a base for comparison. The order of markers for chromosomes 4, 5, and 7 was based on that of barley to correct for the ancestral rearrangement of these chromosomes in wheat and the pericentric inversion in wheat chromosome 4A (Naranjo et al. 1987; Anderson et al. 1992). Loci were included in the consensus map only when their order agreed among maps within a species and when they were present in at least two linkage maps for particular homeologous chromosomes in any of the species. Additional markers detected with probes unique to one of the maps from the species mentioned above—but also mapped in rice, maize, or oat—were included in the consensus maps. The relative position of markers was determined by the location of markers detected with common probes in the individual linkage maps. A proportion of probes detected markers that were not mapped in wheat, *Ae. tauschii*, or barley but were assigned to wheat chromosome arms and mapped in rice, maize, or oat. When these markers were mapped to homeologous segments between Triticeae and these species, and were assigned to the same wheat chromosome arm as the homeologous segment, their position on the Triticeae consensus map was estimated based on relative position in the species being compared. For simplicity, we refer to the resulting consensus maps as Triticeae consensus maps.

These consensus maps efficiently combine genetic information accumulated for related grass species (wheat, *Ae. tauschii*, and *Hordeum* spp.) for comparison to more distantly related species (rice, maize, and oat). They help circumvent problems of low polymorphism between mapping parents by providing relative marker location information across several maps. For example, 116 markers on the consensus map for group 1 chromosomes provide relative order information for 288 unique markers from the combined individual linkage maps (Van Deynze et al. 1995a). Such maps do not accurately estimate recombination, and species-specific rearrangements cannot be included. Only 12 inconsistencies were reported in the order of 116 markers detected with common probes from seven *Triticum*, four barley, and two rye maps for group 1 chromosomes of Triticeae (Van Deynze et al. 1995a). In this study, the relative order of loci among linkage maps of different species was given more weight than was difference in recombination. The relative distance between markers detected with probes common to more than one linkage map was used to position markers on the consensus maps. Although these maps include mostly molecular-genetic linkage data, information from classical and physical maps may be used as well.

Comparison of Grass Species with Triticeae

The proportion of the Triticeae consensus maps examined was: 85% using 160 probes mapped in rice, 92% using 211 probes mapped in maize, and 92% using 192 probes mapped in oat (Table 1).

Table 1. Genome comparisons with Triticeae and oat, rice, and maize.

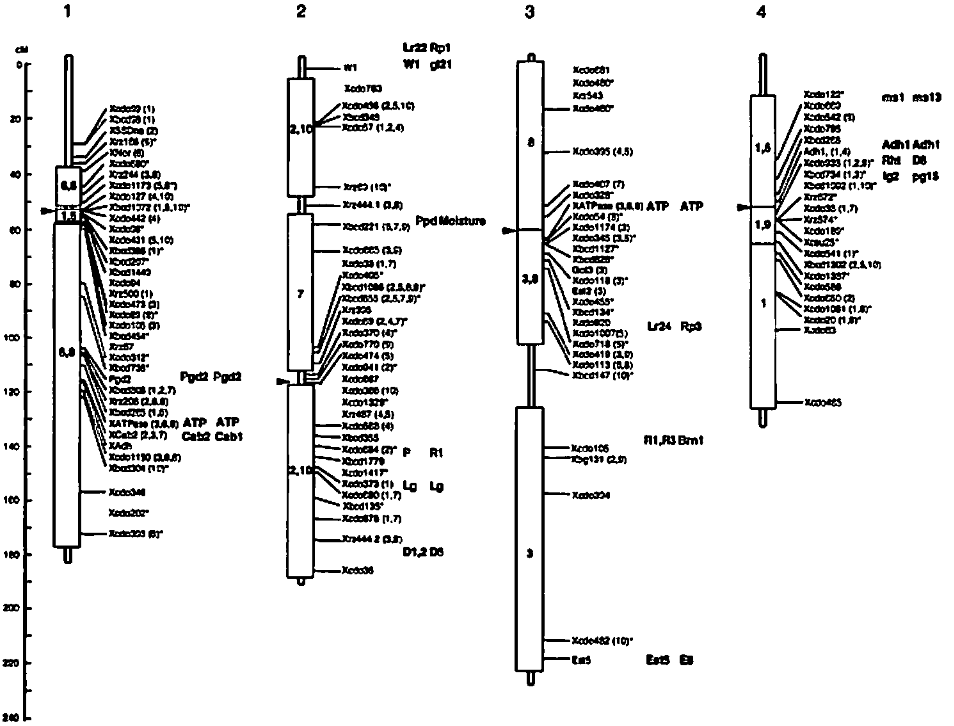
Species	Genome coverage in Triticeae[1] (%)	Genome covered by Triticeae (%)	Genome conservation (%)	Number of regions conserved	Size of conserved regions (cM)
Oat	92	99	94	16	5–215
Rice	85	95	93	16	3–225
Maize	92	98	92	20	3–120

[1] Includes data from aneuploids and linkage maps (Nelson et al. 1995abc; Van Deynze et al. 1995).

From the alternative perspective, probes mapped in wheat, *Ae. tauschii*, or barley represented 95%, 98%, and 99% of the length of the rice (Causse et al. 1994), maize, and oat linkage maps (Van Deynze et al. 1995b), respectively.

Rice

Except for rice chromosome 12, each rice chromosome has a substantial region conserved in one or more of the homeologous Triticeae chromosomes (Fig. 1). Only four out of 10 probes hybridizing to loci located on rice chromosome 12 detected loci on Triticeae species, and these are found on Triticeae chromosomes 1S and 5S. The linkage maps for the entire rice chromosomes 1 and 9 were conserved relative to the linkage maps of chromosomes 3 and part of chromosome 5 of Triticeae, respectively. Except for insertions from other rice chromosomes near the centromeres of homeologous Triticeae chromosomes, the orders of loci on rice chromosomes 4, 5, and 6 of rice were also conserved relative to Triticeae chromosomes. The order for the majority of orthologous loci between rice and Triticeae chromosomes is conserved, but some internal discrepancies exist, usually involving markers that map at LOD <2.0. As indicated in the distal regions of Triticeae chromosomes 1, 5, and 6, conservation in the order of loci relative to rice breaks down near the ends of these chromosomes.



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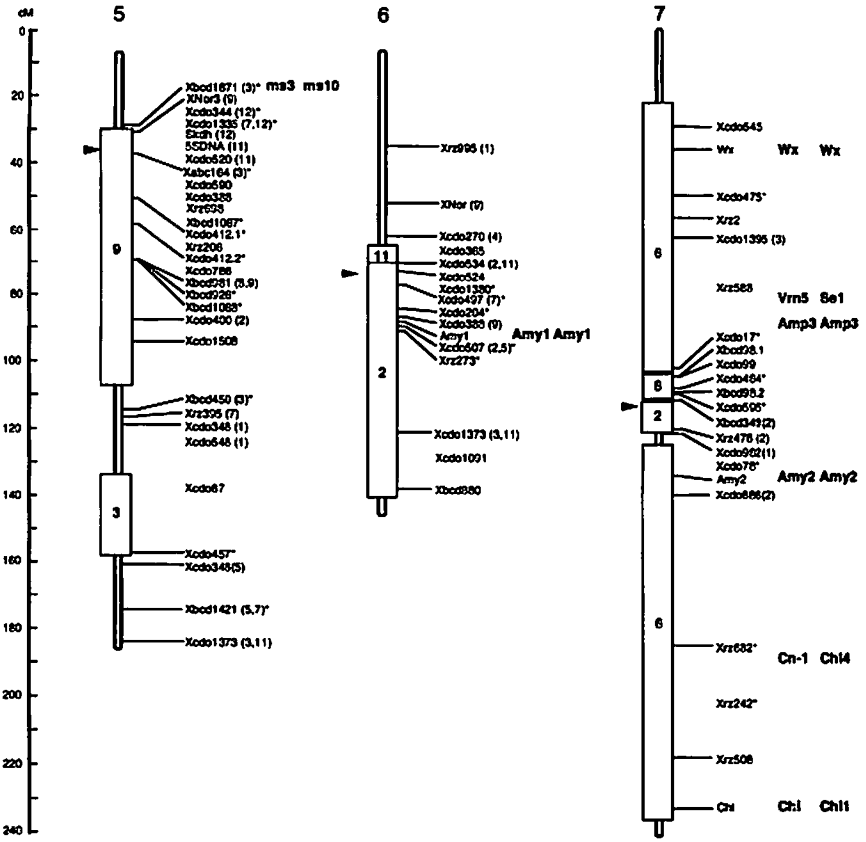
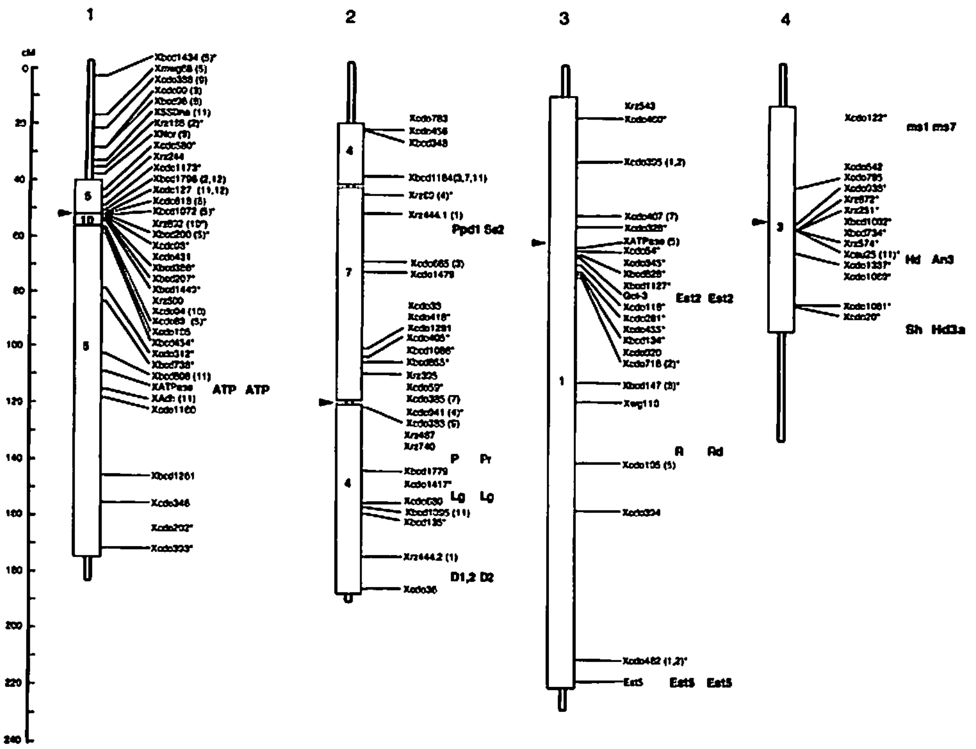


Figure 1. Consensus map of Triticeae species (*T. aestivum*, *Ae. tauschii*, *Hordeum* spp.), with conserved segments from homeologous rice chromosomes superimposed. The length of the bar for each chromosome represents the full length of the linkage group. Markers without tick marks were assigned to wheat chromosome arms, but not placed on a linkage map of Triticeae spp. Their position was estimated based on their relative location in rice. The order of markers outlined by the boxed area is conserved relative to the rice chromosome indicated within it. Numbers in parentheses following markers indicate their homeologous location(s) in rice. Marker loci not followed by parentheses are mapped in the homeologous chromosome of rice indicated in the boxed region. The arrowheads indicate centromere locations in Triticeae. Asterisks indicate single copy probes in wheat. Boldface symbols to the right of the linkage maps represent putative gene loci in Triticeae (Hart et al. 1993; Hockett and Nilan 1985) and rice (Kinoshita 1993). *Se2* was mapped in rice by M. Maheswaran, IRRI. *Hd3a* (chromosome 4) is a quantitative trait locus (QTL) for heading date in rice (Li et al. 1995).

Rice and wheat contribute complimentary information to comparative mapping. Most single-copy clones selected for good genome coverage of a rice linkage map (Causse et al. 1994) also detect single- or low-copy sequences in Triticeae species, maize, and oat (stringency=0.5X SSC at 65 °C). Wheat nullisomic (Sears 1966), ditelosomic (Sears and Sears 1978), and deletion stocks (Werner et al. 1992) are especially valuable for comparative mapping, because virtually every DNA fragment can be assigned to a wheat chromosome arm or portion of an arm (Anderson et al. 1992). By combining this information with linkage data for wheat, erroneous conclusions about chromosome or single locus inconsistencies can be avoided, because essentially all copies of the DNA sequences detected by a probe can be located in the wheat genome. In comparative mapping, the estimated degree of genome conservation with species other than wheat may be underestimated, because information about probes is limited to the polymorphism between mapping parents.

Maize

For the Triticeae genome map represented by loci mapped in maize, 92% is conserved between the two species (Table 1 and Fig. 2). Many of the Triticeae chromosomes were homeologous to at least two maize chromosomes. Maize chromosomes 1 and 5, 1 and 9, 2 and 7, 2 and 10, 3 and 8, 4 and 5, 6 and 8, and 6 and 9 were homeologous to portions of the same Triticeae chromosome, thus confirming the duplication of chromosome segments within the maize genome observed in comparative maps between maize and rice (Ahn and Tanksley 1993), sorghum (Whitkus et al. 1992), and oat (Van Deynze et al. 1995a, b). A segment delimited by *Xcdo1380* and *Xcdo772* on Triticeae chromosome arm 6L is inverted in homeologous maize chromosome 4, but not maize 5. A paracentric inversion in maize chromosome 9 relative to Triticeae chromosome arm 7S was detected, delimited by *npi253* and *wx1* as reported by Devos et al. (1994).



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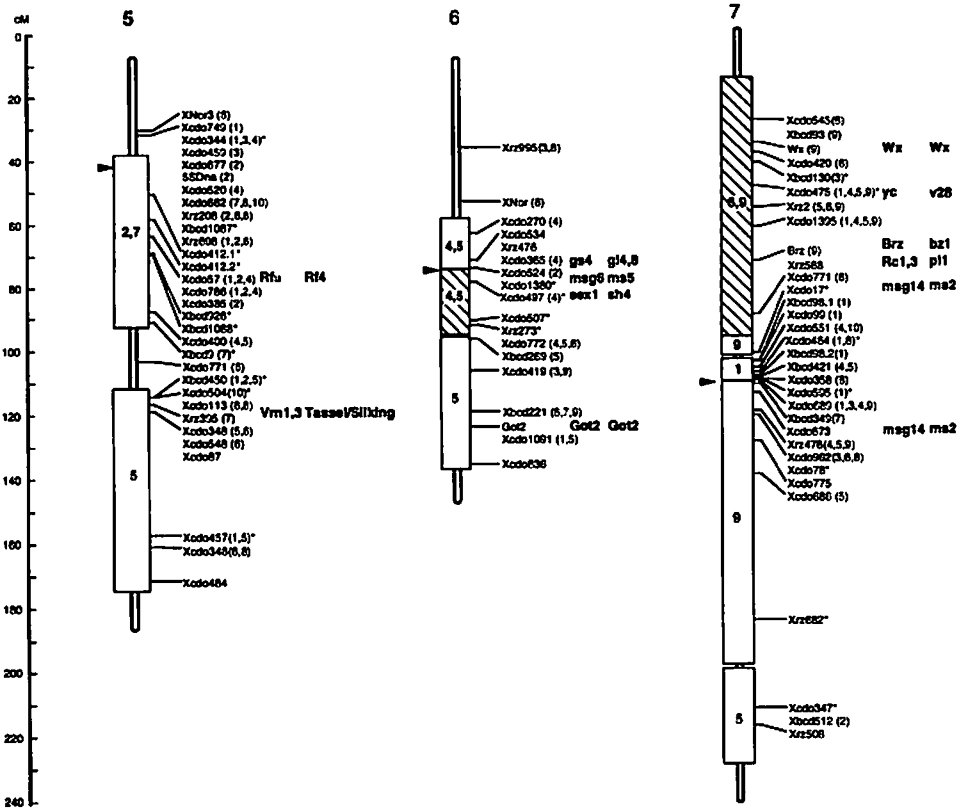


Figure 2. Consensus map of Triticeae species (*T. aestivum*, *Ae. tauschii*, *Hordeum* spp.), with conserved segments from homeologous maize chromosomes superimposed. The length of the bar for each chromosome represents the full length of the linkage group. Markers without tick marks were assigned to wheat chromosome arms, but not placed on a linkage map of Triticeae spp. Their position was estimated based on their relative location in maize. The order of markers outlined by the boxed area is conserved relative to the maize chromosome indicated within it. Numbers in parentheses following markers indicate their homeologous location(s) in maize. Marker loci not followed by parentheses are mapped in the homeologous chromosome of maize indicated in the boxed region. The hatched boxes on Triticeae chromosomes 6 and 7 represent inversions in chromosomes 4 and 9 of maize, respectively, relative to Triticeae. The arrowheads indicate centromere locations in Triticeae. Asterisks indicate single copy probes in wheat. Boldface symbols to the right of the linkage maps represent putative gene loci in Triticeae (Hart et al. 1993; Hockett and Nilan 1985) and maize (Coe et al. 1993). Moisture (chromosome 2) and tassel/silking (chromosome 5) are QTL for the respective traits (Phillips et al. 1992).

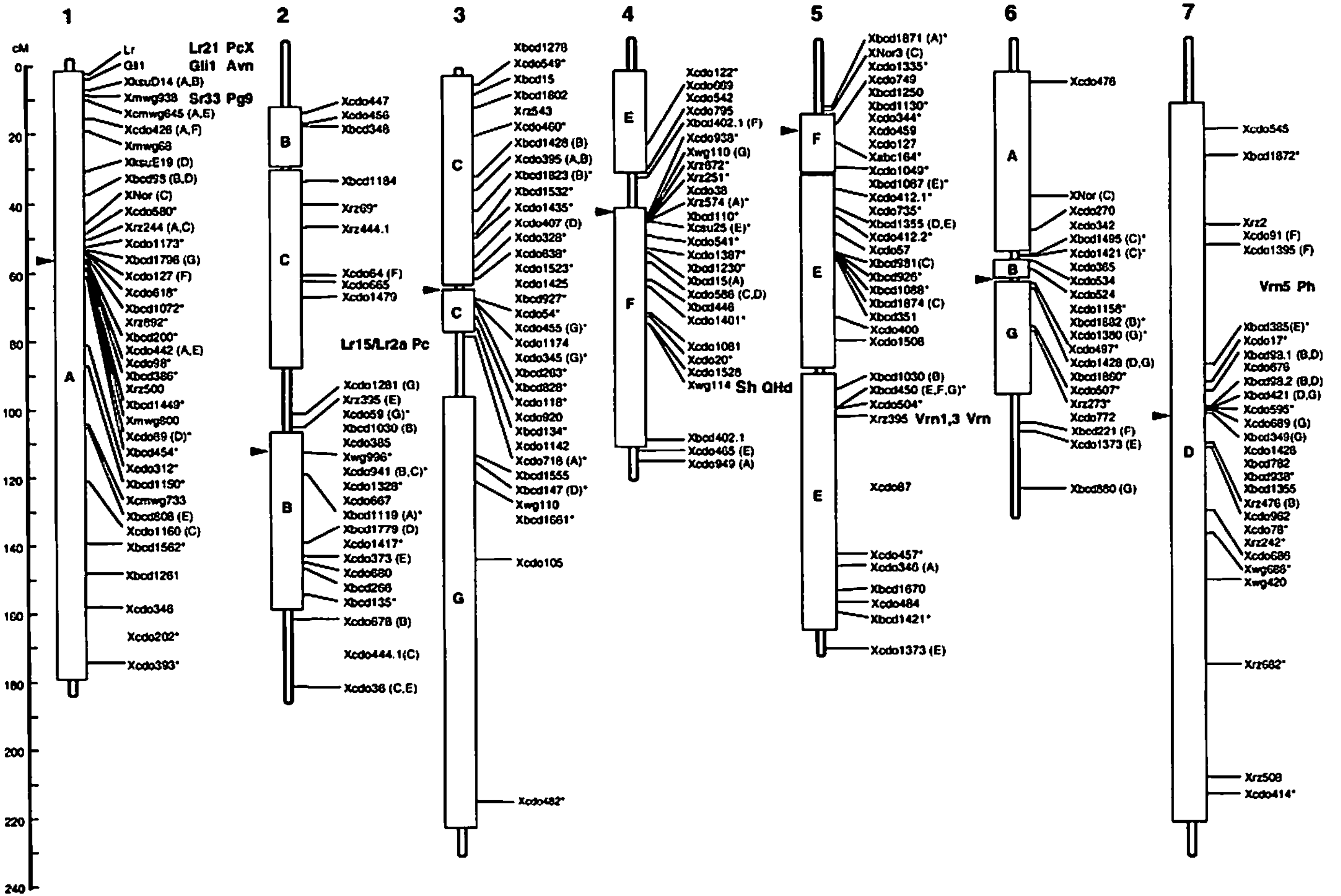
Oat

Of the 192 probes common to Triticeae and diploid oat, 130 were positioned on a linkage map of the Triticeae species studied. Sixteen regions ranging from 5 to 215 cM in Triticeae were conserved between oat and Triticeae (Table 1 and Fig. 1). At least part of each oat chromosome showed conservation in the order of DNA markers detected with the same probes relative to Triticeae. The entire Triticeae chromosomes 1 and 7 are conserved relative to oat chromosomes A and D, respectively. Gross rearrangements are evident between homeologous chromosomes of Triticeae 2 and oat B, and Triticeae 5 and oat E. Smaller rearrangements, insertions, or single locus inconsistencies are evident, but may be a consequence of the resolution of the linkage maps or duplicate loci.

Putative Orthologous Gene Loci

The positions of putative orthologous gene loci for simply- and quantitatively-inherited traits and biochemical markers between Triticeae and rice, maize, and oat (Figs. 1 to 3) were determined based on their relative positions on classical (Hart et al. 1993; Hockett and Nilan 1985; Kinoshita 1993, Coe et al. 1993) and molecular maps. The relative positions in homeologous chromosome segments were conserved for traits such as resistance to leaf and stem rust, leaf and endosperm waxiness, kernel, pericarp, coleoptile and seedling color, dwarfing, awn morphology, male sterility, male-fertility restoration, shrunken endosperm, vernalization, and photoperiod response. The nuclear organizer region (*Nor*) locus on rice chromosome 9 is putatively orthologous to *Nor3* on wheat and *Ae. tauschii* chromosome arm 5DS, and the *Nor* locus on maize chromosome 6 is putatively orthologous to the *Nor1* locus on wheat chromosome arm 1BS. The *Nor* locus in oat does not appear to be orthologous to *Nor* loci of Triticeae.

Figure 3 (overleaf). Consensus map of Triticeae species (*T. aestivum, Ae. tauschii, Hordeum* spp.) with conserved segments from homeologous oat chromosomes superimposed. The length of the bar for each chromosome represents the full length of the linkage group. Markers without tick marks were assigned to wheat chromosome arms, but not placed on a linkage map of Triticeae species. Their position was estimated based on their relative location in oat. The order of markers outlined by the boxed area is conserved relative to the oat chromosome indicated within it. Numbers in parentheses following markers indicate their homeologous location(s) in oat. Marker loci not followed by parentheses are mapped in the homeologous chromosome of oat indicated in the boxed region. The arrowheads indicate centromere locations in Triticeae. Asterisks indicate single copy probes in wheat. Boldface symbols to the right of the linkage maps represent putative gene loci in Triticeae (Hart et al. 1993; Hockett and Nilan 1985) and homeologous chromosome segments in hexaploid oat (Siripoonwiwat 1995). *PcX, Avn,* and *Pg9* (chromosome 1) were mapped by Chong et al. (1994) and *Pc* (chromosome 2) was mapped by Bush et al. (1994).


1
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cM
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60
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100
120
140
160
180
200
220
240
Lr
Gli1
XksuD14 (A,B)
Xmwg938
Xcmwg645 (A,E)
Xcdo426 (A,F)
Xmwg68
XksuE19 (D)
Xbcd93 (B,D)
XNor (C)
Xcdo580*
Xrz244 (A,C)
Xcdo1173*
Xbcd1796 (G)
Xcdo127 (F)
Xcdo618*
Xbcd1072*
Xrz692*
Xbcd200*
Xcdo442 (A,E)
Xcdo98*
Xbcd386*
Xrz500
Xbcd1449*
Xmwg800
Xcdo89 (D)*
Xbcd454*
Xcdo312*
Xbcd1150*
Xcmwg733
Xbcd808 (E)
Xcdo1160 (C)
Xbcd1562*
Xbcd1261
Xcdo346
Xcdo202*
Xcdo393*
Lr21 PcX
Gli1 Avn
Sr33 Pg9
A
B
C
B
Xcdo447
Xcdo456
Xbcd348
Xbcd1184
Xrz69*
Xrz444.1
Xcdo64 (F)
Xcdo665
Xcdo1479
Lr15/Lr2a Pc
Xcdo1281 (G)
Xrz395 (E)
Xcdo59 (G)*
Xbcd1030 (B)
Xcdo385
Xwg996*
Xcdo941 (B,C)*
Xcdo1328*
Xcdo667
Xbcd1119 (A)*
Xbcd1779 (D)
Xcdo1417*
Xcdo373 (E)
Xcdo680
Xbcd266
Xbcd135*
Xcdo678 (B)
Xcdo444.1(C)
Xcdo36 (C,E)
C
C
G
Xbcd1278
Xcdo549*
Xbcd15
Xbcd1802
Xrz543
Xcdo460*
Xbcd1428 (B)
Xcdo395 (A,B)
Xbcd1823 (B)*
Xbcd1532*
Xcdo1435*
Xcdo407 (D)
Xcdo328*
Xcdo638*
Xcdo1523*
Xcdo1425
Xbcd927*
Xcdo54*
Xcdo455 (G)*
Xcdo1174
Xcdo345 (G)*
Xbcd263*
Xbcd828*
Xcdo118*
Xcdo920
Xbcd134*
Xcdo1142
Xcdo718 (A)*
Xbcd1555
Xbcd147 (D)*
Xwg110
Xbcd1661*
Xcdo105
Xcdo482*
E
F
Xcdo122*
Xcdo609
Xcdo542
Xcdo795
Xbcd402.1 (F)
Xcdo938*
Xwg110 (G)
Xrz872*
Xrz251*
Xcdo38
Xrz574 (A)*
Xbcd110*
Xcsu25 (E)*
Xcdo541*
Xcdo1387*
Xbcd1230*
Xbcd15(A)
Xcdo586 (C,D)
Xbcd446
Xcdo1401*
Xcdo1081
Xcdo20*
Xcdo1528
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Sh QHd
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Xcdo465 (E)
Xcdo949 (A)
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XNor3 (C)
Xcdo1335*
Xcdo749
Xbcd1250
Xbcd1130*
Xcdo344*
Xcdo459
Xcdo127
Xabc164*
Xcdo1049*
Xbcd1067 (E)*
Xcdo412.1*
Xcdo735*
Xbcd1355 (D,E)
Xcdo412.2*
Xcdo57
Xbcd981(C)
Xbcd926*
Xbcd1088*
Xbcd1874 (C)
Xbcd351
Xcdo400
Xcdo1508
Xbcd1030 (B)
Xbcd450 (E,F,G)*
Xcdo504*
Xrz395
Vrn1,3 Vrn
Xcdo87
Xcdo457*
Xcdo346 (A)
Xbcd1670
Xcdo484
Xbcd1421*
Xcdo1373 (E)
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Xcdo476
XNor (C)
Xcdo270
Xcdo342
Xbcd1495 (C)*
Xcdo1421 (C)*
Xcdo365
Xcdo534
Xcdo524
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Xbcd1682 (B)*
Xcdo1380 (G)*
Xcdo497*
Xcdo1428 (D,G)
Xbcd1890*
Xcdo507*
Xrz273*
Xcdo772
Xbcd221 (F)
Xcdo1373 (E)
Xbcd380 (G)
D
Xcdo545
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Xrz2
Xcdo91 (F)
Xcdo1395 (F)
Vrn5 Ph
Xbcd385(E)*
Xcdo17*
Xbcd93.1 (B,D)
Xcdo676
Xbcd98.2 (B,D)
Xbcd421 (D,G)
Xcdo595*
Xcdo689 (G)
Xbcd349(G)
Xcdo1428
Xbcd782
Xbcd938*
Xbcd1355
Xrz476 (B)
Xcdo962
Xcdo78*
Xrz242*
Xcdo686
Xwg686*
Xwg420
Xrz682*
Xrz508
Xcdo414*

Conserved Segments Among Species

Comparisons of the conserved regions in the linkage maps of rice, maize, and oat relative to the consensus maps for Triticeae chromosomes (Figs. 1–3) indicate that certain segments have been conserved. For example, there are three segments for homeologous chromosomes of Triticeae chromosome 2 conserved across the three species studied (Fig. 4). Segment 1 on the distal portion of Triticeae chromosome arm 2S is homeologous to rice chromosome 4, maize 2 and 10, and oat B. A more proximal segment (segment 2) on Triticeae chromosome arm 2S is homeologous to rice 7, maize 7, and oat C, and Triticeae chromosome arm 2L (segment 3) includes a segment homeologous to rice 4, maize 2 and 10, and oat B. However, the breakpoints between segments do not appear to be conserved among the species. The breakpoint between segments 1 and 2 of rice is not clearly defined between *Xrz69* (rice chromosome 4) and *Xbcd1184* (rice 7). Maize and oat clearly have different breakpoints between these segments. The breakpoints between the segments on 2S and 2L (segments 2 and 3) are conserved relative to rice and maize only. Segment 3 on oat chromosome B consists of a small region homeologous to Triticeae 2S, which is not present in homeologous segments of rice or maize. The orientation of regions homeologous to segments 1 and 3 is conserved in Triticeae, rice, and maize, but not oat. Conserved segments across all four species can be detected for regions homeologous to each of the Triticeae chromosomes.

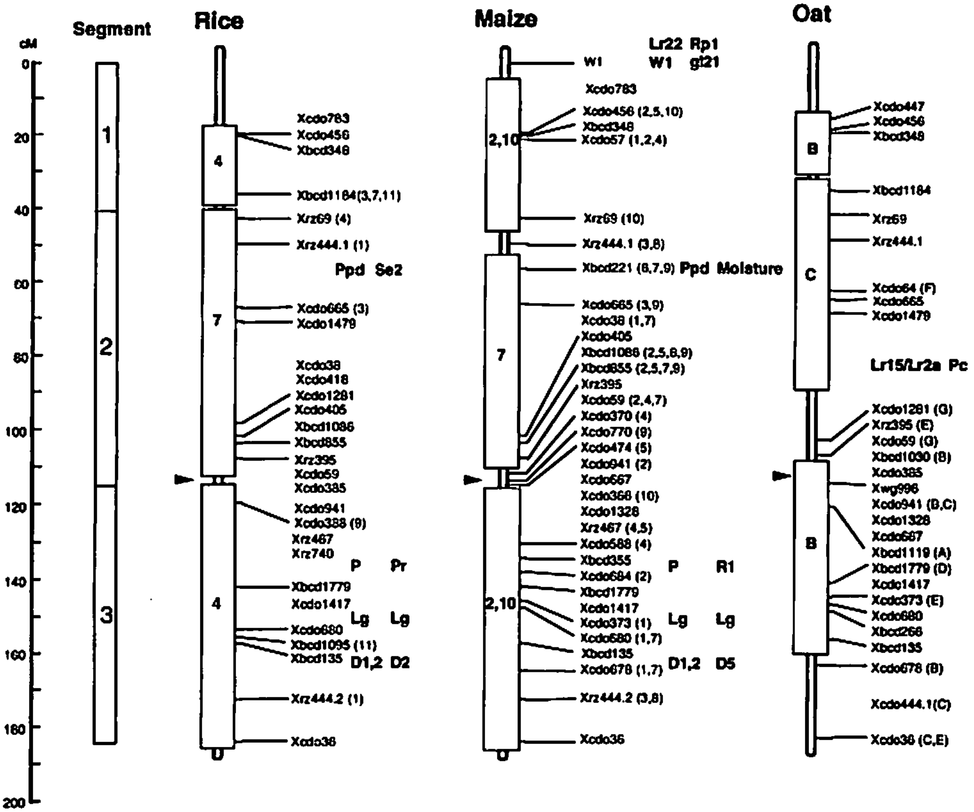

Figure 4. Consensus maps for group 2 chromosomes of Triticeae species (*T. aestivum*, *Ae. tauschii*, *Hordeum* spp.) with conserved segments from homeologous rice, maize, and oat chromosomes superimposed. Segments 1, 2, and 3 are conserved in all species compared. Boldface symbols to the right of the linkage maps represent putative gene loci in Triticeae (Hart et al. 1993; Hockett and Nilan 1985) (left symbols) and homeologous chromosome segments in rice (Kinoshita 1993; M. Maheswaran, IRRI, personal communication), maize (Phillips et al. 1992; Coe et al. 1993) and hexaploid oat (Bush et al. 1994; Siripoonwiwat 1995) (right symbols).

Genome Conservation and Evolution

At the current resolution of comparative maps among Triticeae species, rice, maize, and oat (Ahn and Tanksley 1993; Ahn et al. 1993; Kurata et al. 1994b; Van Deynze et al. 1995a, b), there does not appear to be a great deal of structural divergence. The degree of conservation based on genome map length ranges from 62% between rice and maize (Ahn and Tanksley 1993) to 94% between Triticeae and oat (reported in this study). This is in contrast to the 30% genome conservation between *Brassica oleracea* and *Arabidopsis thaliana* of the Cruciferae family (Kowalski et al. 1994). Comparative maps using common probes with Triticeae (this study; Ahn et al. 1993), oat (Van Deynze et al. 1995a, b), and between maize and rice (Ahn and Tanksley 1993) indicate that specific chromosomal segments appear to be conserved among all species. Examples of chromosomes homeologous to Triticeae chromosomes 1 (Van Deynze et al. 1995a), 2 (see results section) and 5 (Van Deynze et al. 1995b) have been described. The order of loci detected by common probes mapped in maize, sorghum (Whitkus et al. 1992), and sugarcane (Grivet et al. 1994) indicates that these same segments may be conserved among these species. It appears that large translocations and inversions are the mechanisms of evolution for chromosomes of Gramineae species. Smaller rearrangements and single locus inconsistencies are also evident, as well as differences in copy number for particular loci. The loss of gene order conservation is most apparent near the ends of the chromosomes.

Relevance of Comparative Maps to Triticeae Species

Comparative maps can be used for improvement of Triticeae species by combining information accumulated in other species not previously accessible using classical techniques. They may be used to saturate species-specific maps in a targeted region, or simply to develop linkage maps for species, such as wheat, that may have a low level of polymorphism among parental lines. To construct a new map in durum, for example, both consensus maps and comparative maps can be used to chose the most appropriate probes. Such probes can be chosen to provide genome coverage at the desired resolution and for close linkage to previously mapped agronomic trait loci.

The information gained by identifying orthologous loci for the numerous previously characterized mutants and expressed sequence tags in rice (Kinoshita 1993; Uemda et al. 1994; Kurata et al. 1994a) and maize (Coe et al. 1993) may advance genetic research in Triticeae species. The relationships between gene products and physiology of plants for particular traits must be known to define orthologous loci between species. Genes affecting what appear to be totally different characters may be the result of orthologous gene loci that differ in expression or interaction with other genes (Darling and Abbott 1992). These must be distinguished from traits which appear to be similar based on phenotype but controlled by unrelated genes. Comparative mapping may be of limited value for traits that are unique to a species. Also, certain characters such as male sterility may be affected by numerous loci found throughout the genomes of many species. The use of comparative mapping for polygenic traits will require sophisticated QTL analysis. A number of putative orthologous loci for various traits between Triticeae and rice, and maize and oat, are indicated in Figures 1–3. Van Deynze et al. (1995b) reports the location of putative orthologous loci for storage proteins and resistance to leaf rusts between oat and Triticeae, as well as for vernalization and photoperiod response loci among Triticeae species, rice, maize, and oat. Putative orthologous loci for pericarp color (*P*, *Pr*, *R1*), lack of ligules (*Lg*), and dwarfing (*D1-2*, *D2*, *D5)* are located on homeologous chromosomes Triticeae 2L, rice 4, and maize 2 or 10, respectively (Figs. 1 and 2). These highly conserved orthologous loci are good candidates for further characterization based on comparative mapping. Isolating genes for further study, plant transformation, or genetic manipulation in a large complex genome such as wheat may be facilitated by studying or cloning orthologous gene loci in a smaller, well-characterized genome such as rice.

It is clear that numerous chromosomal segments are conserved among Triticeae species, rice, maize, and oat. The putative orthologous loci for traits indicated in this study are only the first examples of the practical potential of comparative mapping for the advancement of genetic research of Gramineae species.

Acknowledgments

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Utilization of PCR Primers for Wheat Storage Protein Genes in Population Genetic Analysis

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Abstract

Increasing knowledge concerning gene sequence and structure is providing new and more vigorous tools for analyzing variations in natural populations. Sequences of *Triticum* genes coding for seed storage proteins were used to study the genetic structure of 25 populations of *Triticum turgidum* subsp. *dicoccoides* from the Fertile Crescent. A surprisingly wide within-population variation was detected, in addition to the variation among populations already documented. Diversity was found to be associated with environmental factors such as climate and soil variation, confirming that this variation does not occur at random but is the result of natural selection.

Introduction

Wild emmer, a common progenitor of cultivated tetraploid and hexaploid wheats, is an important source of genetic variation exploitable in breeding programs aimed at improving quality and resistance characteristics. In addition, its occurrence at several sites along the Fertile Crescent provides exciting opportunities for studying the genetic organization of populations and their relationship with environmental factors.

Diversity at the biochemical and molecular levels in *Triticum turgidum* subsp. *dicoccoides* populations has been studied by Nevo and Payne (1987), Nevo et al. (1995), and Pagnotta et al. (1995). Nevo and Payne (1987), by electrophoretically analyzing 231 individuals for protein subunits coding for genes at the *Glu-1* loci, showed that: (1) the two HMW glutenin loci, *Glu-A1* and *Glu-B1*, are, overall, extremely variable, containing a total of 11 and 15 alleles, respectively; (2) variation in some populations is severely restricted, supporting the island population genetic model; and (3) correlation between glutenin diversity and frequencies of specific glutenin alleles and physical (climate and soil) and biotic (vegetation) variables were very significant.

Nevo et al. (1995) and Pagnotta et al. (1995) analyzed the genetic structure of 25 populations for y-type glutenin subunit genes at the *Glu-1* loci, by using PCR methodology. They found a wide genetic diversity among and, more surprising, within populations, and confirmed that glutenin diversity is not random but a result of natural selection as an adaptive environmental strategy.

The results of this analysis led to the following investigations: (1) to characterize genes encoding new allelic variants; and (2) to detect genetic polymorphism for population genetics studies and linkage analysis.

Materials and Methods

Two hundred and forty nine genotypes belonging to 25 populations collected in locations mapped in Figure 1 were used in this study. Values for the following environmental factors were recorded for each collection site: Altitude (Al, measured in m), mean annual evaporation (E, measured in mm), mean humidity at 14.00 (Hu14, measured by %), mean annual humidity (Huan, measured by %), longitude (Ln), latitude (Lt), total solar radiation per year (Rad), mean relative variability of rainfall (Rr, measured by %), mean interannual variability of rainfall (Rv, measured by %), mean number of Sharav days (Sh, i.e. hot and dry), mean August temperature (Ta, measured in °C), seasonal temperature difference (Td, measured in °C), day–night temperature difference (Tdd, measured in °C), mean annual temperature (Tm, measured in °C), mean January temperature (Tj, measured in °C), mean number of tropical days (Trd). Soil characteristics were used as three dummy variables: Basalt (Bs), Terra rossa (Tr), and Rendzina (Rz).

The range of populations considered can be subdivided into central populations (5) and several types of marginal populations. Population No. 1 is in the north, with a cold and dry environment. Population No. 2 is in the southeast, and is warm and dry (8 populations). Population No. 3 is in the west, with a humid environment (10 populations). Population No. 4 is from Turkey, with a warm and semi-dry environment (Nevo and Beiles 1989).

Twenty seeds per genotype were germinated in petri dishes after surface sterilization with sodium hypochlorite (final concentration 1% in water). The etiolated seedlings were used to extract genomic DNA following the Dellaporta et al. (1983) method.

PCR was performed using oligonucleotides synthesized on sequences of *Triticum aestivum* cv Cheyenne (Halford et al. 1987) genes coding for all regions of the 1By HMW glutenin subunits. Since HMW genes contain a very long central repetitive domain, primers were chosen in the regions corresponding to the N- and C-terminal regions. Oligonucleotide primers 5' ATG GCT AAG CGG TTG GTC CT 3' and 5' CTG TGT TAA CAT GGT ATG GGT TGT C 3' were prepared according to D'Ovidio et al. (1994). The PCR mixture per genotype consisted of 250 ng of genomic DNA, 250 ng of each of the two primers, 300 mM each of dATP, dGTP, dCTP, dTTP (Pharmacia), 2.5 units of Taq Polymerase (Boehringer), and water to make up 100 mL. The mixture was covered with two drops of mineral oil (Perkin Elmer). The amplification conditions were as follows: hot start at 94 °C for 2 minutes followed by 35 cycles at 94 °C for 1 minute, 60 °C for 2 minutes and 72 °C for 2 min. The 35 cycles were followed by an extension of 7 minutes at 72 °C. After the amplification cycles were completed, 15 mL of amplified product per sample was run in a 1× TBE buffer (on a 1% agarose mini gel containing 0.5 mg/mL of ethidium bromide) at 80 volts for about 2 hours. The gels were photographed under UV light with Polaroid film 667.

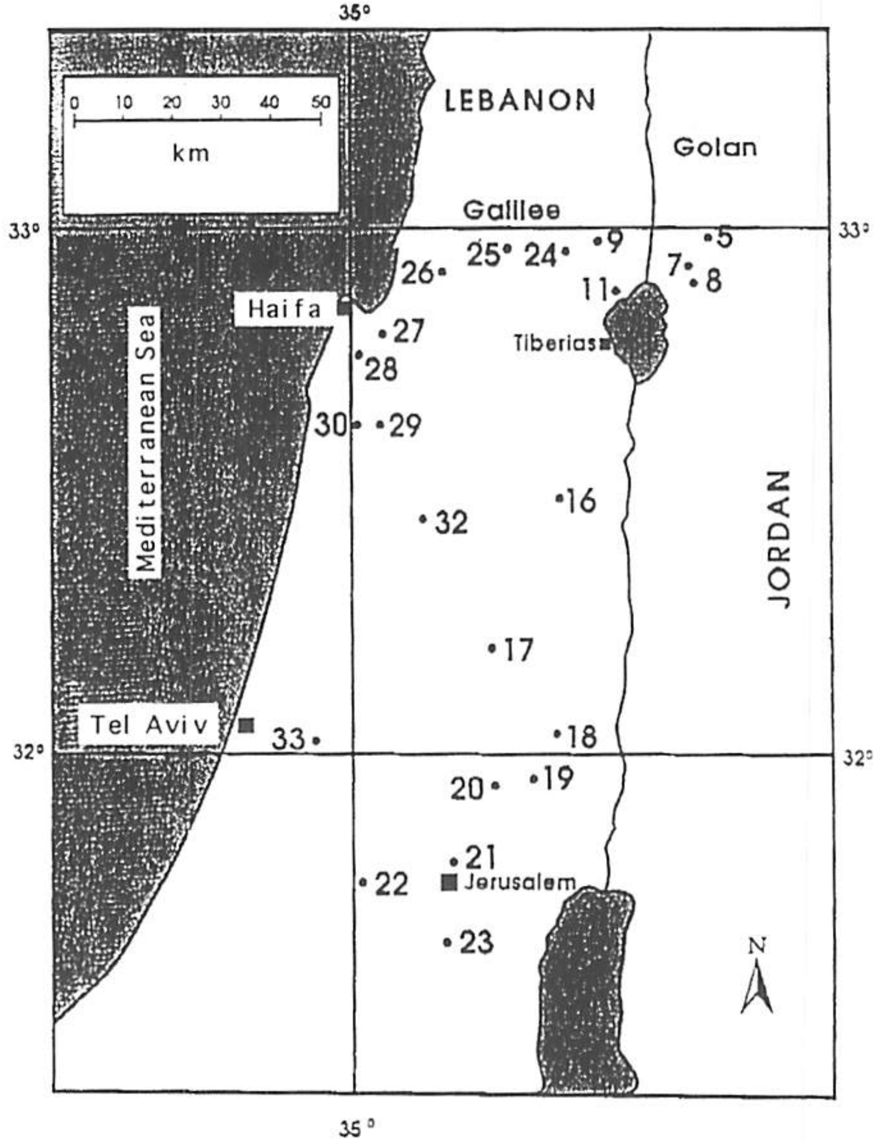

Figure 1. Collection locations for 25 populations of wild emmer wheat in the Fertile Crescent. Numbers correspond to Table 1.

Band dimension was measured using the lambda marker as reference. To get a better measurement and avoid differences in gel-running, gels were re-run, loading different combinations of PCR products. Results were arranged in a matrix, with 0 for absence and 1 for presence of any "possible band." Spearman rank correlations were computed between all variables. Stepwise multiple regression analysis was employed to determine associations between environmental factors and frequency of PCR bands or band series (Pagnotta et al. 1995).

Results

Variability and Polymorphism

PCR amplification identified 29 characteristic patterns in the 25 populations analyzed. There were differences in frequency and/or distribution (Table 1). Some were very rare, such as patterns 5, 9, 12, 13, 17, 19, 23, 25, and 27, which were present in only one of the 249 genotypes. Others were widely distributed, such as patterns 3, 4, and 6, which were present in 33, 40, and 20 genotypes, respectively. Moreover, some populations (5, 29, and 32) did not show variation, whereas others (16 and 26) had a predominant pattern with a small proportion of some second pattern. Some populations (9, 21, and 30) presented five to six different patterns, about equally distributed.

Table 1. Glutenin DNA PCR pattern frequencies and distribution in 25 populations of *Triticum dicoccoides*.

Pat.	1	5	7	8	9	11	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	37
1	0.2				0.1	0.5																			
2	0.8									0.1		0.1	0.8	0.5	0.1										
3		1.0						0.1	0.1			0.3			0.6	0.6					0.6				
4			0.3						0.7			0.3		0.3	0.2	0.3				1.0	0.1			0.8	
5			0.1																						
6			0.4					0.1					0.2	0.2		0.1	0.9				0.1				
7			0.2						0.1	0.2	0.5	0.1													
8				0.1														0.7							
9				0.1																					
10				0.7	0.3																0.1				
11				0.1																		0.1			
12					0.1																				
13					0.1																				
14					0.4																				
15						0.5																			
16							0.9											0.3				0.1			
17							0.1																		
18								0.8														0.8			
19									0.1																
20										0.7															
21											0.5														
22												0.1							0.8		0.1				
23												0.1													
24															0.1								1.0		
25																	0.1								
26																			0.2						
27																								0.1	
28																								0.1	0.4
29																									0.6

Patterns were considered as multilocus structures, with each locus containing two alleles. On this basis, genetic indices (Table 2), genetic diversity within and among populations (Nei 1973) (Table 3), genetic distance (Nei 1978) (Tables 4 and 5), Spearman rank correlations (Table 6), and multiple regressions (Table 7) were computed.

Table 2. Genetic variation based on 12 loci for HMW glutenin in 249 genotypes based on 25 populations of *Triticum dicoccoides*.

Locality	Mean no. of alleles per Locus (A)	Percentage of loci polymorphic	Genetic diversity (He)
1	1.083	8.3	0.028
5	1.000	0.0	0.000
7	1.250	25.0	0.086
8	1.417	41.7	0.104
9	1.417	41.7	0.107
11	1.083	8.3	0.044
16	1.167	16.7	0.032
17	1.250	25.0	0.072
18	1.333	33.3	0.075
19	1.250	25.0	0.078
20	1.167	16.7	0.088
21	1.417	41.7	0.132
22	1.333	33.3	0.112
23	1.333	33.3	0.160
24	1.333	33.3	0.096
25	1.167	16.7	0.058
26	1.167	16.7	0.032
27	1.333	33.3	0.147
28	1.083	8.3	0.028
29	1.000	0.0	0.000
30	1.333	33.3	0.105
31	1.250	25.0	0.047
32	1.000	0.0	0.000
33	1.500	50.0	0.131
37	1.083	8.3	0.042
Mean	1.230	23.0	0.069

Polymorphism for each population ranged from uniformity (5, 29, and 32) to 50% (33), with an average of 23% (Table 2). Genetic diversity varied among populations, ranging from 0.000 (5, 29, 32) to 0.160 (23). Mean polymorphism (23%), together with mean number of alleles per locus (1.230) and mean genetic diversity (0.069), was comparable to the results found by Nevo and Beiles (1989) for the same parameters using 42 allozyme loci (22%, 1.252, and 0.059, respectively).

Genetic Differentiation Within and Among Populations (Gst Analysis)

Average among populations represented 68.7% of the total diversity (0.218) showed by the 12 "possible bands" (Table 3).

Table 3. Genetic diversity among and within populations for 12 HMW glutenin loci in 25 *Triticum dicoccoides*.

Bands[†]	Gene diversity (Ht)	Genetic diversity within populations (Hs)	Genetic diversity between populations (Dst)	Genetic diversity % between populations (Gst)
H2310	0.0472	0.0194	0.0279	59.01
H2290	0.4984	0.2238	0.2746	55.09
H2200	0.0080	0.0073	0.0008	09.64
H2090	0.1483	0.0387	0.1096	73.89
H2070	0.4953	0.1391	0.3562	71.92
H2030	0.3267	0.0674	0.2593	79.38
H2010	0.2188	0.0443	0.1745	79.76
H1850	0.0472	0.0274	0.0198	41.93
H1780	0.1207	0.0371	0.0836	69.27
H1750	0.0921	0.0144	0.0777	84.33
H1740	0.4667	0.1584	0.3083	66.05
H1730	0.1483	0.0418	0.1064	71.78
Mean	0.2181	0.0683	0.1499	68.71

[†] Digits indicate the base pair number.

The absolute and relative contributions of single bands to differentiation of genetic diversity among populations (Table 3) indicate that the bands with the highest values (which could be population-specific) were H1750, H2010, and H2030, accounting for 84, 80, and 79% of interpopulation genetic diversity, respectively.

Genetic and Geographic Distance

Genetic distance between populations (Table 4) ranged from 0.000 (between populations 18 and 33) to 0.607 (between populations 37 [Turkey] and 32). Genetic distance did not match geographic distance. Some populations were geographically close, such as 5 and 7 (about 10 km apart) and 5 and 9 (about 50 km apart) with genetic distances of 0.089 and 0.387, respectively. On the other hand populations 5 and 18, and populations 24 and 23, which are more than 200 km apart, showed genetic distances equal to 0.057 and 0.025, respectively.

Table 4. Matrix of genetic distance between 25 populations of *Triticum dicoccoides*.

Pop.	1	16	17	18	19	20	21	22	23	24	25	26	28	29	30	31	32	33	27	5	7	8	9	11
1	****																							
16	0.240	****																						
17	0.157	0.063	****																					
18	0.210	0.172	0.155	****																				
19	0.082	0.313	0.198	0.231	****																			
20	0.250	0.319	0.193	0.181	0.085	****																		
21	0.170	0.197	0.130	0.009	0.163	0.128	****																	
22	0.006	0.212	0.124	0.014	0.043	0.160	0.104	****																
23	0.052	0.158	0.109	0.042	0.078	0.133	0.027	0.018	****															
24	0.136	0.195	0.096	0.023	0.161	0.137	0.007	0.082	0.025	****														
25	0.194	0.216	0.127	0.013	0.191	0.137	0.001	0.123	0.042	0.003	****													
26	0.375	0.278	0.315	0.078	0.216	0.141	0.086	0.258	0.131	0.146	0.107	****												
28	0.455	0.350	0.403	0.176	0.296	0.228	0.145	0.342	0.220	0.247	0.211	0.094	****											
29	0.251	0.186	0.196	0.003	0.297	0.242	0.026	0.185	0.066	0.045	0.030	0.089	0.188	****										
30	0.205	0.238	0.134	0.025	0.160	0.101	0.002	0.125	0.048	0.010	0.002	0.089	0.174	0.050	****									
31	0.170	0.056	0.002	0.204	0.202	0.206	0.177	0.141	0.139	0.142	0.180	0.353	0.432	0.252	0.184	****								
32	0.251	0.186	0.239	0.174	0.351	0.360	0.206	0.228	0.168	0.183	0.225	0.294	0.367	0.182	0.255	0.252	****							
33	0.216	0.153	0.140	0.000	0.213	0.159	0.008	0.143	0.042	0.026	0.013	0.057	0.160	0.007	0.020	0.185	0.181	****						
27	0.159	0.134	0.081	0.171	0.223	0.231	0.146	0.135	0.121	0.112	0.163	0.335	0.337	0.208	0.170	0.088	0.063	0.171	****					
5	0.188	0.272	0.115	0.057	0.187	0.136	0.026	0.123	0.066	0.009	0.013	0.186	0.296	0.087	0.013	0.168	0.288	0.058	0.164	****				
7	0.272	0.220	0.210	0.021	0.188	0.112	0.024	0.177	0.067	0.059	0.034	0.017	0.113	0.033	0.029	0.255	0.232	0.012	0.234	0.089	****			
8	0.319	0.378	0.248	0.174	0.152	0.092	0.130	0.217	0.162	0.143	0.132	0.126	0.244	0.222	0.086	0.278	0.389	0.137	0.284	0.138	0.119	****		
9	0.163	0.361	0.350	0.338	0.104	0.242	0.299	0.139	0.171	0.311	0.347	0.275	0.373	0.387	0.303	0.340	0.361	0.297	0.328	0.387	0.294	0.157	****	
11	0.078	0.206	0.273	0.193	0.114	0.277	0.195	0.071	0.073	0.215	0.247	0.216	0.285	0.211	0.255	0.275	0.211	0.189	0.243	0.322	0.198	0.341	0.113	****
37	0.475	0.560	0.385	0.284	0.255	0.177	0.230	0.350	0.285	0.249	0.225	0.207	0.345	0.335	0.189	0.424	0.607	0.239	0.467	0.223	0.210	0.200	0.475	0.509

The genetic distance between different types of marginality is lower than the distance between the five central populations (0.216). Likewise, distances within regions were similar to the distances between regions (Table 5), emphasizing the fact that distance is not the main factor of population differentiation (Wright 1943).

Table 5. Matrix of Nei's unbiased genetic distance coefficients, averaged by marginality.

Marginality	No. of populations	1	2	3	4
1. North	1				
2. Southeast	8	0.146	0.139		
3. West	10	0.241	0.155	0.157	
4. Central	5	0.204	0.174	0.200	0.216
5. Turkey	1	0.475	0.316	0.329	0.324

Environmental Association

Some bands and some patterns were found to be statistically associated with environmental conditions, particularly with temperature and humidity (Tables 6 and 7).

Table 6. Spearman rank correlation.

	Al	Td	Sh						
High 2030	0.481*	0.454*	0.675***						
	Ln	Td	Tdd	Hu14	Huan	Trd	Ev	Rv	Rr
High 1780	0.410*	0.480*	0.422*	0.544**	0.511**	0.460*	0.461*	0.566	0.586**
	Rad								
High 1730	0.432*								
	Ln	Lt	Td						
Pattern h1	0.446*	0.456*	0.499*						
	Al	Tdd	Sh						
Pattern h2	0.627***	0.411*	0.693***						
	Tdd	Rn	Rd	Hu14	Dw	Ev	Rad		
Pattern h1	0.423*	0.482*	0.596**	0.417*	0.564**	0.507*	0.694***		

Stepwise multiple regression (carried out using PCR variables as dependent variables and ecological variables as independent variables) shows that a combination of temperature, water-availability, and soil variables accounts for a significant proportion of the variation in band frequencies (Table 7). For example, the variance Pattern h1 is explained by a three-variable combination: seasonal temperature difference, humidity at 14:00, and January mean temperature (R=0.599***; Table 7).

Table 7. Coefficients of multiple regression (r^2).

	Rz	Rz Rv	Rz Rv Ta
High 2090	0.276**	0.382**	0.441**
	Al	Al Trd	Al Trd Ev
High 2070	0.107ns	0.322*	0.413*
	Td	Td Tdd	Td Tdd Tm
High 2030	0.231*	0.380**	0.533**
	Hu14	Hu14 rZ	
High 1780	0.207*	0.270*	
	Tm	Tm Trd	Tm Trd Tj
High 1740	0.072ns	0.353*	0.442**
	Td	Td Hu14	Td Hu14 Tj
Pattern h 1	0.194*	0.322*	0.599***
	Al	Al Tdd	Al Tdd Trd
Pattern h 2	0.362**	0.423**	0.532**
	Ev	Ev Rv	
Pattern h 4	0.101ns	0.308*	
	Dw		
Pattern h 7	0.326**		
	Trd	Trd Tr	Trd Tr Lt
Pattern h 16	0.191*	0.342*	0.395*

Discussion

The primers identified from the published sequence of genes coding for the HMW glutenin subunits have detected a wide polymorphism in *T. dicoccoides*. The analysis corroborates the evidence of genetic diversity and divergence in natural populations of *T. dicoccoides* already found by Nevo and Payne (1987), Nevo et al. (1982), Nevo and Beiles (1989), Carver and Nevo (1990), Pagnotta et al. (1995), and Nevo et al. (1995). The populations of *T. dicoccoides* show a great variability between and, more surprising, within populations. Although present results show minor variability within populations—compared with allozyme differentiation (Nevo and Beiles 1989), where interpopulation allozymic diversity was 60% (0.165)—it is enlightening to compare the variability among population (68.7%) with that within populations (31.3%). *Triticum dicoccoides* is hence an important wild wheat relative for future breeding programs as a source of genes for wheat improvement.

This high glutenin diversity between and within populations is probably due to microgeographic differentiation—either edaphic (Nevo et al. 1988a) or climatic (Nevo et al. 1988b)—which plays an important role in genetic differentiation of wild emmer, including glutenin differentiation (Nevo and Payne 1987; Nevo et al. 1995). Temperature and humidity are the most important environmental factors associated with particular genetic constitutions, which is in agreement with the findings of Peacock (this workshop), who identifies temperature as having the greater effect in the Fertile Crescent. This appears to contrast with results which found that altitude was the major environmental factor discriminating among populations (e.g. Ciaffi et al. 1993; Damania et al. 1995). Unfortunately, altitude is often the only environmental factor recorded in germplasm collections; as a result, it appears very relevant. Altitude is clearly related in some way with temperature and humidity, but it cannot be the main factor, per se.

Population divergence does not follow the isolation by distance model of Wright (1943). By contrast, it is quite often more easy to find greater genetic difference among close populations than among populations far apart. This confirms the island population genetic model of wild emmer (Nevo and Beiles 1989). This patchy genetic distribution appears to reflect the underlying ecological heterogeneity, suggesting that natural selection is a major factor determining evolution differentiation. Selection seems to be the major determinator of genetic differentiation, while genetic drift and migration seem to have a minor effect;. Nevertheless, in very small populations, founder effects may predominant (Nevo and Beiles 1989). The high polymorphism and genetic variation found between populations may be explained by ecological factors (and not by geographic distance alone), which determine glutenin differentiation both at the protein (Nevo and Beiles 1987) and DNA (Pagnotta et al. 1995) levels, as predicted by the environmental theory of genetic differentiation (Nevo 1988). Moreover, micro-environmental variation, coupled with limited migration of *T. dicoccoides*, can explain the variation within populations.

Considering the high variability found within populations, germplasm collectors should also consider micro-environmental factors. Likewise, large germplasm collections should be reduced into core collections (Frankel 1984; Brown 1989) in order to decrease the large amount of material to multiply. Although a core collection will not replace the original collection, it will make the latter more accessible. There are several ways (in terms of variables considered and/or data analysis) in which core collections can be assembled; accurate ecological and geographic data from the collection sites, analyzed via multivariate analysis (Peeters and Martinelli 1989; Peeters et al. 1990; Charmet et al. 1994) is one of the most powerful bases for this type of germplasm reduction.

The following conclusions are useful for future germplasm collection:

- It is useless to collect on the basis of geographical distance only.
- It is essential to collect data on soil, environmental, and ecological factors, together with seed (or plant).
- Core collections should be obtained on the basis of ecogeographical data and molecular marker analysis.

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Use of PCR-analysis in Durum Wheat to Screen for Quality Parameters

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Abstract

Specific primers for the PCR amplification of low molecular weight glutenins and gliadins, and high molecular weight glutenins were carried out. The amplified fragments for LMW glutenins varied and allowed classification of lines into the group carrying LMW-1 and LMW-2 genes. Amplified fragments for gliadins allowed the differentiation of genotypes with γ-gliadin 42 and γ-gliadin 45. No polymorphism was found for HMW glutenins. Two hundred and sixty seven genotypes (including checks) of the 1994 advanced durum yield trials were analyzed; 244 lines amplified for the LMW-2 genes, and only 23 for LMW-1. Two hundred and sixty three lines from the same trial were analyzed for gamma gliadins. Two hundred and thirty five showed an amplified fragment indicating a γ-gliadin 45 type, and 28 a γ-gliadin 42 type. Putative recombinants were identified. Comparison with SDS-PAGE must be carried out to confirm recombination.

Introduction

The use of restriction fragment length polymorphisms (RFLPs) to construct genetic linkage maps was first proposed by Botstein et al. (1980). Since then, extensive linkage maps have been constructed for several agricultural crops such as maize (Helentjaris 1987), tomato (Bernatzky and Tanksley 1986), rice (McCough et al. 1988), wheat (Liu and Tsunewaki 1991), barley (Graner et al. 1991; Heun et al. 1991), etc. Linkage maps can be used to increase selection efficiency for qualitative and—more importantly—quantitative trait loci (Lander and Botstein 1989; Paterson et al. 1988; Kleinhofs et al. 1993). Since then, however, the application of RFLP technology in breeding programs has been limited. The main reasons for the cautious use of the technology are the high cost of setting up the technology, the technical skills required, and lack of successful examples of marker-assisted selection in a breeeding program. DNA amplification by polymerase chain reaction, or PCR (Saiki et al. 1985; Williams et al. 1990), is a promising alternative to the use of RFLP in breeding programs. Setting up PCR technology is less costly and more easily automated to screen the large populations required for selection in plant breeding. As only a small amount of DNA is required, mini DNA extraction protocols can be used. Using PCR analysis, durum wheat cultivars can be differentiated according to their storage protein composition (D'Ovidio et al. 1992).

The two major storage protein groups in the wheat endosperm are the prolamins (α-, β-, γ-, and ω-gliadins) and the glutenins. The glutenin proteins have important effects on the physical properties of wheat dough, and their presence is associated with the technological properties of flour. In durum cultivars, the LMW-1 and LMW-2 glutenin subunits are responsible for differences in gluten viscoelasticity. The presence of the LMW-2 glutenin subunit in durum genotypes confers a superior quality. Originally, this property of LMW-2 glutenins was attributed to the presence of gliadins, especially the component γ-45. As the gliadins are monomeric proteins, they do not have an important function for the formation of gluten. Pogna et al. (1988) established that LMW glutenin subunits are the actual cause of strong gluten viscoelaticity, based on the intralocus recombination of cv Berillo at the Gli-B1 locus, reported by Margiotta et al. (1987). It is possible to distinguish good and poor technological flour properties by analysis of PCR products of LMW glutenins, γ-gliadin, and HMW glutenins (D'Ovidio et al. 1992). The objective of this study was to determine if PCR amplification of the LMW-1 and LMW-2 glutenins is a more informative selection criterion in a breeding program than γ-gliadins, HMW glutenins, and conventional quality analysis.

Materials and Methods

Total genomic DNA was isolated from fresh leaves using the protocol described by Saghai-Maroof et al. (1984), with minor changes. The extract was treated with RNase to degrade the RNA. DNA was quantified using a spectrophotometer (Beckman DU-61). The quality of the extracted DNA was checked visually on a 1% agarose gel. The DNA stock was kept at -45 °C.

PCR analysis was conducted on a thermocycler Perkin Elmer 9600 system. The reaction mixture (50m) contained 1x PCR buffer (10 mM TRIS-HCl, pH 8.3, 1.5 mM MgCl2, 50 mM KCl, 0.1 mg/mL gelatine, 100 mM each of dNTP, 1 unit of Taq polymerase [all products from Boehringer Mannheim], 200 mM of primer, and 50 ng of template DNA. The mixtures were subjected to the following cycle: one cycle of 94 °C for 2 minutes, followed by 40 cycles of 92 °C for 1 minute, 60 °C for 2 minutes, and 72 °C for 2 minutes, followed by one cycle of 72 °C for 7 minutes. The PCR products were electrophoretically separated in a 1.2% agarose gel in 1x TBE buffer and made visible by ethidium bromide staining.

The primer used was previously described by D'Ovidio et al. (1992) and Pagnotta et al. (1993), according to published sequences for LMW (Colot et al. 1989) and gamma gliadins (Scheets and Hedgcoth 1988).

Plant Materials Used

Omrabi 5, Haurani, Mrb 9/Haucan, and Bicre 3/Cham 1/Gta/Stk possess LMW-2 glutenins; Cham 1 (Waha) and Chahba 88/Mrb 11 possess LMW-1. Lines 204 (Omlahn), 220 (Omrabi 3/Khabur 2), 822 (Haurani), 1012

(Gd/Fg/5/Sapi/4/Rabi/3/Ld390//Belle/Tc.2), and 1313 (Heider/ Src1) are also advanced durum lines from the 1993/94 durum yield trials.

LMW Glutenin Primer Sequences

5'-ATG AAG ACC TTC CTC GTC TT-3'

5'-C AAC GCC GAA TGG CAC ACT A-3'

Gamma–Gliadin Primer Sequences

5'-ATG AAG ACC TTA CTC ATC CT-3'

5'-ACA TAC ACG TTG CAC ATG G-3'

High Molecular Weight Glutenin Primer Sequences

5'-TCT CAA GAT CCT ATG TTA AT-3-

5'-TGC CCA TAT TGT CTT GCG AC-3-

Results and Discussion

Polymorphism for LMW Glutenins (Glu-B3 Locus)

PCR amplification of low molecular weight glutenin sequences showed the presence of three amplified fragments, ranging between 900 and 1200 base pairs. The two smaller fragments were uniform in all analyzed genotypes. The size of the long fragment differed between genotypes possessing LMW-1 and LMW-2 glutenin subunits (Fig. 1a). The amplification product in genotypes possessing the LMW-2 subunits (Omrabi 5, Haurani, Mrb 9/Haucan, and Bicre 3/Cham 1/Gta/Stk) was about 50 bp longer than that found in genotypes possessing LMW-1 subunits (Cham 1 [Waha] and Chahba 88/Mrb 11). The differentiation of genotypes by specific primers for LMW-1 and LMW-2 can be confirmed by conventional protein electrophoresis.

Polymorphism for Gamma Gliadins (Gli-B1 Locus)

For gamma gliadin primers, five major fragments between 750 and 1000 bp were amplified (Fig. 1b). No variation between genotypes was found for two smaller-sized fragments (800 and 830 bp) and one larger-sized fragment (970 bp). Two fragments at 900 and 950 bp showed polymorphism between durum wheat genotypes. Cultivars showing the amplified fragment at about 900 bp correspond to genotypes possessing gliadin 42, whereas those with the amplified fragment at about 950 bp belong to genotypes possessing γ-gliadin 45. The two groups identified as γ-42 and γ-45 genotypes based on PCR amplification correspond exactly with the electrophoretic pattern of the gamma gliadin protein fraction in SDS-PAGE analysis (results not shown). Waha and Chahba 88/Mrb 11 belong to the group of genotype γ-42 varieties, whereas Omrabi 5, Haurani, Mrb 9/Haucan, and Bicre 3/Cham 1/Gta/Stk belong to the group of genotype γ-45.

HMW Glutenin Amplification (Glu-1)

The primers used for amplification of HMW glutenins (Fig. 1c), revealed only one amplified fragment at about 900 bp, which was monomorphic for all analyzed genotypes.

Some 267 genotypes (including checks) from the 1994 advanced durum yield trials were analyzed (Fig. 1). Two hundred and forty four of the lines amplified for the LMW-2 genes, and only 23 for LMW-1. Neither O alleles or any other alleles, previously described for T. dicoccoides (Pagnotta et al. 1993), were found. The poor variation found for LMW glutenins in durum varieties and advanced lines confirm the results of others (D'Ovidio et al. 1992). It also indicates the usefulness of the PCR marker for selection.

Some 263 lines (including checks) of the 1994 advanced durum yield trials were analyzed for gamma gliadins. Some 235 showed an amplified fragment at 950 bp (corresponding to 235 lines with γ-gliadin 45), and 28 at 900 bp (corresponding to 28 lines with γ-gliadin 42). Putative recombinants have been identified. Comparison with SDS-PAGE must be carried out to confirm the recombination.

The durum wheat breeding program at ICARDA routinely analyzes quality parameters such as SDS, SDSM, PROT%, TKW (g), and VIT% for the selection of parental lines to be used the following year. These analyses are complemented by SDS-PAGE protein and farinogram analysis. Four hundred and fifty six genotypes of the advanced durum yield trials (1994) were analyzed. The best lines for each of the parameters tested were compared with the result of PCR amplification of LMW glutenins or γ-gliadins (Table 1). In the group of lines with the highest protein content, lines with LMW-2 and lines with LMW-1 were found. Also in the group of lines with the highest SDS values, lines with LMW-2 and lines with LMW-1 were found. Only in the group of lines with modified SDS value (SDSM) were no genotypes with LMW-1 found. Accordingly, SDSM is the best parameter to predict the potential for a strong gluten. In cases where amplification with LMW-1 and LMW-2 was not carried out, amplification for γ-42 or γ-45 was used to indicate the presence of LMW-1 or LMW-2. The γ-gliadins 42 and 45 were found to be genetic markers of quality (Pogna et al. 1990), whereas allelic variation for LMW glutenin subunits—mainly encoded at the Glu-B3 locus, which is about 2 cM from the Gli-B1 locus 1.7 (Singh and Shephard 1988)—is primarily responsible for differences in gluten viscoelasticity properties (Pogna et al. 1988, 1990).

The analysis shows that many of the advanced durum lines developed for the temperate climate at ICARDA already possess the genes for strong gluten. To know whether LMW-1 or 2 and γ-gliadin 42 or 45 genes are present in the durum germplasm is an additional important parameter, which will be included in the routine analysis of durum germplasm at ICARDA.

Table 1. Quality trait analysis in advanced durum lines.

Line	SDS	SDSM	Prot%	TKW	LMW/Gliadin[1]
			With highest protein content		
204	35	4.7	16.2	39.9	2
1,316	33	5.5	16.5	32.9	1
			With highest SDS values		
822	38	4.7	12.3	37.2	2
1,012	36	5.3	15.9	44.8	1
			With highest SDSM values		
220	36	5.7	16.2	39.9	2
1,313	37	5.9	15.9	37.7	950

[1] 90.8% of the lines in advanced durum yield trials had LMW-2.

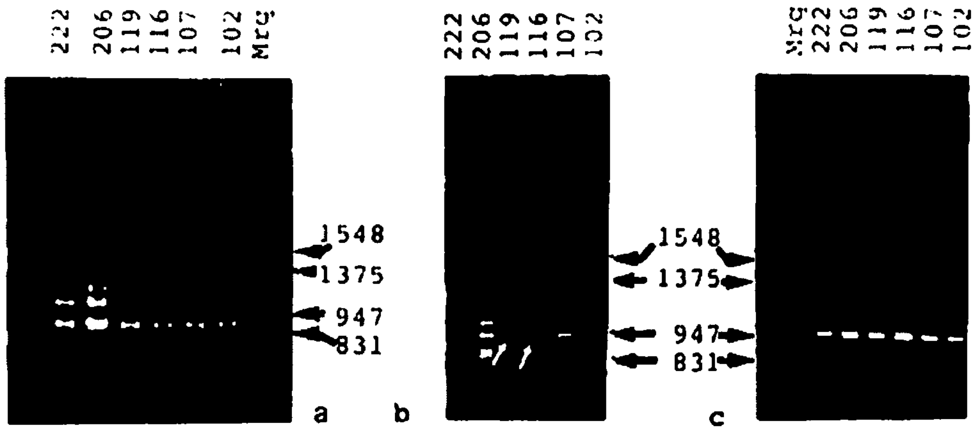

Figure 1. PCR amplification of genomic DNA of six durum genotypes with primers: (a) LMW glutenin, (b) gliadin, and (c) HMW glutenin. From right to left: Mr=size marker, λ-EcoRI-HindIII digest from Boehringer Mannheim, Omrabi 5 (102), Haurani (107), Waha (116), Chahba 88/Mrb 11 (119), Mrb 9/Haucan (206) and Bicre/Cham 1/Gta/Stk (222).

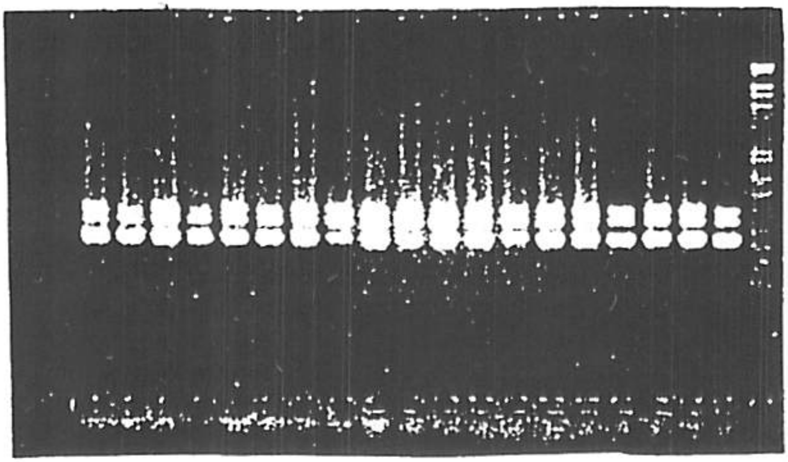

Figure 2. Screening of durum lines with LMW PCR primers for presence of LMW-1 and LMW-2. From left to right: Lanes 2 and 4 show amplification for LMW-1, all other lanes show amplification for LMW-2, lane 20 size marker.

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Molecular Studies of Genes Involved in Quality and Drought Tolerance: Development of Probes

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Abstract

Proteins accumulated in early and late phases of seed maturation are storage proteins, globulin, and late embryogenesis proteins. The storage proteins, glutenin, and gliadin (rich in cysteine), play a major role in technological pasta quality in wheat by producing a larger viscoelastic gluten. The globulins (low molecular weight, cysteine-rich proteins), may also contribute to quality parameters such as the surface state of cooked pasta, loaf volume, and crumb structure. Many DNA sequences of these proteins have already been characterized. Corresponding proteins were classified in two groups: one group of purithionin (5.5 kDa) and lipid transfer proteins (7–9 kDa) with eight cysteines, and a group of chloroform/methanol proteins (12–15 kDa) and puroindolines (13 kDa) with 10 cysteines. Other proteins, the group of NADP-dependent thioredoxin-h, also play a major role in pasta quality. Three proteins were characterized in this group: NADP, thioredoxin reductase, and thioredoxin-h (12 kDa). The LEA proteins may contribute to seed stability and protect embryos and plants against desiccation. In this family, dehydrins are the major proteins. Four clones of cDNA sequences were characterized (see Labhilili et al., this proceedings), and the protein sequences were similar to dehydrins and RAB proteins of mono- and dicotyledons plant.

Introduction

The proteins which make the major contribution to wheat quality are well known to be gliadins and glutenins. These proteins are the major constituents of gluten, and the viscoelastic properties of gluten determine to a great extend the quality of the end products. In each of the proposed gluten structure models, much is generally made of the importance of the S-S bonds, but until recently no mechanism has been proposed to explain the exchange reaction between intra- and intermolecular bonds.

According to Osborne's classification, albumins and globulins also exist beside the storage proteins. These fractions contain mainly enzymes and structural proteins. Members of these protein families, low molecular weight, cysteine rich proteins (LMW-CRP) are considered important for quality parameters such as surface state of cooked pasta (Kobrehel et al. 1989a, b, 1991), loaf volume, and crumb structure.

The NADP-dependent thioredoxin system (NTS) reduces both storage proteins and LMW-CRP, after which all reduced proteins are able to interact to realize the gluten network.

There are two main phases of grain filling: the first lasts up to the desiccation phase and the second starts with grain desiccation to mature grain (Fig. 1).

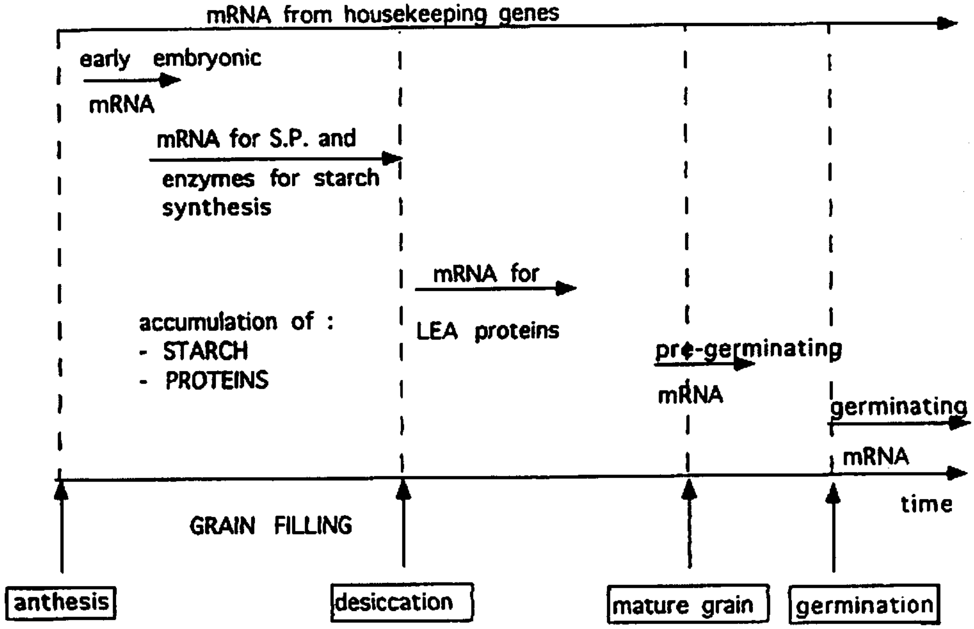

Figure 1. LEA gene expression during seed maturation.

It is well known that storage proteins are synthesized during the first phase, as well as most of the albumins and globulins. Not much research has been devoted to the proteins which are synthesized during the second phase. There is some evidence that proteins synthesized during this period are more susceptible to environmental conditions than during the first phase (Blumenthal et al. 1993).

This paper, summarizing the results obtained in our laboratory, is presented in two parts. The first concerns proteins involved in technological quality, which are synthesized mainly during the first phase of grain development; the second one concerns proteins synthesized during the second phase of grain maturation.

Low Molecular Weight Cysteine-rich Protein and NADP-dependent Thioredoxin System: Implications on Technological Quality

Low molecular weight Cysteine-rich Protein

The different LMW-CRPs are listed in Figure 2. Beginning from the lowest molecular weight, they are:

- The purothionins: with a molecular weight of 5.500 kDa and 8 cysteine residues, they have been known for many years, and it is believed that they have bactericide and fungicide properties.
- The lipid transfer proteins (LTPs): these range from 7 to 9 kDa in wheat. They also have 8 cysteine residues and *in vitro* they are able to transfer lipids from membranes to membranes (Dieryck et al. 1992).
- The chloroform/methanol proteins (CM): with a molecular weight ranging from 12 to 15 kDa. They have 10 cysteine residues (Gautier et al. 1989, 1990, 1991. Joudrier et al. 1995; Lullien et al. 1991a, b). They are members of the tetrameric α-amylase inhibitor family and probably play some role against insect damage.
- The puroindolines: recently discovered, they have a molecular weight of 13 kDa, 10 cysteine residues (Blochet et al. 1993), and are believed to play a role in seed friability. They have very interesting emulsifying properties. The possibility of using these proteins as an additive in the food industry is under study. Recent results show that they have antifungal properties (Marion et al. 1995).

PROTEINS	MOLECULAR WEIGHT		CYSTEINE NUMBER	ROLE
THIONINS	α-1	5500 Da	8	BACTERICIDE FUNGICIDE
	α-2	5500 Da	8	
LIPID TRANSFER PROTEINS = LTPs	LTP-7	7000 Da	8	LIPID TRANSFER
	LTP-9	9000 Da	8	
EXOGENOUS α-AMYLASE INHIBITORS	CM1†	12000	10	RESISTANCE AGAINST INSECTS
	CM2		10	
	CM3		10	
	CM16	TO	10	
	CM17†		10	
	0.28		10	
	0.19		10	
	0.53	15000 Da	10	
PUROINDOLINES†	a	13000 Da	10	GRAIN FRIABILITY
	b	13000 Da	10	

† Proteins specific to T. aestivum

Figure 2. Low molecular weight cysteine-rich wheat proteins.

The NADP-dependent Thioredoxin-h System (NTS)

An oxidoreduction system, the NADP-dependent Thioredoxin System (NTS), has recently been under study in wheat (Kobrehel et al. 1991). This system is composed of three different components: NADP, NADP-thioredoxin reductase, and thioredoxin-h itself.

Thioredoxins are proteins, typically with a molecular weight of 12 kDa, that are widely distributed in the animal, plant, and bacterial kingdoms. The active site is usually highly conserved for all proteins with the consensus sequence -W-C-G-P-C-. Thioredoxins undergo a reversible redox change through a disulfide group (S-S→2SH).

The seed is the only tissue for which NTS has been ascribed a function in plants. Previous studies have shown that thioredoxin is able to reduce the purothionins already mentioned. More recently it has been demonstrated that NTS is also capable of reducing members of other protein families, such as the α-amylase and trypsin inhibitors, as well as storage proteins (Kobrehel et al. 1992). Present evidence, obtained mainly with wheat, suggests that thioredoxin-h functions as an early signal in germination. Simultaneously, the major storage proteins and CRP are reduced (in particular, reduction of protein inhibitors neutralizes their activity towards their target enzymes), and the proteins are more susceptible to hydrolysis.

There is now evidence that NTS selectively reduces the intramolecular disulfide bonds of flour proteins (i.e., glutenins, gliadins, and CRP), which then undergo a series of sulfhydryl/disulfide interchange reactions that result in the formation of new intermolecular disulfide bonds. In this manner, NTS appears to promote the formation of the gluten network, thereby enhancing the quality of the final baked product (Buchanan et al. 1992; Wong et al. 1993).

Figure 3 illustrates the possible interactions between the different components and quality parameters. Results obtained with two families of CRP (the CM-proteins and the lipid transfer proteins) illustrate the type of data that can be obtained using molecular biology tools:

- Several cDNA clones encoding these proteins were isolated. These CRPs were synthesized as precursors of larger molecular weight, with a molecular mass of mature proteins of 7–16 kDa. They all contain 8–10 cysteinee residues. These cDNAs could be used as molecular markers (RFLP, maps, QTL).

- During seed development, the probes were used to follow the pattern of accumulation of mRNAs specific for each protein. It was then possible to see that mRNAs corresponding to CM proteins accumulate between 10 to 24 DAF; those corresponding to LTP appear later, between 24 to 34 DAF.

- The isolated cDNAs were used to produce the corresponding protein in heterologous hosts, such as *E. coli* or yeast (Gautier et al. 1994; Lullien-Pellerin et al. 1994). It is also possible to modify the sequence by site-directed mutagenesis, after which the recombinant proteins are used for structural analysis (such as RMN or X-ray crystallography) and for structure–function study.

The object of this work is to have both a better knowledge of the *in vivo* role of these proteins, and to prepare the way to transgenic cereals with improved technological properties.

Besides those CRP families believed to be directly involved in the technological quality of wheat, other families of protein synthesized at the end of grain maturation are of interest. These late embryogenesis-abundant proteins (LEAs) are synthesized when the desiccation phase starts (Fig. 1), that is to say, when the accumulation phase is finished. If these proteins are implicated in hydric stress tolerance, it is also probable that, depending on their level of synthesis during the desiccation phase, they could influence technological quality.

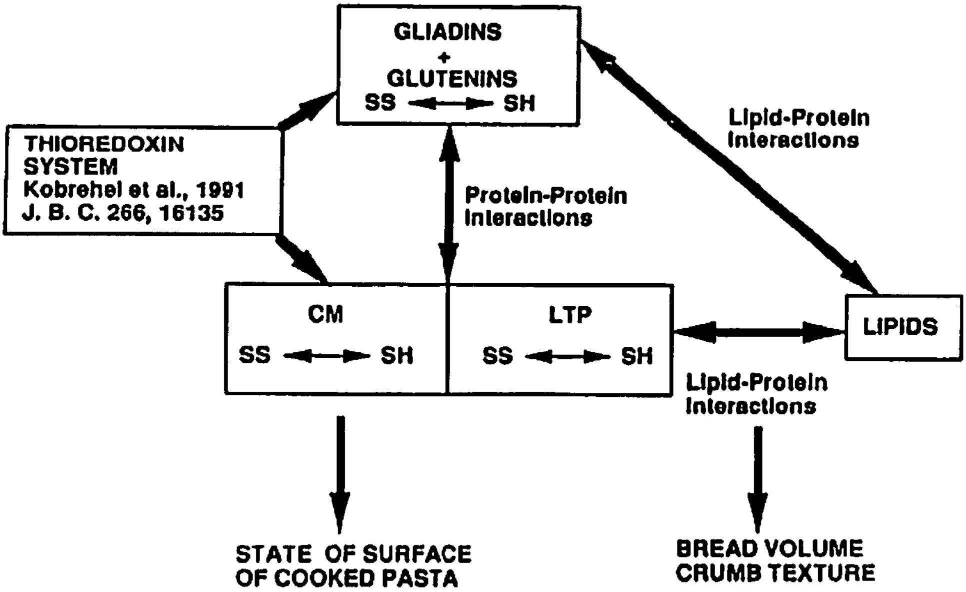

Figure 3. Role of low molecular weight cysteinee-rich protein in the technological quality of wheat.

Late Embryogenesis-abundant Proteins: the Dehydrins

Of the cultivated plants, *Triticum turgidum* subsp. *durum* (often grown under limited water conditions) provides an attractive system for studying drought tolerance because of its natural genetic variation for this character. In order to understand the physiological mechanisms of adaptation to drought and identify molecular markers, a study of genes induced by water stress was undertaken in *T. durum*. Attention was focused on genes coding for dehydrins belonging to group 2 of LEA proteins.

Four cDNA clones coding for dehydrin (TdDHN) were isolated and characterized. TdDHN15 and 16 showed sequence similarity with *T. aestivum* RAB, and TdDHN9.6 showed sequence similarity with *T. aestivum* COR proteins. More details concerning the identified clones are given in Mustapha Labhilili's paper.

Comparison of the accumulation of dehydrin transcripts in *T. durum* cultivars varying for tolerance to drought shows that drought-sensitive cultivars respond faster to dehydration than drought-tolerant cultivars. However, the level of induced transcripts decreases more rapidly when the stress continues.

The application of exogenous ABA induces accumulation of dehydrin transcripts in roots and shoots of sensitive and tolerant cultivars.

Using the PCR technique, we found two dehydrin genes (*Tddhn1* and *Tddhn2*) in diploid ancestor wheats presumed to be donors of genomes A, B, and D. This indicates that *T. durum* dehydrin genes were maintained during the course of wheat evolution. Nevertheless, these two genes have evolved separately.

Perspectives

Research will continue in four main areas:

- The isolation of a full set of water-stress-induced genes by a differential screening of the library is underway. The clones will be classified into different families, and the sequence of one clone from each family will be carried out in order to identify the encoded proteins.
- In order to verify and extend the relationship between the accumulation of the dehydrin transcript and the drought tolerance of *Triticum durum*, a larger number of cultivars and plants at a more advanced stage will be tested.
- The isolated cDNA clones are potential molecular markers for drought tolerance to be used in wheat breeding programs studying the genetic variability (by RFLP) of different cultivars and looking for water-stress gene RFLP alleles.
- If, as hypothesized, LEA proteins play a role in protecting plant tissue during dehydration, the accumulation of protein in water-stressed wheat must be monitored.

Conclusion

The transfer of new and useful genes endows seed with novel properties. Changing gene regulation or introducing genes from other species to raise the level of proteins with the required properties (higher nutritional value, better technological properties) should result in medium-term benefits. If we want to manipulate protein composition by genetic engineering we must find out how protein synthesis is regulated during seed development. To study the regulation of genes coding for these sulfur-rich proteins it is necessary to isolate and characterize the corresponding genes, then to perform promotor analysis. Such studies are underway on a barley gene coding for the CMd protein.

Besides these main aims, the isolated genes could be used as probes for (Fig. 4.):

- The establishment of wheat genetic map.
- The study of genetic variability (by RFLP or RAPD techniques, for example) in different cultivars, and the search for RFLP alleles or QTL in wheat breeding programs.

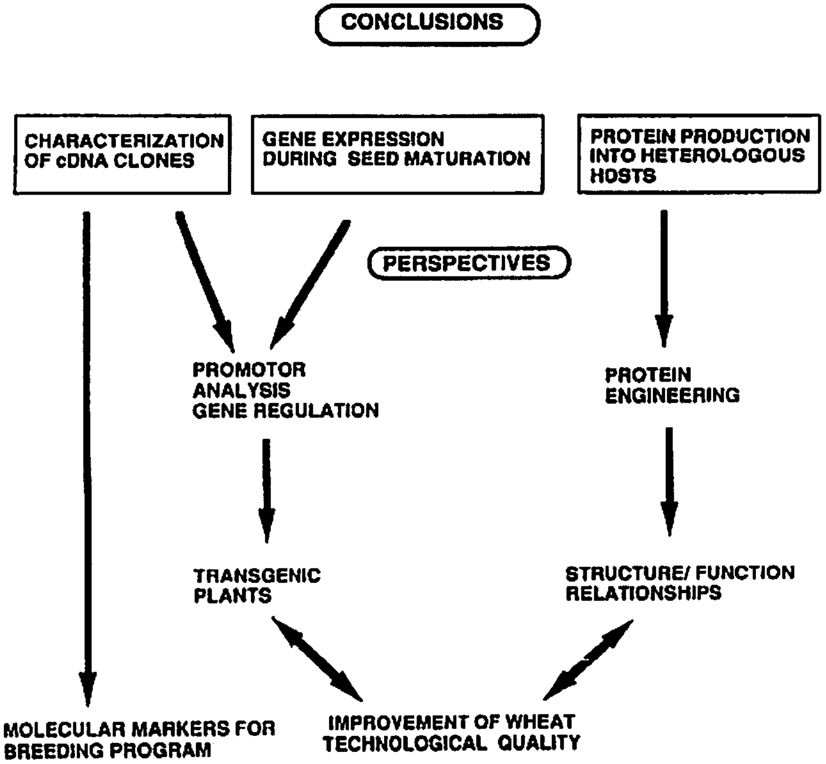

Figure 4. Analytical approach to gene tagging, expression, and transformation.

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Abiotic Stress and Molecular Markers

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Abstract

DNA sequences of durum dehydrin genes pTd27e, pTd16, pTd25a, and pTd38 were characterized and compared with the sequences of dehydrin and RAB genes of other cereal crops. Structure, conformation, and physiological role of durum dehydrin proteins were detailed. Correlations between the expression of dehydrins genes and drought tolerance were studied in three tolerant and one susceptible durum genotypes.

Introduction

Durum is an important crop for food production in the Mediterranean basin. It is used for many local specialities such as couscous, burghul, spaghetti, bread, etc. Durum is grown in arid and semi-arid zones, particularly where drought is the most prevalent abiotic stress.

To select for drought tolerance (or resistance), morpho-physiological traits as well as proline, sugar, and amino acid content, are used as selection tools during growth. Late embryogenesis-abundant proteins (LEAs) and DNA markers (RFLP, PCR) have surfaced recently as important alternative selection tools.

The LEA protein is a major protein, abundant in dry seed (Blackman et al. 1991). Its accumulation in the seed starts during desiccation and ends after germination (Baker et al. 1988; Close et al. 1989). During desiccation, cell embryos develop a high capacity to survive under drought stress (Thomann et al. 1992).

Four groups in the LEA family are characterized below (Galau et al. 1993; Dure et al. 1989):

1. This group includes the *Em* proteins of durum and *D19* of cotton. The nucleic acid sequences of these proteins are similar to each other.
2. This group includes RAB proteins and dehydrins. The proteins in this group have many conserved sequence characteristics.
3. This group includes protein *D7* of cotton and the LEA protein of ricin. The proteins of this group are characterized by a repetitive consensus 11-mer, "TAQAAKQKTSE" sequence.

4. This group includes LEA proteins 5 and 14 of cotton. These proteins are similar to the LEA proteins of group two but the sequences are more hydrophobic.

Group two is the most important. The protein sequences of the family members are similar. There is no cysteinee, no tryptophane, and they are very rich in glycine (more than 20%) and threonine (8–10%). Their molecular weight is variable and their isoelectric point (IP) is basic (Labhilili et al. 1995). These proteins are synthesized during desiccation in seed. In roots, leaves, and embryos they can be produced after drought (Labhilili et al. 1995), or cold stress (Guo et al. 1992; Houde et al. 1992). LEA proteins can be regulated either by abscisic acid (ABA) or by different abiotic stresses (Roberton and Chandler 1992; Skriver and Mundy 1990).

The aim of our study was to compare the expression of durum dehydrin and cereal RAB proteins under drought stress. The correlation between the expression of this durum dehydrin gene and drought tolerance was studied in four durum wheat genotypes.

Materials and Methods

Durum seed was sterilized and germinated in petri dishes for three days and then stressed in a desiccator as described by Close et al. (1989) for different periods of time. The RNA was extracted from 0.5 g of fresh material (coleoptiles and roots) or from 10 g of dry seed (Chirgin et al. 1979; Chomczynski and Sacchi 1987) and separated by agarose gel electrophoresis under denaturing conditions (Kroczek and Siebert 1989). The RNA was bound to Hybond N+ membranes after alkaline transfer. Radioactive labeling of probes was done by random priming (Amercham) with P32. Hybridation and washing of membranes were carried out as described Maniatis et al. (1989).

Poly A+ mRNA was purified by oligo dT cellulose column chromatography. A cDNA library was constructed using the SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning kit (BRL), as per manufacturer's directions. This library was screened with cDNA clone HVDHNB18 of barley (Close et al. 1989). Identified clones were isolated and amplified in E. coli. Plasmid DNA was purified by alkaline lysis (Stephen et al. 1990). DNA sequencing was carried out with the chain terminator reaction technique according to Sanger et al. (1977). The DNA sequence was determined with the automatic sequencer system (Applied Biosystems, ABI 377) and the nucleic sequences were analyzed with Microgenia (Beckman) and PCgene (Intelligenetics) software.

Durum lines were tested in 1985 in 29 locations in the Mediterranean region.

Results

Characterization of Dehydrin Genes in Durum

In durum, four cDNA clones (pTd27e, pTd16, pTd25a, and pTd38) were characterized (Labhilili et al. 1995). The nucleic sequences contain translated and non-translated 3' and 5' domains. The translated sequences start with a methionine codon ATG and finish with TGA or TAA codon stops. Like many plant genes (Heidecker and Messing 1986; Hunt et al. 1987; Messing et al. 1983), and in particular cereal genes (Close et al. 1989; Mundy and Chua 1988; Vilardell et al. 1990), dehydrin genes have one or more potential polyadenylation recognition sequences—AATAAT, AATAAAG, or AATAAA—with a variable distance between recognition site and codon stop (Labhilili et al. 1995). Coding regions also show significant GC (more than 25%), and more than 75% of codons have the XXG/C sequence (Labhilili et al. 1995). According to Feucher (1989) and Murray et al. (1989), codons with the sequence XXG/C are strongly expressed if the percentage of these codons is high in the translated sequences.

Characterization of Durum Dehydrin Proteins

Proteins Tddhn15, Tddhn16, and Tddhn9.6 (Td=*Triticum durum*, Dhn=dehydrin gene) are deduced from the nucleic sequences pTd27e, pTd16, pTd25a, and pTd38 of durum, respectively (Labhilili et al. 1995). These proteins are similar to the LEA proteins of group two. Like all examined dehydrins and RAB proteins of group two, durum dehydrins contain many conserved sequences. We found the consensus sequence ME(F/Y/H)QG in the amino terminal extremity, and we found the consensus sequence TGEKKGIMDKIKEKLPGQH (domains 5 and 6) in the carboxyl-terminal amino acid extremity. Between those extremities we found one consensus sequence, DEYGNP (domain 1), which is characteristic of all dehydrin and RAB proteins. After this sequence, there is frequently one sequence of 7–8 successive serine residues IL(H/Q)RSGSSSSSSSSEDD (domain 2). This sequence is missing in Tddhn9.6 of durum and PEADHN of pea (Labhilili et al. 1995; Roberton and Chandler 1992). Close to this sequence, there is a consensus sequence, GMGGR(R/K)KKG(M/I)(K/M)(D/E)KIKEKLPG(G/-)QH (domains 3 and 4), very similar to the carboxyl-terminal amino acid sequence. Another semi-preserved consensus sequence, TGG(A/T)YGQQGHTG, is present. The copy number of this sequence is variable.

Comparison of Durum Dehydrin Proteins to Dehydrin and RAB Proteins of Others Cereals

Amino acid composition

The dehydrin protein amino acid composition of durum (Labhilili et al. 1995), and protein HVDHN18 of barley (Close et al. 1989), ZMDHM3 and ZMDHM17G of maize (Close et al. 1989; Vilardell et al. 1990), OSRAB21 of rice (Vilardell et al. 1990), and TARAB and TARAB15B of bread wheat (King et al. 1992) is similar (Table 1). These proteins have no cysteinee, no tryptophane, but high contents of glycine. Moreover, TddHn9.6 is very rich in threonine but lacking in tyrosine, arginine, and phenyl alanine. The molecular weight of all these proteins varies and the isoelectric point is usually basic. The isoelectric point is acid and the molecular weight is low only for TDDHN9.6 of durum wheat and PEADHN of pea. The amino acid composition of durum dehydrin and RAB protein of bread wheat is similar.

Similarity between durum dehydrins and dehydrins and RAB proteins of others cereal

The similarity among dehydrin protein sequences (Tddhn15 and Tddhn16) of durum, and dehydrin proteins of barley (HVDHN18, HVDHN8, HVDHN9), maize (ZMDHM3, ZMRAB17G), rice (OSRAB21), and bread wheat (TARAB, TARAB15B) is high (Table 2). This similarity is higher between durum wheat dehydrins and bread wheat RAB proteins, particularly between Tddhn15 and TARAB, and between Tddhn16 and TARAB15B. The similarity of the nucleic acid sequence of cDNA clones pTd27e and TARAB is 89.3%. The translated sequences of these two sequences are identical, except that three guanine bases are inserted at positions 129, 146, and 205 in pTd27e. These insertions induce a structural change of the protein Tddhn15 at positions 25, 60, and 123.

The similarity between the nucleic acid sequences of pTd16 and TARAB15B is 75.6%. The 3' and 5' non-translated sequences are very different. The translated sequences are identical except for the insertion of two sequences (132 and 108 bp) into TARAB15B. The sequences of these two fragments correspond to the repetitive sequence TGGTYGQQGHTGM of TARAB15B. This sequence is repeated seven times in TARAB15B and twice in Tddhn16.

There is a high similarity between HVDHNB18 of barley and durum dehydrins.

Table 1. Amino acid composition (%) of durum dehydrins (Tddhn15, Tddhn16, Tddhn9.6) (Labhilili et al. 1995), barley dehydrin (HVDHN18) (Close et al. 1989), wheat TARAB proteins (TARAB, TARAB15B) (King et al. 1992; Joshi et al. 1992), maize (ZMDHN3 ZMRAB17G) (Close et al. 1989; Vilardell et al. 1991) and rice (OSRAB21) (Mundy and Chua 1988).

	Barley HVDHN18	Maize ZMDHNM3	Maize ZMRAB17G	Rice OSRAB21	Bread wheat TARAB	Bread wheat TARAB15	Durum Tddhn15	Durum Tddhn16	Durum Tddhn9.6
Arg	2.7	3.0	4.2	3.7	7.4	3.5	4.0	2.5	0.0
Cys	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gly	29.8	27.5	27.4	25.2	20.8	29.9	25.3	23.4	23.7
His	7.6	7.8	7.1	6.1	4.7	6.9	5.3	5.7	10.8
Lys	4.9	7.8	7.7	8.0	8.1	5.2	8.7	8.2	9.7
Phe	0.0	0.6	0.6	0.6	1.3	0.4	1.3	1.3	0.0
Ser	3.6	5.4	5.4	5.5	6.7	3.5	6.0	6.3	1.1
Thr	12.9	7.8	8.3	14.1	6.0	14.7	6.0	8.2	17.2
Try	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0
Tyr	4.4	3.0	3.0	1.2	2.0	3.9	2.0	2.5	0.0
MM: Da	22,574	16,955	17,161	16,543	15,766	23,229	15,163	15,958	9662
PI	8.89	9.04	9.67	10.00	10.59	9.68	10.16	9.73	6.95

MM=molecular weight; PI=isoelectrique point.

Table 2. Similarity (%) between durum wheat dehydrins (Tddhn15, Tddhn16, Tddhn9.6) and dehydrin and RAB proteins of barley (HVDHN18, HVDHN8, HVDHN9), wheat (TARAB, TARAB15B), maize (ZMDHM3, ZMRAB17G), and rice (OSRAB21).

	Tddhn15	Tddhn16	Tddhn9.6
Tddhn15	100		
Tddhn16	64.8	100	
Tddhn9.6	66.7	73.1	100
HVDHN18	78.6	84.1	79.6
HVDHN8	73.4	74.1	51.6
HVDHN9	69.2	74.1	57.0
ZMDHM3	76.7	74.7	63.4
TARAB	87.9	67.8	65.6
TARAB15B	78.0	86.0	79.6
OSRAB21	75.4	70.9	66.7
ZMRAB17G	76	72.8	65.6

Alignment of durum dehydrin proteins, and dehydrin and RAB proteins of others cereals.

The alignment of the protein sequences of dehydrins and RAB proteins of cereals shows six highly preserved domains (Fig. 1). The protein sequences consist mainly of domain 1 (DEYGNP), which is typical for dehydrin and RAB proteins, domain 2 (ILHRSGSSSSSSEDD), with six successive serine residues, domain 3 (GMGGRRKKGIKE), which is rich in glycine, threonine, and lysine, domain 4 (KIKEKLPGG), domain 5 (TGEKKGIMD), and domain 6 (KIKEKLPGQH) in the carboxy-terminal extremity.

Domains 2, 3, and 4, and domains 5 and 6 form two blocks with 37 and 9 amino acids, respectively. These blocks are highly preserved in dehydrin and RAB protein of cereal (Close et al. 1989; Guo et al. 1992; Labhilili et al. 1995) and other crops such as cotton (Baker et al. 1988; Due et al. 1989; Galau et al. 1992a, b, 1993), soybean (Chen et al. 1992), and the resurrection plant *Craterostigma plantagineum* (Piatkowski et al. 1990).

Between domains 4 and 5 we found a semi-preserved sequence, TGGTYGQQGHTGM. The copy number of this sequences is variable. This variation characterizes each protein and is also responsible for the variation of the molecular weight of the protein. This sequence is present in Tddhn15 of durum, TARAB of bread wheat, OSRAB21 of rice, and HVDHN8 and HVDHN9 of barley. In Tddhn16 of durum, and ZMRAB17G and ZMDHM3 of maize, it is represented one time. It is represented five times in HVDHN18 of barley and seven times in TARAB15B of bread wheat. The amino terminal extremities of those proteins are similar. The consensus sequence of this extremity is ME(F/Y/H)QGQ.

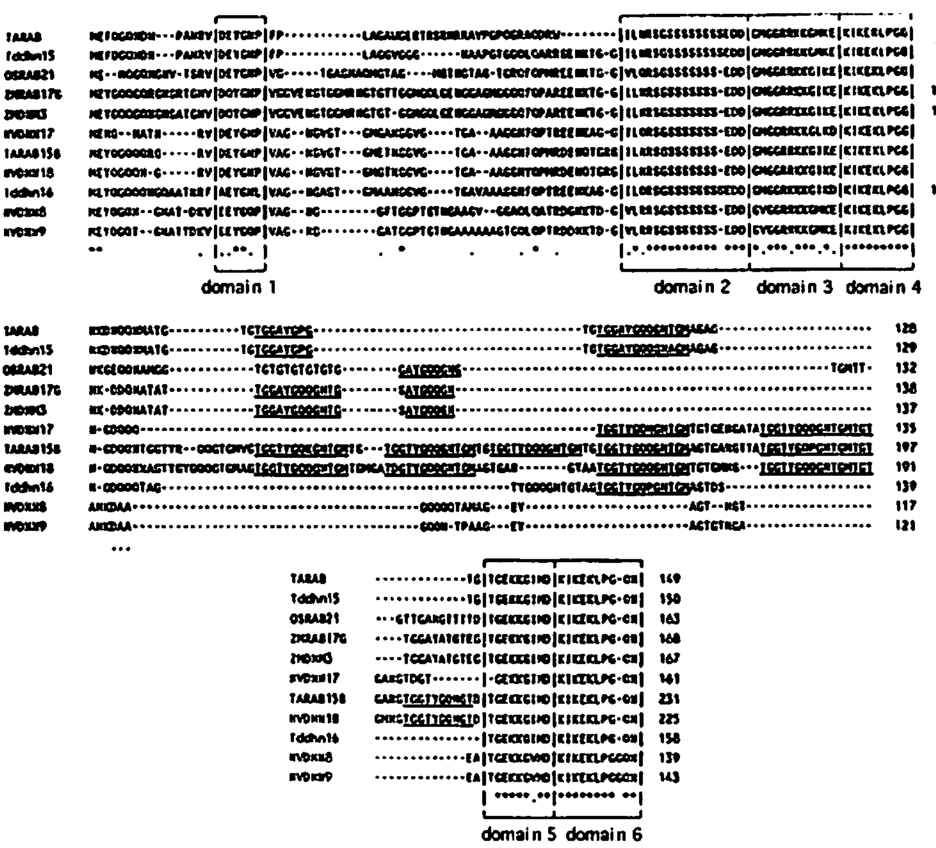

Figure 1. Alignment of durum wheat dehydrin (Tddhn15, Tddhn16) (Labhilili et al. 1995), barley dehydrin (HVDHN8, HVDHN9, HVDHN17, HVDHN18) (Close et al. 1989), wheat TARAB proteins (TARAB, TARAB15B) (King et al. 1992; Joshi et al. 1992), maize (MDHN3 ZMRAB17G) (Close et al. 1989, Vilardell et al. 1991), and rice (OSRAB21) (Mundy and Chua 1988). Domains 1, 2, 3, 4, 5, and 6 in the box show sequences preserved in most dehydrins and RAB proteins of LEA protein group 2. Underlined sequences are semi-preserved. Numbers show position of amino acid in protein; (*) represents amino acid preserved; (.) represents amino acid similar; (-) represents amino acid absent. The similar amino acids are A, S, T; D, E; N, Q; R, K; I, L, M, V; F, Y, W.

Structure and conformation

LEA proteins of group 2 are hydrophile and have no hydrophob extremity. They are soluble in a salt solution (50 mM KCl) and stable at high temperatures. For this reason, this group cannot be a structural part of a cell organelle (Baker et al. 1988). Predictions of the structure of these proteins (Garnier et al. 1978; Chou and Fasman 1979) indicate an α and ß helix, and a turn zone (Goday et al. 1990) (Fig. 2). The α-helix corresponds to the hydrophile sequences (domains 3, 4, and 6), the ß structure to the hydrophob sequences, and the turn zone to domains 1 and 2.

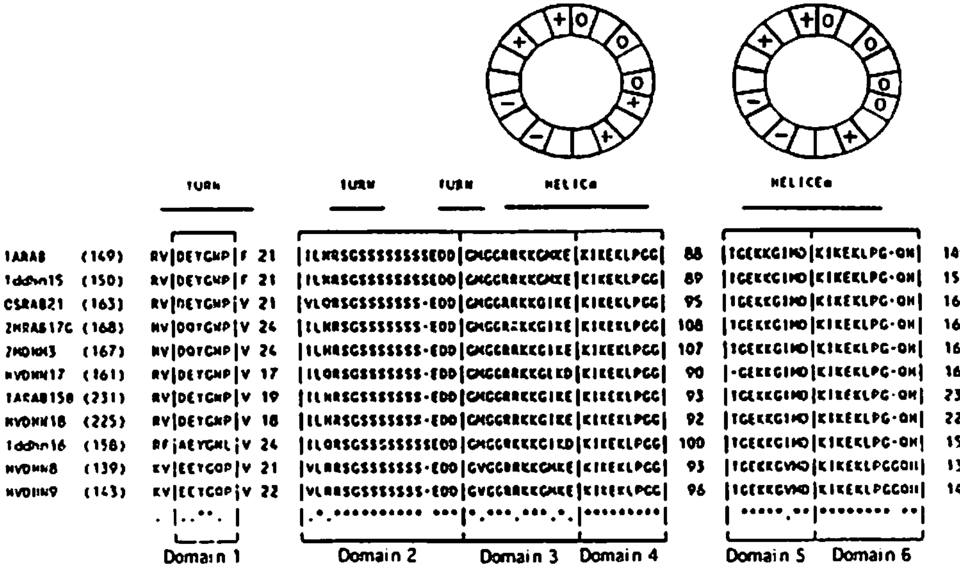

Figure 2. Predicted structure of conserved domains of durum dehydrins (Tddhn16, Tddhn15), barley dehydrins (HVDHN17, HVDHN18, HVDHN8, HVDHN9), maize dehydrin (ZMDHM3), maize RAB protein (ZMRAB7G), and rice RAB protein (OSRAB21). (+) represents amino acid acid; (-) represents amino acid basic; and (0) represents amino acid hydrophobs.

Figure 2 shows the α-helix corresponding to preserved domains 3, 4, 5, and 6 in cereal dehydrin and RAB proteins. These two α-helixes are amphophil (Baker et al. 1988; Due et al. 1989; Goday et al. 1990). The tertiary structure of the protein has not yet been determined. The proteins are presumed to be filamentous (Baker et al. 1988) or amorph (Roberton and Chandler 1992). These proteins are found in cytoplasm and not in membranes.

Study of Durum Dehydrin Gene Expression

The expression of dehydrin genes was studied under the same environmental conditions in roots and shoots of young durum plants four days after desiccation. The accumulation of mRNA (Fig. 3) starts after 30 hours of desiccation and is increased by drought stress. The accumulation of dehydrin mRNA in roots and shoots of the susceptible genotype Tomclair was higher after 30 hours of desiccation than in the resistant genotypes Belikh 2, Durelle, and Waha (Cham 1). However, after 67 hours of desiccation, this accumulation was higher in the resistant genotypes. Comparison of the resistant genotypes shows that the accumulation was higher in Belikh 2 than in Durelle and Waha.

Correlation Between mRNA Accumulation and Field Performance.

The field performance at 29 locations in dry areas was correlated with mRNA accumulation (Fig. 3) in Belikh 2 and Cham 1 (Table 3). The agronomical performance of Belikh 2 in this environment was better than Cham 1. Grain yield and the stability parameters SDS and R were better in Belikh 2 than in Cham 1. Also, the accumulation of mRNA after 67 hours of desiccation was higher in Belikh 2 than in Cham 1 (Fig. 3). From a comparison of field performance and dehydrin mRNA accumulation, we conclude that Belikh 2 is more drought tolerant than Cham 1, Durelle, and Tomclair. Therefore, it seems to be possible to use dehydrin mRNA accumulation as an indirect marker to select drought tolerant and susceptible genotypes.

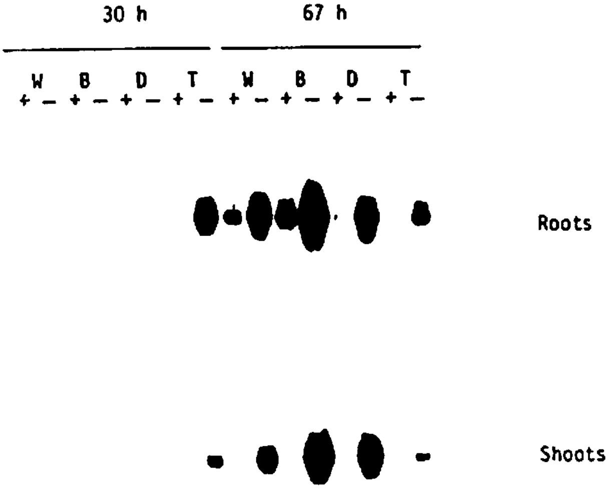

Figure 3. Accumulation of a dehydrin transcript after desiccation in roots and shoots of young plants of tolerant varieties Belikh 2 (B), Durelle (D), Waha (W), and susceptible variety Tomclair (T). Twenty µg of total RNA was loaded in the agarose denatured gel (Kroczek and Siebert 1989). Thirty and 67 hours represent duration of desiccation; (+) represents plants grown under normal conditions; and (-) represents plants grown under stress conditions.

Table 3. Field performance of Belikh 2 and Cham 1 at 29 locations.

Genotype	Grain yield	Stability parameters	
	kg/ha	R	SDS
Belikh 2	4,028	0.8	5.3
Cham 1	3,526	13.2	6.9
Trial means	3,640		
LDS	217		
CV	15.4		
No. of locations	29		

Discussion

Under desiccation, plants lose large quantities of water, particularly at the initial stages. Later, water content in the plant reaches equilibrium with the environment. Desiccation also induces the accumulation of dehydrin transcripts in the roots and shoots of the stressed plants, as well as in the seed. The speed of accumulation is fast in germinating seeds and slower in roots and shoots (but not slow). This difference is probably due to differences in the cell regulation of various tissues. Decrease of dehydrin mRNA accumulation in shoots is linked to cessation of growth or degradation of mRNA (Creelmen et al. 1990) or a cessation of mRNA translation in seeds and roots (Roberton and Chandler 1992).

Durum wheat dehydrins are similar to dehydrins and RAB proteins of other crops. They are identified in monocots and dicots after desiccation, osmotic stress, and cold stress, as well as after treatment of roots, shoots, and seed following germination with ABA, and during seed maturation (Due et al. 1989; Galau et al. 1992b; King et al. 1992a; Kusano et al. 1992; Labhilili et al. 1995; Mundy and Chua 1988; Vilardell et al. 1990). Many sequences are preserved in these proteins.

Accumulation of dehydrin mRNA in seed during maturation, and in the plant after drought or cold stress, increases under stress (Labhilili et al. 1995; King et al. 1992a). This increase is different for susceptible and tolerant genotypes. In tolerant genotypes the accumulation is more important.

Accumulation of dehydrin mRNA is similar in dry areas and under drought stress. Resistant genotypes have good performance and more accumulation of dehydrin mRNA after long stress. From this it is possible to conclude that dehydrin genes are regulated by stress. The product of expression of this gene is correlated with drought-stress tolerance.

The supposed structure of this protein is filamentous or amorphous. The high percentage of glycine gives the protein more flexibility and mobility.

The physiologic role of LEA proteins has not been determined. It is possible that amphophil structures link them to water and other solutes when the plant is under drought stress. This can protect the crop from desiccation and help it to survive drought stress.

Cold stress has the same effect on cell water content. The water freezes and induces desiccation of cells. It is possible that protein induced by cold or desiccation has the same physiologic role: protecting the cell of the plant against desiccation.

We suggest the role of these proteins is as an osmoprotectant against drought and a cryoprotectant against cold.

Treatment with exogenous ABA induces expression of dehydrin genes in roots and shoots (in equal proportion) of susceptible and resistant genotypes (Labhilili et al. 1995). Regulation by ABA is the same in both tolerant and susceptible genotypes, but under drought stress the regulation is different. Vilardell et al. (1991) suggest two ways of gene regulation under stress: one directly controlled by stress, the second under ABA control.

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Storage Proteins in the Mediterranean Durum Landraces

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Abstract

The SEWANA region (southern Europe, western Asia, and northern Africa) is an important area for durum, which covers more than 75% of the land under cultivation. Durum is grown for human consumption as pasta, burghul, couscous, frekeh, and bread. Because of this, high grain quality is essential. Grain quality is affected by the quality and quantity of the gluten proteins: gliadins and glutenins. The paper focuses on the importance of gluten proteins for grain-quality improvement, as well as on a preliminary study of the genetic variability of gliadin components by factorial correspondence analysis (FCA).

Introduction

SEWANA includes all the countries on the Mediterranean basin, a region which is extremely important for durum wheat. In fact, more than the half of the total land under durum cultivation is in this region (Nachit et al. 1987). Durum wheat, *Triticum turgidum* subsp. *durum*, is used primarily for human consumption in products such as pasta, burghul, couscous, frekeh, and bread (El Haramein et al. 1993). Pasta is the main durum product in the world, but not in WANA. Whatever the end product, the same quality properties are required, except for bread.

Durum wheat has a better pasta-making quality than any other *Triticum* species, due to its rheological properties. This is so even if large variability exists between cultivars. Durum grain quality is defined by rheological properties such as consistency, elasticity, flour color, resistance to disintegration, and retention of a firm structure during and after cooking (Feillet 1984; Autran 1985). These important economical characteristics of grain quality are strongly effected by the quantity and quality of the gluten proteins: gliadin and glutenin.

Protein content is another important factor in grain quality. It is affected by environmental conditions such as temperature and rainfall, as well as soil fertility and nitrogen supply. However, high protein-content doesn't mean good pasta-making quality. Therefore, the role played by the proteins is fundamental to a good end-product, because the composition and interaction of the seed storage proteins determine grain quality (Impiglia et al. 1993). During the last two decades, scientists have focused their attention on the composition, variability, and effect of these protein fractions (Kasarda et al. 1976; Porceddu et al. 1983; Shewry et al. 1985; Bietz et al. 1987; Shepherd 1988; Wrigley et al. 1988).

The storage proteins are traditionally divided in two main groups—gliadins and glutenins—according to their solubility in different solvents (Osborne 1907). Gliadins, soluble in alcohol/water mixtures, are monomeric protein molecules which have been divided into four subgroups—a, b, g, and w—according to their relative mobility on polyacrylamide gel at acid pH. They are coded by clusters of tightly associated genes, acting like single genes, located on the short arms of homeologous chromosomes 1 and 6 of the A and B genomes of durum wheat (Lafiandra et al. 1983). Glutenins, soluble in alkaline or acidic solutions, are composed of several polypeptidic subunits joined by intermolecular disulphide bridges. They are divided into high and low molecular weight glutenin subunits (HMW-gs and LMW-gs) according to their molecular weight. Genes coding for HMW-gs are located on the long arms of chromosomes 1A and 1B (Payne et al. 1980). Those for LMW-gs are on the short arms of chromosomes 1A and 1B.

In the past, breeders used an indirect selection method for grain quality improvement. If we look at the frequencies of LMW-2 and gliadin γ-45, which express strong gluten, we see that they are much higher than LMW-1 and gliadin γ-42, which express weak gluten (Figs. 1 and 2).

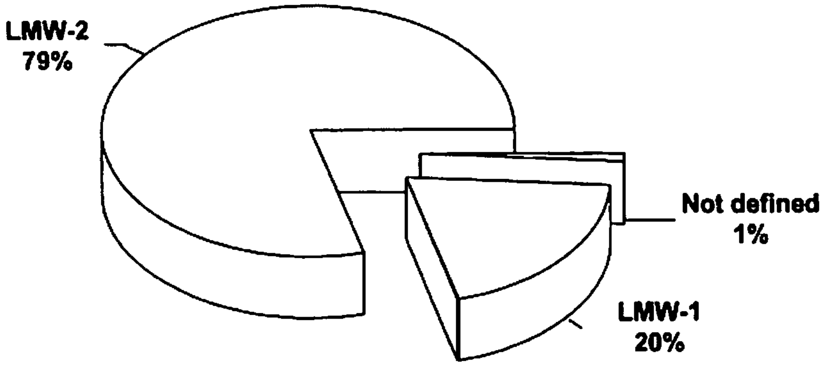

Figure 1. Low molecular weight glutenin subunits (LMW-gs) in a SEWANA durum wheat collection.

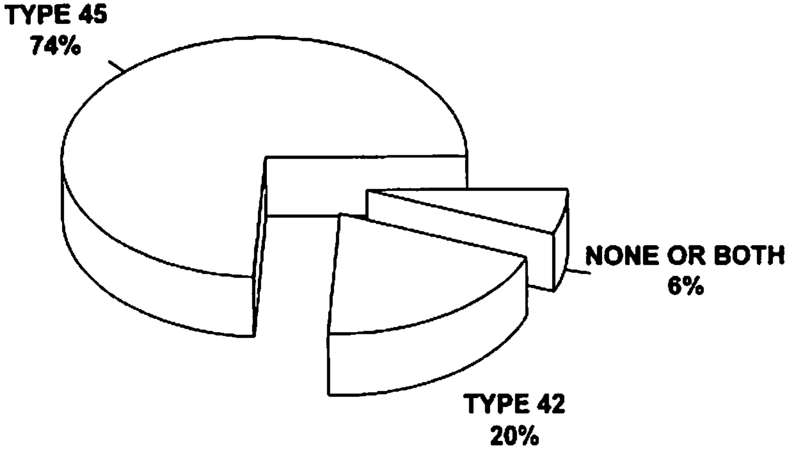

Figure 2. Gliadin components 42 and 45 in a SEWANA durum wheat collection.

Selection for grain quality using polyacrylamide gel electrophoresis (PAGE) methods for storage proteins began in the late 1970s.

Due to their high level of heterogeneity, determined by the genotype, the seed storage proteins can be utilized for various studies: e.g. to measure genetic diversity, to assess variation in populations, to study the migration of species from centers of diversity, to detect off-types in pure seed production, and to improve grain quality in cereals.

Several approaches for the assessment of genetic diversity are available. Morpho-physiological characters show the presence of considerable variation (Porceddu et al. 1984; Spagnoletti Zeuli et al. 1984). Molecular and biochemical markers also show variation in cereal populations (Ciaffi et al. 1993; Lagudah et al. 1987; Nevo et al. 1987), reflecting more directly the independence of the genotype from environmental effects.

In this study we focus our attention on two important aspects of seed storage proteins. First, the importance of seed storage proteins in grain quality improvement. Second, the utilization of polymorphism in the gliadin components to assess variation between populations of *T. turgidum* subsp. *durum* originating in SEWANA.

Materials and Methods

Forty seven populations originating in SEWANA were utilized in this study (Table 1). Gliadin proteins were extracted from single seeds derived from spikes collected at random with 1.5 M dimethylformamide (DMF) and fractionated at pH 3.1 in aluminum lactate buffer by polyacrylamide gel electrophoresis (A-PAGE) according to Khan et al. (1985), with minor modifications. Twenty seeds were analyzed for each population.

The relative mobility of each band was calculated using two reference bands, 42 and 45 of the durum cultivars Waha and Korifla, respectively. Either or both cultivars were used to provide standard reference electrophoregrams for every PAGE run.

Grain quality characters for the 47 populations were evaluated in the Cereal Quality Laboratory at ICARDA for the following traits: 1,000 kernel weight (TKW), vitreousness (Vitr.), flour color (FC), sedimentation SDS test (SDS), sedimentation SDS index (SDSi), and sedimentation SDS modified index (SDSmi).

Table 1. Populations and their origins.

Entry	Landrace	Origin	Entry	Landrace	Origin
1	Guemgoum	Alg	26	Biadi	Syr
2	Pol/Ze.b.	Alg	27	Bal.Hamra	Syr
3	Casoar	Alg	28	Shahba	Syr
4	MBB	Alg	29	Paramo	Spa
5	Hedba	Alg	30	IC 18004	Tun
6	IC 17329	Alg	31	IC 17815	Tun
7	IC 1292	Cyp	32	IC 17729	Tun
8	Pan.s501	Cyp	33	IC 6201	Tun
9	P.M.s5021	Cyp	34	Menceki	Tur
10	IC 12060	Egy	35	IC 5971	Egy
11	Cannizzaralta		36	AD 16	Fra
12	S.Cappelliilta		37	IC 20499	Grc
13	Jor.col.164	Jor	38	IC 20502	Grc
14	IC 20062	Mor	39	IC 20663	Grc
15	IC 19941	Mor	40	IC 20429	Grc
16	IC 20074	Mor	41	IC 20514	Grc
17	IC 20121	Mor	42	IC 20260	Grc
18	IC 19917	Mor	43	IC 20969	Jor
19	IC 19935	Mor	44	Hammari	Syr
20	IC 7682	Prt	45	Granadino	Spa
21	TE 8304	Prt	46	Sari	Tur
22	Javardo	Prt	47	Kazene	Tur
23	Haur.Ayob.	Syr			
24	Akbash	Syr			
25	Nab.Jam.	Syr			

Seed Storage Proteins for Grain Quality

The findings of two recombinant lines at the Gli-B1 locus (Margiotta et al. 1987; Impiglia et al. 1993) have identified the role of the LMW glutenins (which are the smallest subunits taking part in the disulfide cross-linked matrix) in determining pasta-making quality in durum wheat. The only genetic markers closely linked with LMW-1 and LMW-2 are the γ-gliadin components 42 and 45, respectively.

In our study, we analyzed the 47 SEWANA populations electrophoretically for LMW-1 and LMW-2, and also for the major grain-quality traits. The results are shown in Table 2. High values for SDS, SDSi, and SDSmi showed a strong correlation with the presence of LMW-2, while low values were associated with LMW-1.

As for the other grain quality characters analyzed, we observed no significant differences between the two allelic forms of LMW glutenin subunits in the SEWANA durum populations.

Multivariate Statistical Analysis

The migration electrophoretic pattern for the gliadin component of each population was considered as a vector. The outcome matrix was a two-dimensional contingency table (individual × protein component), where each accession was described by a set of 0 and 1, according to relative mobility, and the presence or absence of each component. Factorial correspondence analysis, proposed by Benzecri in 1973 and later described by Barrai (1986) was used as a multivariate statistical approach to study the genetic variability of the gliadin components within and between populations.

FCA is a weighted principal component analysis of a contingency table. It delineates the distribution of the original data as dispersions from a common centroid and establishes the relative position of each original observation. In the present study, the descriminant on the principal components describes, by canonical variables, the variability of the different protein components between populations.

Correspondence analysis allows a graphical representation of the association between the rows and columns of a table, based on the spectral resolution of the matrices. The method can be applied for any kind of data that corresponds to points in a Euclidean space, determined from cell frequencies with any structure and distribution. The objective of FCA is to show the relationship between row and column criteria, or, in our case, between genotype and gliadin components.

Results

Table 2 shows the Eigen values for the principal component (PC) factors. Percentage and cumulative estimation of total variance expressed by each value were computed by FCA using a program developed by G. Nachit.

The first 26 values describe more than 90% of the total variability. This is due to the small contribution of each Eigen value. The X^2 value was significant. The lowest relative frequencies were those with the greatest distance, such as gliadin components 13, 14, 16, 17, 38, 39, 41, and 89.

Because they are usually present on the D genome, the first four gliadin components probably originate from an introgression from bread wheat. However, these eight gliadins are important for their discrimination among populations.

The square of the distance (D^2) may explain how much each population contributes to variability. Tables 3 and 4 give the highest and lowest eight values for the gliadin components and populations, respectively.

Table 2. Correspondence analysis.

Factor	Eigen value	Percent	Cumulative
1	0.17274	8.19	8.19
2	0.16229	7.69	15.88
3	0.13314	6.31	22.18
4	0.12367	5.86	28.04
5	0.12141	5.75	33.80
6	0.10997	5.21	39.01
7	0.09962	4.72	43.73
8	0.09270	4.39	48.12
9	0.08405	3.98	52.10
10	0.07811	3.70	55.80
11	0.07083	3.36	59.16
12	0.06934	3.29	62.45
13	0.06727	3.19	65.63
14	0.06055	2.87	68.50
15	0.05530	2.62	71.12
16	0.05245	2.49	73.61
17	0.04930	2.34	75.95
18	0.04162	1.97	77.92
19	0.03947	1.87	79.79
20	0.03918	1.86	81.64
21	0.03562	1.69	83.33
22	0.03454	1.64	84.97
23	0.03078	1.46	86.43
24	0.02929	1.39	87.82
25	0.02816	1.33	89.15
26	0.02583	1.22	90.37
27	0.02316	1.10	91.47
28	0.02107	1.00	92.47
29	0.02007	0.95	93.42
30	0.01698	0.80	94.22
31	0.01595	0.76	94.98
32	0.01470	0.70	95.68
33	0.01268	0.60	96.28

(continued next page)

(Table 2 continued)

Factor	Eigen value	Percent	Cumulative
34	0.01065	0.50	96.78
35	0.00958	0.45	97.24
36	0.00899	0.43	97.66
37	0.00858	0.41	98.07
38	0.00699	0.33	98.40
39	0.00632	0.30	98.70
40	0.00608	0.29	98.99
41	0.00536	0.25	99.24
42	0.00436	0.21	99.45
43	0.00352	0.17	99.62
44	0.00339	0.16	99.78
45	0.00290	0.14	99.91
46	0.00182	0.09	100.0

X^2=42225.332; df=3450.

Table 3. Eight highest and lowest values for gliadin components.

Gliadin component	Relative frequency	D^2
13	0.00020	50.83420
14	0.00095	52.49733
16	0.00095	43.36364
17	0.00190	23.46524
38	0.00260	13.71501
39	0.00105	17.54218
41	0.00190	18.45250
89	0.00160	23.71279
43	0.02334	0.71305
45	0.03369	0.32749
50	0.02484	0.82636
52	0.02329	0.86635
58	0.02324	0.85826
61	0.02574	0.71748
64	0.02664	0.64352
78	0.02514	0.80130

The correspondence analysis for the dispersion of columns and rows, and the cluster analysis carried out to examine the association between different gliadin components and populations, both show some associated groups. Landraces originating from Cyprus do not show much variability. This may be due to Cyprus' geographical isolation. The Portuguese landraces are the most diverse among the durum germplasm populations analyzed. The Syrian landraces are more diverse than the Moroccan and Greek. The Moroccan populations are all grouped together, whereas some of the Syrian landraces are found among the Greek, Algerian, and Tunisian landraces, and some others are completely out of the group.

Table 4. Eight highest and lowest gliadin values for populations.

Population	Origin	Relative frequency	D^2
Pans 501	Cyp	0.01899	3.61389
IC 12060	Egy	0.01899	2.95907
TE 8304	Prt	0.01899	3.39739
Javardo	Prt	0.01869	7.056076
Biadi	Syr	0.02014	3.35841
Baladi Hamra	Syr	0.02254	3.71488
Menceki	Tur	0.02099	3.01617
IC 20969	Jor	0.01899	3.02599
IC 20121	Mor	0.02489	1.16526
IC 19917	Mor	0.02044	1.03852
IC 7682	Prt	0.02189	0.68086
AD 16	Fra	0.01924	1.02881
IC 20663	Grc	0.02284	0.90606
IC 20429	Grc	0.02699	1.25606
Hammari	Syr	0.02354	1.18931
Kazene	Tur	0.01999	1.03743

Some gliadin components are strongly associated, and may be encoded by the same multigene family. Gliadin components 14 and 17 are tightly linked, as are 82 and 85. Other components are located at opposite quadrants, such as γ-42 and γ-45, confirming them as two allelic forms belong the Gli-B1 locus.

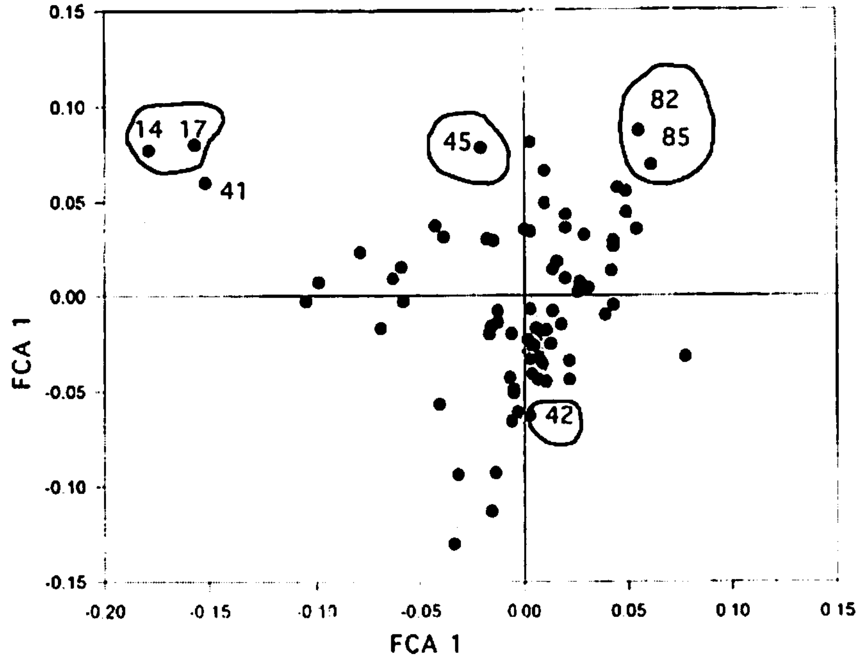

Figure 3. Plot of the gliadin components (factorial correspondence analysis).

Conclusion

Our results confirm the superiority of Mediterranean durum germplasm, and the importance of LMW-2 for selecting high grain quality durums for grain quality improvement in a breeding program.

FCA is a statistical tool with good potential for studying the genetic variability of gliadin components, confirming the view of other scientists (Asins et al. 1989).

Although it is not possible to detect all the alleles at each locus, we can analyze the components independently. This is a promising approach to the assessment of genetic diversity in germplasm collections.

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Use of *Triticum dicoccoides* to Improve Grain Quality in Durum Wheat

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Abstract

Wild emmer has a higher grain protein content than cultivated durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.). From crosses between durum × *Triticum turgidum* subsp. *dicoccoides*, recombinant lines were identified with high protein content, high grain yield, and other desirable traits. The F_6 lines from crosses between durum (Haurani Nawawi, Stork) and *T. dicoccoides* JS were studied. The recombinant lines were grown in two environments. The quality traits measured were grain protein content (%), carotene content (ppm), sedimentation (mL), 1,000 kernel weight (g), vitreousness (%), and grain yield per plant (g). Significant differences for environmental means were observed for most traits. The recombinant lines showed higher protein content than durum × durum lines; similar results were detected for vitreousness and sedimentation. The results demonstrate that *T. dicoccoides* could be a good source to improve durum grain quality.

Introduction

Wild emmer (*T. dicoccoides*, AABB, 2n=28) is used to improve durum grain quality and disease resistance (Nachit 1986; Maali 1990). Wild emmer is the immediate progenitor of all cultivated forms of tetraploid and hexaploid wheat (Feldman and Sear 1983). This species has a higher grain protein content and nutritional value than cultivated wheat (Avivi 1978; Tahir et al. 1983; and Sharma et al. 1981), with reported protein content up to 33.1%. Cook (1913) reported that wild emmer wheat was discovered by Aaronsohn in 1906. He stressed the importance of its agronomic characters and recommended its use in breeding programs. Genes for grain quality, stress tolerance, disease resistance, yield, and adaptation have been transferred from wild relatives to cultivated wheat (Avivi et al. 1983; Tahir 1986; Levy and Feldman 1987; Nachit 1992).

Selection for protein content and other desirable traits from durum × *T. dicoccoides* crosses could be used to transfer and accumulate these genes in new recombinants without sacrificing yield (Nagle 1980). Generally, genes of protein adversely affect yield. Thus it is necessary to monitor both traits simultaneously. With proper selection and testing, it is possible to combine and select lines carrying desirable traits from the *T. dicoccoides* parent. However, a large number of segregants in the subsequent breeding generation carry undesirable genes for tenacious glume and fragile rachis, along with their desirable traits (Tahir and Pashayani 1990).

Consequently, attempts were made to introduce the high protein factors from *T. dicoccoides* into durum wheat through conventional plant breeding techniques (Maali 1990; Nachit 1991). The purpose of this study was: (1) to compare the quality traits of durum cultivars and their progenies with *T. dicoccoides* and durum × *T. dicoccoides* progenies; and (2) to study the effect of site on quality traits.

Materials and Methods

Two durum cultivars—Haurani Nawawi (Jordanian landrace) and Stork (an early variety)—and wild emmer (*T. dicoccoides* JS, collected in the Irbid area, Jordan) were used in this study. Five crosses were made: (1) Stork × Haurani; (2) Haurani × Stork; (3) *T. dicoccoides* × Haurani; (4) Haurani × *T. dicoccoides*; and (5) *T. dicoccoides* × Stork.

F_5 seed of the five crosses and their parents were sown at two sites in Jordan: (1) Mushaqar, a research station of the National Center for Agricultural Research and Technology Transfer (NCARTT), with an average precipitation of 350 mm, latitude 31° 34' N, and longitude of 35° 43' E; and (2) Jubeiha, a research station of the University of Jordan (UOJ), with an average precipitation of 500 mm, latitude 32° 01' N, and longitude 35° 52' E. Sowing was carried out on November 18, 1989, and harvesting in mid-June, 1990.

The experimental design was a randomized complete block with three replicates. The MSTATC package was used to analyze the data.

The following quality traits were determined:

- Grain protein content. The Kjeldahl method was used, with near-infrared quality analyzer (GQA) model 31 E1.
- Carotene content in parts per milliliter. A scale was developed at ICARDA for rapid visual evaluation of color in the ground flour of durum wheat (Williams et al. 1986). The scale consists of a wooden board carrying seven round holes 6.5 cm in diameter, covered with clear plexiglas. The cells are filled with ground durum flour samples ranging from 2 to 8 ppm yellow pigment, as determined by water-saturated butanol extraction. The yellow pigment content of the unknown sample is recorded to the nearest 0. 5 ppm.
- Sedimentation test (SDS). The sample is shaken in the presence of lactic acid and sodium dodecyl sulfate (SDS). Proteins with good hydration capacity and good oxidation give stable suspensions. After a standard shaking procedure and standing period, the suspension is related to the strength and baking potential of the wheat flour.

- Thousand kernel weight (TKW). This is the weight of 500 seeds x 2.
- Vitreousness (%). This is determined by counting the number of vitreous kernels in a given sample and dividing by the total number of seeds in the sample.
- Grain yield (g/plant). Yield components (fertile tillers x number of kernels/spikelet x number of spikelets/spike x TKW/1000) are used to estimate this parameter.

Results and Discussion

Significant differences between site means were observed for grain protein content. Means were 16.4 and 13.0% for Mushaqar and Jubeiha, respectively. These results are in agreement with those of Williams et al. (1988), who found that protein content is affected by growing season, site, fertilizer, and other factors. Similar results were found for vitreousness, sedimentation, kernel weight, and grain yield. However, no differences between the two sites were detected for carotene content (Table 1). These results corroborate our earlier findings (Nachit and Asbati 1987; Nachit and Sayegh 1989).

Table 1. Means of quality traits for crosses between durum wheat and wild emmer *(Triticum dicoccoides)* for F_6 generations at two sites, Mushaqer and Jubeiha, in Jordan (1989/90).

Site	Trait					
	PC	Vitr.	CC.	TKW	SDS	GY
Mushaqar	16. 4	85. 8	7. 8	39. 0	37. 9	4. 4
Jubeiha	13. 0	78. 3	7. 8	36. 6	23. 8	3. 0
LSD (.05)	0. 6	2. 2	0. 3	1. 3	3. 2	0. 6
CV (%)	8. 6	16. 0	14. 4	7. 3	21. 7	31. 9

PC=protein content (%); Vitr.=vitreousness(%); CC=carotene content (ppm); TKW=1000 kernel weight (gm); SDS =sedimentation test (mL); GY =grain yield (gm/plant).

Significant differences were detected in parental material for protein content. *Triticum dicoccoides* showed the highest values at both sites; protein content was 22.9% at Mushaqar and 20.0% at Jubeiha. Stork had the lowest values, followed by Haurani, at both sites.

As for the crosses, *T. dicoccoides*-derived crosses had higher values for protein content than cultivated wheats and their derived crosses. Similar trends were found for vitreousness and sedimentation (Tables 2 and 3). In contrast, *T. dicoccoides* showed the lowest values for kernel weight and grain yield (Tables 2 and 3). However, *T. dicoccoides*-derived crosses showed reasonable kernel weight and grain yield. This suggests that *T. dicoccoides* could be useful as parental material to improve quality traits in durum cultivars. These results are in agreement with the findings of other researchers (Avivi 1978; Tahir 1986; Levy and Feldman 1987;

Nachit 1989; Maali 1990). In addition, these results demonstrate that using appropriate recombination techniques—such as backcrossing and simultaneous selection for quality and yield—incorporation of desirable genes from *T. dicoccoides* into high yielding durum cultivars could be achieved.

Table 2. Means of quality traits for crosses between durum wheat and wild emmer (*Triticum dicoccoides*) for F_6 generations, Mushaqer (1989/90).

	Trait					
	PC (%)	Vitr.	CC	TKW	SDS	GY
			Parent			
Haurani (Hrn)	14. 8	62. 0	5. 8	43. 3	38. 7	4. 8
Stork (Stk)	12. 5	97. 0	3. 7	28. 0	35. 0	1. 6
T. dicoccoides (Dic)						
			Cross			
Har. x Stk	14. 8	72. 2	5. 9	46. 2	23. 9	5. 5
Stk x Har.	13. 3	75. 0	5. 0	38. 0	35. 0	6. 4
Har. x Dic.	17. 8	97. 3	3. 9	36. 9	46. 3	4. 3
Dic. x Har.	17. 7	98. 7	3. 8	35. 7	54. 7	3. 4
Dic. x Stk.	16. 2	92. 9	4. 9	38. 3	36. 3	3. 2
Mean	16. 4	85. 8	4. 8	39. 0	37. 0	4. 4
CV (%)	8. 0	13. 3	10. 7	6. 0	20. 9	27. 8
LSD (.05)	2. 1	19. 2	0. 9	4. 1	13. 4	2. 1

PC=protein content (%); Vitr.=vitreousness (%); CC=carotene content (ppm); TKW=1000 kernel weight (gm); SDS=sedimentation test (mL); GY=grain yield (gm/plant).

Table 3. Means of quality traits for crosses between durum wheat and wild emmer (*Triticum dicoccoides*) for F_6 generations, Jubeiha (1989/90).

	Trait					
	PC%	Vitr.	C	TKW	SDS	GY
			Parent			
Haurani (Hrn)	10. 5	40. 0	4. 9	39. 3.	21. 6.	3. 2
Stork (Stk)	9. 8	58.0	5. 0	44. 0.	17. 7.	1. 7
T. dicoccoides (Dic)	20. 0	98. 3	4. 8	24. 7	36. 3	3. 2
			Cross			
Hrn. x Stk.	10. 9	66. 0	5. 8	41. 8	11. 3	4. 8
Stk. x Har.	11. 5	55. 3	5. 5	42. 0	17. 7.	4. 5
Hrn. x Dic.	14. 9	96. 3	4. 2	32. 0	32. 3	2. 1
Dic. x Hrn	13. 5	90. 7.	3. 5	33.3	36. 0	2. 8
Dic. x Stk	12.1	88. 0	4. 8	38. 0	18. 7	2. 0
Mean	13. 0	78. 3	4. 8	36. 6	23. 8	3. 0
CV (%)	9. 5	19. 3	16. 0	16. 6	22. 0	27. 9
LSD (.05)	2.1	25. 0	1. 3	4. 0	8. 9	1. 4

PC=protein content (%); Vitr.=vitreousness (%); CC=carotene content (ppm); TKW = 1000 kernel weight (gm); SDS=sedimentation test (mL); GY =grain yield (gm/plant).

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Recent Advances in Breeding for Black Point Resistance in Durum Wheat

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Abstract

The economically important black point discoloration is related to furrow, pericarp, aleurone layer, and germ of durum wheat kernel. Phenolic compounds, and particularly ferulic acid derivatives, are related to blackpoint. We found evidence that black point development was related to humid conditions of the growing and mature kernel, even in the absence of pathogenic living agents (for example, on autoclaved sterile kernels). Such *in vitro* test conditions provide a good prediction of field resistance.

Introduction

Among industries based on semolina products (pasta, couscous) and cooked kernels (burghul), there is a wide consensus about color criteria. Generally, a yellow amber color is requested. Red and brown discoloration is a negative attribute, and specks of any color are not accepted by the consumer.

Some discoloration appears during the pasta-making process. For example, yellow-colored carotenoids can be oxidized by lipoxygenases during semolina tempering before extrusion. The development of reddish and brown discoloration may be favored by high-temperature drying conditions. However, we have not yet developed the ability to breed directly for the adaptation to specific industrial processes. The best results have focused on the improvement of mature kernel endosperm and pericarp color characteristics.

Recent Advances in Blackpoint Studies

Extensive work on blackpoint in durum was initiated in 1990 by a French group. Blackpoint is not specific to durum wheat, and is present in many bread wheat samples. However, lower granulometry of flour particles (<150 μ) allows a better tolerance for black or brown specks issued from blackpointed kernels.

Blackpoint is related to the pericarp and bran cell layer, and particularly to the pigmented layer in the kernel ridge (Zee and O'Brien 1970).

Factors of Blackpoint Development

Fungal diseases are commonly believed to be implicated in blackpoint outbreaks in India (Adlakha and Joshi 1974) and North America (Wiese 1987; Conner and Davidson 1988; Maloy and Specht 1988; Conner et al. 1992). *Alternaria alternata* (or *A. tenuis*), *Cochliobolus sativus* (or *Helminthosporium sativum*) and different species of *Fusarium* are frequently cited. French scientists did not find any fungus specifically related to blackpoint (Grignac 1981; ITCF, various). Robert (1984) confirms than thrip-infested durum kernels are more frequently blackpointed.

Climatic factors during grain development are clearly implicated (Adlakha and Joshi 1974, 1974; Robert 1984). Humid conditions after flowering favor blackpoint development (Kilpatrick 1968; Conner 1987; Statler et al. 1975; Grignac 1981; Robert 1984, Régnier 1994).

Genetic factors are important in durum susceptibility to blackpoint. Empirical multilocational registration trials have long since shown that durum cultivars differ considerably in blackpoint susceptibility (Geves 1994).

Histological, Cytological, and Biochemical Events Related to Blackpoint

Numerous works on plant cells and tissues suggest that many brown discoloration reactions may be due to phenolic compound oxidation. Key enzymes in phenolic compound synthesis are phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL). Other enzymes, such as peroxydases (PO) and polyphenoloxydases (PPO), are important in cereal polyphenol oxidization and browning, but it is equally well-established that these enzymes and their substrates are generally situated in different cell compartments (vacuole, organelles, membranes, cell wall), and that the breaking off of cell compartments in senescent tissues could be at least as important as the presence and content of specific enzymes and phenolic compounds.

With cereal endosperm and pericarp (flour, semolina, and bran products), the main phenolic compound is ferulic acid esterified and related to the cell wall, particularly in pericarp tissues (Pussayanawin and Wetzel 1987).

A better knowledge of discoloration phenomena may be obtainable through dynamic studies of phenolic compound metabolism during grain development, filling, and drying. Unhappily, such studies are scarce. McCallum (1989) and McCallum and Walker (1990) demonstrate that phenolic soluble and insoluble compound content are at a maximum at the milk stage in bread wheat. Tabusse (1986) reports similar results in durum wheat.

Recent studies by Régnier (1994) on healthy mature kernels of blackpoint-susceptible variety Arbois confirm the importance of ferulic acid content (Table 1). Blackpointed seed of different samples was found to be richer in insoluble esterified ferulic acid than healthy kernels.

Table 1. Ferulic acid content of different parts of healthy durum kernels.

Ferulic acid (µg/kernel)	Soluble fraction	Insoluble fraction
Embryo	trace	0.24
Endosperm	trace	trace
Aleurone layer	2.01	2.79
Pericarp	0.10	2.07

The application of mist under field conditions during kernel growing is related to higher blackpoint frequency in susceptible Néodur and Arbois cultivar checks (Kaan et al. 1993; Régnier 1994) compared to Arcour and Primadur resistant genotypes (Table 2). Under mist conditions, enzymatic PAL, TAL, PO, and PPO activity peaks were observed earlier in kernel development. PAL, TAL, and PO peaks appear to be higher, but in this experiment no significant genotypic differences were observed for enzymatic activities or ferulic acid content (Régnier 1994).

Table 2. Relation between mist and field blackpoint development (blackpoint computed as the percentage of blackpointed seeds).

Variety	Check 1991	Mist 1991	Check 1992	Mist 1992
Arcour	0.5	5.0	1.1	3.0
Primadur	0.2	4.2	4.7	3.9
Arbois	0.6	5.3	15.3	12.8
Néodur	1.2	9.6	6.6	9.6

Modelization of Blackpoint under *In Vitro* Abiotic Conditions

It was hypothesized that the predominance of decompartmentalization of cells under excessive moisture stress leads to blackpoint. This was tested on different kernel phenotypes and genotypes.

After relatively unsuccessful *in vitro* development of immature living kernels with excised embryos due to fungus and bacteria pollution, we turned to devitalized mature autoclaved kernels, using the following standard protocol:

- A sample of 50 healthy kernels of good size, without any visible symptoms of blackpoint on the embryo or furrow zone, is put in a closed tube, then autoclaved at 121 °C (1 bar pressure) for 10 minutes.
- Ten kernels are place, under sterile conditions (laminar flow sterilized air), in a petri dish on gelose medium (6 g Agar/L of twice-distilled water) with the furrow facing up.
- The petri dishes are placed for 21 days at 25 °C under 5,000 lux fluorescent light.

The development of characteristic blackpoint-like lesions on kernel furrows was observed under these conditions (Kaan et al. 1993). Development of discoloration in the furrow zone was noted, using an empirical scale of 1–5.

Genetic and Environmental Effects on Durum Kernels Submitted to Abiotic *In Vitro* Blackpoint Test

The blackpoint reaction of mature kernels placed *in vitro* after autoclave sterilization was highly related to the blackpoint field reaction for the different genotypes (Table 3). Other tests confirm this. The blackpoint susceptibility rating was significantly correlated (0.69) with our abiotic *in vitro* tests for 22 recently registered varieties.

Table 3. Blackpoint intensity on mature autoclaved durum wheat kernels on gelose medium.

Cultivar	Intensity of spotting	Class of significance (Duncan test)	GEVES evaluation of blackpoint resistance
Arbois	3.87	a	4
Néodur	2.79	b	4
Primadur	2.07	c	7
Arcour	1.92	d	8

Recent cooperative trials suggest that kernel production conditions could be involved in the development of blackpoint-like symptoms. Genotype comparisons should be carried out on seed originating from the same crop.

Conclusion

These results clearly indicate that humid, confined conditions may be implied in blackpoint development in the absence of living pathogenic organisms, and even in the absence of living kernel cells.

In contrast to other works about blackpoint, our experience suggests that fungal or insect disease is not necessary for blackpoint damage to the wheat kernel furrow. Humid field conditions or abiotic humid *in vitro* conditions may lead to blackpoint-like symptoms related to the genotypic field reaction.

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Recent Applications of Near-infrared Spectroscopy to Evaluate Durum Wheat Grain Quality

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Introduction

Near-infrared spectroscopy (NIRS) technology has been in routine use in North America for about 22 years for the determination of protein and moisture content in wheat. The success of NIRS technology has paralleled advances in computer technology, which became available in the late 1970s. Near-infra light is found in the electromagnetic spectrum between the visible and the medium-infrared wavelengths (700–2,500 nm). A NIRS instrument detects electronic signals diffusely reflected from a sample surface that is irradiated by the instrument with the light of many wavelengths. The signals at wavelengths specific to the parameters to be determined are amplified and translated by the instrument into composition data such as protein or moisture content. NIRS analysis offers many advantages. It is simple, rapid, non destructive, and environmentally safe. It requires little or no sample preparation and can analyze samples for multiple parameters simultaneously.

Because of the high demand for simple and fast methods to evaluate early-generation germplasm produced annually by the breeding programs at the International Center for Agricultural Research in the Dry Areas (ICARDA), the Neotec 31-EL grain quality analyzer was installed in 1979 and used mainly for protein determination in cereal and legume grain. The Pacific Scientific feed quality analyzer model 51-A was acquired in 1981 to cover a wider range of applications. Since 1992, the NIRSystems Model 5000 scanning monochromator instrument has brought significant improvement to ICARDA's quality test methods. This paper summarizes recent applications of NIRS to simultaneously test for protein content, sedimentation volume, and yellow pigment content in durum wheat whole grain and whole-meal flour. These are among the most important quality parameters used in early generation screening of durum wheat.

Materials and Methods

Samples of durum wheat were assembled from advanced yield, and on-farm trials grown in Syria over the 1992, 1993, and 1994 growing seasons.

The standard test for determination of protein content in wheat and flour is the Kjeldahl test, described in the Approved Method No. 46-12 of the American

Association of Cereal Chemists (AACC), where the total nitrogen is measured and multiplied by the factor 5.7 to give the protein value. The SDS sedimentation test is determined on 3 g of flour obtained by sieving cyclone-ground meal through a 100-mesh (149 micrometer) stainless steel sieve for five minutes (Axford et al. 1978). Yellow pigment is determined by Approved Method No. 14-50 of the AACC.

A NIRSystems Model 5000 scanning NIR spectrophotometer was used for all NIR analysis.

Results and Discussion

Determination of Protein Content in Durum Wheat

Protein content is the most commonly-used criterion for durum grain quality. It plays a major role in determining the durum price in the export market. The potential of semolina/flour in relation to its end-use is due to aspects of protein quantity and quality.

Protein content in durum wheat covers a range from about 8 to 20%, depending in part on variety but more particularly on environmental factors during growth (rainfall and temperature during grain filling for wheat), fertilizer, agronomic practices, biotic stresses, time and amount of irrigation, etc.

In the early 1970s, when NIRS started to be used successfully in the grain industry, all applications required some form of grinding, and attention was drawn to the importance of granularity in the accuracy of NIRS testing (Williams and Thompson 1978). At ICARDA, NIRS has been applied to the determination of protein in durum since July 1980, using the GQA 31-EL and UDY cyclone mill to grind the samples. During 1980, the first commercial NIRS instrument designed specifically for application to whole seed analysis was introduced (Trebor Model 9000). The instrument worked in transmittance mode over the 850–1,050 nm wavelength range. Whole grain analysis eliminated the errors incurred in ground-grain testing by NIRS. The first genereration of NIRS transmittance instruments was not suitable for the analysis of early generation genotypes, since the test procedure required more than 100 g of grain. Improvement in durum wheat protein content determination without grinding the sample was achieved at ICARDA in 1992 using the NIRSystems Model 5000. Table 1 summarizes the analysis of whole and ground durum grain for protein content.

Prediction of Sedimentation Volume in Durum Wheat

Based on the Zeleny sedimentation test, Axford et al. (1978) introduced the sodium dodecyl sulfate (SDS) sedimentation test for predicting baking quality by estimating gluten characteristics. Proteins with good hydration capacity and good oxidation status give higher suspensions. Guidelines are given in Table 2.

Table 1. Accuracy of prediction of protein content in whole and ground durum wheat by NIRSystems Model 5000.

Sample/treatment	r^2 [†]	SEP	High	Low	N
Durum, whole grain	0.98	0.32	20.8	9.4	36
Durum, ground	0.99	0.21	20.8	9.4	46

[†] r^2=coefficient of determination; SEP=standard error of prediction; N=number of observations.

Table 2. Guidelines for gluten strength, SDS sedimentation.

Sediment height(mL)	Gluten strength
over 70	Exceptionally strong
60–69	Very strong
50–59	Strong
40–49	Medium strong
30–39	Fairly weak
20–29	Weak
Less than 20	Very weak

The SDS sedimentation test has been found to be excellent for assessing gluten strength in durum wheat (Dexter et al. 1980). At ICARDA, high correlations were found between SDS sedimentation volume and durum wheat dough strength (judged fat dividing and sheeting stages of two-layered flat [2LF] bread) and spaghetti cooking quality (Williams et al. 1989).

In 1988, Williams et al. first reported a preliminary study on the prediction of several gluten strength parameters, including SDS sedimentation in bread wheat using NIRS, in a cooperative study between the Grain Research Laboratory, Winnipeg, Canada and ICARDA. The coefficient of correlation between NIRS and SDS sedimentation volume was 0.75, and the standard error of prediction was 5.6 mls.

In 1992 the cereal quality laboratory at ICARDA developed promising calibrations using a NIRSystems Model 5000 near-infrared spectro-photometer to predict SDS sedimentation volume in whole grain and ground durum wheat. The efficiency of this study is summarized in Table 3.

Table 3. Accuracy of prediction of SDS sedimentation volume by NIRSystems Model 5000.

Sample/treatment	r^2 [†]	SEP	High	Low	N
Durum, whole grain	0.85	3.66	57	13	36
Durum, ground	0.83	3.70	57	13	37

[†] r^2=coefficient of determination; SEP=standard error of prediction; N=number of observations.

Prediction of Yellow Pigment in Durum Wheat

Yellow pigment is one of the major quality factors in durum endosperm. It is often identified with carotenoids, which could include both carotene and xanthophylls. A high yellow pigment content is essential for the preparation of pasta products, burghul, couscous, and semolina. Classifications are given in Table 4.

Table 4. Guidelines for yellow pigment content.

Yellow pigment (whole meal flour)	Classification
< 5.0 ppm	Low pigment
5.0–7.0 ppm	Medium pigment
> 7.0 ppm	High pigment

The reference method for yellow pigment calls for the use of water-saturated n-butanol. This powerful solvent has an unpleasant odor, and some people are allergic to it. This encouraged ICARDA scientists to develop a visual method for a rapid and direct evaluation of color in ground durum wheat (Williams et al. 1986). The color scale consists of a wooden board (55x8.5x1.5 cm) containing seven holes of 6.5 cm diameter, covered with clear plexiglass. The seven cells are filled with ground durum wheat samples varying in yellow pigment content from 2 to 8 ppm, as determined by water-saturated n-butanol extraction. To use the color scale, tins of ground durum wheat are simply compared visually with the seven cells on the board, and the yellow pigment content of the unknown sample recorded to the nearest 0.5 ppm. After several years, some difficulties were encountered in determinating pigment, mainly because some new materials were grayish or reddish, and were not compatible with the scale.

To avoid the above mentioned problem, NIRS technology was tried, and has been found very promising in the prediction of pigment content in whole grain and ground durum wheat.

At the Grain Research Laboratory, Winnipeg, Canada, Williams and Sobering reported in 1993 that the extension of wavelength range down to the visible area has been shown to be beneficial to the determination of color parameters, including yellow pigment. The NIRSystems 6500 scanning monochromator, with a wavelength range from 400 to 2500 nm, was used in their study. Some of their results are reproduced in Table 5.

Table 5. Accuracy of prediction of pigment content by NIRSystems Model 6500.

Sample/treatment	r^2 †	SEP	High	Low	N
Durum, whole grain	0.84	0.39	10.4	6.4	36
Durum, ground	0.92	0.28	10.4	6.4	36

† r^2=coefficient of determination; SEP=standard error of prediction; N=number of observations (Williams and Sobering 1993).

At ICARDA, a study was conducted in 1994 for the prediction of pigment content in durum flour, using a NIRSystems Model 5000, which scans the wavelength range from 1,100 to 2,500 nm. A total of 108 samples were selected, milled into whole-meal flour, scanned in the NIRS and analyzed by chemical method. Pigment content ranged from 2.7 to 8.9 ppm. Sample sets were assembled for calibration and validation on the basis of reference data at a ratio of 3:1. The best prediction for pigment content in durum flour gave r^2=0.66 and SEP=0.79.

Model 5000 and the ICARDA color scale were compared for efficiency in predicting pigment content. Statistically, the Model 5000 gave a higher correlation with actual pigment content (r=0.83) compared with the scale prediction (r=0.72). Despite the fact that the wavelength range of the Model 5000 does not extend to the visible wavelength region, the results obtained were considered to be sufficiently accurate, compared with the color scale, for screening durum genotypes for pigment content, and were much more efficient.

Conclusion

NIRS can be successfully used to analyze several important quality parameters in durum germplasm. There are no significant differences in accuracy using whole durum grain or ground durum for the determination of protein content and SDS sedimentation volume.

The whole grain technique requires only 20–25 g of grain, which allows early generation materials to be tested for protein content and SDS sedimentation without any grinding. Subsequently, the same samples can be planted.

For yellow pigment content, more accurate results were obtained with ground durum wheat. Ground grain calibration can be used with as little as 2–5 g. The data suggest that use of a NIRS scanning monochromator with a visible wavelength range would improve the results of yellow pigment prediction.

Finally, NIRS greatly improves the efficiency of screening durum wheat germplasm for quality. The reduction in the time required to analyze durum germplasm enables testing of about 250 samples per day for the three important parameters described. Using traditional wet chemical methods, testing of the same number of samples for the same parameters would require 16 working days.

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