GERMPLASM PROGRAM
LEGUMES

Annual Report for [illegible]

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1. INTRODUCTION

The crop improvement research on cereals and legumes at the International Center for Agricultural Research in the Dry Areas (ICARDA) is done by the Germplasm Improvement Program. Among the cereals, it covers barley, durum wheat and bread wheat, while amongst the legumes it covers lentil, chickpea, faba bean, forage legumes and pea. ICARDA has a global mandate for the improvement of barley, lentil and faba bean, and a regional mandate for the improvement of durum wheat, bread wheat, chickpea, pea and forage legumes. The improvement of durum and bread wheat is done jointly with the International Maize and Wheat Improvement Center (CIMMYT), Mexico, which has a global mandate for wheat improvement. Similarly, chickpea improvement is done jointly with the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), India, which has a global mandate for this crop.

To fulfill the global mandate for the improvement of barley, ICARDA has posted a barley breeder in CIMMYT-Mexico to address the needs of barley improvement for Latin America. CIMMYT has placed a durum breeder and a spring bread wheat breeder at ICARDA with a regional responsibility for West Asia and North Africa (WANA). Winter and facultative bread wheat breeding is based in Ankara (Turkey) with a strong backup at the headquarters. In the case of chickpea, ICRISAT posted a chickpea breeder at ICARDA to address the needs of the crop in WANA.

The overall objective of the Germplasm Improvement Program is to increase the productivity and sustainability of the farming systems which include barley, lentil, faba bean, durum wheat, bread wheat, chickpea, grasspea, pea and forage legumes in partnership with NARS, NGO and farmers.

This objective is being pursued through methodologies emphasizing specific adaptation through decentralized breeding, gender-sensitive participatory approaches, use of biotechnology, use of inputs compatible with the preservation and improvement of the resource base, maintenance and enhancement of agricultural biodiversity, and ultimately alleviation of poverty.

The base for most of the research work is at Tel Hadya, where ICARDA's headquarters are located and where additional environments are created by different planting dates and plastic houses. However, research is also conducted in other sites in Syria (Breda, Bouider, Jindiress, Latakia and farmers' fields) and Lebanon (Terbol and Kfardan). All these sites are directly managed by ICARDA. High elevation sites of the national programs of Syria, Turkey, Russia, Iran and Maghreb countries are used, in a collaborative mode, for developing improved winter and facultative barley, bread and durum wheat, lentil, chickpea and forage legumes adapted to cold environments. The research sites and facilities of the national programs of about 50 countries in the five continents, are used jointly for developing breeding material with specific resistance to some key biotic and abiotic stress factors because of the presence of ideal screening conditions and/or expertise there. The process of decentralization of breeding work is being continued and extended with the help of national programs.

The weather conditions during the 1995/96 season are shown in Figure 1.1 for two typically dry sites (Bouider and Breda) with long term rainfall of 235 and 268 mm, respectively, and in Figure 1.2 for two typically wet sites (Tel Hadya and Terbol) with long term average rainfall of 329 and 555 mm respectively. In all four locations, the total seasonal precipitation in the 1995-96 cropping season was higher than the long term average (316, 360, 404 and 578 mm, respectively). In Bouider there was a dry start to the season followed by five months with rainfall well above the average, particularly in March. Breda showed a similar pattern except that starting from November every month was wetter than usual except February. Tel Hadya had a dry start and a dry finish with a severely cold February and an exceptionally wet March. Terbol had a very irregular distribution with a dry spell between December and February. In all the four sites grain filling coincided with maximum temperatures higher than usual, but this affected negatively yields more in Tel Hadya and Terbol than in Breda and Bouider because of rainfall distribution.

One of the major events in 1996 has been the new agreement between CIMMYT and ICARDA for Wheat Improvement in West Asia and North Africa (WANA), signed by the two Directors General in El Batan on September 18, 1996. The agreement recognizes that ICARDA has a regional responsibility for wheat improvement in WANA, that wheat improvement activities in WANA are a joint CIMMYT/ICARDA Wheat Improvement Program, and that the program leader of ICARDA's Germplasm Improvement Program has the overall responsibility of implementing the joint program in consultation with the Director of the CIMMYT's Wheat Program (Fig. 1.3).

Another major event was the Center Commissioned External Review (CCER) of the legume projects, held in Aleppo in early March 1996. Overall, the CCER produced an excellent, well-balanced report which showed a depth of understanding of our research on legumes and gave constructive ideas for change. The CCER made the following four key recommendations regarding legume research:

1. Establish a single, well-resourced, well-coordinated Food Legume Improvement Project to include chickpea, lentil, faba bean and peas.
2. Re-establish the faba bean project at ICARDA.
3. Remodel food legume improvement projects to emphasize decentralized breeding methodology. Some projects such as

lentil, as indicated in the CCER report, are already using a decentralized breeding mode. It was suggested that the improvement projects of the other legumes move in this direction.

4. Focus forage legume work on innovative types of *V. sativa*, species for high altitudes, and low ODAP-*Lathyrus sativus*.

The program accepted these recommendations and has started to make the necessary changes during 1996.

Most of 1996 went into the preparation of the Medium Term Plan 1998-2001, while during the latter part of 1996 the cereal projects as well as the plant protection group made the necessary arrangements for the Center Commissioned External Review of cereals and integrated pest and disease management projects.

During the year the following changes in staff occurred:

a. in January 1996, Dr. S. Ceccarelli was appointed Acting program leader of the program and Dr. W. Erskine associate program leader;
b. in April 1996, Dr. M. El-Bohussini was appointed as consultant Entomologist and Dr. S. Khalil was appointed as consultant for Faba Bean improvement.
c. in October 1996, Dr. M. Tahir, the winter and facultative winter barley breeder, took a new position in Teheran (Iran) as leader of the Iran/ICARDA collaborative project. As a consequence of this, the spring barley project and the winter and facultative barley project were *de facto* merged at the end of 1996;
d. in the fall of 1996 Dr. J. Peacock, the crop physiologist, was appointed Regional Coordinator for the Arabian Peninsula;
e. Dr. K. B. Singh, the chickpea breeder, completed his consultancy, and left ICARDA after nearly 10 years service

in the Center and Dr. R.S. Malhotra was appointed acting chickpea breeder.

More than 70 scientists from 20 different countries spent between few days and few months in the Germplasm Improvement Program. Their activities varied from discussions with staff members to research projects in collaboration with specific scientists. Their contributions to the achievements of the Program are reported in details in the specific sections.

The following special projects were operational during 1996:

1. **Use of DNA-markers in selection for disease resistance genes in barley**, supported by BMZ and in collaboration with Technische Universität München, Lehrstuhl für Pflanzenbau und Pflanzenzüchtung, Munich, Germany (person in charge M. Baum)
2. **DNA Marker assisted breeding and genetic engineering of ICARDA mandated crops** supported by BMZ and in collaboration with University of Hannover, Prof. Dr.H.J. Jacobsen and University of Frankfurt, Prof. Dr. G. Kahl *(person in charge, F. Weigand)*
3. **The use of Biotechnology for Development of ICARDA Mandated crops** supported by UNDP (person in charge F. Weigand)
4. **Improving Yield and Yield Stability of Barley in Stress Environments**, supported by the Government of Italy (person in charge S. Grando)
5. **Farmer Participation and Use of Local Knowledge In Breeding Barley For Specific Adaptation** supported by BMZ and in collaboration with University of Hohenheim (person in charge S. Ceccarelli)
6. **Increasing the Relevance of Breeding to Small Farmers: Farmer Participation and Local Knowledge in Breeding**

Barley for Specific Adaptation to Dry Areas of North Africa supported by IDRC and in collaboration with IRESA (Tunisia) and INRA (Morocco) (person in charge S. Ceccarelli)

7. **Resistance to nematodes in lentil and chickpea,** in collaboration with the Institute of Nematology of Bari, (persons in charge R.S. Malhotra)
8. **Development of Chickpea Resistant to Biotic and Abiotic Stresses using Interspecific Hybridization and Genetic Transformation** supported by the Government of Italy and in collaboration with ENEA, University of Napoli and the University of Tuscia in Viterbo (person in charge R.S. Malhotra)
9. **Fusarium Wilt in Chickpea**, supported by the Government of Spain and in collaboration with INIA (person in charge R.S. Malhotra)
10. **Wheat Adaptation Studies for Wheat in WANA and Australia**, supported by Grains Research Development Council (GRDC) Australia, in collaboration with the University of Sydney (person in charge G. Ortiz-Ferrara)
11. **International Durum Wheat Improvement**, supported by Grains Research Development Council (GRDC) Australia, in collaboration with the New South Wales Department of Agriculture (person in charge M. Nachit)
12. **Coordinated Improvement Program for Australian Lentils**, supported by Grains Research Development Council (GRDC) (person in charge W. Erskine)
13. **Improvement of drought and disease resistance in lentils in Nepal, Pakistan and Australia**, supported by the Australian Centre for International Agricultural Research (ACIAR) (person in charge W. Erskine)
14. **Central and West Asia Rusts Network-enhanced Regional Food Security Through the Development of Wheat Varieties with**

Durable Resistance to Yellow Rust (person in charge O. Mamluk)

15. **West Asia and North Africa Dryland Durum Improvement Network (WANADDIN)** supported by IFAD (person in charge M. Machit)
16. **Faba Bean in China**, supported by the Australian Centre for International Agricultural Research (ACIAR) and in collaboration with the Genetic resources Unit (person in charge L. Robertson)
17. **Integrated Management of Pest and Diseases**, supported by BMZ (person in charge K. Makkouk)
18. **Durum Wheat Improvement** supported by the Australian Centre for International Agricultural Research (ACIAR)(person in charge M. Nachit)
19. **Kabuli Chickpea** supported by the Australian Centre for International Agricultural Research (ACIAR)(person in charge R.S. Malhotra)

In addition the program is actively involved in the activities of the six Regional Programs and in the following special projects:

Mashreq and Maghreb (M&M) Project
Mediterranean Highland Project
Barley Improvement Project in Ethiopia
Problem-solving Regional Network Project in Egypt Ethiopia, Sudan and Yemen
Matrouh Resource Management Project in Egypt

This report is published in two sections, one with the results of cereal crops improvement work and one with results of the legume crops improvement work.

Most of the results reported in the two sections were obtained during the 1995-96 season, although work done in earlier years is also reported when considered important. The training and network activities, the scientific publications of the program's staff and an updated list of varieties released by national programs are also reported.

As mentioned earlier, much of the work reported here has been done in collaboration with our colleagues in the national programs in WANA and other developing countries and in some institutions in the industrialized countries. Space limitations prevent to mention all our collaborators individually, but to all of them goes our most sincere appreciation. Eventually, the program is greatly indebted to the support staff at the headquartes as well as in various substations: without their hard work, competence and dedication none of the work reported here would have been possible.

(S. Ceccarelli)

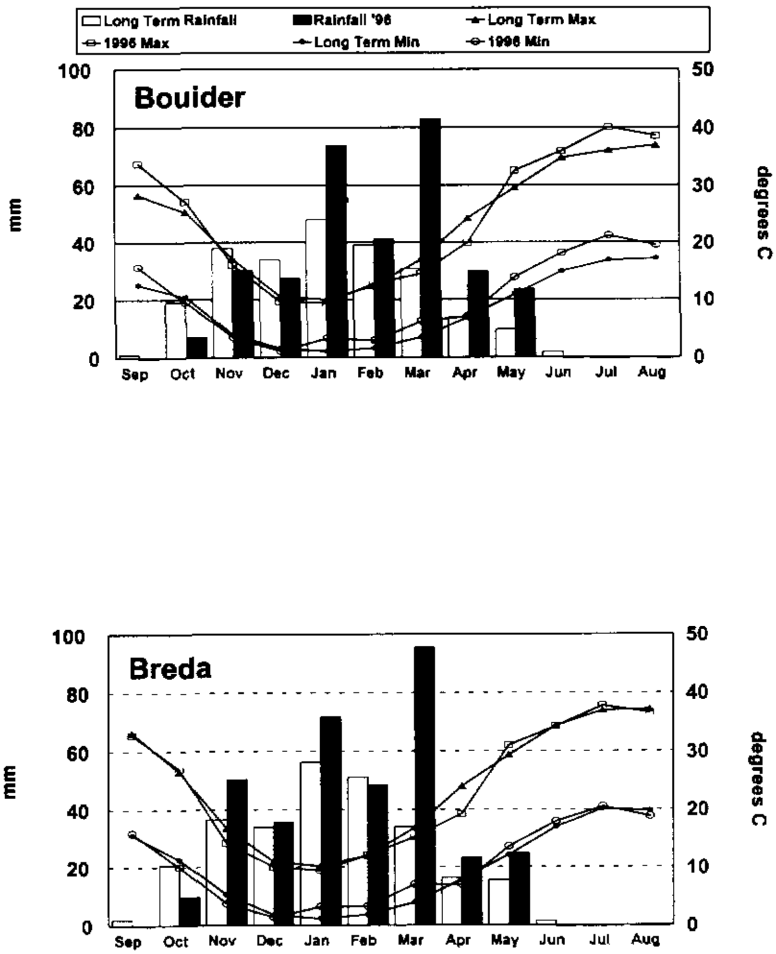

Fig. 1.1. Weather conditions at Bouider and Breda during 1995-96.

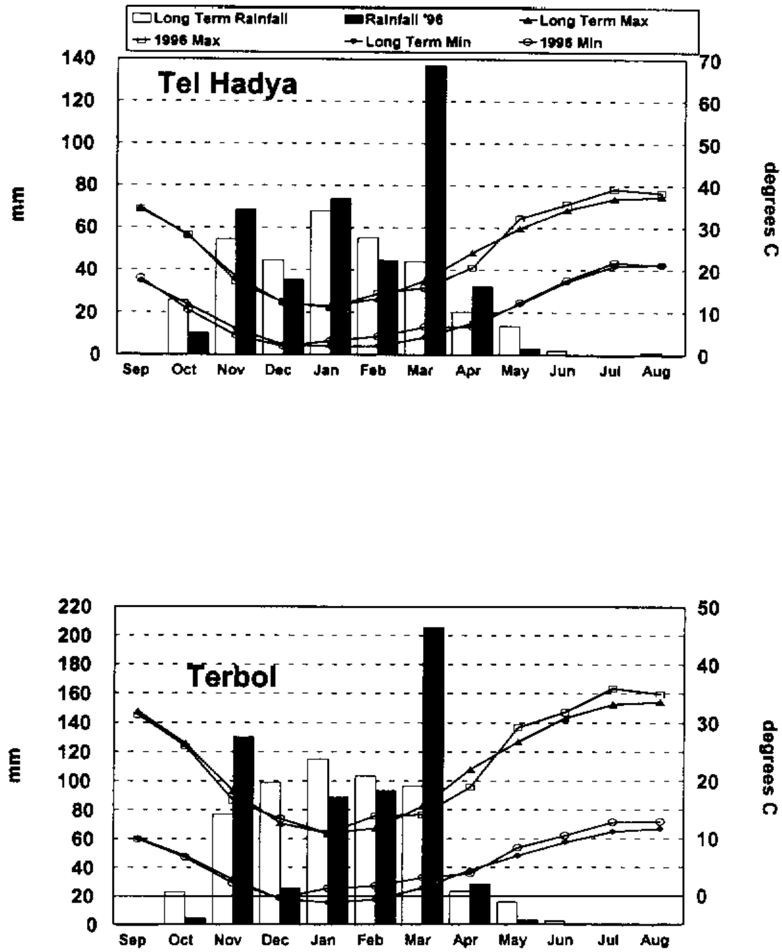

Fig. 1.2. Weather conditions at Tel Hadya and Terbol during 1995-96.

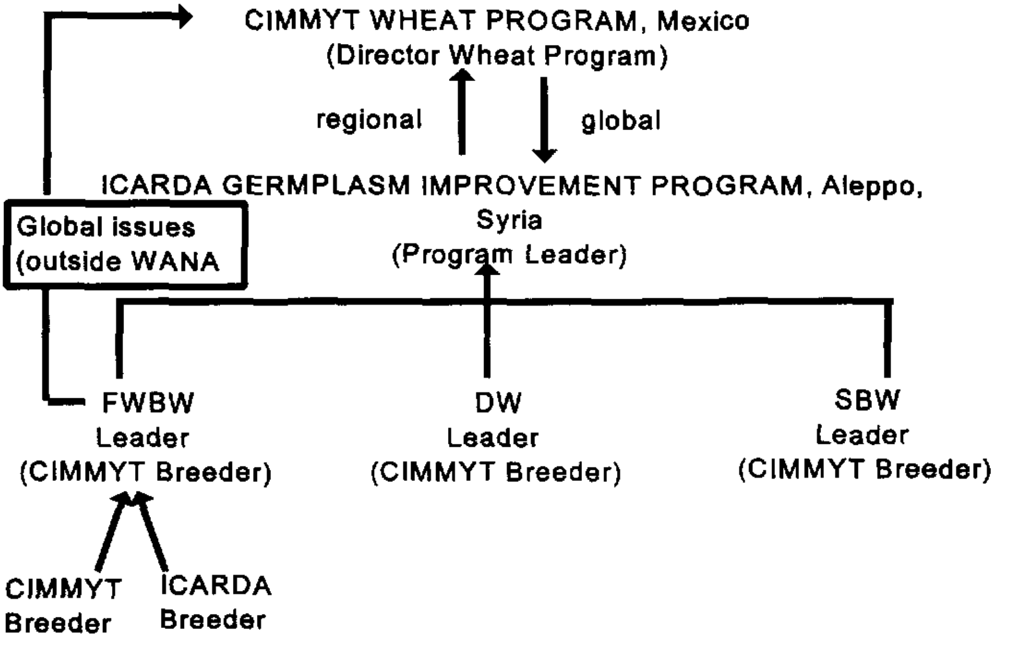

Fig. 1.3. Reporting arrangements for CIMMYT/ICARDA Scientists in the joint Wheat Improvement Program

2. LENTIL IMPROVEMENT

Introduction

Average lentil yields are low because of poor crop management and the low yield potential of landraces. In South Asia and East Africa, diseases are also a major constraint to production. Accordingly an integrated approach to lentil improvement is being pursued at ICARDA covering the development of both improved production technology and genetic stocks. A high priority has been placed on transferring to national programs the results of research on lentil harvest mechanization systems to reduce the high cost of harvesting by hand in the West Asia and North Africa region.

2.1. Lentil Breeding

2.1.1. Base Program

2.1.1.1. Lentil Adaptation and Breeding Scheme

The lentil is an under-exploited and under-researched annual legume. From the onset at ICARDA, we studied the variation in the world germplasm collection to understand factors affecting lentil adaptation to direct the breeding program. Such diverse factors as winter-hardiness, efficiency of iron uptake, phenology as related to the length of the growing season, the sensitivity of flowering to temperature and photoperiod, response to irrigation and disease resistance have all contributed to the pattern of variation found in the world germplasm collection.

Additional information on the specificity of adaptation within the crop has come from collaborative yield trials of common entries selected in different locations. For example, the North African Regional yield trial on lentil was established in 1990 to comprise the best lines selected in Algeria, Libya, Morocco and Tunisia. The results of this regional yield trial showed that lentils selected in the various countries of N. Africa differ substantially in phenology, indicative of the need for specific adaptation to the range of environments in the region. The requirements of Libya, Tunisia, and lowland Algeria are met by lines emanating from the ICARDA West Asian breeding program. However, late-maturing material is required for high altitude areas of Algeria and early-maturing lines are required in Morocco with resistance to rust.

Armed with this understanding of the specific adaptation of the lentil crop and the various consumer/end-use quality requirements of different geographic areas, we have designed the base breeding program as a series of separate, but finely targeted, streams linked closely to national breeding programs.

The three major target agro-ecological regions of production of lentil are 1. S. Asia and E. Africa 2. Mediterranean low to medium elevation and 3. High elevation area of West Asia and North Africa. These correspond to the maturity groups of early, medium and late maturity. Within each of these major regions there are specific target areas. Thus, for example, within the Mediterranean low to medium elevation region, specific target areas are 1. the major production area of 300-400 mm annual rainfall, 2. arid areas with < 300 mm annual rainfall, 3. Morocco, where there is the additional problem of rust and 4. Egypt, where lentil is irrigated. Each of these target areas has slightly different blends of key traits for recombination. The target areas/regions and key traits for selection/recombination are tabulated in Table 2.1.

Based on the premise that local selection for adaptation to a specific target area is the most efficient selection method, selection at ICARDA in West Asia is limited to adaptation to the home region - Mediterranean low to medium elevation - and for traits where we have a comparative advantage such as vascular wilt resistance. As a result, the breeding program has decentralized to work closely with national programs.

Table 2.1. Target agro-ecological regions of production of lentil and key breeding aims.

Region	Key traits for recombination
Mediterranean low to medium elevation	
1. 300-400 mm ann. rainfall	Biomass (seed + straw), attributes for mechanical harvest & wilt resistance
2. <300 mm ann. rainfall	Biomass, drought escape thru' earliness
3. Morocco	Biomass, attributes for mechanical harvest & rust resistance
4. Egypt	Seed yield, response to irrigation, earliness & wilt resistance
High elevation	
1. Anatolian highlands	Biomass & winter hardiness
2. N. African highlands	Seed yield & low level of winter
South Asia and E. Africa	
1. India, Pakistan, Nepal & Ethiopia	Seed yield, early maturity, resistance to rust, ascochyta and wilt
2. Bangladesh	Seed yield, extra earliness & rust resistance

For the home region, the breeding program uses a bulk-pedigree system with off-season generation advancement (at Terbol, Lebanon 950 m elevation), single plant selection in the F_4 generation, and selection of progeny rows for vascular wilt resistance in the F_5 generation. For the other regions, crosses are agreed with cooperators and made at Tel Hadya; the generations advanced in the off-season and the segregating populations shipped to national cooperators for local selection. We started making specific crosses in 1985 and since then have made specific crosses for Algeria, Bangladesh, India, Iraq, Jordan, Libya, Morocco, Nepal, Syria and Turkey. A total of approximately 200 crosses are made annually. One avenue for the distribution of segregating material is through these country-specific crosses. The international trial network provides another system whereby these crosses can be tested sub-regionally. Selections made by NARS are fed back into the international trial system for further distribution. The results from this decentralized system are described in section 2.1.2.

2.1.1.2. Yield Trials

Selections from the breeding program for the Mediterranean low to medium elevation region are tested at three locations varying in their annual average rainfall, namely Breda (long-term average annual rainfall total *ca* 260 mm) and Tel Hadya (*ca* 335 mm) in Syria and Terbol (*ca* 550 mm) in Lebanon in preliminary yield trials in the F_7 generation and in advanced yield trials the following generation and season. The lines are also re-tested synchronously for vascular wilt resistance in the wilt-sick plot at Tel Hadya (*see Section* 2.1.1.3) to ensure that only high-yielding, wilt resistant lines are advanced in the breeding program.

In 1995/96 season, Tel Hadya and Breda received totals of 405 and 360 mm rainfall, respectively, which were higher than the long-term average annual rainfall total in both locations. However, at Terbol there was 525 mm rainfall, less than the long-term average rainfall. The winter was less cold than normal in Syria with 32 frost d at Breda and

28 frost d at Tel Hadya. By contrast winter was cooler and longer at Terbol in Lebanon, where there were 63 frost events during 1995/96 crop season.

A total of 240 entries were tested at Tel Hadya, 100 entries at Terbol and 125 entries at Breda in different trials (Table 2.2). The overall evaluation for seed yield showed that the percentage of entries significantly exceeded the best check was more in Breda than at the other two sites. However a considerable number of test entries out-yielded the best check in all the locations with respect to both seed and biological yield. The average seed yield over trials varied from 1974 kg/ha at Terbol, through 1500 kg/ha at Tel Hadya to 758 kg/ha at Breda. The corresponding biomass yields were 6.8 t/ha in Terbol, 5.9 t/ha in Tel Hadya and 5.0 t/ha in Breda. The harvest index (HI) was strikingly lower in Breda, at HI = 0.15, compared to Tel Hadya (HI = 0.25) and Terbol (HI = 0.28). Although the HI in Breda was similar to that of last season, the average seed and biological yield were much higher than in the 1994/95 crop season. The mean co-efficient of variation for seed yield over trials was highest at Breda (18.7%), followed by Tel Hadya (15.9%) and Terbol (8.3%), the converse of rainfall.

Selection of best entries was done on the basis of mean percent over the grand mean across locations and their performance was compared with the best of the checks. The selected lines from preliminary yield trials constituted advanced yield trials for 1996/97 season and the entries selected from advanced yield trials were included in international yield trials.

(W. Erskine and A. Sarker)

Table 2.2. Results of the lentil yield trials (preliminary and advanced) for seed (S) and biomass (B) yields (kg/ha) at three contrasting rainfed locations, Terbol (Lebanon), Tel Hadya and Breda (Syria) during the 1995/96 season.

Location	Terbol		Tel Hadya		Breda	
	S	B	S	B	S	B
Number of trials	4	4	10	10	5	5
Number of test entries*	100	100	240	240	125	50
% of entries sig. ($P<0.05$) exceeding best check**	2.3	0	1.0	11.2	5.5	11.8
% of entries ranking above best check (excluding above)	26.1	17.0	18.6	42.0	26.4	16.4
Yield of top entry (kg/ha)	2464	8707	1999	8653	1226	6085
Best check yield (kg/ha)	2173	7466	1711	5943	880	4820
Location mean (kg/ha)	1974	6845	1500	5916	758	5039
Mean C.V. (%) over trials	8.3	10.6	15.9	12.9	18.7	8.4
Mean % advantage of lattice over RCB analysis across trials	12.3	23.2	10.7	5.6	7.7	26.8
Mean % advantage of NNA*** over RCB analysis across trials	59.7	31.7	34.2	16.9	35.2	36.5

* Entries common over locations.
** Large-seeded checks: ILL 4400-long-term, ILL 5582-improved; small-seeded checks: ILL 4401-long-term, ILL 5883-improved.
*** NNA = Nearest neighbor analysis

2.1.1.3. Screening for Vascular Wilt Resistance

Vascular wilt caused by *Fusarium oxysporum* f. sp. *lentis* is the major fungal disease of lentil in the Mediterranean region. As chemical control for the disease is not feasible, host plant resistance is the most practical method of disease management. Aspects of biocontrol of vascular wilt and the host range of *Fusarium oxysporum* f. sp. *lentis* are covered in Section 2.2.

<u>Evaluation of a large-seeded lentil germplasm for resistance to wilt</u>

Sources of resistance to the disease have been more commonly identified in a small-seeded background than among large-seeded lentils (See Ann. Report 1995). However, systematic screening for resistance among large-seeded accessions in the ICARDA lentil collection has not been conducted. As wilt symptoms appear only after the onset of flowering, an investigation of the relationship between the timing of the onset of flowering and resistance was warranted.

Accordingly, a collection of 880 large-seeded accessions from 29 countries was planted in the wilt sick plot at Tel Hadya farm on January 11, 1996. Forty seeds of each accession were sown in rows, 50 cm long, 30 cm apart with a susceptible check (ILL 4605) repeated after every four test entries. A randomized complete block design with three replicates was used.

Percentages of wilted plants/row were recorded four times during the growing season with the dates selected according to the disease progression, which is strongly environmentally influenced.

Grand means for percentage of wilted plants at the four scoring dates were: 9%, 21%, 29% and 45%, respectively, clearly indicating the overall progression of the disease with time. However, the rate of disease development varied over genotypes with some genotypes showing early wilting during reproductive growth, whereas others exhibited symptoms late. This is illustrated by the plot of the genotype means at Score 1 on April 25 and their score (4) on May 15 (Figure 2.1).

Analysis of variance for the 880 accessions permitted the identification of 129 resistant accessions (% wilted plants < 20%), among which 30 accessions were highly resistant (% wilted plants < 10%). The resistance of these new sources will be re-tested next season.

To understand the association between wilt incidence and time to flowering, the accessions were classified into four groups (Table 2.3). Most accessions flowered between 116 and 125 days after sowing. The correlation between time to flower and wilt incidence was $r = -0.26$ ($P < 0.01$), indicating that in general early flowering accessions are

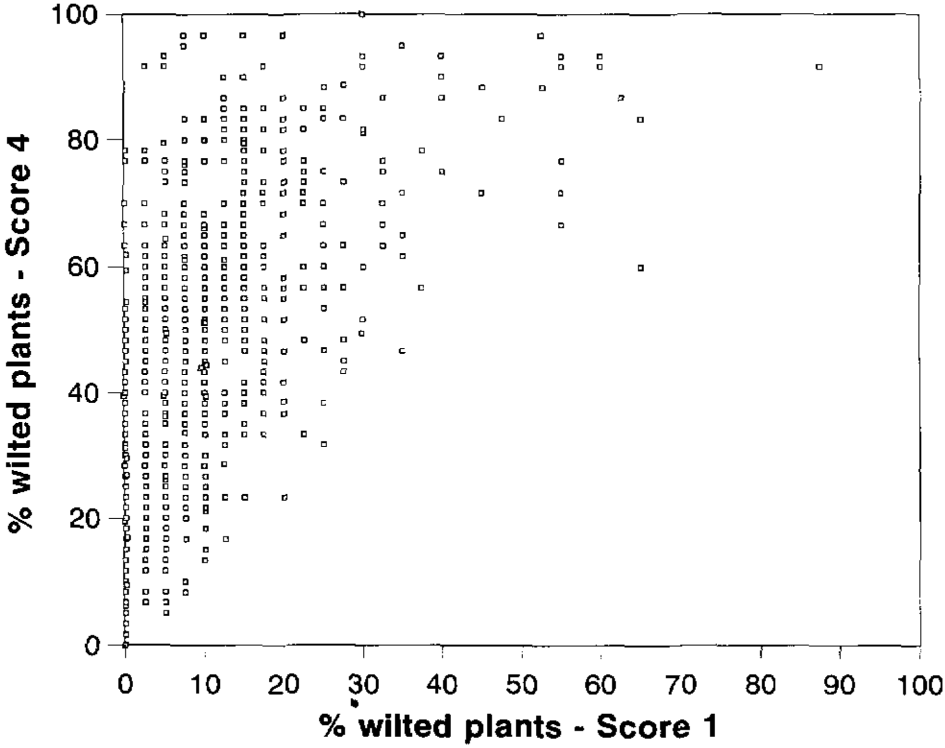

Figure 2.1 Point graph illustrating the mean of percent wilted plants among 880 large-seeded lentil germplasm accessions at Score 1 (April 25) and Score 4 (May 15).

more susceptible to wilt than the late flowering ones. However, the percentage of variation in wilt incidence accounted for by time to flower was only 7% and the distribution of both traits in Figure 2.2 shows the presence of many exceptions - wilt resistant accessions in a late flowering background.

Table 2.3. Distribution of the 880 accessions based on days to 50% flowering (DFLR).

DFLR/days	99-105	106-115	116-125	126-136
No. of accessions	28	269	571	12
% in each group	3.2	30.6	64.9	1.4

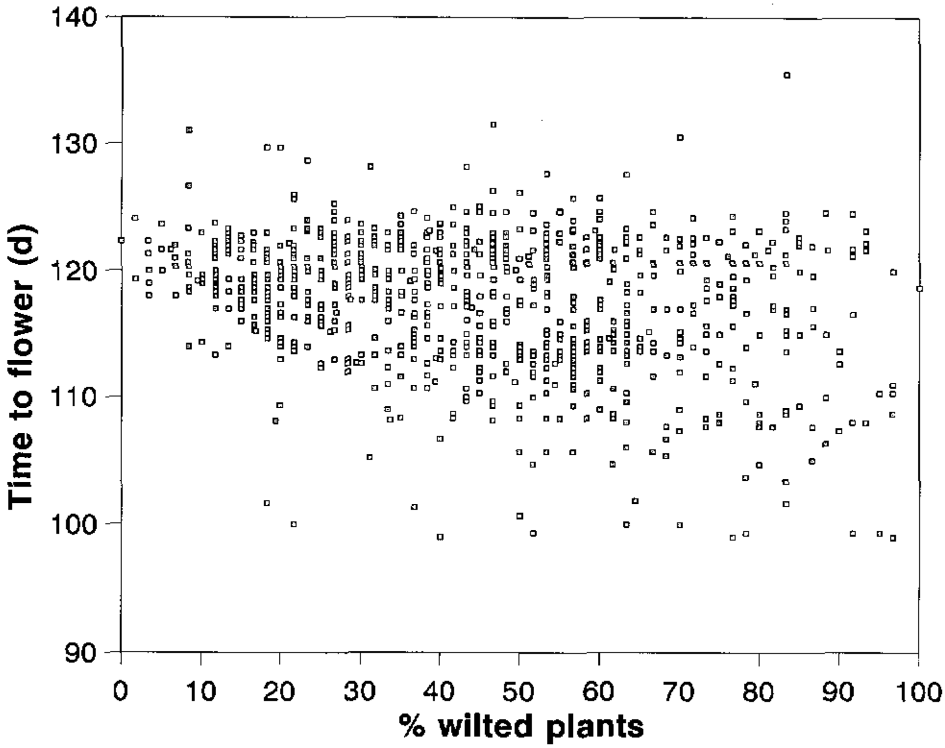

Fig. 2.2. Distribution of accession means for time to flower and percentage of wilted plants of large-seeded lentil germplasm.

Among the large-seeded accessions, the correlation coefficient between seed weight and wilt incidence was low and non-significant at $r = 0.05$ ($P > 0.05$).

The association between country of origin and wilt incidence was found highly significant in a one-way ANOVA by country of origin. There were a total of 11 countries represented by more than five large-seeded accessions per country (in a sub-set of 278 accessions). Among such countries, the 65 resistant accessions were concentrated in five countries of origin, namely Chile, Iran, Greece, and India and Turkey (Table 2.4). These results correlate well with an earlier evaluation of a lentil core collection (all seed sizes), in which resistance was found particularly in accessions collected from Chile, Iran, Greece and India.

Table 2.4. Distribution of resistance to vascular wilt based on country of origin (subset of 278 accessions of known origin (>5 accessions/country) and seed size).

Country	No. entries tested	No. resistant accessions	% of resistant accessions
Chile	125	50	40
Iran	90	7	7.8
India	7	4	57
Greece	14	1	7.1
Turkey	42	3	7.2

Screening of breeding material for wilt resistance

In addition to the identification of new sources of resistance, 410 lines of breeding material were screened in the wilt sick plot at Tel Hadya in the 1995/96 season. This follows the screening of 897, 1229 and 1061 breeding lines in the 1992/93, 93/94 and 94/95 seasons, respectively. The tested lines may be grouped into two categories: Cycle I - new untested lines and Cycle II - lines tested previously (confirmation of their resistance in the previous season). On the basis of mean over replicates, the percentage of entries with a highly resistant (0-5% wilted plants, mean over replicates) or resistant (>5-20% wilted plants, mean over replicates) response to lentil vascular wilt were 67.5% for Cycle I and 90.4% for Cycle II, indicating the reliability of the screening method. However for breeding purposes, we have selected for advancement a specific subset of the resistant lines - those which have a maximum plot score of < 20% wilted plants in the last score. This screening is a key and integral part of the breeding program; this is elucidated in section 2.1.1.1.

(B. Bayaa (Aleppo University) and W. Erskine)

Inheritance of resistance to lentil vascular wilt

In the only reference to the genetics of resistance to lentil vascular wilt, Kamboj *et al.* (1990) reported that, in India, the inheritance was controlled by five independently segregating genes, based on the reaction of individual plants.

We investigated the inheritance of resistance to wilt using six parents contrasting in disease reaction in seven cross combinations. The segregation in five of the crosses was examined in trays under artificial inoculation in the plastic house. The results of the F_2-derived F_3 generation progeny test showed a segregation pattern among progenies of 3 resistant to 1 susceptible for the three crosses involving the susceptible parent (Ann. Report 1995). This is indicative of a single recessive gene for susceptibility.

The segregation pattern of individual F_2 plants was also studied. However, on an individual plant basis the distinction between the moderately resistant parents and the susceptible parent was small, opening the possibility of some individual plants being mis-classified for disease reaction. Consequently row data, such as that of the F_2-derived F_3 generation progenies, may be considered reliable, whereas data based on individual plants, even in artificially-inoculated soil in trays, is considered problematic.

Two of the crosses were between the highly resistant parent and a moderately resistant line. These differences in the level of resistance were significant. The broad-sense heritability values for resistance in these two crosses were 88.7 % and 84.1 %. Thus, although susceptibility is controlled by a single recessive major gene, there are also significant differences in the level of resistance and such differences are controlled by polygenes. The relatively high heritability values suggest that some response to selection among the resistant types for a high level of resistance may be expected.

Another cross (L92-17-2 x L692-16-1(S)) of lentil between wilt-resistant and susceptible parents was examined in the field in ICARDA's wilt-sick plot in three replicates in 1995 as F_2-derived F_4 progeny rows and in 1996 as F_8 recombinant inbred lines (derived from single seed descent). In the field, wilt incidence was assessed as the percentage of wilted/dead plants in a plot (row of 40 plants).

In 1995, the resistant parent had 3.3 % wilted plants with a standard error of ± 16.0, whereas the susceptible parent had 58.3 % wilted plants (Fig. 2.3). There was a discontinuous distribution *of the F_2-derived F_4 progenies with* no progenies rated between 31 and 40 % wilted plants. The segregation pattern of the replicated F_2-derived F_4 progenies fitted a 3:1 ratio of resistance to susceptibility ($\chi^2 = 0$), confirmation of a single recessive gene for susceptibility.

In 1996, the parents and F_8 recombinant inbred lines from single seed descent of the same cross were grown in the same manner and wilt-sick plot. The resistant parent had 1.8 % wilted plants and the susceptible parent had 48.3 % wilted plants (Fig. 2.3). The results showed a continuous distribution among F_8 recombinant inbred lines for resistance, which contrasts with the clear Mendelian segregation pattern observed in the previous season. We will run a quantitative trait locus analysis of the 1996 data to estimate the number of loci involved and repeat the assessment of parents and F_8 recombinant inbred lines for resistance vascular wilt in the field in the 1996/97 season. Efforts are also underway to identify linked DNA markers for this important trait.

(B. Bayaa, A. Abbas (Aleppo *University*), I. Eujayl, W. Erskine and M. Baum)

2.1.1.4. Screening for Winter Hardiness

Lentil is currently sown in spring in Iran and Turkey at

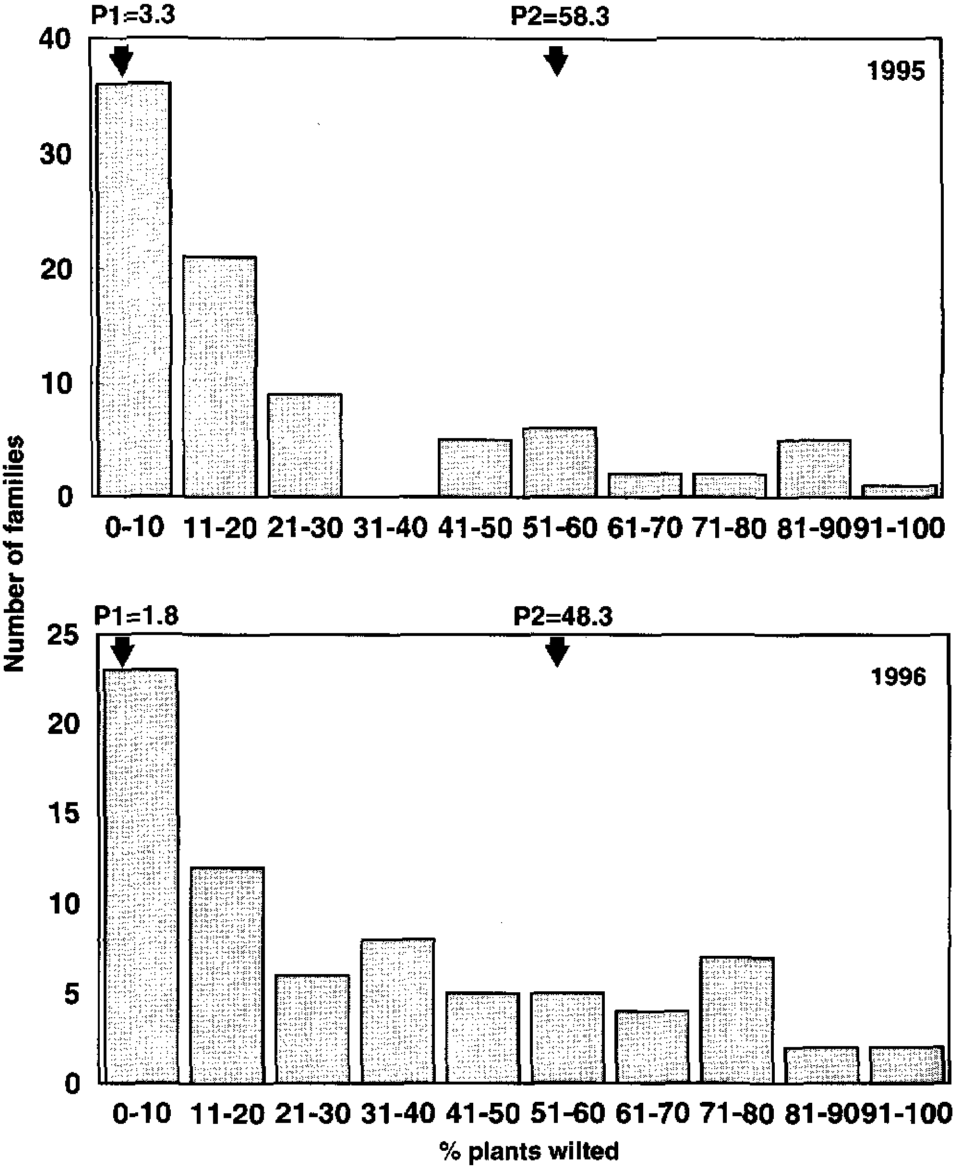

Fig. 2.3. Distribution of mean percentage wilted plants of F_2-derived F_4 progenies in 1995 and F_8 lines from single seed descent in 1996 of a single cross field-tested against lentil vascular wilt in a sick-plot.

elevations above approximately 850 m elevation on *ca* 400,000 ha. Research in Turkey has indicated that yields may be increased by up to 50 % by early sowing in late autumn with winter hardy cultivars. However, the use of such cultivars is not yet widespread in Turkey, because at elevations above *ca* 850 m the level of winter hardiness in the current cultivars is inadequate in cold winters.

A major program to recombine yield with the necessary winter hardiness is underway at the Central Research Institute for Field Crops, Ankara, Turkey through field screening. Two complementary approaches are being followed. In the first approach, winter-type germplasm collected from South-East Anatolia is being selected (Table 2.5). In the second approach, crosses with winter-hardy germplasm sources and early generation material are being produced by the ICARDA program in Aleppo, Syria and then segregating populations are selected under severe winter conditions in highland Turkey (see Section 2.1.1.1).

Table 2.5. Selection of Turkish lentil germplasm for winter-hardiness (WH) at Central Research Institute for Field Crops, Turkey from 1990-1997.

Year	Activity
1990/91	Collection of 152 land race populations from S.E. Anatolia
1991/92	5604 single plant selections made - selection for WH
1992/93	880 progeny rows - selection for WH
1993/94	340 preliminary yield trials, 1 location - weak sel. for
1994/95	WH
1995/96	325 yield trials, 1 location - selection for WH
1996/67	172 yield trials, 4 locations - selection for WH
	45 yield trials, 4 locations

In the 1995/96 winter season, 172 selected red-cotyledon lines from germplasm were tested at Hymana, Konya, Sivas and Yozgat (Turkey). Screening for winter-hardiness was only possible at Haymana, where the absolute minimum temperature reached -16 ^{0}C. At the other sites the susceptible control was undamaged in the relatively mild winter. The mean yields of the winter yield trials varied from 2977 kg/ha at Yozgat to 721 kg/ha at Haymana. Next season a selected set of 45 lines will be sown at the same sites. This lines will form the germplasm base of highland winter lentil technology. Research on the agronomy of these new winter-hardy lines is also underway particularly on the key issue of weed control.

In Iran screening for winter-hardiness has been undertaken at Gazvin by the Seed and Plant Improvement Institute. The best large-seeded selections are ILL 590 and 857. On-farm trials of winter sowing are in progress in the area surrounding Gazvin.

In addition, as an aid to the field-based selection for winter-hardiness, we are exploring the use of marker-assisted selection. Recombinant inbred lines (RIL) (1084 total) at the F_6 generation have been prepared at USDA/ARS, Washington State University for a total of 10 crosses segregating for winter-hardiness. These will be used for a quantitative trait locus analysis of winter-hardiness in collaboration with the Turkish program. In a related activity at Tel Hadya, Syria, the parents and 87 F_8 RIL derived by single seed descent from a single cross (L92-17-2 (P_1) x L692-16-1(S)(P_2)) were planted in Dec. 1995. In January 1996 following a period of relatively warm winter weather (daily max. & min. temp. = 14 & 3.7 °C), the temperature dropped at night to an absolute minimum of -6.6 °C after 34 days of sowing. There were clear differences between the parents in susceptibility to cold. The parents and RIL were scored for severity of damage on a 1-9 scale with 1 = no damage and 9 = plants killed. The parents were scored as $P_1 = 1$ and $P_2 = 5$. The RIL population showed a discontinuous segregation pattern of 46 resistant and 41 susceptible lines, giving $\chi^2 = 0.184$ ($P < 0.05$) for a single gene segregation (Fig. 2.4). Initial analysis for linked DNA-markers at a stringent LOD score and recombination frequency showed linkage between a RAPD marker (OPS16b) and cold tolerance at 9.1 cM in a data set of 127 segregating loci. We will now undertake a quantitative trait locus analysis for the trait and re-test the population against winter cold. But it is clear from this preliminary research that markers for cold-related traits are possible to find in lentil, encouraging the focus on finding markers for winter-hardiness at high elevations.

(N. Aydin, A. Aydogan (Central Research Institute for Field Crops, Ankara, Turkey), and I. Eujayl, W. Erskine and M. Baum)

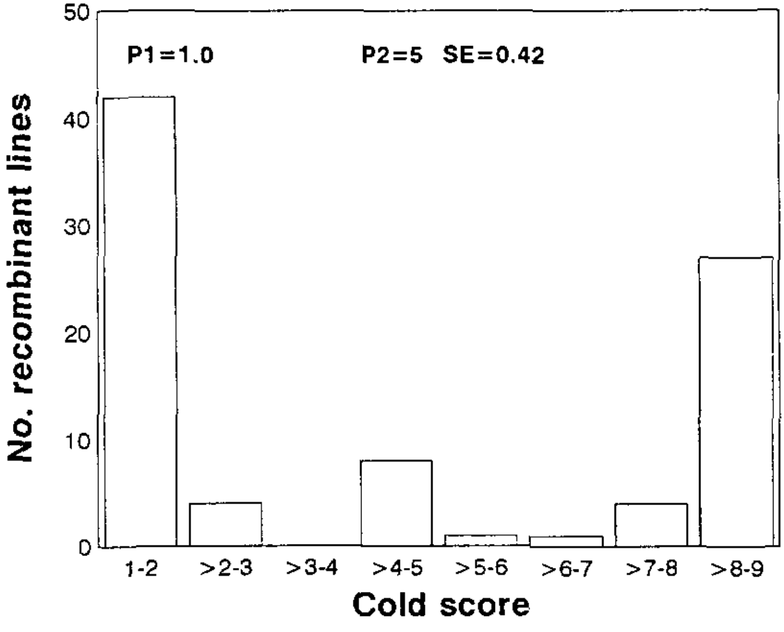

Fig. 2.4. Distribution of mean cold damage rated on a 1-9 scale (1 = no damage; 9 = all plants killed) of F_8 lines from single seed descent of a single cross grown at Tel Hadya, Syria in 1995/96.

2.1.1.5. A Bottleneck in Lentil: Widening the Genetic Base in South Asia

Lentil (*Lens culinaris* Medikus) was developed from *Lens culinaris* spp. *orientalis* (Boiss.) Ponert in the Eastern

Mediterranean region as one of the first domesticated plants (see map far early archaeological remains Fig. 2.5). The crop spread eastward into the Indo-Gangetic Plain around 2000 BC. Today, approximately half of the worlds area (48%) of lentil is in South Asia, where indigenous lentils exhibit a marked lack of variability. This bottleneck has limited breeder's program in this region.

We briefly review evidence of a bottleneck in lentil in South-Asia and then detail progress in widening the genetic base in that region.

According to Barulina (1930), lentil in South Asia is of an endemic group, named grex *pilosae*, characterized by strong pubescence on the vegetative organs giving a grey-green color to the foliage. Additionally, *pilosae* lentils are characterized by short/rudimentary tendrils, a recessive allele at the tnl locus. These two qualitative characters clearly differentiate the lentil of South Asia from other cultivated lentils.

Land race accessions from South Asia are characterized by early flowering and maturity, low biological yield, short stature and small seeds. Quantitative variation among nine characters was assessed and expressed as mean co-efficient of variation (Table 2.6). India and Pakistan lie among those countries with lowest quantitative agro-morphological variation.

Table 2.6. Average sown area (ha) and mean co-efficient of variation (CV%) over nine morpho-agronomic characters.

Country	Area (1000 ha)	No. of accessions	CV (%)
India	1183	100	15.7
Pakistan	62	14	16.6
Iran	139	100	19.0
Syria	92	31	19.0
Turkey	747	77	19.1
S.E.D.			2.63
World	3298		

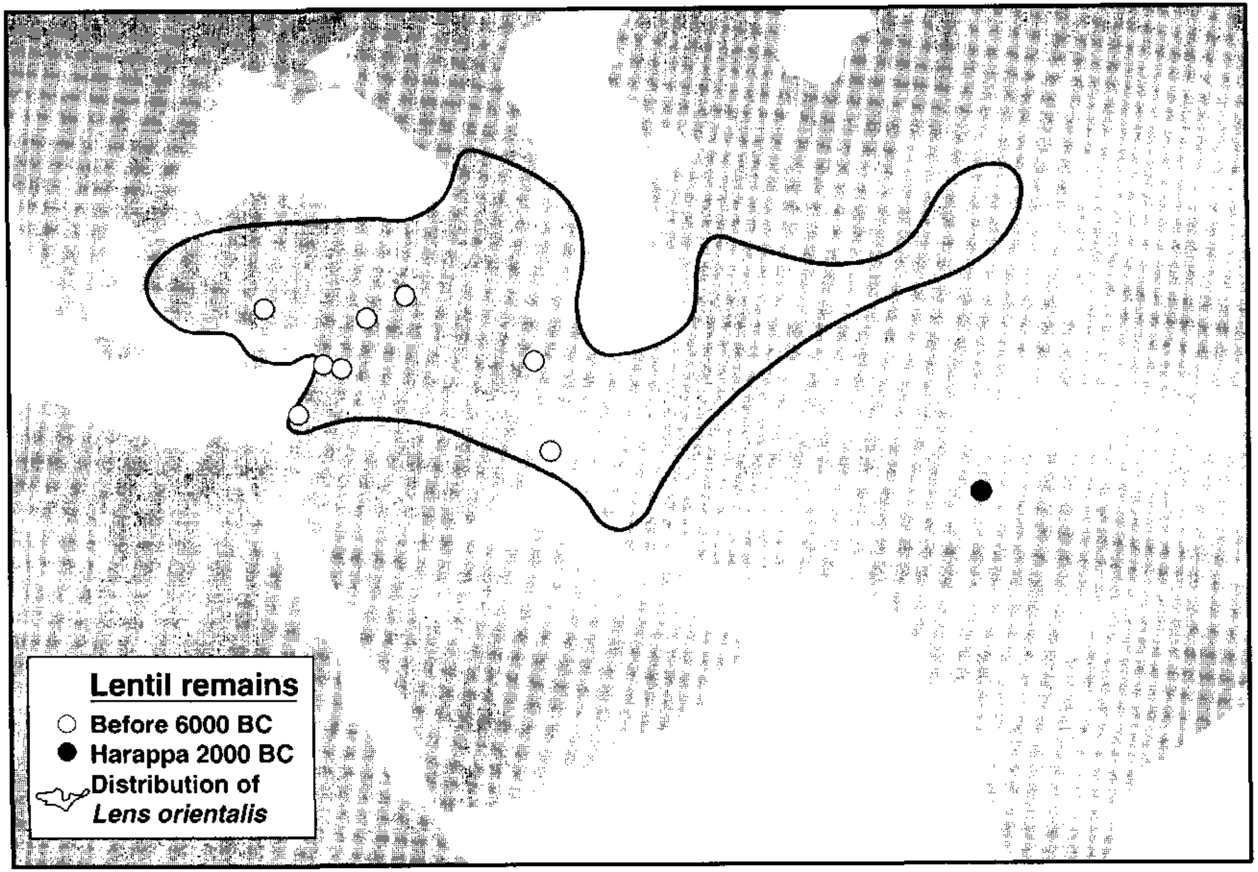

Fig. 2.5. Map illustrating the current distribution of *Lens orientalis*, early (pre-6000 B.C.) archaeological finds of lentil and the earliest lentil finds in South Asia.

Following selection for local adaptation, the lentil of South Asia also differs physiologically from that of West Asia. Germplasm from South Asia is more sensitive to temperature control of flowering, less responsive to photoperiod and more cold susceptible than material from West Asia. Considering adaptation to the edaphic factors of iron (Fe) and boron (Bo) deficiencies, land races from South Asia are tolerant to boron deficiency but susceptible to iron deficiency; whereas, West Asian germplasm is predominantly iron efficient but boron inefficient.

Together, the individuality of S. Asia germplasm and the fact that, lentil germplasm from India is among the least variable among the lentil-producing countries, despite India being the largest producer in the world are the evidence of a genetic bottleneck in South Asia. It is probable that the founder population adapted to the new environment in S. Asia was small in number and had low variability.

A start was made with the introduction into lowland S. Asia of "Precoz" (ILL 4605), an early flowering, large seeded (4.5 g/100 seeds) lentil. It has been released for commercial cultivation as "Manshera 89" in the wetter region of Pakistan. Its early flowering synchronizes with the flowering of South Asia germplasm, allowing artificial crossing. "Precoz" and its derivatives have been included in every crossing block in India with the result that 18 (15%) of 89 lines tested in the trials of the All-India Coordinated Improvement Project in the 1993/94 season were from "Precoz" crosses.

For the highlands of S. Asia, plant introduction from West Asia has proved useful. Genotypes ILL 5865 with cold tolerance has been registered as ShirAZ-96 in Baluchistan, Pakistan.

The genetic base may also be widened through hybridization. Asynchrony in flowering between exotic material from West Asia and *pilosae* lentil enforced reproductive isolation. This was partly broken by the arrival of the early flowering "Precoz". However, a major systematic effect to wider the genetic base available to breeders in South Asia has been made by ICARDA by extensive crossing of *pilosae* lentils with germplasm from other origins since 1981. Segregating populations were then distributed to National Programs and selection were made locally for adaptation and disease resistance. Moreover, stable lines developed from such specific crosses were sent to the National Programs through International Trials Nurseries. As a result, Bangladesh has released two varieties, namely "Barimasur - 2" and "Barimasur - 4" from ILL 4353 X ILL 353 and L5 X FLIP 84 - 112L crosses, respectively (Figure 2.6). In Pakistan, "Masoor 93" was released in Punjab Province (Figure 2.6). It comes from the cross 18-12 (local) * ILL 4400 (ex Syria) made at Faisalabad, Pakistan, using hybridization techniques learned at ICARDA.

Mutation breeding played a third role in broadening the genetic base of lentil in the sub - continent. At Indian Agricultural Research Institute, India and Nuclear Institute for Agriculture and Biology, Pakistan, a wide range of chlorophyll and morphologic mutation were developed using physical and chemical mutagens. Several varieties with a high yield potential, early maturing, reduced plant height, upright growth habit, have been identified and included in national trials in Pakistan.

The above examples show the widening of the genetic base of the lentil in South Asia and the rupture of an ancient bottleneck. Additionally, they illustrate a decentralized approach to breeding.

(W. Erskine, S. Chandra, M. Chaudhry, I.A. Malik, S. Sarker, B. Sharma, M. Tufail and M.C. Tyagi)

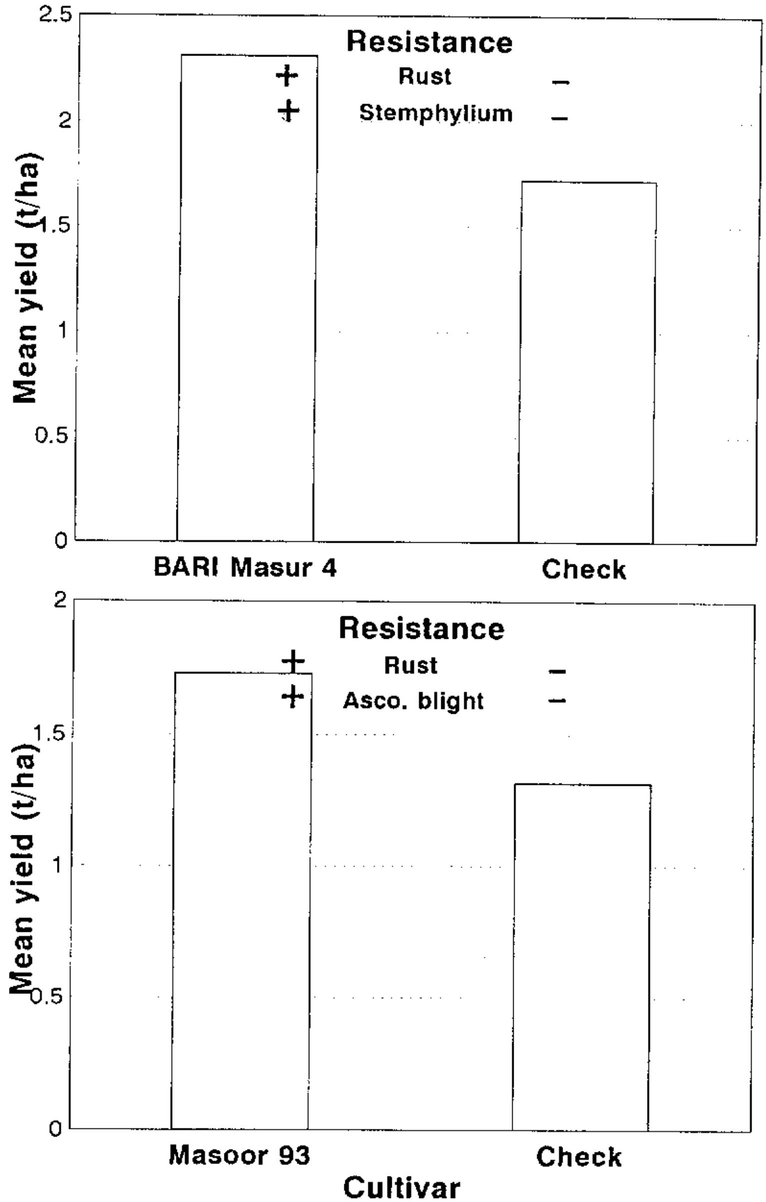

Fig. 2.6. Mean yield (kg/ha) from yield trials of Masur-95 in Pakistan and BARI Masur 4 in Bangladesh, together with their known resistances in comparison with the respective local control cultivars.

2.1.1.6. Inheritance of Time to Flower

The timing of flowering is a key to adaptation and hence productivity. Lentils from southern latitude countries flower early compared to West Asian material. Breeding of early maturing varieties in S. Asia is an important objective in view of the increasing importance of intensive crop sequences. Information on the inheritance of flowering and the associated mode of gene action governing flowering would be useful in devising suitable breeding procedures to develop early genotype of lentil.

Seven parental lines (*ILL 5773, ILL 6037, ILL 4605, ILL* 2501, ILL 7557, ILL 7665, ILL 7555) comprising lines from both West and South Asia were used to develop a half-diallel set of populations. The parents, F_1s and F_2s were grown in a randomized complete block design with two replications at Tel Hadya, Syria and at the Indian Agricultural Research Institute, New Delhi, India. Observations on time to first flower were recorded on individual plants. The F_2 observations were first analyzed in a Mendelian manner. The F_2 data for time to flower from Delhi showed continuous distribution. However, in the Tel Hadya data four crosses (ILL 5773 x ILL 6037, ILL 5773 x ILL 4605, ILL 6037 x ILL 2501 and ILL 4605 x ILL 2508) out of the 20 F_2 populations showed discontinuous distributions with *Mendelian ratios of* 3 *late:* 1 *early* (Figure 2.7). This finding will be checked from F_3 studies (F_2-derived F_3 families) in 1996/97. This preliminary data suggests that time to flower, as indicated by the four early x late crosses, is controlled by a single gene with late flowering dominant over earliness. Also, the parents ILL 4605 and ILL 6037 possess the same gene for earliness. Quantitative analysis of Delhi data and the rest of the crosses from Tel Hadya will be carried out.

(B. Sharma, M.C. Tyagi (Indian Agricultural Research Institute), A. Sarker and W. Erskine)

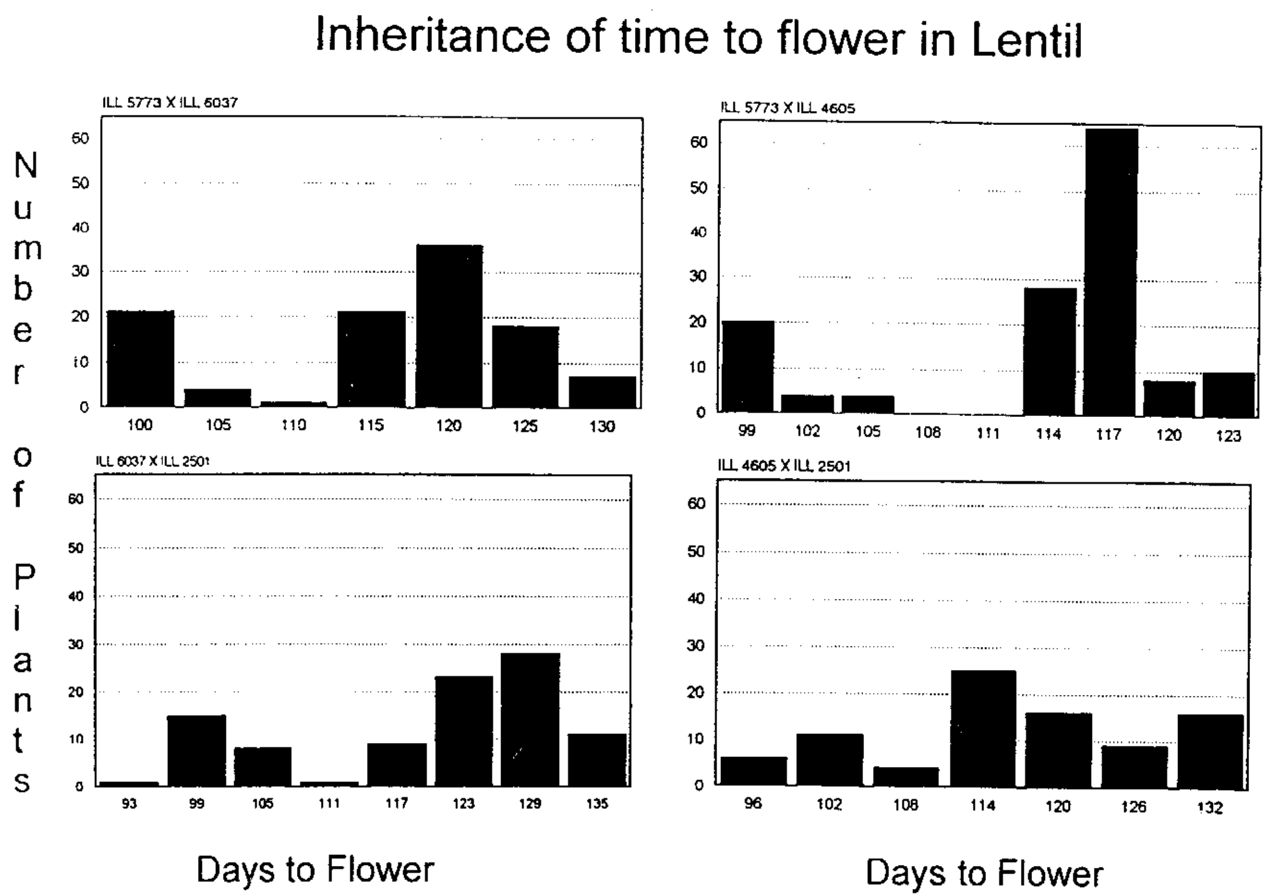

Fig. 2.7. Discontinuous distribution of time to flower (d) of F_2 populations from four lentil crosses.

2.1.2. Use of Lentil Germplasm by NARSs

2.1.2. Use of Lentil Germplasm by NARSs

2.1.2.1. Advances for the Mediterranean Region

The ICARDA base program provides segregating populations and breeding lines to national programs in North Africa and West Asia for elevations below c. 850 m around the Mediterranean Sea. To date, more use has been made by NARSs of lines than segregating populations and few lentil crosses are made outside ICARDA in North Africa and West Asia.

Table 2.7. lists lentil lines released as cultivars and Table 2.8. gives those lines selected for pre-release multiplication and/or on-farm trials by NARSs.

In Syria the red-cotyledon line ILL 5883 awaits submission to the variety release committee following its testing in on-farm trials over the last five years, where it yielded significantly more grain than the local check in different geographic regions and rainfall zones. Additionally, it has improved standing ability for harvest mechanization over the local check and resistance to vascular wilt disease, the most important disease of lentil in Syria.

Large-seeded material occupies an estimated 20 % of the lentil area in Syria, which is dominated by red-cotyledon small-seeded lines. The spread in Syria of the earlier-registered, large-seeded line Idlib 1, which has good standing ability and yield, has been monitored through surveys. In 1992/93 only one percent of producers were using the new lentil cultivar; whereas in 1996 this had risen to 12%.

In Lebanon results from an adoption study indicate that Talya 2 is starting to spread in the Beqaa valley and that yellow cotyledon is the preferred seed type in southern Lebanon. Accordingly, FLIP 86-2L (ILL 5988), a yellow cotyledon line out-yielding Talya 2 in on-farm trials, has been registered for cultivation in the south of the country.

Table 2.7. Lentil cultivars released by national programs

Country	Cultivar name	Year of release	Specific features
Algeria	Syrie 229	1987	Yield, seed quality
	Balkan 755	1988	Yield, seed quality
	ILL 4400	1988	Yield, seed quality
Argentina	Arbolito (ILL 4605x-4349)	1991	Yield, tall & early
Australia	Aldinga (FLIP-80L)	1989	Yield
	Digger (FLIP84-51L)	1993	Yield, red cotyledon
	Cobber (FLIP84-58L)	1993	Yield, red cotyledon
	Matilda (FLIP84-154L)	1993	Yield, yellow cotyledon
	Northfield (78S 26013)	1995	High yield, Ascochyta blight res.
Bangladesh	Falguni - BARI Masur 2 (Sel.ILL4353xILL353)	1993	Rust res. & yield
	BARI Masur 4 (Sel. L5 x FLIP84-112L)	1995	Rust and Stemphylium resistance
Canada	Indianhead (ILL 481)	1989	Green manure
	CDC Redwing (Eston x ILL5588)	1994	Ascochyta res.
	CDC Matador (Indianhead) x (Eston x PI179310)	1994	Ascochyta res.
Chile	Centinela (74TA470)	1989	Rust res. & yield
China	FLIP87-53L	1988	Yield in Qinghai Province
Ecuador	INIAP-406 (FLIP 84-94L)	1987	Rust res. & yield
Egypt	Precoz (ILL 4605)	1990	Intercropping in sugarcane
	Sinai 1 (sel ILL 4605)	1996	Early, high protein for N. Sinai
	Giza 51 (FLIP84-51L)	1996	Tol. High soil moisture
Ethiopia	R 186	1980	Yield
	Chalew (ILL 358)	1984	Rust res. & yield
	Chikol (NEL 2704)	1994	Rust res. & yield
	FLIP84-7L	1994	Rust resistant, high yield
	Gudo (FLIP84-78L)	1995	Rust resistant, high yield
	Ada'a (FLIP86-41L)	1995	Rust resistant, high yield
Iraq	Baraka (78S26002)	1994	Yield, standing ability
Jordan	Jordan 3 (78S 26002)	1990	Yield, standing ability
Lebanon	Talya 2 (78S 26013)	1988	Yield, standing ability
	Toula (FLIP86-2L)	1995	High yield, large seeds
Libya	El Safsaf 3 (78S26002)	1993	Yield, st. ability in E. Libya
Morocco	Precoz (ILL 4605)	1990	Rust res. & yield
Nepal	Sikhar (ILL 4402)	1989	Yield
N Zealand	Rajah (FLIP87-53L)	1992	Yield, red cotyledon
Pakistan	Manserha 89 (ILL 4605)	1990	Ascochyta & rust res.
	Masur-95 (18-12 x ILL 4400)	1995	Ascochyta & rust res.
	ShirAZ-96 (ILL 5865)	1996	Winter-hardy
Sudan	Aribo 1 (ILL 818)	1993	Yield in Jebel Mara
	Rubatab 1 (ILL 813)	1993	Yield in N. Sudan
Syria	Idleb 1 (78S 26002)	1987	Yield, reduced lodging
Tunisia	Nsir (ILL 4400)	1986	Large seeds & yield
	Nefza (ILL 4606)	1986	Large seeds & yield
Turkey	Firat 87 (75Kf 36062)	1987	Small seeds & yield
	Erzurum 89 (ILL 942)	1990	Spring sowing & yield
	Malazgirt89 (ILL 1384)	1990	Spring sowing & yield
	Sazak 91 (NEL 854)	1991	Winter sowing, red cotyledon
	Sayran 96 (ILL 1939)	1996	High yield & harvestability
U.S.A.	Crimson (ILL 784)	1991	Yield in dry areas

Table 2.8. Lentil lines in on-farm testing or pre-release multiplication by NARSs.

Mediterranean region	
Algeria	ILL 468
Egypt	FLIP84-112L
Iraq	ILL 5883, FLIP87-56L
Jordan	FLIP84-147L, FLIP88-6L
Lebanon	ILL 2126, FLIP84-59L, FLIP85-38L, FLIP87-56L
Morocco	*FLIP86-15L, FLIP86-16L, FLIP87-19L, FLIP87-22L*
Syria	ILL 5883, ILL 7012
Tunisia	78S26002, FLIP 84-58L
High elevation	
Iran	ILL 590, ILL 707, ILL 857, ILL 975, ILL 4400
S. Latitudes	
Ethiopia	FLIP87-74L
Nepal	ILL 2580, ILL 4402
Sudan	ILL 813, FLIP88-43L
Yemen	ILL 4605, FLIP84-14L

In Jordan the national program is in the process of releasing two lentil lines (FLIP88-6L & FLIP84-147L) which performed well in on-farm trials.

In South-East Turkey, where winter red lentil is widely grown, Sayran-96 (ILL 1939) was registered during 1996 on the basis of testing by the S. E. Anatolian Regional Research Station.

In Iraq the large-seeded, yellow cotyledon line 78S 26002 was registered in 1992 as Baraka. The red cotyledon lines ILL 5883 and FLIP87-56L (ILL 6246) are being tested in on-farm trials in Iraq. To fuel the demand from Iraqi consumers for both red and yellow cotyledon lentil, the crop's area has been estimated to have grown to approximately 30,000 ha. To a large extent based on the new cultivar. A lentil adoption study is now being mounted in Iraq.

In Libya the line El Safsaf 3 (78S26002), released in 1993 for cultivation for the East of the country, continues to perform well in the East, but also has given high yields under central-pivot, irrigated conditions in Central Libya at Meknosa.

Lentil production and area continue to decline in Algeria but the lines ILL 468, ILL 4400, LB Redjas, Setif 618 and Balkan 755 are in seed production for future use by farmers.

In Morocco there are several lentil lines in catalogue trials, namely: FLIP86-15L (ILL 6001), FLIP86-16L (ILL 6002), FLIP86-19L (ILL 6005), FLIP86-21L (ILL 6007), FLIP87-19L (ILL 6209) and FLIP87-22L (ILL 6212), all with field resistance to rust. Rust screening under controlled conditions was started at Meknes and the lines ILL 5480 and FLIP 88-32L (ILL 6456) were confirmed as rust resistant.

The North African Regional yield trial on lentil was re-initiated for the 1996/97 season under the coordination of the Moroccan lentil breeder.

In Egypt the line Shami 1 (ILL 4605) is becoming popular in the north Sinai region, because its early maturity avoids drought stress under the low prevailing rainfall conditions. Giza 51 (FLIP84-51L) with small seeds was registered during 1996 because of its tolerance to high moisture conditions.

(National Agricultural Research Systems)

2.1.2.2. Advances for Southern Latitude Region

This region comprises the sub-continent of India and Ethiopia where an early flowering habit is required together with resistance to rust, ascochyta blight and wilt. The importance of foliar pathogens contrasts with other major areas of lentil production.

There are three strong lentil breeding programs in Pakistan with two in Faisalabad and the remaining program in Islamabad. Over the last five years ICARDA has worked closely with these programs in joint selection as the focus of a thrust to broaden the genetic base of lentils in South Asia. The cultivar Masur 95, with ICARDA parentage, is proving popular with farmers in the Sialkot region on account of its rust resistance and high yield (Figure 2.6).

The major production problems in Bangladesh addressable through breeding are rust and Stemphylium blight. We have been making targeted crosses for Bangladesh of rust resistance sources with the local susceptible cultivar 'L5' in the base program at Tel Hadya. Selections have now been made in Bangladesh of adapted rust resistant plants from segregating populations. As a result, Falguni (BARI Masur 2) was released in 1993 as the first rust resistant lentil cultivar in Bangladesh. Another rust-resistant line (ILX 87247), locally selected from the cross of L5 x FLIP84-112L (ILL 5782), was released as BARI Masur 4 in 1995 (Figure 2.6). It also has resistance to Stemphylium blight and an erect plant stature suitable for inter-cropping in sugarcane, and mixed cropping with mustard, which is a widespread production practice for lentil in Bangladesh. Another four lines having high yield potential and combined resistance to rust and Stemphylium blight are in pre-release stage. Two of them have been identified for late planting condition (about 1 month late) for medium high-lands after harvest of autumn rice. The other two lines are for the main lentil growing season.

India has a strong lentil breeding program coordinated under the All India Coordinated Pulse Improvement Project of the Indian Council of Agricultural Research (ICAR). In India, the genetic base of the crop has been widened by the use of the introduced 'Precoz' in crossing. As result of the widening of the genetic base (Section 2.1.1.5), the All-India Coordinated Program now has a new extra-bold nursery and an extra early nursery. Rust resistance, selected in Morocco, is holding in Kanpur. We have established cooperation with Pantnagar Agricultural University on screening for rust resistance in breeding lines, the wild germplasm and the possibility of collaboration in the search for markers for rust resistance. Our vascular wilt resistance lines are being widely used as source parents within India.

Nepal grew around 170,000 ha of lentil spread from the Terai area adjacent to India to the lower Mid-Hills last season. ICARDA has been requested for specific targeted crosses by Nepali program. ILL 2580 and ILL 4402 are among entries being considered for release.

Bilateral interaction - ICARDA directly with the NARSs of S. Asia - has been strong in the fields of the exchange of germplasm and in the development of tailored breeding material. The value to NARSs of such bilateral interaction has fueled the felt need for more support to regional activities on lentil improvement. At an ICARDA/ICAR sponsored seminar on 'Lentil in S. Asia' held in Delhi in 1991, participants from S. Asia were enthusiastic about the need and value of a regional network on lentil improvement and its potential for the development of the crop in their individual countries. Recently, we were catalytic in securing funding for a project entitled 'Improvement of drought and disease resistance in lentils from the Indian Sub-continent' from the Australian Centre for International Agricultural Research (ACIAR).

In Ethiopia Gudo = FLIP84-78L (ILL 5748) and Ada'a = FLIP86-41L (ILL 6027) were registered in 1995. Ada and Akaki are the areas where the released line NEL 358 is becoming very popular and a study on its impact is planned.

In Sudan, where lentil cultivation has grown from nothing to self-sufficiency, to underpin the change the cultivar Rubatab 1 (ILL 818) was released for cultivation in the Northern Province and Jebel Mara Region in 1993/94. The program identified FLIP88-43L (ILL 6467) as promising in the Northern Province.

(National Agricultural Research Systems)

2.1.2.3. Advances for High Altitude Region

The high altitude region primarily consists of those regions of Afghanistan, Iran, Pakistan and Turkey where lentil is normally grown as a spring crop because of the severe winter cold. In Iran the line ILL 857 selected at Gazvin on the basis of winter-hardiness is in the pre-release stage (See Section 2.1.1.4). Progress in highland Turkey is given in the same section.

In Baluchistan (Pakistan) the lentil cultivar ShirAZ-96 (FLIP-85-27L) was released to farmers in 1996. The line was selected at the Arid Zone Research Institute, Quetta, on the basis of its cold tolerance and a larger seed size than the local cultivar.

(National Agricultural Research Systems)

2.1.2.4. Advances in Other Areas

The lentil industry in New Zealand has declined to a point where only 1500-2000 M.T. of cultivar 'Rajah' (FLIP87-53L) will be produced in 1996. The successful commercialization of 'Rajah' has helped to keep the lentil industry alive in New Zealand for the last few years. This is primarily due to its increased resistance to Ascochyta blight in the field compared to 'Titore'. Crops of 'Rajah' have not required foliar application of fungicide compared to up to three applications with 'Titore'. The New Zealand industry is under pressure from the emerging Australian competition.

In Australia there is now considerable interest in lentil. Prior to the testing of germplasm from ICARDA, lentil assessment in Australia was limited to a few lines representing phenological extremes - extra early and extra late flowering and maturity. ICARDA Mediterranean-adapted material has fitted in well into the vacuum. In Victoria the red-seeded cultivars Digger = FLIP84-51L (ILL 5722) and Cobber = FLIP84-58L (ILL 5728) and the green cultivar Matilda = FLIP84-154L (ILL 5823) are forming the basis of a viable lentil industry. In 1995 the estimates were of a lentil crop of 8-10,000 tonnes a pool return of A$400 - a four million dollar industry based on ICARDA germplasm. In New South Wales the lines FLIP84-51L (ILL 5722) and FLIP86-16L (ILL 6002) are in multi-location testing following their selection at Tamworth over several seasons. Northfield (78S 26013) was released in S. Australia in 1996. In Western Australia during 1994, the first commercial crops of lentil were grown. Twelve farmers grew a total of around 200 ha with average yields of 0.8 t/ha and a top yield of 1.8 t/ha from ICARDA derived lines.

In response to epidemics of lentil Ascochyta blight in the Canadian Province of Saskatoon, the Crop Development Centre, University of Saskatoon has released the first two resistant Canadian lentil cultivars 'CDC Redwing' (Eston x ILL 5588) and 'CDC Matador' (Indian head (alias ILL 481) x (Eston x PI179310); both of which have ICARDA parentage and the former has resistance from an ICARDA parent (ILL 5588). Further East in Manitoba (Canada), the lentil crop suffers from Anthracnose blight. The only known resistant source to the disease is ICARDA germplasm ILL 481 = 'Indianhead'.

In the USA the variety Crimson (ILL 784) is now being grown on about 1000 ha in the Palouse area of Washington State. Markets have been slow to develop. Apparently the buyers want plumper seeds ('Turkish seed-type').

(National Agricultural Research Systems)

2.2 Lentil Pathology

2.2.1. Host Range of *Fusarium oxysporum* f.sp. *lentis* within some Cereal, Food and Forage Legumes

Lentil vascular wilt caused by *Fusarium oxysporum* f.sp *lentis* is the most devastating disease of lentil in many parts of the world. The causal agent persists in the soil and plant debris as chlamydospores. Forma speciales of *Fusarium* spp. are known to be host specific. However, some f. sp. infect plants other than their restrictive hosts.

To understand the epidemiology of the disease and to contribute to its control via cultural practices (crop rotation), this study aimed to identify alternate hosts for *Fusarium oxysporum* f.sp *lentis* within some Mediterranean cereal, and food and forage legume crops.

Twenty one species belonging to the Graminae (*Triticum aestivum*, *T. durum*, and *Hordeum vulgare*) and the Leguminosae (*Cicer arietinum*, *Lathyrus ciliolatus*, *L. ochrus*, *L. sativus*, *Medicago noeana*, *M. rigidula*, *M. rotata*, *M. polymorpha*, *Pisum sativum*, and *Vicia ervilia*, *V. faba*, *V. narbonensis*, *V. palestina*, *V. pannonica*, *V. sativa*, *V. sativa amphicarpa*, *V. villosa dasycarpa*) were tested as possible hosts for *Fusarium oxysporum* f.sp. *lentis*.

The experiment was conducted in plastic pots filled with sterilized soil-sand mixture under plastic house conditions. Seeds were scarified, as needed, and surface sterilized with 5% solution of "clorox". Ten seeds of the test entry were sown around the periphery of each pot and four seeds of the susceptible lentil control (ILL 4605) were sown in its center. Entries were arranged in a randomized complete block design with five replicates and watered as needed. Two weeks after planting, pots were inoculated by adding 250 ml/pot of a liquid inoculum (2 x 10^6 microconidia/ml). Plants were checked for wilt symptoms eight weeks after inoculation. Samples from both the vegetative and root systems were taken periodically for plating onto potato dextrose agar to detect *F. oxysporum*.

The fungus was successfully isolated from the roots and stems of both *Vicia sativa and V. pannonica*. This indicates a systemic-type of infection on these two hosts, showing that they are possible alternate hosts for the fungus.

The fungus was isolated from the roots alone of *Vicia villosa, V. palestina, V. ervilia,* and *Medicago rotata* which may be considered as carriers.

The other plant species were unaffected by the fungus.

2.2.2. Biocontrol of *Fusarium oxysporum* f.sp. *lentis*

A total of 31 soil samples were collected from the main lentil growing areas in Syria and processed for their microflora composition using the dilution plate technique. The resulting microorganism(s) were screened and their antagonistic activity determined by confronting their colonies with those of *Fusarium oxysporum* f. sp. *lentis* on the surface of PDA medium using the following formula:

$$\% \text{ of inhibition} = ((R2 - R1)/R2)100$$

where $R2$ = the largest diameter of the fungal colony and $R1$ = the smallest diameter of the colony (facing bacterial colony)

A total of 35 bacterial colonies, all *Bacillus* spp., with inhibition rates from 52 to 77% were identified. The six most antagonistic isolates (# 1, 9, 17, 21, 26, 27) were used in subsequent tests.

These isolates were tested for their *in vitro* antagonistic activity on PDA. For this test, lentil seeds were surface-sterilized (0.525% sodium hypochlorite for 5 m), soaked for 10 m in 8 x 10^6 bacterial cell suspension in 0.2% dextrin, air-dried and plated at equal distance on the surface of PDA in a petri dish. Forty eight hours later, a 5 mm disc taken from the periphery of an actively growing colony of *F. oxysporum* f. sp. *lentis* was placed in the center of the dish. The growth of the fungus was recorded a week after incubation at 20 ± 2 °C.

Seed germination rate was unaffected by soaking seeds in bacterial suspension and the growth of the fungal colony was greatly reduced.

The *in vivo* antagonistic activity of the bacterial isolates was investigated in plastic pots in the plastic house. Five surface-sterilized lentil seeds of the vascular wilt-susceptible cultivar - ILL 4605 (0.525% sodium hypochlorite for 5 m) were soaked for 10 m in 8 x 10^6 bacterial cell suspension in 0.2% dextrin, air-dried and planted, equally distanced, at the periphery of a pot containing soil mixture (2 soil: 1 sand) sterilized at 150 °C for 24 hours. The fifteen treatments in this and the subsequent *in vivo* field trial comprised six bacterial strains used as monocultures (see list above); six simple mixtures of isolates (# 1+9; 1+26; 1+27; 9+21; 17+26; and 26+27) and three 3-component mixtures (# 9+17+21; 1+17+26; and 21+26+27) with controls (Dextrin only; Benlate only). Two weeks after planting, pots were inoculated each with 60 ml of *F. oxysporum* f. sp. *lentis* spore suspension (2.5 x 10^6 spore ml^{-1}). The experiment was in a randomized complete blocks design with three replications.

Seed germination rate was unaffected by treatment and was 100% throughout. Disease severity means (67 days after planting on 1-9 scale) were < 5 for plants arising from seed treated with the following isolates (# 1, 9, 21, 26, 27, 17+26, 26+27) and > 5 in the rest, as compared with a rating 9 for the check. Treating seeds with the biological agents led to an increase in the number of pods per plant as well as an increase in the biological yield per plant, compared to the check.

The *in vivo* antagonistic activity was then

investigated under field conditions in a wilt-sick plot. Two lentil lines were used: ILL 4605 (highly susceptible) and ILL 7136 (moderately susceptible). Lentil seeds were surface sterilized for 5 m in 0.525% sodium hypochlorite, rinsed three times in sterilized distilled water, and air-dried on Whatman filter paper. Seeds were then coated with 0.2% dextrin solution at a rate of 0.4 ml per 100 g seed and were treated as follows:
(I) soaked for 10 m in the bacterial suspension of each of the most powerful antagonistic bacteria and their combinations (15 treatments); (ii) Soaked for 10 m in benlate suspension (1 g ai kg^{-1} seed); and (iii) soaked for 10 m in sterilized distilled water. Seeds of all treatments were then dried under the laminar airflow. Planting was on January 11, 1996 in a wilt-sick plot. Each experimental plot consisted of three 50 cm-long rows with 50 seeds per row. The untreated susceptible control was planted after every fourth row. The experiment was in a split plot design with three replications.

After 127 and 136 days of planting, wilt incidence was recorded as % wilted plants, for which there was a significant genotype by treatment interaction. The moderately susceptible genotype - ILL 7136 had significantly reduced wilt incidence in all the bacterial-treated plots compared to the untreated controls. The highly susceptible line - ILL 4605 had significantly lower mean wilt incidence in the plots treated with isolates # 9, 17 & 21 than the untreated control. There were significant increases in biomass and straw yield in ILL 7136 when inoculated with isolates # 1, 21, 27 and (1+9) compared to the control. They out-yielded the check by 20-50%. The interaction was non-significant for grain yield. The experiment is being repeated next season.

The effect of antagonistic bacteria on the growth of *Rhizobium leguminosarum* was assessed. The six antagonistic bacterial isolates (# 1, 9, 17, 21, 26, and 27) were confronted with lentil rhizobial strains (Le-719, Le-726 and Le-735) on Yeast Mannitol Agar medium. Rhizobial growth was unaffected by the presence of the antagonistic bacteria after incubation for one week at 20 ± 2 °C.

The effect of some fungicides commonly used for seed dressing on the growth of the antagonistic bacteria was studied. Benlate (benomyl) and Tecto 60 (thiabendazole) were used at 0.25, 0.50 and 1 g ai l^{-1} to produce poisoned-PDA media. The six antagonistic bacteria were streaked on the surface of PDA in petri dishes. After 40 h, the center of each petri dish was inoculated with 5 mm-diameter disc of *F. oxysporum* f. sp. *lentis* and incubated for a week at 20 ± 2 °C. The control treatment consisted of inoculating the fungus and antagonistic bacteria on un-poisoned PDA.

Results revealed that neither fungicide affected the growth of the antagonists after incubation for 48 h. However the growth of isolates 1 + 21 was weak on PDA amended with benlate at 1 g ai l^{-1}. The growth of the fungus on PDA amended with either fungicides was null.

(S. El Hassan, B. Bayaa (Aleppo University) and W. Erskine)

2.3. Lentil Mechanization

During the first decade of ICARDA, a major drive was made to develop economic machine harvest systems for lentil production (FLIP Annual Reports 1986-1990; Legume Program Annual Reports 1991-93). Following the introduction and use by farmers in Syria and Turkey of such systems, a moratorium was placed on further technical research at ICARDA pending the completion of an adoption survey of producers in Syria.

The joint survey was conducted with the Universities of Aleppo and Cukurova, Adana. A total of 79 lentil producers spread among the major lentil growing regions of Syria were interviewed using a questionnaire format to elucidate the importance of lentil in the farming enterprise and issues related to harvest. In addition, a similar survey was initiated in the Urfa Province of South-East Anatolia, Turkey, which has covered 29 farmers to-date.

In Syria a major contrast was observed between the average area of lentil grown by farmers in different Provinces. The mean area of lentil in El Hassake Province was 32 ha, compared to 4 ha in Aleppo Province and 0.6 ha in Deraa Province. Farmers reported altering their lentil areas for two main reasons: economics and crop rotation.

Ninety percent of farmers grew lentil on flat, deep soil free of stone problems. Sowing by hand-broadcast has become rare outside Deraa Province. Twelve percent of farmers were growing the new cultivar - Idlib 1; compared to only 1 % in the 1992/93 season.

For harvest, the use of hand labour (94 % of farmers) was widespread in Aleppo, Idlib, Hama and Deraa Provinces. However, in El Hassake Province 57 % of farmers harvested by machine. Two years ago, only 19 % of farmers had adopted mechanized harvest; clearly the practice is spreading. Eighty one percent of the machine harvest was done with a combine, 13 % of farmers used a large (4 m-wide) self-propelled mower-swather and 3 % of farmers used a small self-propelled mower. The double-knife mower was not found. Among those farmers using machinery for harvest, 91 % rented the equipment; the remainder used their own machinery.

In Urfa Province, Turkey the initial survey of 29 farmers revealed that *c.* 70 % harvested by machine. Among equipment used, 80 % were tractor-drawn double-knife mowers. Clearly, at present the adoption of lentil harvest mechanization is considerably more advanced in Urfa province, Turkey than in Syria. The survey in Turkey will be continued to cover more farmers and other areas of production.

(Y. El Saleh, T. Özcan (Çukurova University, Adana, Turkey) S. Barbara (Aleppo University) and W. Erskine)

2.4. Lentil Entomology

2.4.1. Sitona in Lentil

Several insecticides were tested at Tel Hadya against *Sitona crinitus* Herbst: Promet(10, 15 and 25cc/kg) , Gaucho(3 and 5 g/kg), Confidor(6 and 12 kg/ha)and Carbofuran(10 and 20 kg/ha). The best of these chemicals was Promet (25CC/kg), which had the least percent nodule damage (11%) compared to the control (68%). The other insecticides tested were not as effective to reduce significantly nodule damage by Sitona. However, grain yield differences between the different treatments were not significant (Table 2.9).

Table 2.9. Effect of Promet, Gaucho, Confidor and Carbofuran on percent nodule damage by Sitona, biological yield and grain yield of lentil, 1995/96.

Treatment	%Nodule damage (13 April)	Biological yield (kg/ha)	Grain yield (kg/ha)
PROMET 10 CC/KG	28.25	6790.46	1490.50
PROMET 15 CC/KG	14.03	6555.29	1455.00
PROMET 25 CC/KG	11.05	6425.88	1339.50
GAUCHO 3 G/KG	45.28	6330.93	1368.75
GAUCHO 5 G/KG	37.70	6585.34	1503.00
CONFIDOR 6 KG/HA	52.50	6073.72	1426.00
CONFIDOR 12 KG/HA	72.50	6350.16	1557.00
CARBOFURAN 10 KG/HA	31.00	6209.83	1527.75
CARBOFURAN 20 KG/HA	28.08	6166.40	1326.75
CONTROL	68.00	5923.88	1335.50
LSD AT 5%	24.83	832.60	235.01

We also evaluated the residual effect of the Carbofuran on Sitona infestation. This experiment has been carried out for the last five years. The results showed that there is no residual effect of the Carbofuran; the percent nodule damaged was similar to that of the control.

A lot of information has been generated on the chemical control of Sitona in lentil. In the future, more emphasis will be put on biological control and on host plant resistance.

(M. El Bouhssini, N. Sharaf Eldin, and A. Joubi)

2.5. Boron Toxicity Tolerance

B toxicity can cause a large reduction in growth and yield of lentil as shown in a pot experiment in 1994/95. Results in barley showed that B-toxicity tolerance is needed to be drought tolerant in high B soils, and variation in B-toxicity tolerant can cause genotype by environment interaction in grain yield (see ICARDA 1997). In order to find out whether there is variation in B-toxicity tolerance among materials in the lentil breeding project, 77 lentil lines from the following four international nurseries: LIYT-Early, LIABN, LICTN, and LIFWN were screened. The experiment was conducted under a plastic house in soil mixed evenly with boric acid at the rate of 50 mg B/kg soil (giving a hot water extract of around 14 ppm B). Three replications were used. Visual growth and foliar B-toxicity symptom scores were taken 4 and 5 weeks after sowing, respectively.

There were highly significant differences between entries in both growth and B-toxicity symptom scores. The two traits had a moderately negative correlation, meaning that entries with poor growth tends to have less symptoms and vice versa. Table 2.10 shows the entries with the best and worst scores. As there is evidence that genotypes relatively tolerant to boron toxicity are also relatively susceptible to boron deficiency and vice versa (Nable et al. 1989), the boron-toxicity sensitive entries are expected to be tolerant to boron deficiency.

(S.K. Yau and W. Erskine)

Table 2.10. Lentil lines in the 1995/96 screening test having the best or the worst performance in a soil to which 50 mg B/kg soil was added.

Entry Abbreviation	Growth score[1]	Symptom score[2]
B-toxicity tolerant:		
ILL 5883	2.3	0.3
ILL 2439	2.3	2.3
ILL 7127	3.3	1.7
B-toxicity sensitive:		
ILL 5597	4.3	6.0
ILL 6816	7.0	3.0
ILL 6260	6.7	3.7

[1] 1 to 9 scale: 1 = good growth
[2] 0 to 9 scale: 0 = no symptoms

2.6. International Testing Program

The international testing program on lentil is a vehicle for the dissemination of genetic materials and improved production practices, in the form of international nurseries and trials, to the national programs in and outside the WANA region. The genetic materials comprise early segregating populations in F_3 and F_4 generations, and elite lines with wide or specific adaptation, special morphological or quality traits, and resistance to common biotic and abiotic stresses. Nurseries are only sent on request and often include germplasm specifically developed for a particular region or a national program. A list of trials supplied in the 1996/97 season is given in Table 2.11.

The *testing program* helps *in* identification of genotypes with specific and wide adaptation. The performance data permit assessment of genotype x environment interaction and help in targeting breeding efforts for specific agro-ecological conditions.

The salient features of the 1994/95 international nursery results, received from cooperators are presented here. The stability analyses of some of the trials were done using Eberhart and Russell (1966) model.

In lentil there are three different yield trials. The Lentil International Yield Trial-Large (LIYT-L) was reported from 21 locations in 13 countries. A number of test entries exceeded the local check by a significant margin ($P \leq 0.05$) at Suffit in Libya; Sidi El Aidi in Morocco; Cordoba in Spain; Breda, Gelline, Hama, Heimo and Idleb in Syria; and Beja in Tunisia. The five heaviest yielding entries across the locations were FLIP 92-48L, 78S 26002, FLIP 90-3L, FLIP 91-12L, FLIP 89-51L with seed yields of 1633,1501, 1495, 1462 and 1437 kg/ha, respectively. The stability analysis revealed that the entries, FLIP 87-15L, and ILL 323 were relatively stable across environments as compared to others.

Table 2.11. Distribution of Legume International Nurseries to cooperators for the 1996/97 season.

International Trial/Nursery	No. of sets
Yield Trial, Tall (LIYT-L-97)	51
Yield Trial, Small (LIYT-S-97)	34
Yield Trial, Early (LIYT-SL-97)	20
Screening Nursery, Large-Seed (LISN-L-97)	38
Screening Nursery, Small-Seed (LISN-S-97)	27
Screening Nursery, Early (LISN-SL-97)	20
Screening Nursery, Drought Tolerance (LISN-DT-97)	43
F_3 Nursery, Large Seed (LIF_3N-L-97)	9
F_3 Nursery, Small Seed (LIF_3N-S-97)	10
F_3 Nursery, Early (LIF_3N-SL-97)	10
F_3 Nursery, Cold Tolerance (LIF_3N-CT-97)	9
Cold Tolerance Nursery (LICTN-97)	26
Ascochyta Blight Nursery (LIABN-97)	20
Fusarium Wilt Nursery (LIFWN-97)	30
Rust Nursery (LIRN-97)	12
Total	359

For the Lentil International Yield Trial-Small (LIYT-S) data were analyzed for seed yield for 15 locations in 8 countries. At some of the locations including, Terbol in Lebanon; and Breda, Idleb, Hama, and Gelline in Syria; some of the test entries exceeded the respective local check by a significant margin. The five best entries across locations were FLIP 92-28L, FLIP 90-41L, 81S 15, FLIP 92-37L and FLIP 92-19L with seed yield of 1829, 1794, 1761, 1740 and 1724 kg/ha, respectively. The stability analysis for seed yield revealed that the entries, FLIP 92-28L, FLIP 92-37L, FLIP 92-19L, FLIP 89-20L, FLIP 92-15L, FLIP 90-25L, FLIP 92-18L, FLIP 92-27L, FLIP 84-43L and FLIP 90-22L, were relatively adaptable across environments as compared to others.

For Lentil International Yield Trial-Early (LIYT-E) data were analyzed for seed yield for 13 locations. At Shendi in Sudan; Malawi in Egypt; Habak in India; Maru in Jordan; and Beja in Tunisia 2,7,1,1, and 2 of the test entries exceeded the respective local check in seed yield by a significant margin ($P \leq 0.05$). On basis of mean across locations the five heaviest yielding lines were FLIP 86-39L, FLIP 89-53L and FLIP 86-38L. At all the locations a large number of entries were earlier in flowering than the respective local checks. Stability analysis for seed yield revealed that only two of the 23 entries, namely FLIP 86-39C and FLIP 89-53L exhibited specific adaptation to the high yielding environments, and another four namely, FLIP 86-38L, FLIP 87-66L, FLIP 92-52L and ILL 7163 exhibited general adaptation over different environments. The remaining 17 entries were unpredictable across environments.

For Lentil International Screening Nursery - Large (LISN-L), Small (LISN-S), and Early (LISN-E), the data for seed yield were reported from 13, 12, and 9 locations, respectively. The analyses of data revealed that at 7 locations in LISN-L (Terbol in Lebanon; Elvas in Portugal; Piestany in Slovakia; and Aleppo, Gelline, Hama and Heimo in

Syria), 7 locations in LISN-S (Sid and Gemmiza in Egypt; Elvas in Portugal; Aleppo, Gelline, Heimo and Izra'a in Syria), and only one location (New Delhi in India), in LISN-E, some of the test entries exceeded the respective local check by a significant margin ($P \leq 0.05$). At most of the locations, the test entries flowered either at the same time or earlier than the respective local check. The five heaviest yielding lines across locations in these nurseries are given in Table 3.12.

For the first time, one Lentil International Drought Tolerance Nursery was initiated and supplied to 30 locations. Although 11 locations sent the data back, but only six locations were worth analyzing. Out of these only at two locations namely, Aleppo and Hama in Syria some of the entries exceeded the local check by a significant margin. On the basis of average over locations, the five best entries were selected and given in Table 3.12.

Table 3.12. The five heaviest yielding lines across locations in different lentil screening nurseries, 1994/95.

Rank	LISN-L	LISN-S	LISN-E	LISN-DT
1	FLIP 85-18L	FLIP 95-49L	FLIP 93-47L	FLIP 92-48L
2	FLIP 95-11L	FLIP 95-36L	FLIP 95-63L	FLIP 93-47L
3	FLIP 88-10L	FLIP 95-48L	FLIP 93-46L	FLIP 86-38C
4	FLIP 95-15L	FLIP 84-112L	FLIP 95-61L	FLIP 91-1C
5	FLIP 95-10L	FLIP 95-39L	FLIP 95-62L	FLIP 86-39L

The results of Lentil International F_6-Nursery Large (LIF_6N-L), F_6-Nursery Small (LIF_6N-S), F_6-Nursery Early (LIF_6N-E), and F_6-Nursery Cold Tolerance ($LIF N_6CT$) were received from 10, 6, 6 and 4 locations, respectively. At 6, 3, 2 and 1 locations the cooperators made some individual plant selections for their use.

The results of Lentil International Cold Tolerance Nursery were reported from seven locations. There was no cold at Ghinchi, and Chefe Donsa in Ethiopia, and Hymana in Turkey. None of the test entries at Almora in India, 13 test entries at Toshevo in Bulgaria, all the test entries at Tel Hadya in Syria and Erzurum in Turkey exhibited tolerance reaction (rating ≤4).

The results of Lentil International Ascochyta Blight Nursery were received from 5 locations. All the test entries at Toshevo in Bulgaria showed a reaction of ≤ 5 (on 1-9 scale, where 1=no infection and 9=killed). At Jammu in India, all the test entries with the exception of 2 showed a *reaction of ≤ 3. At Tel Hadya in Syria,* all the test entries were rated as 1 and susceptible check as 2, and at Guelma in Algeria and Jema'a Shain in Morocco there was no disease infestation.

The results of Lentil International Rust Nursery were reported from 7 locations. The test entries showed a reaction of 1-3 at Akaki in Ethiopia, 1-5 at Pant Nagar in India, 3-9 at Jammu in India, 1-7 at Kanpur in India, and 1-5 at Jema'a Shain in Morocco. The tolerant lines with highest frequency across locations included, UJL 81-129, Lenka, FLIP 84-76L, ILL 5883, FLIP 86-16L, FLIP 87-17L, FLIP 87-74L, and FLIP 88-32L.

The results of Lentil International Fusarium Wilt Nursery were reported from 10 locations. There was no disease infestation at Guelma in Algeria and Heimo in Syria. At Toshevo in Bulgaria, Debre Zeit and Akaki in Ethiopia, Pant Nagar in India, Khumaltar in Nepal, Piestany in Slovakia, Izra'a and Hama in Syria, 8, 19, 26, 21, 4, 28, 25 and 29 entries, respectively, showed resistance reaction (rating ≤ 5). Five entries (FLIP 85-33L, FLIP 86-38L, FLIP 89-60L, FLIP 90-7L and FLIP 90-22L) were resistant across 7 out of 8 locations.

(R.S. Malhotra, W. Erskine and NARSs)

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3. KABULI CHICKPEA IMPROVEMENT

The kabuli chickpea improvement is a joint program with ICRISAT, India. The main objective of the program is to increase and stabilize kabuli chickpea production in the developing world. Of the five main regions, where chickpea is grown, the Mediterranean region and Latin America produce mostly kabuli-type chickpea. Five to ten percent of the area in the other three main production regions (Indian subcontinent, East Africa, and Australia) is also devoted to the production of the kabuli type. The kabuli chickpea is also grown at high elevation areas (>1000 m elevation) in West Asia, especially in Afghanistan, Iran, Iraq, and Turkey; and in the Atlas mountains of North Africa.

Ascochyta blight and fusarium wilt are the two major diseases of chickpea. Leaf miner in the Mediterranean-region and pod borer in other regions are major insect-pests. Drought is the major abiotic stress throughout the chickpea growing areas. Cold assumes importance in Mediterranean environments and the temperate region especially for winter sowing. The kabuli chickpea is mainly grown as a rainfed crop in the wheat-based farming system in areas receiving between 350 mm and 600 mm annual rainfall in the West Asia and North Africa (WANA) region. In Egypt and Sudan, the crop is only grown with supplemental irrigation and in South Asia, West Asia and Central America, a small part of area is grown with supplemental irrigation.

In WANA, where the crop is currently spring-sown, yield can be increased substantially by advancing sowing date from spring to early winter. With the introduction of winter sowing, chickpea cultivation can be extended to lower rainfall (to 300 mm) regions. Increasing plant density and reducing row width can also increase yield significantly, especially in winter-sown crop. Winter sowing allows the crop to be harvested by machine.

Major efforts are underway to stabilize chickpea productivity by breeding cultivars resistant to various stresses, such as the diseases (ascochyta blight and fusarium wilt), insect-pest (leaf miner), parasite (cyst nematode), and abiotic stresses (cold and drought). The exploitation of wild *Cicer* species for transfer of genes for resistance to different stresses and transfer of yield genes is another area receiving high research priority at the Center. DNA fingerprinting in *Ascochyta rabiei* is being pursued with great promise.

During 1996, several collaborative projects continued to operate. Fusarium wilt resistance screening was done in association with the Departamento de Patologia Vegetal, Cordoba, Spain. Studies on leaf miner resistance and application of restriction fragment length polymorphism (RFLP) in characterization of chickpea genotypes and *Ascochyta rabiei* isolates are carried out in collaboration with the University of Frankfurt, Germany. Research on the development of irrigation-responsive cultivars is being conducted with the Agriculture Research Centre, Giza, Egypt.

3.1. Chickpea Breeding

Major objectives of the breeding are (1) to develop cultivars and genetic stocks with high and stable yield and segregating populations to support National Agricultural Research Systems (NARSs) and (2) to conduct strategic research to complement objective 1. Specific objectives in the development of improved germplasm for different regions are:

1. **Mediterranean region**: (a) Winter sowing: resistance to ascochyta blight, tolerance to cold, suitability for machine harvesting, medium to large seed size; (b) Spring sowing: cold tolerance at seedling stage, resistance to ascochyta blight and fusarium wilt, tolerance of drought, early maturity, medium to large seed size.

2. **High elevation areas:** Spring sowing, cold tolerance at seedling stage, resistance to ascochyta blight, terminal drought tolerance, early maturity, and medium to large seed size.
3. **Indian subcontinent and East Africa:** Resistance to ascochyta blight and/or fusarium wilt, drought tolerance, early maturity, small to medium seed size, response to supplemental irrigation.
4. **Latin America:** Resistance to fusarium wilt and root rot, and large seed size.

Major strategic research projects are:

1. Exploitation of wild *Cicer* species for (a) transfer of genes for resistance to cold and cyst nematode and (b) transfer of "yield genes".
2. Pyramiding of genes for resistance to ascochyta blight.
3. Identification of races of fusarium wilt in the WANA region.
4. Increasing shoot biomass yield in chickpea.

(R.S. Malhotra)

3.1.1. Use of Improved Germplasm by NARSs

3.1.1.1. International Nurseries/Trials and Other Breeding Lines

During 1996, 403 sets of different international nurseries and special requests of seeds comprising 17,532 chickpea entries were shipped to NARS to 58 countries (Table 3.1).

(R.S. Malhotra and M.C. Saxena)

3.1.1.2. On-farm Trials in Syria

On-farm trials were conducted in many countries including Algeria, Iraq, Jordan, Lebanon, Morocco, Syria, Tunisia, and

Turkey. Results have been encouraging as demonstrated by a large number of releases of cultivars and their adoption by farmers. The results of on-farm trials in Syria are presented and discussed:

Three chickpea lines (FLIP 88-85C, FLIP 89-29C, and FLIP 90-96C) along with an improved check Ghab 3 were selected by the Directorate of Agriculture and Scientific Research (DASR), Ministry of Agriculture and Agrarian Reforms, from the ICARDA/ICRISAT international trials for conduct of on-farm trials in Syria. These lines were evaluated in on-farm trials at 15 locations (8 at Agricultural Research Stations, 6 at the farmers fields, and 1 at Tel Hadya) throughout Syria. The data were reported from 12 locations and are presented in Table 3.2. The analyses of the data for seed yield revealed significant differences between the entry means at only 4 locations, namely, Al-Ghab, Alkamia, Hasoud and Idleb. The seed size (100-seed weight) was largest in FLIP 88-85C and FLIP 90-96C (33 g) followed by FLIP 89-29C (30 g) and Ghab 3 (28 g). The average ascochyta blight reaction (on 1 to 9 scale where 1 = no visible symptoms, 9 killed) revealed that FLIP 90-96C was the most tolerant (with rating of 2.9), followed by Ghab 3 (3.3), FLIP 88-85C (3.4) and FLIP 89-29C (5.0). The entry FLIP 89-29C was inferior to other cultivars in seed yield at most of the locations. FLIP 88-85C had 16.5% larger seed size than Ghab 3, is tall like Ghab 3 and is suitable for combine harvest. This line has potential to produce over 4 t ha^{-1} seed yield under favorable conditions.

In addition chickpea demonstration trials (using Ghab 2, Ghab 3 and local) were conducted in 8 locations (2 in Der'aa, 1 in Kuneitra, 2 in Hama, 2 in Idleb and 1 in Aleppo). Out of these 8 locations, the Directorate of Extension organized 3 field days in Der'aa, Aleppo and ICARDA, and these field days were well received by the farmers.

(NARSs Scientists and R.S. Malhotra)

Table 3.1. Number of entries furnished in the form of international yield trials and specific nurseries in 1996.

Country	No.of sets trial/nursery	No.of entries	Breeding lines (No.)	Total no. of entries
Algeria	19	705	100	805
Afghanistan	13	639	2	641
Australia	9	361	-	361
Azerbaijan	5	320	-	320
Bangladesh	2	89	-	89
Bhutan	2	128	-	128
Bulgaria	2	65	-	65
Canada	3	114	-	114
Chile	2	74	-	74
Colombia	10	308	-	308
Cyprus	1	25	-	25
Denmark	-	-	6	6
Ecuador	3	115	-	115
Egypt	14	561	-	561
Eritrea	4	164	-	164
Ethiopia	6	222	-	222
France	2	76	-	76
Germany	-	-	4	4
Greece	1	25	-	25
India	21	940	76	1016
Iran	30	1393	1	1394
Iraq	4	192	30	222
Italy	7	302	-	302
Japan	-	-	2	2
Jordan	2	73	2	75
Kazakhstan	1	64	-	64
Kyrghyztan	1	64	-	64
Lebanon	6	242	-	242
Lesotho	2	66	-	66
Libya	6	244	-	244
Lithuania	2	102	-	102
Mexico	7	271	-	271
Morocco	24	1132	4	1136
Nepal	5	251	-	251
Nigeria	-	-	10	10
Oman	-	-	2	2
Pakistan	20	891	2	893
Palestine	5	219	6	225
Peru	4	148	-	148
Portugal	5	213	-	213
Romania	4	146	-	146
Russia	4	181	-	181
Saudia Arabia	6	238	-	180
Slovakia	5	167	-	167
South Africa	2	50	-	50
Spain	9	345	27	372
Sri Lanka	4	171	4	175
Sudan	3	91	-	91
Sweden	1	29	-	29
Total	403	17144	3882	17532

Table 3.2. Seed yield (kg/ha) and other agronomic traits of chickpea entries in the on-farm trials conducted jointly with the Directorate of Agriculture and Scientific Research, Syria and ICARDA during 1995/96.

Entry	Seed yield (kg/ha)									
	Afees	Al-Ghab	Alkamia	Gellin	Hasoud	Hama	Heimo	Homs	Idleb	Izra'a
FLIP 88-85C	1869	2285	2222	577	1184	2025	1930	2635	2271	287
FLIP 89-29C	1762	1634	476	579	687	1593	1855	2384	1791	188
FLIP 90-96C	1590	2141	2334	469	1175	1766	2002	2632	2520	199
Ghab 3	1885	2105	2347	597	1788	1935	1859	2230	2393	315
Location mean	1776	2041	1845	555	1208	1830	1911	2470	2243	247
LSD at P=0.05	N.S.	410	1078	N.S.	684	N.S.	N.S.	N.S.	379	N.S.
C.V. (%)	6.86	6.32	18.37	25.07	17.79	6.79	2.74	18.87	5.31	20.98

Cont. .../

Entry	Seed yield (kg/ha)			100-SW (g)	PLHT (cm)	DFLR	DMAT	AB score	Cold score[a]
	Sqelbia	Tel Hadya	Mean						
FLIP 88-85C	1338	1790	1701	33	58	107	157	3.4	1
FLIP 89-29C	1100	1379	1285	30	60	107	155	5.0	2
FLIP 90-96C	1488	1631	1662	33	57	111	158	2.9	1
Ghab 3	1171	1561	1682	28	56	109	155	3.3	2
Location mean	1274	1590	1583	31	58	108	156		
LSD at P ≤0.05	N.S.	N.S.	194						
C.V. (%)	8.23	10.3	3.85						

PLHT = plant height; DFLR = days to flower; DMAT = days to mature; [a] Scale: 1-9, where 1 = free, 9 = killed; recorded at Tel Hadya; N.S. = not significant.

3.1.1.3. Pre-release Multiplication of Cultivars by National Programs

A large number of lines (Table 3.3) have been chosen by different NARSs during 1995/96 from the chickpea international trials for on-farm testing and pre-release multiplication. This is an incomplete list because we do not have full information. Most of the new lines have tolerance

Table 3.3. Chickpea lines identified for on-farm testing and pre-release multiplication by NARSs in recent years.

Country	Lines
Afghanistan	ILC 482, ILC 3279, FLIP 82-4C, FLIP 82-9C, FLIP 82-16C, FLIP 82-20C
Algeria	FLIP 82-150C, FLIP 83-49C, FLIP 83-71C, FLIP 84-17C, FLIP 84-109C, FLIP 84-145C, FLIP 85-17C
Cyprus	FLIP 85-10C
Egypt	FLIP 80-30C
France	FLIP 84-188C
Iraq	FLIP 82-150C, FLIP 86-6C, FLIP 88-85,FLIP 89-29C, FLIP 90-76C, FLIP 90-96C, FLIP 90-179C
Jordan	FLIP 84-15C, FLIP 85-5C
Lebanon	FLIP 82-150C, FLIP 86-6C, FLIP 88-85C
Libya	FLIP 84-79C, FLIP 84-93C, FLIP 84-144C
Mexico	FLIP 81-293C
Morocco	FLIP 83-48C, FLIP 84-79C, FLIP 84-145C, FLIP 84-182C
Syria	FLIP 88-85C, FLIP 89-29C, FLIP 90-96C
Tunisia	FLIP 83-47C
Turkey	FLIP 81-70C, FLIP 82-74C, FLIP 82-161C, FLIP 82-269C, FLIP 83-31C, FLIP 83-41C, FLIP 83-47C, FLIP 83-77C, FLIP 84-79C, FLIP 85-13C, FLIP 85-15C, 87AK 71112.

to ascochyta blight and cold. They have large seed size, thus they meet consumers' demands. If grown in winter, they attain a minimum height of 40 cm and can be thus harvested by machine. Seeds of some of the promising lines are being multiplied at ICARDA to meet the initial basic demand of NARSs.

(NARS Scientists, R.S. Malhotra and M.C. Saxena)

3.1.1.4. Release of Cultivars by NARSs

To date, NARSs in 22 countries have released 71 lines as cultivars from the improved germplasm furnished by ICARDA (Table 3.4). Forty-nine of these have been released for winter sowing in the Mediterranean region, 14 for spring sowing including four in China, and six for winter sowing in more southerly latitudes. With these releases chickpea cultivars bred at the Center have been released in the four major regions of the chickpea production.

(NARS Scientists, R.S. Malhotra and M.C. Saxena)

Table 3.4. Kabuli chickpea cultivars released by national programs.

Country	Cultivar released	Year of release	Specific features
Algeria	ILC 482	1988	High yield, blight resistance
	ILC 3279	1988	Tall, blight resistance
	FLIP 84-79C	1991	Cold, blight resistance
	FLIP 84-92C	1991	Blight resistance
China	ILC 202	1988	High yield, for Ginghai pr.
	ILC 411	1988	High yield, for Ginghai pr.
	FLIP 81-71C	1993	High yield
	FLIP 81-40WC	1993	High yield
Cyprus	Yialousa (ILC 3279)	1984	Tall, blight resistance
	Kyrenia (ILC 464)	1987	Large seeds
Egypt	Giza 195	1993	High yield under irrigation
France	TS1009 (ILC 482)	1988	Blight resistance
	TS1502 (FLIP 81-293C)	1988	Blight resistance
	Roye Rene (F 84-188C)	1992	Cold, blight resistance
India	Pant G 88-6 (derived from a cross with ILC 613)	1996	Botrytis grey mould resistance released for Tarai area
Iran	ILC 482	1995	High yield, blight resistance
	ILC 3279	1995	High yield, blight resistance
	FLIP 84-48C	1995	High yield, blight resistance
Iraq	Rafidain (ILC 482)	1991	Blight resistance, high yield
	Dijla (ILC 3279)	1991	Tall, blight resistance
Italy	Califfo (ILC 72)	1987	Tall, blight resistance
	Sultano (ILC 3279)	1987	Tall, blight resistance
	Pascia (FLIP 86-5C)	1995	Blight resistance, high yield
	Otello (ICC6306/NEC206)	1995	Blight resistance, desi, feed
	Jubeiha 2 (ILC 482)	1990	High yield, blight resistance
Jordan	Jubeiha 3 (ILC 3279)	1990	High yield, blight resistance
	Janta 2 (ILC 482)	1989	High yield, wide adaptation
Lebanon	Baleela (FLIP 85-5C)	1993	Green seed consumption
	ILC 484	1993	High yield, blight resistance
Libya	ILC 195	1987	Tall, blight resistance
Morocco	ILC 482	1987	High yield, blight resistance
	Rizki (FLIP 83-48C)	1992	Large seed, blight resistance
	Douyet (FLIP 84-92C)	1992	Large seed, blight resistance
	Farihane (FLIP 84-79C)	1995	Large seed, blight resistance
	Moubarak (FLIP 84-145C)	1995	Large seed, blight resistance
	Zahor (FLIP 84-182C)	1995	Large seed, blight resistance
Oman	ILC 237	1988	High yield, Irrigated conditions
	FLIP 87-45C	1995	High yield, blight resistance

Cont.../

Table 3.4. **Cont'd./...**

Country	Cultivars released	Year of release	Specific features
	FLIP 89-130C	1995	High yield, blight resistance
Pakistan	Noor 91 (FLIP 81-293C)	1992	High yield, blight resistance
Portugal	Elmo (ILC 5566)	1989	Blight resistance
	Elvar (FLIP 85-17C)	1989	Blight resistance
Spain	Fardan (ILC 72)	1985	Tall, blight resistance
	Zegri (ILC 200)	1985	Mid-tall, blight resistance
	Almena (ILC 2548)	1985	Tall, blight resistance
	Alcazaba (ILC 2555)	1985	Tall, blight resistance
	Atalaya (ILC 200)	1985	Mid-tall, blight resistance
	Athenas (ILC 2 x CA2156)	1995	Large seed, blight resistance
	Bagda (ILC 72 x CA 2156)	1995	Large seed, blight resistance
	Kairo (ILC 72 x CA 2156)	1995	Large seed, blight resistance
Sudan	Shendi	1987	High yield, irrigated conditions
	Jebel Marra-1 (ILC 915)	1994	High yield, irrigated conditions
	Wad Hamid-1 (FLIP 89-82C)	1996	High yield, Large seeded
Syria	Ghab 1 (ILC 482)	1986	High yield, blight resistance
	Ghab 2 (ILC 3279)	1986	Tall, blight resistance
	Ghab 3 (FLIP 82-150C)	1991	High yield, cold & blight res.
Tunisia	Chetoui (ILC 3279)	1986	Tall, blight resistance
	Kassab (FLIP 83-46C)	1986	Large seeds, blight resistance
	Amdoun 1 (Be-sel-81-48)	1986	Large seeds, wilt resistance
	FLIP 84-79C	1991	Blight, cold resistance
	FLIP 84-92C	1991	Large seed, blight resistance
	ILC 195	1986	Tall, blight resistance
Turkey	Guney Sarisi 482	1986	High yield, blight resistance
	Damla (FLIP 85-7C)	1994	Blight resistance
	Aziziye (FLIP 84-15C)	1994	Blight resistance
	Akcin (87AK71115)	1991	Tall, blight resistance
	Aydin 92 (FLIP 82-259C)	1992	Large seed, blight resistance
	Menemen 92 (FLIP 85-14C)	1992	Large seed, blight resistance
	Izmir 92 (FLIP 85-60C)	1992	Large seed, blight resistance
USA	Dwelley (Surutato x FLIP 85-58C)	1994	Blight resistance
	Sanford (Surutato x FLIP 85-58C)	1994	Blight resistance

3.1.2. Screening for Stress Tolerance

3.1.2.1. Cultivated Species

3.1.2.1.1. Wilt Resistance

Fusarium wilt induced by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f.sp. *ciceri* (Padwick) Snyd. & Hans. is the second most important disease of chickpea worldwide. In WANA, it is prevalent in parts of North Africa and in the Nile Valley. *F. oxysporum* f. sp. *ciceri* is both soil-borne and seed transmitted. Breeding for Fusarium wilt-resistance has been one of the main objectives in chickpea improvement. In this effort, the major bottleneck has been the presence of different races of the pathogen.

Very recently we have developed about 1.3 hectare of Fusarium wilt-sick plot at Tel Hadya so that we can screen and develop chickpeas resistant to Fusarium wilt. The results of screening of 2174 Kabuli breeding lines (developed at ICARDA) against Fusarium wilt during 1994/95 and 1995/96 are presented in Table 3.5. The results revealed that 7 lines were completely free from damage (rating 1 on 1 to 9 scale) by Fusarium wilt and 188 lines showed a resistant reaction (rating 2 to 4).

The preliminary screening of 867 lines revealed that 77 lines were tolerant with rating between 2 and 4. These lines will be evaluated next year for confirmation of resistance.

The Chickpea International Fusarium Wilt Nursery (CIFWN) with 31 test entries, the fusarium wilt differential, and preliminary yield trial winter (PYT-W) with wilt resistant lines were grown during 1995/96 season in wilt-sick plot. The results for evaluation for fusarium wilt resistance in CIFWN, differential and PYT-W (Table 3.5) showed that 16, 8, and 43 lines, respectively, showed a tolerant reaction (rating $\leq$ 4).

(R.S. Malhotra)

Table 3.5. Reaction of FLIP (breeding) lines, germplasm lines, entries in the Chickpea International Fusarium Wilt Nursery (CIFWN), in diferetial set and in the Preliminary Yield Trial (PYT-W) in Tel Hadya.

Rating scale	% of plants killed	Type of material				
		FLIP lines	Germplasm lines	CIFWN	Differential	PYT-W
1	0	7	0	3	2	23
2	1-5	73	35	7	3	12
3	6-10	72	25	4	2	5
4	11-20	43	17	2	1	3
5	21-40	198	50	0	1	4
6	41-60	330	63	4	0	0
7	61-80	453	198	5	0	3
8	81-99	258	93	5	0	6
9	100	740	386	1	2	8
Total		2174	867	31	11	64

3.1.2.2. Segregating Material

The breeding material comprising five F_2 crosses, 14 F_4 bulk populations, and 86 F_5 progenies were grown in wilt-sick plot for selection of breeding material resistant to Fusarium wilt. Out of these 696 plants in F_4 were selected, and 22 progenies in F5 were bulked.

In addition, 5 crosses in F_2 and 10 crosses in F_4 made for drought tolerance were also grown in wilt-sick plot. Out of these 5 crosses in F_2 and 445 resistant plants in F_4 were selected for advancement.

The Fusarium wilt sick plot will help in development of breeding materials at ICARDA. We are now using these sources with higher level of resistance in our breeding program for improvement of Fusarium wilt resistance in chickpea.

Table 3.6. Reaction of breeding materials to Fusarium wilt grown in sick plot at Tel Hadya, 1995/96.

Generation	Reaction on 1-9 scale									
	1	2	3	4	5	6	7	8	9	Total
A. Fusarium wilt										
F_5 Progenies	82	3	1	0	0	0	0	0	0	86
F_4 Bulk	0	5	5	4	0	0	0	0	0	14
F_2 Bulk	0	2	1	1	1	0	0	0	0	5
B. Drought and Fusarium wilt										
F_4 Bulk	0	0	4	4	0	1	0	1	0	10
F_2 Bulk	0	0	1	1	2	1	0	0	0	5
Total	82	10	12	10	3	2	0	1	0	120

3.1.2.2.1. Ascochyta Blight

Ascochyta blight caused by *Ascochyta rabiei* is the most serious foliar disease of chickpea in WANA region, particularly where low temperatures (15-25^0 C) prevail during the crop season. Its occurrence is irregular and is weather dependent. However, a good season for the chickpea crop is often favorable to ascochyta blight. Winter sowing of chickpea provides an opportunity to increase chickpea yield by almost 100%; unfortunately, it also increases the risk of ascochyta blight devastation. Therefore, control of ascochyta blight is essential to increase chickpea production and yield stability. Host resistance is the most practical and economic way to manage the ascochyta blight problem, but its level has to improve.

3.1.2.2.1.1. Evaluation of Segregating Populations for Resistance to Existing Race Population in Debris and Artificial Inoculation of a Mixture of Six Races of Ascochyta Blight under Tel Hadya Conditions

The reaction of F_2 to F_5 generations to the existing race populations and a mixture of six races of ascochyta blight sprayed in the field at Tel Hadya is given in Table 3.7. The disease developed in epidemic form as was evident from the death of check lines throughout the nursery. The isolation of ascochyta blight pathogen from the debris in field in the previous season revealed that there are three pathotype groups, pathotype I, II and III. Pathotypes I and II represent the earlier mentioned six races, and pathotype III is new and more virulent. All the three pathotypes were present in the field. No progeny was rated 1, 2 or 3, but 136 and 1168 progenies in F_5 were rated 4 and 5. Another 2719 F_5 progenies were rated 6. These results indicate that there is

Table 3.7. Reaction of F_2 to F_5 generations to Ascochyta blight at Tel Hadya, 1995/96.

Generation	Reaction on 1-9 scale									
	1	2	3	4	5	6	7	8	9	Total
F_2 Bulk	0	0	0	0	1	10	19	22	2	54
F_4 Bulk-Large	0	0	0	0	18	25	19	0	0	62
F_4 Bulk-Tall	0	0	0	0	1	6	1	0	0	8
F_4 Bulk-Tall	0	0	0	2	12	39	30	2	0	85
F_5 Progenies-L	0	0	0	0	22	201	157	72	12	464
F_5 Progenies-T	0	0	0	49	502	1290	1191	407	61	3500
F_5 Progenies-E	0	0	0	3	271	382	210	73	6	945
F_5 Progenies	0	0	0	84	373	846	550	194	32	2079
Total	0	0	0	138	1200	2799	2177	770	113	7197

Scale: 1 = free from damage; 2 = highly resistant; 3 = resistant; 4 = moderately resistant; 5 = intermediate; 6 = moderately susceptible; 7 = susceptible; 8 = highly susceptible; and 9 = all plants killed.

an immediate need to change the strategy to develop resistant materials using genotypes resistant to more virulent pathotype III. This will only help to develop materials from which high yielding lines could be selected.

(R.S. Malhotra)

3.1.2.2.1.2. Evaluation of Breeding Lines for Resistance to Existing Pathotypes I, II, and III Populations in Debris and Inoculum Spray of a Mixture of Six Races

Lines included in different trials were evaluated in the ascochyta blight field during the 1995/96 season (Table 3.8). None of the lines exhibited rating 3 or less. One line had a rating of 4, and 39 lines had a rating of 5. Likewise, 110, 232, 89 and 7 lines were with rating 6, 7, 8 and 9, respectively, indicating that the majority of lines identified resistant in the past were susceptible to the increasing population of pathotype III in the field.

(R.S. Malhotra)

Table 3.8. Reaction of breeding lines in different trials to the existing ascochyta blight inoculum in the debris (pathotypes I, II and III) and artificial inoculum spray of mixture of *six races of A. rabiei* at Tel Hadya, 1995/96.

Trial Name	Disease Reaction on 1-9 scale									
	1	2	3	4	5	6	7	8	9	Total
CIYT-W-MR	0	0	0	0	8	9	4	0	0	21
CIYT-SPR	0	0	0	0	0	4	12	5	0	21
CIYT-SL1	0	0	0	0	2	7	11	1	0	21
CISN-W	0	0	0	0	1	18	31	11	0	61
CISN-SP	0	0	0	0	4	12	34	11	0	61
CISN-SL1	0	0	0	1	8	7	20	10	0	46
PYT	0	0	0	0	16	53	120	51	7	247
Total	0	0	0	1	39	110	232	89	7	478

3.1.2.2.1.3. Reaction of the Entries in Chickpea International Ascochyta Blight Nursery (CIABN) to Ascochyta Blight at Tel Hadya

The CIABN comprised 50 test entries. The susceptible check sown after every two test entries was uniformly killed throughout the nursery. Out of 50 test entries, one line (ICC 13729) had a rating of 2, 7 lines (FLIP 94-508C, FLIP 94-510C, ICC 3912, ICC 3919, ICC 3991, ICC 4475 and ICC 12004) had a rating of 3, 3 lines (FLIP 92-179C, FLIP 94-509C, ICC 14903) had rating of 4, and 13 lines (FLIP 84-182C, FLIP 88-83C, FLIP 90-85C, FLIP 91-196C, FLIP 92-45C, FLIP 92-155C, FLIP 92-164C, FLIP 92-174C, FLIP 92-175C, FLIP 92-190, FLIP 92-194C, FLIP 93-160C, FLIP 93-174C) had rating of 5. In addition, 18 and 8 lines, respectively had a rating of 6 and 7.

In the gene pyramiding project for combining sources of resistance to ascochyta blight, the sources of resistance of diverse origin were crossed. Five crosses made during 1994/95 were advanced in off-season to get F_2 bulks. These 5 F_2 bulks alongwith 201 progenies in F_3, and 243 progenies in F_4 were grown in field for evaluation for Ascochyta blight during the season. The results (Table 3.9) revealed that 137 progenies

Table 3.9. Reaction of segregating populations/lines to ascochyta blight in pyramiding of genes (resistant x resistant crosses) experiment at Tel Hadya, 1995/96.

Generation	Reaction on 1-9 scale									
	1	2	3	4	5	6	7	8	9	Total
F_4 Progenies	0	17	120	49	35	21	1	0	0	243
F_3 Progenies	0	0	0	5	64	74	41	17	0	201
F_2 Bulk	0	0	1	2	0	0	0	1	1	5
Total	0	17	121	56	99	95	42	18	1	449

Ascochyta blight rating: 1 = free, 9 = all plants killed.

in F_4, none in F_3, and 1 bulk population in F_2 were rated as 2 or 3 (on 1 to 9 scale, where 1 = free, 9 = killed). Another 2 F_2 bulks, 5 F_3 and 49 F_4 progenies were tolerant (rating = 4) to ascochyta blight.

(R.S. Malhotra)

3.1.2.2.2. Drought Tolerance

Drought causes severe yield loss in chickpea. Lack of appropriate screening technique and rating scale for drought tolerance have restricted plant breeders from making progress. Based on the study initiated in 1990 a screening technique and a rating scale to evaluate germplasm for drought tolerance were developed.

Based on this screening technique involving (1) delayed sowing by three weeks during spring at a relatively dry site, and (2) preliminary evaluation of materials on 1 (=resistant) to 9 (=susceptible) scale to discard susceptible lines. A total of 1000 new germplasm lines were evaluated this season, only 19 of these lines with rating 4 were drought tolerant (Table 3.10).

Another 40 lines in Chickpea International Drought-Tolerant Nursery (CIDTN) were evaluated for drought tolerance. Based on these criteria, 31 lines with rating 3 and 4 were drought tolerant (Table 3.10). The lines with 3 rating included ILC 5371, ILC 6119, FLIP 87-5C, FLIP 87-7C, FLIP 87-8C, and FLIP 88-42C.

At ICARDA, we are utilizing these lines to generate material with high yield, drought tolerance and disease resistance.

(R.S. Malhotra and C. Johansen)

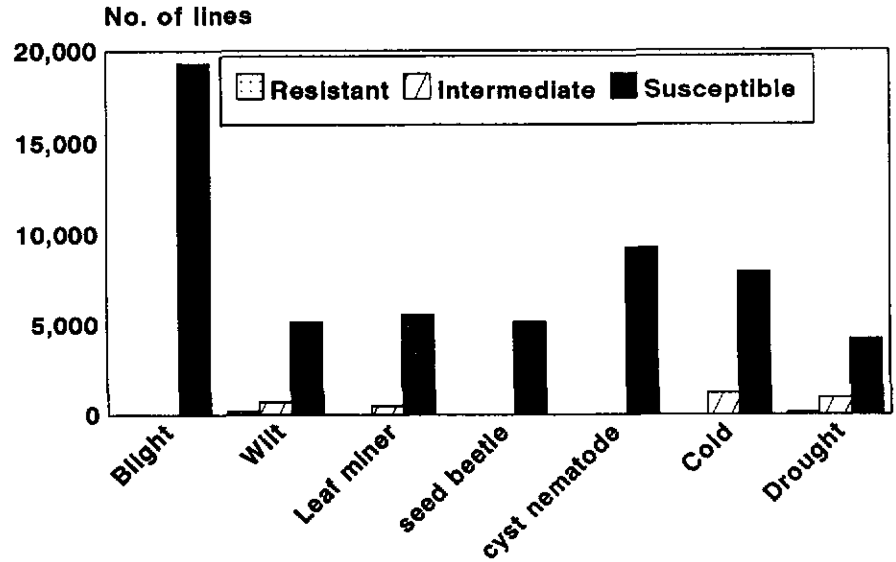
Table 3.10. Evaluation of CIDTN-96 and chickpea germplasm lines for drought tolerance at Tel Hadya, 1995/96.

Visual score*	CIDTN-96	Germplasm
1	0	0
2	0	0
3	6	0
4	25	19
5	9	107
6	0	535
7	0	277
8	0	53
9	0	9
Total	40	1000

* where 1 = resistant, early flowering, very good early plant vigor, 100% pod setting; 9 = highly susceptible, lack early plant vigor, no flowering, no pod setting.

3.1.2.2.3. Combined Evaluation to Seven Stresses

The number of lines evaluated between 1978 and 1996 for different stresses are shown in Figure 3.1. The results include evaluations in the 1995/96 season for wilt and drought. Resistant sources have been identified for all stresses except to seed beetle and cyst nematode. Further evaluation of lines will continue only for wilt and drought because we believe there is scope to identify improved sources of resistance to these stresses.



Resistant	32	187	8	0	0	13	135
Intermediate	9	704	485	0	20	1,191	916
Susceptible	19,329	5,150	5,532	5,153	9,237	7,891	4,154

Fig. 3.1. Reaction of chickpea germplasm accessions to biotic and abiotic stresses at Tel Hadya, 1978-1996.

3.1.2.3. Wild Species

3.1.2.3.1. Fusarium Wilt

In search for higher level of resistance we have also evaluated 173 accessions from 7 wild *Cicer* species (Table 3.11) and observed that 9 accessions from *C. bijugum*, 26 from *C. judaicum*, and 11 from *C. pinnatifidum*, were completely free from the damage by Fusarium wilt. None of the accessions from *C. echinospermum* and *C. pinnatifidum* were resistant to Fusarium wilt.

(R.S. Malhotra)

Table 3.11. Reaction of wild *Cicer* species to Fusarium wilt at Tel Hadya, during 1994/95 and 1995/96 seasons.

Cicer	Rating scale*									Total
	1	2	3	4	5	6	7	8	9	
bijugum	9	0	1	1	7	4	1	0	2	25
cuneatum	0	0	0	0	1	1	0	0	0	2
echinospermum	0	0	0	0	0	0	0	0	9	9
judaicum	26	0	1	1	4	5	4	2	4	47
pinnatifidum	11	0	0	1	3	5	4	1	12	37
reticulatum	0	0	0	0	0	1	1	1	47	50
yamashitae	0	0	0	0	0	0	0	0	3	3
Total	46	0	2	3	15	16	10	4	77	173

* 1 = free from damage, 9 = all plants killed.

3.1.2.3.2. Drought Tolerance

Preliminary screening for drought tolerance in annual wild *Cicer* species was carried out with the goal of improving adaptability of the cultigen by exploiting its wild relatives. Two-hundred forty-five annual wild *Cicer* accessions were screened for few phenological and agronomic traits following late spring sowing in a replicated trial. Out of eight wild species *C. judaicum* (several accessions) and *C. pinnatifidum* (few accessions) continued to flower and to set pods when remaining species were since long dried-up. Significant variation was observed among and within species based on field performance of agronomic traits. Among the annual wild *Cicer* species two accessions each of *C. bijugum* (ILWC 34, ILWC 65) and *C. reticulatum* (ILWC 36 and ILWC 116) gave highest seed yield under drought conditions.

(B. Ocampo and R.S. Malhotra)

3.1.2.3.3. Cold Tolerance

Forty two accessions of wild *Cicer* species were evaluated for confirmation of their reaction to cold. *C. bijugum* exhibited highest level of cold tolerance followed by *C. reticulatum, C. pinnatifidum and C. judaicum* (Table 3.12).

Table 3.12. Reaction of wild *Cicer* species to cold under field conditions "reconfirmation" at Tel Hadya, 1995/96.

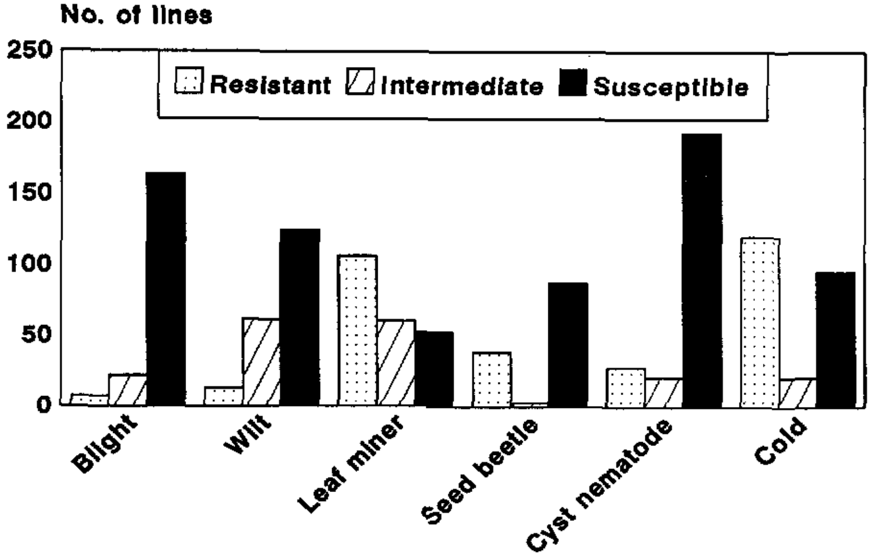
Cicer species	Cold rating				Total
	1	2	3	4	
bijugum	6	4	0	0	10
echinospermum	0	0	4	0	4
judaicum	0	0	0	1	1
pinnatifidum	0	0	1	1	2
reticulatum	0	23	2	0	25
Total	6	27	7	2	42

Cold rating: 1-9; 1 = free, 9 = all plants killed.

(R.S. Malhotra)

3.1.2.3.4. Evaluation for Multiple Stresses

Evaluation of eight annual wild *Cicer* species has been conducted to identify sources of resistance to multiple stresses. The results are summarized in Figure 3.2. Sources of resistance were found for all six stress factors. Wild species were the only source of resistance so far found to seed beetle and cyst nematode and had higher level of resistance than the cultivated species for fusarium wilt, leaf miner, and cold. The most important source for resistance to different stress factors was found in *C. bijugum*, while *C. yamashitae* was the least important.



Resistant	7	13	107	39	28	121
Intermediate	22	62	61	3	21	21
Susceptible	164	125	53	88	193	96

Fig. 3.2. Reaction of wild Cicer accessions to six stresses at Tel Hadya, 1987-1996.

3.1.2.4. Development of Sources of Resistance to Biotic and Abiotic Stresses in Chickpea and *Cicer* species

Known sources of resistance are shown in Table 3.13. Sources of resistance are available individually to all seven stresses for which research is being conducted. Sources with multiple stress-resistance have been identified in *Cicer* species. These sources have been shared with NARS through the distribution in the Legumes International Testing Program. They have been used in crossing programs at ICARDA and many national programs. High yielding lines with combined resistance to ascochyta blight and cold have been bred at ICARDA and shared with the national programs. Research at ICARDA has acted as a catalyst and many national programs are engaged in resistance breeding.

(R.S. Malhotra, K.B. Singh, S. Weigand, M.C. Saxena, M. Omar (ICARDA), M.V. Reddy, C. Johansen (ICRISAT, India), A. Porta-Puglia, N. Greco and M. Di Vito (Italy), H. Halila (Tunisia), R. Jimemez-Diaz (Spain))

3.1.3. Germplasm Enhancement

3.1.3.1. Improvement in Shoot Biomass Yield

Low shoot biomass in the Mediterranean basin is an important reason among others for poor yield. Our previous results show that seed yield in chickpea is highly correlated with biomass yield (above ground plant parts). A project to increase the biomass yield was initiated during 1989/90. As a result of hybridization between parents involving high yield and biomass the progenies were bulked and evaluated for biomass. Ninety four progenies were tested in three different trials with 35, 35 and 24 test entries during 1995/96. The results are summarized in Tables 3.14, 3.15 and 3.16.

The trial mean for biomass yield was 7109 kg/ha in PYT-1, 6234 kg/ha in PYT-2 and 6260 kg/ha in PYT-3 as compared to the respective biomass yields of 5592, 5980, and 4647 kg/ha for the standard check (ILC 3279). The highest biomass yield was 8.776 tons in PYT-1 (for Sel 95TH 3725), 7.456 tons (for S 92260) in PYT-2 and 7.517 tons (for Sel 95TH 30302) in PYT-3. A large number of lines in PYT-1, 7 lines each in PYT-2 and PYT-3, produced more than 2 ton/ha seed yield.

Table 3.13. Resistance sources to biotic and abiotic stresses in chickpea developed at ICARDA.

Stresses	Sources of resistance
Single stresses	
Ascochyta blight (AB)	ILC (202, 6482) ICC (4475, 6328, 12004) FLIP 90-98C, 91-2C, 91-18C, 91-22C, 91-24C, 91-46C, 91-50C, 91-54C)
Fusarium wilt (FW)	ILC (267, 1278, 1300), FLIP (86-93C, 87-33C, 87-38C)
	ILWC 34, ILWC 66 (*C. bijugum*); ILWC 44, ILWC 192 (*C. judaicum*); ILWC 82, ILWC 149 (*C. pinnatifidum*)
Leaf miner (LM)	ILC 3800, ILC 5901, ILC 7738
Seed beetle (SB)	ILWC 39, ILWC 181
Cyst nematode (CN)	ILWC 292
Cold (CO)	ILC 8262, ILC 8617
	ILWC 66, ILWC 220, ILWC 240, ILWC 241, ILWC 243 (*C. bijugum*)
Drought (DR)	FLIP 87-59C
Multiple stresses	
AB, FW, CO	FLIP 91-178C, FLIP 93-53C, FLIP 93-98C
AB, LM, CN	ILWC 250 (*C. pinnatifidum*)
AB, LM, CO	ILWC 37 (*C. cuneatum*)
AB, FW, SB, CN, CO	ILWC 70 (*C. bijugum*)
AB, SB, CN, CO	ILWC 62, ILWC 70, ILWC 73 (*C. bijugum*)
FW, LM, SB	ILWC 98, ILWC 102 (*C. judaicum*)
LM, SB,CO	ILWC 39 (*C. echinospermum*)
SB, CO	ILWC 112 (*C. reticulatum*); ILWC 179, ILWC 181 (*C. echinospermum*)

Table 3.14. Seed yield (SYLD), biomass (BYLD), plant height (PTHT), 100-SW, and harvest index (HI) of high yielding lines in PYT-Biomass-1 at Tel Hadya, 1995/96.

Sel. 95TH	Parentage	SYLD	BYLD	PTHT	100SW	HI
4097	(FLIP 89-63C x FLIP 85-45C) x FLIP 85-18C	2776	7735	72	30.2	35.9
3742	(FLIP 88-62C X FLIP 85-15C) X S 91167	2700	7796	74	34.6	34.6
6096	S 91167 X FLIP 84-164C	2633	7939	73	35.8	33.2
3851	(FLIP 88-70C X FLIP 85-15C) X S 91167	2447	7857	74	39.0	31.1
3764	(FLIP 88-62C X FLIP 85-15C) X S 91167	2420	6980	76	33.6	34.7
3744	(FLIP 88-62C X FLIP 85-15C) X S 91167	2408	6245	74	32.7	38.6
10266	FLIP 84-92C X FLIP 88-39C	2388	6653	69	33.7	35.9
4344	(FLIP 85-5C X FLIP 89-28C) X FLIP 82-11C	2373	8735	72	39.6	27.2
3727	(FLIP 88-62C X FLIP 85-15C) X S 91167	2371	8306	74	34.4	28.5
3725	(FLIP 88-62C X FLIP 85-15C) X S 91167	2296	8776	73	34.8	26.2
3828	(FLIP 88-70C X FLIP 85-15C) X S 91167	2229	7735	74	36.5	28.8
3864	(FLIP 88-70C X FLIP 85-15C) X S 91167	2192	7714	75	36.9	28.4
3947	(FLIP 88-70C X FLIP 85-15C) X S 89TH 78998	543	8061	73	37.9	6.7
Check	ILC 3279	1906	5592	71	28.5	34.1
S.E.		286	776			
Mean		1939	7109	74	35.0	27.3
LSD at P = 0.05		822	2226			
C.V. (%)		20.9	15.4			
Significance		*	NS			

Table 3.15. Seed yield (SYLD), biomass (BYLD), plant height (PTHT), 100-SW, and harvest index (HI) of high yielding lines in PYT-Biomass-2 at Tel Hadya, 1995/96.

Entry name	Parentage	SYLD	BYLD	PTHT	100SW	HI
S 95053	S 91151 X FLIP 85-18C	2394	6864	76	35.6	34.9
S 92307	(FLIP 85-122C X FLIP 85-137C) X FLIP 85-18C	2177	6655	74	33.9	32.7
S 95074	S 91168 X FLIP 85-18C	2141	7433	77	36.9	28.8
S 92260	(FLIP 85-62C X FLIP 81-293C) X FLIP 85-62C	2133	7456	81	37.7	28.6
S 92306	(FLIP 85-122C X FLIP 85-137C) X FLIP 85-18C	2121	6240	74	30.7	34.0
Ghab 1	ILC 482	2112	5116	55	30.5	41.3
S 95078	ICCV 89853 X S91208	2018	6984	77	35.7	28.9
S 95044	S 91208 X ILC 4641	1946	6794	75	35.6	28.6
S 92262	(FLIP 85-91C X ILC 3856) X FLIP 85-91C	1917	5710	70	33.3	33.5
S 95048	S 91151 X FLIP 85-18C	1741	7379	76	42.1	23.6
S 92217	(FLIP 85-18C X FLIP 85-90C) X FLIP 85-18C	1681	7185	80	38.1	23.4
S 95063	S 91167 X FLIP 84-33C	1293	7265	78	38.0	17.8
S 95066	S 91167 X FLIP 84-33C	1074	6826	77	38.6	15.7
Check	ILC 3279	2011	5980	75	27.2	33.6
S.E.		169	431			
Mean		1740	6234	76	35.2	28.1
LSD at P = 0.05		493	1256			
C.V. (%)		13.7	9.8			
Significance		*	*			

Table 3.16. Seed yield (SYLD), Biomass (BYLD), plant height (PTHT), 100-SW, and harvest index (HI) of high yielding lines in PYT-Biomass-3 at Tel Hadya, 1995/96.

Sel. 95TH	Parentage	SYLD	BYLD	PTHT	100SW	HI
30264	(FLIP 85-62C X FLIP 81-293C) X FLIP 85-62C	2465	6159	76	40.1	40.0
30330	ILC 72 X TT 2	2293	6689	68	32.3	34.3
30316	ILC 72 X TT 2	2253	7008	71	38.1	32.1
30310	ILC 72 X TT 2	2139	6684	71	37.7	32.0
30254	(FLIP 85-18C X FLIP 85-90C) X FLIP 85-18C	2131	6882	75	42.0	31.0
30302	ILC 72 X TT 2	2054	7517	73	36.7	27.3
30268	(FLIP 85-62C X FLIP 81-293C) X FLIP 85-62C	2009	6649	75	40.3	30.2
30314	ILC 72 X TT 2	1988	6583	76	36.5	30.2
30300	ILC 72 X TT 2	1924	6912	77	38.0	27.8
30276	FLIP 86-77C X FLIP 82-22C	1864	6563	65	40.6	28.4
30284	ILC 72 X LPB 1	1521	6680	75	37.9	22.8
Check	ILC 3279	1696	4647	66	27.9	36.5
SE		149	508			
Mean		1938	6260	69.7	36.9	31.0
LSD at P = 0.05		446	1524			
C.V. (%)		109	11.5			
Significance		NS	NS			

3.1.4. Development of Improved Germplasm

3.1.4.1. Breeding Methods:

Four breeding schemes are *operating to* develop improved germplasm for different agroclimatic zones. They are:

1. **The bulk-pedigree method of breeding for blight- and cold-resistance**. This method was adopted during the mid 1980s and it takes four years from crossing to bulking of new lines. It allows effective screening for cold and ascochyta blight during the main season. This type of material is suitable primarily in WANA for winter and spring sowing. Some of these materials are also useful in the Indian subcontinent.

2. **Two cycle selection for ascochyta blight and fusarium wilt**. After the development of wilt-sick plot at Tel Hadya, we have developed a two-cycle selection for ascochyta blight and fusarium wilt resistance. It allows selection for blight in one generation and for wilt in the next. This type of material is in demand for North Africa.

3. **Bulk-pedigree method of breeding for wilt- and drought-resistance**. This breeding scheme consists in delayed sowing in wilt-sick plot and harvesting in off-season when part of the crop has matured. Output from this project benefits East Africa.

4. **Generation of large-seeded, wilt-resistant germplasm**. We sieve to select large-seeded material while advancing method through bulking up to F_4 generation. The focus of this project is Latin America.

3.1.4.2. Performance of Newly Bred Lines at ICARDA Sites

Two hundred and forty eight newly-bred lines were evaluated in four preliminary yield trials (PYTs) at two locations (Tel Hadya and Terbol) and in two seasons (winter and spring). Several lines were numerically superior to the check Ghab 1 (the check in winter) and ILC 1929 (the check in spring) in seed yields. But the seed yields of only 106 lines at Tel Hadya and 128 lines at Terbol were significantly superior to check in winter and none of the test entries was superior to the check in spring. (Table 3.17).

The 1995/96 was a normal season, and the seed yield in winter and spring plantings were good. The winter chickpea produced 99% and 61% more seed yield than the spring chickpea at Tel Hadya, and Terbol, respectively with an average of 76% increase over the two locations. The overall mean seed yield for these locations for the last thirteen years gave 72% increase in yield in winter over spring planting (Figure 3.3).

(R.S. Malhotra)

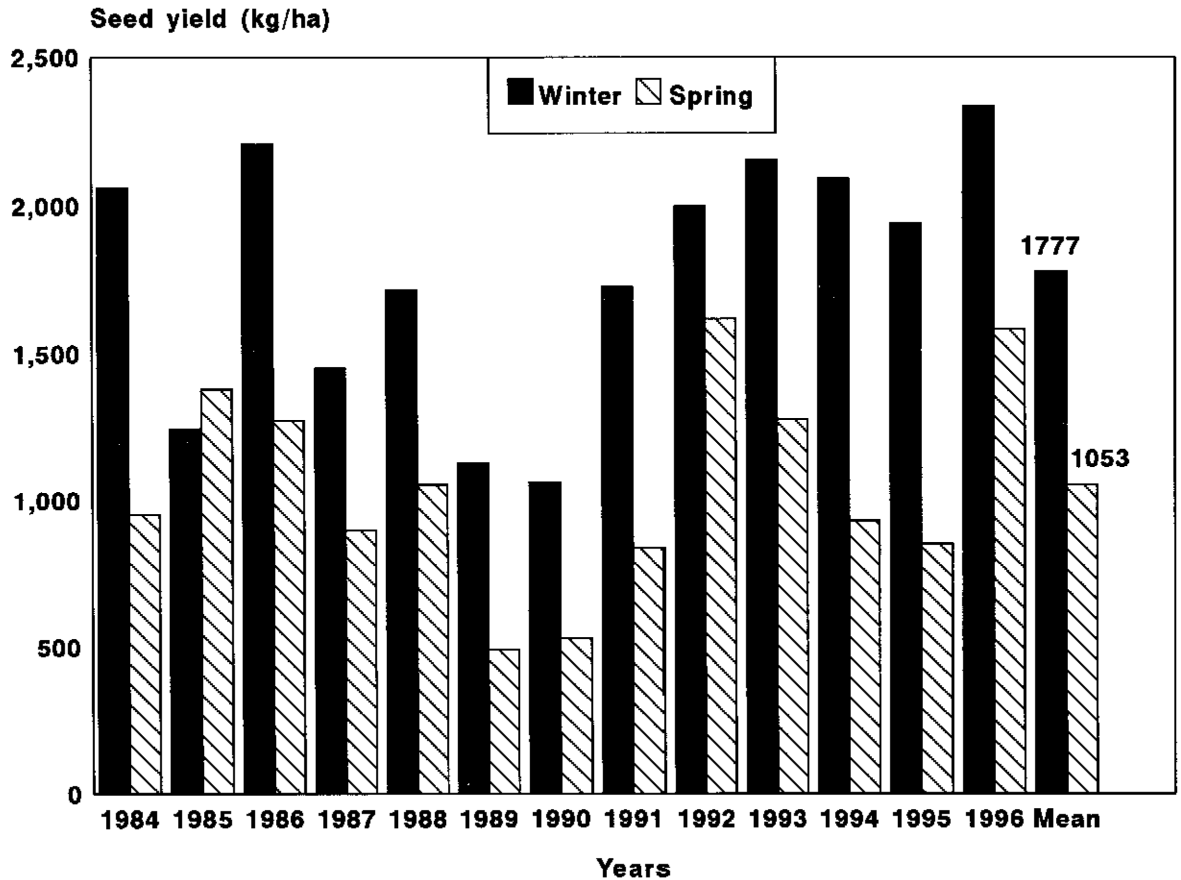


Fig. 3.3. Mean seed yield (kg ha^{-1}) of chickpea grown in winter and spring at three locations (Tel Hadya, Jindiress and Terbol) in eleven years (1984-94) and two locations (Tel Hadya and Jindiress) in the last two years (1995-96).

Table 3.17. Performance of newly developed lines during winter and spring plantings at Tel Hadya and Terbol, 1995/96.

Location and season	No. Of trials	No. Of entries			Yield		Range for	
		Tested	Exceeding check	Sig. Exceeding check	Mean of Location	Mean of Highest yielding entry (kg ha^{-1})	C.V. (%)	LSD ($P \leq 0.05$) (kg ha^{-1})
<u>Tel Hadya</u>								
-Winter	4	248	106	23	2119	2695	10-13	408-556
-Spring	4	248	0	0	1063	1742	14-17	286-397
<u>Terbol</u>								
-Winter	4	248	128	80	2546	3258	9-12	485-629
-Spring	4	248	0	0	1581	2308	10-12	301-402

3.1.5. Strategic Research

3.1.5.1. Development of Early Flowering and Podding Lines under Low Temperature

Chickpea is highly sensitive to low temperature as compared to faba bean, lentil and pea. The latter crops start flowering and podding much before chickpea when planted at the same time. This may be a distinct disadvantage to chickpea especially during dry seasons or seasons with low rainfall during the spring months. Therefore, an effort has been underway to develop early flowering lines under low temperatures at Tel Hadya since 1992/93. To achieve this goal, we made intraspecific and interspecific crosses and also made selections from early flowering germplasm. Sixty nine F_4 progenies from eleven crosses were grown during 1995/96. Thirty six progenies flowering 10 days earlier than ILC 482 (early flowering check) were selected and harvested individually.

An early flowering trial with 29 test entries and 4 checks was grown for yield evaluation. The seed yield and days to flowering of the top 10 lines of the material sown for the development of early lines under low temperature is given in Table 3.18. All these lines flowered at least 10 days earlier than the improved check ILC 482 and were almost flowering at the time as that of earliest flowering check (ILC 6104). These lines also exhibited increase in seed yield as compared to both checks (ILC 6104 and ILC482). Two lines namely, FLIP 84-92C and FLIP 91-119C gave significantly higher yield than ILC 482 and were also earlier in flowering.

Table 3.18. Seed yield of top ten lines from the material sown for the development of early lines under low temperature at Tel Hadya, 1995/96.

Entry name	DFLR	Yield (kg/ha)
FLIP 84-92C	115	3472
FLIP 91-119C	114	3187
FLIP 89-83C	112	2823
FLIP 91-117C	111	2807
FLIP 92-92-IC	114	2741
FLIP 91-117C	111	2722
FLIP 91-126C	112	2579
FLIP 92-92-2C	115	2522
FLIP 91-122C	111	2491
FLIP 89-83C	111	2481
Check 1 (ILC 6104)	112	1449
Check 2 (ILC 482)	128	2281
S.E.	0.76	255
Mean	115.3	1971
LSD at P = 0.05	2.19	734
C.V. (%)	0.93	18.3

3.1.6. Interspecific Hybridization

So far we have succeeded in crossing between the cultigen and two other species namely, *C. reticulatum* and *C. echinospermum*. In our interspecific hybridization work we have been involving only kabuli types from the cultigen. We thought probably this may be a barrier in interspecific crossing. Thus recently, in the year 1995/96 to exploit other wild species for introgression of desirable resistance genes we reviewed our efforts and in our crossing program we substituted the kabuli types with the desi types and succeeded in getting about 163 seeds from the crosses with *C. bijugum, C. pinnatifidum, and C. judaicum*. These crossed seeds are being evaluated for confirmation of their hybridity. If this turns to be true we will be in a position to introgress the resistances from other wild species.

(R.S. Malhotra and B. Ocampo)

3.1.6.1. Introgression of Wild Genes to the Chickpea Cultigen

Wild species have significantly contributed to the crop improvement mainly for incorporation of biotic and abiotic stress resistance from the wild to the cultigen. At ICARDA most of the accessions from 8 wild annual *Cicer* species have been evaluated for various biotic and abiotic stresses and some of these have been observed to have higher level of tolerance to one or more stresses. So far at ICARDA we succeeded to cross the kabuli cultigen only with the two wild *Cicer* species, *C. reticulatum* and *C. echinospermum*. Thus attempts have been made to introgress the tolerance available only in these two crossable species. We have succeeded in introgressing cyst nematode resistance from *C. reticulatum*, and cold tolerance from *C. reticulatum* and *C. echinospermum* to the cultigen. A large number of uniform lines with resistance to nematode and cold are now available and are being increased for their yield and agronomic evaluation. During this process of interspecific hybridization an attempt was also made to increase seed yield and results of nine years study are summarized here.

3.1.6.1.1. Cold Tolerance: Yield Data for Cold for Intraspecific Crosses

Seven derived lines from intraspecific crosses for improvement of cold tolerance with 3 checks, 2 tolerant and one susceptible were put in an RCB experiment with 3 replications at Tel Hadya. The seed yield under October plantings revealed that Sel 95TH 1802 was the highest yielder with 3987 kg ha^{-1} (Table 3.19) and was followed by Sel 95TH 1803, -1806, -1805, -1807, and -1801 with seed yields more than 2.5 tons as compared to 446 kg ha^{-1} of ILC 482 the most adapted line and 1.87 ton ha^{-1} of ILC 8262 the most cold tolerant germplasm accession.

Table 3.19. Seed yield and yield related characters of 7 improved cold tolerant lines derived from intraspecific crosses at Tel Hadya, 1995/96.

Sel 95TH	Parentage	DFLR	DMAT	HGT cm	BYLD kg/ha	SYLD kg/ha	HI %	SW g	CTR
1802	FLIP 83-72C X FLIP 85-81C	190	245	69	13481	3987	30	29.4	3
1803	FLIP 84-93C X FLIP 85-49C	191	245	69	14352	3369	23	28.8	3
1806	FLIP 84-92C X ILC 3465	194	246	63	11129	3039	27	26.9	3
1805	FLIP 84-92C X ILC 3465	194	245	67	11241	2704	24	27.5	3
1807	FLIP 83-66C X FLIP 86-44C	191	245	75	10129	2606	26	23.0	3
1801	FLIP 83-72C X FLIP 85-49C	190	244	66	9611	2580	27	27.2	3
1804	FLIP 85-122C X ILC 3465	191	245	67	9556	2015	21	31.0	4
ILC 8262 (T)	-	198	245	70	10056	1870	18	24.5	2
ILC 482 (MT)	-	191	238	43	1962	446	21	24.7	6
ILC 533 (S)	-	-	-	-	759	215	27	16.7	7
S.E.		0.90	0.37	0.76	773.05	307.5	2.8	0.94	
Mean		192	244	65	9228	2283.0	24.5	26.0	
L.S.D. at P = 0.05		2.69	1.12	2.28	2296.7	913.7	8.4	2.80	
C.V. (%)		0.81	0.26	2.01	14.51	23.33	19.9	6.29	

DFLR = days to flower, DMAT = days to maturity, HGT = plant height, BYLD = biological yield, SYLD = seed yield, HI = harvest index, SW = 100-seed weight, CTR = cold tolerance rating.

3.1.6.1.2. Introgression of Nematode Resistance from Wild Species to the Cultigen

Heterodera ciceri is one of the important nematodes causing severe damage to chickpea in West Asia and North Africa WANA). Although it can be effectively controlled by the nematicides and other management practices but both are expensive. The control through nematicides is also damaging to the environment. Thus use of resistant cultivars is the best alternative to combat nematode problem. Screening of 9000 chickpea germplasm accessions maintained at ICARDA revealed that all were susceptible. However, several accessions of wild species revealed the presence of variability for resistance. A resistant accession from *Cicer reticulatum* (ILWC 119) was crossed with two high yielding cultivars (ILC 482 and FLIP 87-69C) at Tel Hadya, Syria during 1989/90. The F_1 progenies were grown in the summer nursery at Terbol in the Beqa'a Valley of Lebanon to produce F_2 seeds. These F_2 seeds were grown in pots in the plastic house at Tel Hadya to evaluate their reaction to a Syrian population of the cyst nematode using an inoculum level of 20 eggs per gram of soil. Single seed was first sown in a small jiffy pot containing inoculum free soil. Three such pots were then sunk in a larger plastic pot containing nematode infesting soil and were arranged on benches in the plastic-house at a temperature 20 ± 2°C. Plants were allowed to reach flowering to early podding stage when they were uprooted to examine the nematode infestation on roots growing out of the jiffy pots in the infested soil. Root infestation was recorded on 0-5 scale (where 0=free of nematodes and 5=more than 50 females/plant). Plants with a score of 0-2 were considered as resistant. Such plants, with their major root system intact in the jiffy pot, were transplanted in larger pots containing nematode free soil for seed increase. The generation of the selected lines was advanced by using summer nursery in Terbol and the next generation again evaluated for reaction to nematode infestation in plastic-house at Tel Hadya. Using this technique 11 F_9 progenies have been developed showing uniformity in resistance to the nematode and good agronomic characters. Their seeds are now being multiplied for yield evaluation. This is the first example of successful transfer of nematode resistance from the wild to the cultigen in chickpea.

3.1.6.1.3. Yield Improvement

Fifteen lines derived from interspecific crosses between carefully chosen parents were evaluated for cold tolerance and seed yield during 1995/96 at Tel Hadya and Terbol. On the basis of mean over Tel Hadya plantings in winter and autumn, and Terbol plantings in Autumn, 4 lines namely Sel 93TH 24405, 93TH 24416, 95TH 1704, 95TH 1734 gave seed yields better than the cultigen check and also exhibited higher level of cold tolerance.

Nineteen F_7 promising and uniform progenies selected from the crosses between the cultigen (ILC 482 and FLIP 87-69C), and the wild *Cicer* species, *C. reticulatum* (ILWC 118 and ILWC 182) were evaluated alongwith their parents for seed yield and other traits (Table 3.21). The highest biomass and seed yield was obtained by the line 12 which also exhibited higher seed size than the cultigen parent involved. All the derived lines were uniform in maturity and had a semi-spreading growth habit like the cultigen parents. All the derived lines were kabuli type except the line nos. 5, 6, 14 and 15, which were desi types with brown color seed (Table 3.22).

There were no differences in the derived lines from straight and reciprocal crosses.

Table 3.20. Seed yield and yield related characters of 15 F_7 lines derived from interspecific crosses involving *C. arietinum* (ILC 482, FLIP 87-69C) and *C. reticulatum* (ILWC 118, ILWC 182) and four parents at Tel Hadya sown during autumn(TH-A), winter(TH-W) and at Terbol during autumn(Ter-A), 1995/96.

Ent. no.	Sel.no.	Parentage	Seed yield (kg/ha)				100-SW	CTR
			TH-W	TH-A	Ter-A	Mean		
1	93TH24405	ILC482XILWC118	2421	1831	3012	2421	25.3	2
2	93TH24406	ILC482XILWC118	2512	1333	2951	2265	25.3	2
3	93TH24410	FLIP87-69CXILWC118	2244	1956	1753	1984	26.4	3
4	93TH24411	FLIP87-69CXILWC118	1388	1661	1543	1531	25.0	2
5	93TH24416	ILC482XILWC182	2310	2748	3259	2772	26.3	2
6	93TH24444	NEWC35XILC482	1811	1914	3160	2295	24.5	2
7	93TH24448	ILC482XNEWC36	2298	2165	1802	2088	24.9	3
8	95TH1704	FLIP87-69CXILWC118	2613	2454	2296	2454	25.0	2
9	95TH1710	ILWC118XFLIP87-69C	1702	1682	1630	1671	24.0	3
10	95TH1716	ILC482XILWC182	2170	2432	2000	2201	27.3	2
11	95TH1722	ILWC182XILC482	2365	1962	1704	2010	32.3	2
12	95TH1734	FLIP87-69CXILWC219	3180	2388	2605	2724	31.3	2
13	95TH1740	FLIP87-69CXILWC229	2080	1896	2667	2214	35.9	3
14	95TH1744	ILC482XNEWC36	1844	1554	2691	2030	28.1	1
15	95TH1745	ILC482XNEWC36	1922	1621	2827	2123	30.2	1
16	ILC482		2883	1450	2062	2132	28.5	6
17	FLIP87-69C		2675	2590	1765	2343	38.7	3
18	ILWC118		1018	807	617	814	15.4	2
19	ILWC182		296	211	235	247	14.5	2
SE			220.7	393.8	268.3	-	-	-
CV (%)			18.2	42.4	21.3	-	-	-

Table 3.21. Seed yield and yield related characters of 15 F_7 lines derived from interspecific crosses involving *C. arietinum* (ILC 482, FLIP 87-69C) and *C. reticulatum* (ILWC 118, ILWC 182) ranked in order of decreasing seed yield at Tel Hadya, 1995/96.

Line	Cross	DFL[d]	DMA	HGT cm	BYD kg/ha	SYD kg/ha	HI %	SW g
12	AR	138	187	58	9438	3180	34	31.3
482[a]	A	135	185	52	6845	2883	42	28.5
87-69[b]	A	136	184	62	6941	2675	39	38.7
8	AR	140	185	44	7064	2613	37	25.0
2	AR	148	186	55	6900	2512	36	25.3
1	AR	148	186	50	6104	2421	40	25.3
11	RA	133	185	55	7243	2365	33	32.3
5	AR	136	181	37	5034	2310	46	26.3
7	AR	135	184	47	5473	2298	42	24.9
3	AR	139	186	55	5802	2244	39	26.4
10	AR	136	184	48	6295	2170	41	27.3
13	AR	138	190	59	7956	2080	26	35.9
15	AR	137	183	37	5528	1922	35	30.2
14	AR	137	184	38	5103	1844	36	28.1
6	RA	136	183	38	4691	1811	39	24.5
9	RA	138	188	46	6842	1702	35	24.0
4	AR	138	189	47	5789	1388	24	25.0
118[c]	R	136	182	15	3086	1018	33	15.4
182[c]	R	137	182	14	1029	296	29	14.5
S.E.		1.04	0.78	1.80	583.6	220.7		0.74
C.V.		1.23	0.71	6.08	16.22	18.20		4.93

[a] ILC line; [b] FLIP line; [c] ILWC line

A = *C. arietinum*, R = *C. reticulatum*, AE = A × E and AR = A × R.

[d] DFL = days to flower, DMA = days to maturity, HGT = plant height, BYD = biological yield, SYD = seed yield, HI = harvest index, SW = 100-seed weight.

Table 3.22. Wild species related characters of 15 lines derived from interspecific crosses involving *C. arietinum* (ILC 482, FLIP 87-69C)) and *C. reticulatum* (ILWC 118, ILWC 182) at Tel Hadya, 1995/96.

Line	Cross	Characters evaluated					
		UMAT[d]	SSH	STY	SCOL	SW g	GRH
12	AR	u	owl	K	beige	31.3	ss
482[a]	A	u	owl	K	beige	28.5	ss
87-69[b]	A	u	owl	K	beige	38.7	ss
8	AR	u	owl	K	beige	25.0	ss
2	AR	u	owl	K	beige	25.3	ss
1	AR	u	owl	K	beige	25.3	ss
11	RA	u	owl	K	beige	32.3	ss
5	AR	u	owl	D	brown	26.3	ss
7	AR	u	round	I	beige	24.9	ss
3	AR	u	owl	K	beige	26.4	ss
10	AR	u	owl	K	beige	27.3	ss
13	AE	u	owl	K	beige	35.9	ss
15	AR	u	owl	D	brown	30.2	ss
14	AE	u	owl	D	brown	28.1	ss
6	RA	u	owl	D	brown	24.5	ss
9	RA	u	owl	K	beige	24.0	ss
4	AR	u	owl	K	beige	25.0	ss
118[c]	R	nu	owl	D	brown	15.4	s
182[c]	R	nu	owl	D	brown	14.5	s
S.E.						1.31	
C.V.						8.32	

[a] ILC line; [b] FLIP line; [c] ILWC line
[d] UMAT = uniformity in maturity, u = uniform, nu = non-uniform, SSH = seed shape, STY = seed type, SCOL = seed color, SW = 100-seed weight, K = kabuli, D = desi, I = intermediate, GRH = growth habit, ss = semi-spreading, s = spreading.

3.1.6.2. Cyst Nematode Resistance

The evaluation of 9257 accessions of the cultigen to resistance to cyst nematode didn't reveal any resistance in the cultivated species. The evaluations of wild *Cicer* accessions, however, revealed that among the crossable species only one accession of *C. reticulatum* was found resistant to cyst nematode. Since cyst nematode is a serious problem in parts of the chickpea growing area and crosses between cultigen and resistant accession of *C. reticulatum* were made to transfer genes for cyst nematode resistance to cultigen. The earlier work on nematode resistance revealed that the straight crosses didn't result in good seed size and other seed related traits in later generations. Thus the backcrosses to the cultigen were affected to improve the seed quality.

During 1995/96, 2822 plants were evaluated for cyst nematode resistance (Table 3.23). Two hundred and four plants in F_2, 204 plants in F_3 backcrosses, 495 plants in F_4 backcrosses, and 217 plants in F_9 with resistance behaviour were selected.

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3.1.6.3. Cold Tolerance

Materials sown during 1995/96 for cold tolerance study included 51 entries in Chickpea International Cold Tolerance Nursery, 42 resistant sources and 42 wild *Cicer* species. Some segregating populations of intraspecific crosses including 47 F_6, 96 F_5, 41 F_4, 76 F_3 were planted. Out of these 54, 91, 23 and 43 plants were selected from F_6, F_5, F_4, and F_3, respectively. Among interspecific crosses, 351 F_8-F_3 progenies planted, 4 F_8, 23 F_7, 72 F_6, 5 F_5, 91 F_4 and 9 plants in F_3 were selected. Also out of 115 plants in F_6 of backcross one, 56 plants were selected. In addition, selections were made on 2 F_3 bulks, 5 F_4 bulks, 13 F_3 third backcross one, and 15 F_1 and F_2 diallel crosses.

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of agronomic importance having resistance to major chickpea constraints, including that to the chickpea cyst nematode.

As it occurs for other diseases, nematode populations may belong to different pathotypes whose knowledge is necessary for any breeding program. Therefore studies on the occurrence of pathotypes in *H. ciceri* started in 1994 and continued in 1995/1996.

3.1.8.1. Screening of Chickpea Breeding Material to *Heterodera ciceri*

This is reported in section 3.1.6.1.2.

3.1.8.2. Investigation on the Occurrence of Pathotypes in *Heterodera ciceri*

Three populations of the nematode, one each from Jordan, Lebanon and Syria were reared on chickpea and cysts extracted with a large can, kept separately and used to infest soil to obtain a population density of 20 eggs of the nematode/g. Plastic pots were then filled 1000 cm^3 of this potting soil and sown with seeds of the 22 cultivars or lines, belonging to twenty plant species, reported in Tables 3.24, 3.25 and 3.26. Twenty one were leguminous plants. There were 220 pots each nematode population (ten each entry) maintained in the same plastic-house.

Half of the pots, five for each plant species, were uprooted 50 days after germination, when they were at flowering to early podding stage, washed free of adhering soil, ground in a blender and nematode life stages extracted by the centrifugation method and counted.

The remaining pots were left for one more month to allow different nematode young stages to become adults. Then the soil was thoroughly mixed and a 200 g subsample used to extract nematode cysts according to Fenwick can and Seinhorst's flotation methods. Cysts were then counted and crushed to determine numbers of eggs.

All populations of *H. ciceri* reproduced well on chickpea, lentil, grasspea and pea. Populations from Jordan and Lebanon reproduced also on the line ILWC 119 of *C. reticulatum*, in which resistance to the nematode was reported. Nematode soil population declined to very low level in pots planted with the other plant species or lines (Tables 3.24 and 3.25). Nematode juveniles penetrated roots of all

Table 3.24. Number of eggs of *Heterodera ciceri* /g of soil from pots inoculated with three populations of the nematode from different geographical areas and planted with 22 cultivars or lines belonging to 20 plant species. (Nematode population density at sowing 20 eggs/g soil).

Cultivar or line	Nematode population*		
	1	2	3
Chickpea (ILC 482)	117	51	107
Lentil (Idleb 1)	34	77	20
Grasspea (IFLA 347)	43	36	25
Pea (IFPI 83)	82	62	32
Faba bean (ILB 163)	1.8	2.3	2.4
Bean (Local Syrian)	2.5	3.2	4.5
Cowpea (Local Syrian)	0.5	2.4	1.3
Lupin (Rabatab Sudan)	6.4	6.8	3.7
Soybean cv Evans	1.0	3.4	1.3
Vetch cv Lolita	1.5	2.6	1.2
Annual medics (IFMA 811)	4.3	2.8	1.8
Alfalfa cv Casalina	1.7	2.0	0.4
Spanish espercet cv Grimaldi	1.1	2.4	0.8
Crimson clover cv Diogene	2.7	1.4	0.4
Red Clover cv Collestrada	0.5	1.8	0.9
White clover cv Comunali	0.7	0.7	0.5
Cicer reticulatum (ILWC 119)	18	40	63
C. bijugum (ILWC 62)	13	5.8	12
C. bijugum (ILWC 71)	6.9	6.9	7.8
C.pinnatifidum (ILWC 213)	1.4	5.9	1.9
C.pinnatifidum (ILWC 252)	4.4	3.3	2.6
Carnation (Local Lebanese)	4.7	4.4	3.6

* 1 = Idleb (Syria); 2 = Jordan; 3 = Lebanon.

plant species (Table 3.26) but only on the mentioned plant species a large number of juveniles reached the adult stage. On the other plant species females and cysts were nil or few. The largest number of nematode specimens in the roots was observed in all cicer species and in pea, grasspea and lentil and the least in carnation, cowpea, lupin and soybean.

Table 3.25. Reproduction of *Heterodera ciceri* in pots inoculated with three populations of the nematode from different geographical areas and planted with 22 cultivars or lines. (Nematode population density at sowing 20 eggs/g soil).

Cultivar or line	Nematode population*		
	1	2	3
Chickpea (ILC 482)	+	+	+
Lentil (Idleb 1)	+	+	+
Grasspea (IFLA 347)	+	+	+
Pea (IFPI 83)	+	+	+
Faba bean (ILB 163)	-	-	-
Bean (Local Syrian)	-	-	-
Cowpea (Local Syrian)	-	-	-
Lupin (Rabatab Sudan)	-	-	-
Soybean cv Evans	-	-	-
Vetch cv Lolita	-	-	-
Annual medics (IFMA 811)	-	-	-
Alfalfa cv Casalina	-	-	-
Spanish espercet cv Grimaldi	-	-	-
Crimson clover cv Diogene	-	-	-
Red Clover cv Collestrada	-	-	-
White clover cv Comunali	-	-	-
Cicer reticulatum (ILWC 119)	-	+	+
C. bijugum (ILWC 62)	-	-	-
C. bijugum (ILWC 71)	-	-	-
C.pinnatifidum (ILWC 213)	-	-	-
C.pinnatifidum (ILWC 252)	-	-	-
Carnation (Local Lebanese)	-	-	-

* 1 = Idleb (Syria); 2 = Jordan; 3 = Lebanon.
** + = Final population/initial population Pf/Pi > 1; - = Pf/Pi $\leq$ 1.

According to the results of this test and of that conducted the previous year, although there is no clear evidence of the presence of pathotypes within populations of *H. ciceri*, nevertheless some variation has been observed which deserve more attention, especially regarding the reproduction on ILWC 119, to ascertain whether this is due to differences in nematode virulence or to heterogeneity occurring in the population of this line.

Table 3.26. Number of total specimens of *Heterodera ciceri*/ root of 22 cultivars or lines grown in pots containing soil infested with three different populations of the nematode. (Nematode population density at sowing 20 eggs/g soil).

Cultivar or line	Nematode population*		
	1	2	3
Chickpea (ILC 482)	2646	3575	3002
Lentil (Idleb 1)	659	817	351
Grasspea (IFLA 347)	638	833	852
Pea (IFPI 83)	937	2019	1156
Faba bean (ILB 163)	98	355	116
Bean (Local Syrian)	164	256	165
Cowpea (Local Syrian)	22	1	1
Lupin (Rabatab Sudan)	39	18	22
Soybean cv Evans	46	75	35
Vetch cv Lolita	180	819	137
Annual medics (IFMA 811)	42	105	82
Alfalfa cv Casalina	120	133	87
Spanish espercet cv Grimaldi	104	151	254
Crimson clover cv Diogene	112	257	133
Red Clover cv Collestrada	111	185	187
White clover cv Comunali	94	129	200
Cicer reticulatum (ILWC 119)	959	1213	1223
C. bijugum (ILWC 62)	678	464	490
C. bijugum (ILWC 71)	490	484	544
C.pinnatifidum (ILWC 213)	889	487	459
C.pinnatifidum (ILWC 252)	673	404	729
Carnation (Local Lebanese)	4	2	0

* 1 = Idleb (Syria); 2 = Jordan; 3 = Lebanon.

(N. Gireco, M. Di Vito, R.S. Malhotra, M.C. Saxena)

3.1.9. International Testing Program

The international testing program on Kabuli chickpea is a vehicle for the dissemination of genetic materials and improved production practices, in the form of international nurseries and trials, to the national programs in and outside the WANA region. The genetic materials comprise early segregating populations in F_3 and F_4 generations, and elite lines with wide or specific adaptation, special morphological or quality traits, and resistance to common biotic and abiotic stresses. Nurseries are only sent on request and often include *germplasm specifically* developed for a particular region or a national program. A list of trials supplied for the 1996/97 season is given in Table 3.27.

Table 3.27. Distribution of Legume International Nurseries to cooperators for the 1996/97 season.

International Trial/Nursery	No. of sets
Yield Trial Spring (CIYT-Sp-97)	47
Yield Trial Winter, Medit. Region (CIYT-W-MR-97)	57
Yield Trial Southerly Latitudes-1 (CIYT-SL1-97)	8
Yield Trial Southerly Latitudes-2 (CIYT-SL2-97)	10
Yield Trial Latin America (CIYT-LA-97)	16
Screening Nursery Winter (CISN-W-97)	43
Screening Nursery Spring (CISN-Sp-97)	39
Screening Nursery, South. Latitudes-1(CISN-SL1-97)	8
Screening Nursery, South. Latitudes-2(CISN-SL2-97)	6
Screening Nursery, Latin America (CISN-LA-97)	9
F_4 Nursery, Mediterranean Region (CIF_4N-MR-97)	18
F_4 Nursery, Southerly Latitudes (CI_4N-SL-97)	5
Ascochyta Blight Nursery: Kabuli (CIABN-A-97)	29
Ascochyta Blight Nursery: K. & D. (CIABN-B-97)	25
Fusarium Wilt Nursery (CIFWN-97)	28
Cold Tolerance Nursery (CICTN-97)	28
Drought Tolerance Nursery (CIDTN-97)	42
Total	418

The testing program helps in identification of genotypes with specific and wide adaptation. The performance data permit assessment of genotype x environment interaction and help in targeting breeding efforts for specific agro-ecological conditions.

The salient features of the 1994/95 international nursery results, received from cooperators are presented here. The stability analyses of some of the trials were done using Eberhart and Russell (1966) model.

The Chickpea International Yield Trial-Spring (CIYT-SP) was reported from 19 locations in 7 countries. A number of test entries exceeded the respective local check by a significant margin ($P \leq 0.05$) at Akaki in Ethiopia; Maragheh in Iran; Al-Ghab, Hama, Heimo, Jableh and Tel Hadya in Syria; and Amasya, Diyarbakir and Izmir in Turkey. The five heaviest yielding entries across the locations were FLIP 91-186C, FLIP 91-203C, FLIP 90-137C, ILC 482 and FLIP 91-202C, respectively, with seed yield of 1450, 1422, 1395, 1390 and 1382 kg/ha. The stability analysis revealed that the entries, FLIP 91-203C, FLIP 91-186C, FLIP 91-188C, FLIP 92-142C, FLIP 91-202C and FLIP 91-26C were relatively stable across environment as compared to others.

For Chickpea International Yield Trial-Winter-Mediterranean (CIYT-W-MR) data were reported from 21 locations in 10 countries. At Maru in Jordan; Elvas in Portugal; Al-Ghab, Gelline, Heimo, Homs, Izra'a and Tel Hadya in Syria; Beja and Oued Meliz in Tunisia; and Izmir in Turkey, some of the test entries exceeded the respective local check by a significant margin ($P \leq 0.05$). The five heaviest yielders across locations included FLIP 91-60C, FLIP 91-63C, FLIP 91-222C, FLIP 92-162C, and FLIP 90-96C and gave 2270, 2251, 2224, 2223, and 2192 kg/ha, respectively. The stability analysis revealed that the entries FLIP 91-60C, FLIP 92-162C, FLIP 92-155C, FLIP 92-169C, FLIP 91-61C, FLIP 91-220C, and FLIP 91-219C, with above average yield were superior to other lines in adaptation.

The results of Chickpea International Yield Trial Southerly Latitudes-1 (CIYT-SL1) were reported from 6 locations in 5 countries. At locations in southern latitudes none of the test entries exceeded the local check by a significant margin The five heaviest yielding entries across locations included, FLIP 92-179C, FLIP 90-63C, FLIP 92-172C, FLIP 92-95C and FLIP 90-27C with respective seed yields of 1963, 1963, 1883, 1803 and 1793 kg/ha. The data on days to flowering revealed that at most locations some of the entries flowered either at the same time or earlier than the respective local check. The stability analysis for seed yield revealed that the entries namely, FLIP 92-179C, FLIP 90-63C, FLIP 92-172C, FLIP 92-95C, FLIP 92-96C, ILC 482, FLIP 90-14C, FLIP 90-100C, FLIP 91-33C, FLIP 92-59 and FLIP 92-136C were adaptable across environments.

The results for Chickpea International Yield Trial Southerly Latitudes-2 (CIYT-SL2) were reported from 8 locations in 6 countries. Only one test entry exceeded the local check in seed yield by a significant margin at Akaki in Ethiopia and Hudeiba in Sudan. The five heaviest yielding entries across locations included FLIP 90-126C, FLIP 90-125C, FLIP 82-150C, FLIP 91-35C and FLIP 90-28C with seed yields of 2252, 1962, 1883, 1865, and 1860 kg/ha, respectively. The data on days to flowering revealed that at most locations some of the entries flowered earlier or at the same time when the respective local check flowered. The stability analysis for seed yield revealed that 13 entries namely, FLIP 90-126C, FLIP 90-125C, FLIP 82-150C, FLIP 91-35C, FLIP 90-28C, FLIP 89-82C, FLIP 89-117C, FLIP 92-7C, FLIP 91-75C, FLIP 91-77C, ILC 482, FLIP 91-193C, and FLIP 91-88C, were adaptable across environments.

The results for Chickpea International Yield Trial Latin America (CIYT-LA) were reported from 5 locations in 4 countries. The ANOVA for seed yield revealed that at only one location in Latin America namely, Chillan in Chile and two other locations namely, Bornova and Izmir in Turkey, 10, 18, and 2 test entries exceeded the respective local check in seed yield by a significant margin ($P \leq 0.05$). The five heaviest yielders across locations included FLIP 91-98C, FLIP 90-16C, FLIP 90-158C, FLIP 89-20C and FLIP 90-19C with seed yields of 1980, 1817, 1749, 1703 and 1702 kg/ha, respectively. The stability analysis for seed yield revealed that a few lines namely, FLIP 91-98C, FLIP 90-16C, FLIP 89-20C, FLIP 90-19C, FLIP 85-5C, and FLIP 88-6C with above average mean seed yield and non-significant deviation from regression were adaptable across environments.

The data on seed yield of Chickpea International Screening Nurseries -Winter (CISN-W), -Spring (CISN-SP), -Southerly Latitudes-1 (CISN-SL1), -Southerly Latitudes-2 (CISN-SL2) and -Latin America (CISN-LA) were reported from 19, 19, 4, 2, and 6 locations, respectively. Some of the test entries exceeded the local check by significant margins at 12, 8, 2, 1 and 3 locations, in CISN-W, CISN-SP, CISN-SL1, CISN-SL2 and CISN-LA, respectively. The five heaviest yielding entries across locations are given in Table 3.28.

Table 3.28. The five heaviest yielding lines across locations in different chickpea screening nurseries, 1994/95.

Rank	CISN-W	CISN-SP	CISN-SL1	CISN-SL2	CISN-LA
1	F[a] 93-93C	F 93-58C	F 93-93C	F 93-206C	F 90-17C
2	F 93-186C	F 93-39C	ILC 482	F 93-213C	F 85-5C
3	F 93-147C	F 93-47C	F 93-133C	F 93-229C	ILC 464
4	F 93-149C	F 93-79C	F 93-169C	F 93-200C	F 93-76C
5	F 93-139C	F 93-51C	F 93-46C	F 93-227C	F 93-84C

[a] F = FLIP

Chickpea International F_4 Nurseries for Mediterranean (CIF$_4$N-MR) and for Southerly Latitudes (CIF$_4$N-SL) were supplied to cooperators at 15 and 10 locations, respectively. Only 5 and 1 location in CIF$_4$N-MR and CIF$_4$N-SL, respectively, reported the usefulness of these nurseries under their own environmental conditions and made individual plant selections for use in their own breeding materials.

The results for Chickpea International Ascochyta Blight Nursery (CIABN) Kabuli (A) were reported from 4 locations and for CIABN desi+kabuli (B) from 7 locations. None of the entries (kabuli or desi) was tolerant to Ascochyta blight infestation across all locations. Considering the frequency of occurrence of an entry among the tolerant group (with rating ≤ 5 on 1-9 scale) in Kabuli types, entries FLIP 91-23C, FLIP 93-146C, and FLIP 93-176C showed tolerance at 9 out of 11 locations and appeared best, and these were followed by ILC 200, FLIP 84-182C, FLIP 90-85C, FLIP 91-8C, FLIP 92-132C, FLIP 92-189C, FLIP 93-62C, and FLIP 93-160C, which occurred 8 times. Similarly, among desi lines two entries, ICC 12004, and ICC 13269 were tolerant at 4 out of 7 locations. These entries thus exhibited relatively broad-based resistance to Ascochyta blight as compared to others in this nursery. The differential reaction of lines at various places further revealed the presence of variability in the pathogen. This nursery has been very useful in the identification of resistant sources to ascochyta blight and several NARSs have used these resistant sources in their breeding programs.

The Chickpea International Drought Tolerant Nursery (CIDTN) was initiated for the first time in 1995. The nursery was sent to 29 locations but results were reported from 3 locations only. On the basis of drought tolerance scores (where 1 = free from damage, and 9 = killed or no yield), three lines namely, ILC 574, ILC 4515, and ILC 4527 were tolerant to drought at all the locations, and were followed by ILC 1337, ILC 1577, ILC 1850, ILC 1988, ILC 2166, ILC 2173, ILC 2466, ILC 2882, ILC 2929, ILC 6119, FLIP 87-5C, FLIP 87-59C, FLIP 87-85C, and 88-42C, which were tolerant at 2 out of 3 locations.

The results of Chickpea International Fusarium Wilt Nursery (CIFWN) were reported from 9 locations. None of the entries was resistant (≤ 20% plants killed). Some of the lines namely, ILC 240, ILC 3300, UC 27, Be Sel 81-48, and Be Sel 81-103 were scored as resistant at 6 out of 9 locations, and UC 15 and ICCV-2 at 5 out of 9 locations.

The results of Chickpea International Cold Tolerance Nursery (CICTN) were reported from 3 locations (Nishabour in Iran, Tel Hadya in Syria and Terbol in Lebanon). Seventeen entries were rated as tolerant ($\leq$ 4 rating on 1 to 9 scale where 1 = free, 9 killed) at all the three sites.

(R.S. Malhotra and NARSs Scientists)

3.2. Chickpea Pathology

Diseases form a major biotic constraint to the production of chickpea in the WANA region. Ascochyta blight caused by *Ascochyta rabiei* is the most serious foliar disease of chickpea in the region, particularly where low temperatures of 15-25°C prevail during the cropping season. Its annual epidemics is not regular and is usually weather-dependent. A good season for the chickpea crop is often favourable for ascochyta blight development. With the increased adoption of winter chickpea, the risks of frequent Ascochyta blight epidemics continue to increase. It is therefore absolutely necessary to control or manage Ascochyta blight if advantage is to be taken of winter-sown chickpea to increase and stabilise yields. A major emphasis in chickpea pathology is therefore given to identify durable and stable sources of resistance to Ascochyta blight for use in the hybridization program.

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is the most important soil-borne disease. Other soil-borne diseases such as black root rot (*Fusarium solani*), and wet root rot (*Rhizoctonia solani*) that are favoured by high moisture conditions are important in some areas in Ethiopia and irrigated fields in Egypt and Sudan. Dry root rot

(*Rhizoctonia bataticola*), collar rot (*Sclerotium rolfsii*) and stem blight (*Sclerotinia sclerotiorum*) also occur on chickpea in the region but overall, they are less important than Fusarium wilt.

The objectives of the chickpea pathology research are to: (1) screen chickpea germplasm for identification of sources of resistance to the major diseases using laboratory, greenhouse and field screening techniques; (2) share the resistant accessions with national programs through international disease nurseries; (3) collect information on disease prevalence and severity in the WANA region in collaboration with the national scientists; (4) study the epidemiology and pathogenic variability of the major diseases; (5) develop integrated disease management strategies for the control of the major diseases; and (6) develop research collaboration with national programs and advanced institutions in the management of the major diseases in the region.

3.2.1. Field Survey of Chickpea Diseases

The objective of the disease survey was to evaluate the disease situation in Syria and to assess the disease reaction of some promising lines (F90-96, F88-85, and F89-29) in on-farm trials, on-station trials and demonstration plots in farmers fields. The performance of these lines and their reaction to Ascochyta blight as compared to Ghab 3, the currently widely grown cultivar, was of particular interest.

Disease incidence and severity on chickpea was surveyed in Dara'a, Homs, Hama, Aleppo, Idleb and Hassakeh provinces in Syria. In total, 28 locations, including off-station and on-farm trials were visited and the disease incidence and severity at the different locations assessed.

Results of the survey on the reaction of all the on-farm entries to Ascochyta blight during 1996 are summarised on Table 3.29. Among the three new enteries, F 90-96 generally performed better than Ghab 3 across all locations. It recorded an overall mean disease severity rating of 3.1 as compared to Ghab 3 that rated 3.6. Being the 4th year of this entry in the on-farm trial, it should be a strong candidate for release by the Syrian NARS to complement Ghab 3 which is presently the only recommended winter chickpea variety in Syria.

(C. Akem, M. Bellar and NARS Scientists from Syria)

Table 3.29. Incidence of ascochyta blight on chickpea genotypes in on farm trials in Syria, 1996.

Research station	FLIP 88-85	FLIP89-29	FLIP90-96	Ghab-3
Dara'a- Ebta'a	1	2	1	1
Dara'a Jelleen	5	6	1	3.5
Homs	2.5	2.5	1.5	2
Lattakia Jableh	2	3	2	2
Al Ghab	5.5	7.5	5	6
Hama	4.5	5.5	4	5.5
Sakilbieh	2	2.5	2	1.5
Hama	4.5	5.5	4	5.5
Idleb-Telsandal	3	4.5	3	3.5
Idleb-Afes	3.5	5	1.5	1
Aleppo -Tel Hadya	5	6	4.5	4
Aleppo, Izaz-Alkamieh	4.5	7	3.5	5
Hassakeh Tel-Ayloul	1	4	1	1.5
Hassakeh Tel-Hassoud	5.5	6	5	6
Hassakeh- Kamishly-Himo	1	3.5	2.5	1
Hassakeh-Malkieh	9	9	9	9
Mean	3.7	5.0	3.1	3.6

3.2.2. Ascochyta Blight

Host plant resistance continue to be the backbone of Ascochyta blight disease management at ICARDA. Screening for

Ascochyta blight resistance is thus a large component of chickpea pathology research. Evaluations of breeding lines and other selected trials begins in the blight nursery using infected debris and spore suspensions of mixed isolates. Those selected as resistant are retested under controlled conditions in the plastic house using more virulent isolates of the pathogen.

3.2.2.1. Evaluation of F_2 - F_7 Generations

The reaction of the F_2-F_7 segregating populations to Ascochyta blight is presented in Table 3.30.

Out of the 8228 lines evaluated, 2% were resistant with a rating of 2 and 3, 3% were moderately resistant with a rating of 4, and about 16% showed intermediate reactions with a rating of 5.

Most of the resistant entries in the trial were identified in the F_4 progeny lines of RxR crosses which contributed 39% of the total resistant lines.

Among the 50 lines screened in the CIABN trial, only 8 (F94-510, F94-508, ICC 13729, ICC 12004, ICC 4475, ICC 3912, ICC 3919 and ICC 3991) could be rated as resistant, while about half of the entries (48%) showed a susceptible reaction. Most (92%) of the entries in the PYT and AYT trials were susceptible to Ascochyta blight with none rated as resistant. Within the RxR cross population in the gene pyramiding effort, the F4 material had the most lines that were rated resistant. 186 lines or 77% were resistant to moderately resistant with a disease rating of 2-4. Out of this, 17 lines had a disease rating of 2. None of the F_4 and F_5 progeny entries were rated as resistant. The majority were susceptible and only a few (16%) were moderately resistant. In the F_5 progeny tall, 2% were moderately resistant with a rating of 4.

Conditions were particularly favourable for the development and spread of Ascochyta blight in 1995/96. This

Table 3.30. Reactions of chickpea germplasm to Ascochyta blight in field disease nursery, 1996.

Generation	Ascochyta blight reaction on 0-9 rating scale									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
F_5 prog. tall	0	0	0	49	502	1290	1191	407	61	3500
F_5 prog. large	0	0	0	0	22	201	157	72	12	464
F_5 prog. early	0	0	0	3	271	382	210	73	6	945
F_5 progeny	0	0	0	84	373	846	550	194	32	2079
F_4 bulk	0	0	0	2	12	39	30	2	0	85
F_4 bulk large	0	0	0	0	18	25	19	0	0	62
F_4 bulk tall	0	0	0	0	1	6	1	0	0	8
F_2 bulk	0	0	0	0	1	10	19	22	2	54
F_4 R X R	0	17	120	49	35	21	1	0	0	243
F_3 R X R	0	0	0	5	64	74	41	17	0	201
F_2 R x R	0	0	1	2	0	0	0	1	1	5
Inter. PYT lines	0	0	0	1	38	132	257	96	8	532
CIABN	0	1	7	3	13	18	8	0	0	50
Total	0	18	128	198	1350	3044	2484	884	122	8228

included continuous morning drizzles and cool temperatures during the winter months of March and April, which coincided with the flowering and pod filling stages of most of the entries. Thus compared to previous seasons, there was very low expression of resistance in the germplasm screened.

3.2.2.2. Evaluation of Preliminary Yield Trials for Resistance to Race Mixtures of A. Rabiei

Four sets of Preliminary Yield Trials classified according to seed size, maturity and height were evaluated under controlled conditions in the greenhouse for resistance to a race mixture of *A. rabiei*. The six available races were mixed and spray-inoculated unto 4 week old plants in greenhouse pots. Ratings were taken for reactions at seedling stages 2 weeks after inoculations and at podding stages.

Table 3.31 summarises the reaction of the entries in the 4 different trial categories at the podding stage. Among the 64 entries in the PYT-large seeded lines screened, 6 lines (S 95220, S 95177, S 95217, S 95237 and S 95239) had good levels of resistance at the adult stage with disease severity scores of 2 and 3. The rest were either moderately resistant (35%) or susceptible (58%). Within the PYT-Early

Table 3.31. Reactions of PYTs-96 to mixtures of six race isolates of *Ascochyta rabiei* at podding stage in Greenhouse, 1996.

Rating scale	Reaction	Number of entries			
		PYT-L	**PYT-E**	**PYT-T**	**PYT-N**
1	HR	0	0	0	0
2	R	1	0	0	0
3	R	5	1	1	0
4	MR	22	3	8	3
5	T	30	22	43	16
6	MS	6	22	11	31
7	S	0	15	0	12
8	HS	0	0	0	0
9	All killed	1	1	1	0
Total		64	64	64	62

selections evaluated, only 1 line (S 95097) was rated as resistant while most of the others were susceptible. Also, only 1 line (S 95258) was rated resistant in the PYT-Tall entries, with 8 others moderately resistant and the rest susceptible. None of the entries in the PYT-Normal was resistant to the race mixtures. Only 3 were rated as moderately resistant. The entries rated as resistant and moderately resistant will be further challenged with a more aggressive isolate of *A. rabiei* and those confirming their resistances shall be included in the CIABN trials for further multilocation evaluations.

When the reactions at the seedling and podding stages are compared (Fig 3.4), many of the entries rated as resistant at the seedling stages were susceptible at the podding stages. It is thus necessary to take multiple disease scores so as to follow disease development on the different lines and to allow the entries to express their actual reactions under high disease development.

(C. Akem, R.S. Malhotra and S. Kemal)

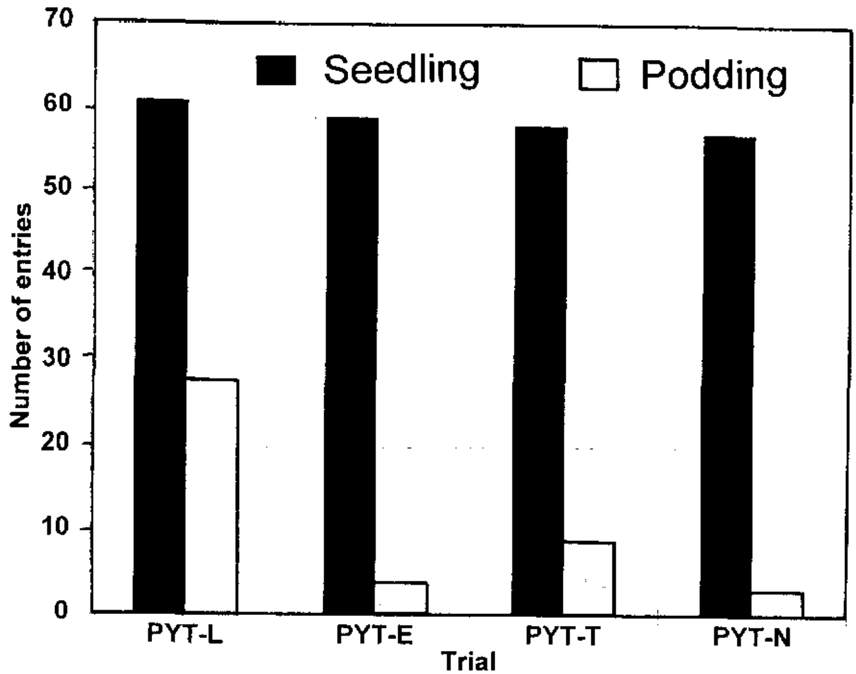

Fig. 3.4. Comparison of chickpea PYT-96 resistance at seedling and podding stages after inoculation with mixture of race isolates of Ascochyta rabiei.

General observations in the Ascochyta blight nursery revealed that disease symptoms on seedlings were widespread by early March even before inoculations with spore suspensions were made. As in previous years, the mist irrigation system was turned on by mid April to quicken disease development eventhough it was apparent that good infection and spread could have still been obtained from the continuous morning drizzles that were characteristic of this period. Spore suspensions from a mixture of culture race isolates was applied 4 times beginning from April 17.

Field observations of an adjacent seed multiplication field planted to Ghab I and 2 showed that the disease severity on the lines in this uninoculated field was greater than could be observed on the lines in the sprayed nursery. The possible explanation to this unexpected observation is that the isolate mixture used to spray-inoculate the nursery was probably not as virulent as the inoculum that came from natural infection due to the favourable weather. The inoculated isolates probably cross-protected the entries from infection by the more virulent natural inoculum that infected the adjacent uninoculated field. This largely supported the suspicion that a larger proportion of the inoculum in the fields is more virulent that the isolates of races 1 to 6 that were used for the inoculations.

Based on this and other observations, some considerations could be made to improve the screening procedure. Field spray inoculations when needed, should be made only with fresh inoculum collected from the field the previous season. The most aggressive isolates should be used for spray inoculations to prevent the cross-protection phenomenon apparent when less virulent isolates are used. To minimize the need or number of spore sprays during the season, infected debris should be spread just after germination to mimic natural conditions and to allow for infection when conditions become favourable, rather than to spread at a routine established time. The frequency of the susceptible check between test entries should be increased, the number of susceptible checks also increased and the checks maintained at a rating of 7 or 8 to enable them provide a secondary source of continuous infection of the entries and a reference rating point. Multiple disease ratings should be made throughout the season when feasible, starting with initial infection to enable separation of entries based on rate induced resistance over time rather than to depend only on discrete timed ratings. The above considerations when incorporated into the nursery development and management could greatly improve the screening procedure.

(C. Akem)

3.3.3. Fusarium Wilt

This was the second year that breeding lines and other advanced trials were evaluated at the newly established wilt sick plot at Tel Hadya farm for resistance to Fusarium wilt. Two blocks of the 3-block, 2-ha plot was ready for use following repeated efforts to establish a uniform inoculum distribution in them. Several trials were evaluated including: new germplasm in the F_2 to F_5 level, preliminary yield trials, International nurseries for wilts and root rots, and reconfirmation of resistance in selected FLIP lines and wild species.

3.3.3.1. New Germplasm and Advanced Trials

Out of 867 new germplasm screened, only 60 showed good levels of resistance to Fusarium wilt with a rating of 2 and 3 on the 1-9 scale. Several were highly susceptible and were uniformly killed with the susceptible check. Table 3.32 shows the reaction summary of the progeny populations evaluated. Among the progeny populations, the F5 entries were highly resistant to wilt. More than 95% of the lines showed no infection. More than 55% of the entries in the Preliminary Yield Trials were highly resistant. Some will be selected for inclosure in the new Chickpea International Fusarium Wilt Nursery (CIFWN) after determining their yield levels in other trials. Table 3.33 shows the reaction of two sets of the International Chickpea Root Rot and Wilt Nursery trials evaluated in the wilt sick plot and in the greenhouse with soil from the sick plot under controlled conditions. In both trial environments, more than 50% of the lines were highly resistant to Fusarium wilt. In the (CIFWN) trial, out of 30 lines evaluated, 12 were highly resistant while 50% of the entries were rated as susceptible. These lines were selected in 1995 from collaborative projects with INRAT Tunisia and

Table 3.32. Reactions of chickpea (cultigens and wild spp) to Fusarium wilt at Tel Hadya, 1996.

Generation	*Wilt reaction on 1-9 rating scale*									
	1	2	3	4	5	6	7	8	9	Total
Differentials	2	3	2	1	1	0	0	0	2	11
PYT (Wilt resistant)	23	12	5	3	4	0	3	6	8	64
CIFWN	3	9	3	1	0	7	5	2	0	30
Wilt resistant lines (reconfirm.)	2	5	2	0	1	2	1	1	0	14
ICRRWN	32	9	5	2	1	0	0	1	0	50
ICRRWN (plastic house)	1	25	14	5	1	1	0	0	3	50
FLIP lines (Reconfirm.)	86	51	19	39	26	23	11	5	4	264
New germplasm	0	35	25	17	50	63	198	93	386	867
Wild *Cicer* spp	45	2	4	3	0	0	0	0	0	54
F5 *progeny*	82	3	1	0	0	0	0	0	0	86
Total	276	154	80	71	84	96	218	108	403	1490

the University of Cordoba in Spain. Their apparent differential reactions in the wilt sick plot suggests that the race or races of *Foc* in the sick plot is different from those *in Tunisia and Spain.*

3.3.3.2. Reconfirmation of Resistance

Table 3.32 also shows the summary *reactions of some* FLIP lines and wild *Cicer* species selected in 1995 as having resistance to Fusarium wilt. About 80% of the 60 wild species were highly resistant to the disease showing good resistance stability in the wild lines. Among the 264 other lines selected as resistant in 1995, only 50% were highly resistant. About 30% showed moderate levels of resistance while the rest were rated as susceptible. Assuming uniformity

Table 3.33. Reaction of chickpea international trials to Fusarium wilt.

Rating scale	Reaction	Number of entries			
		% plants killed	**ICCRWN Field**	**ICCRWN Greenhouse**	**CIFWN Field**
1	Immune	0	32	1	3
2	HR	1-5	9	25	9
3	R	6-10	5	14	3
4	MR	11-20	2	5	1
5	I	21-40	1	1	0
6	MS	41-60	0	1	7
7	S	61-80	0	0	5
8	HS	81-99	1	0	2
9	All killed	100	0	0	0
Total			50	47	30

of inoculum distribution in the plots as shown by the uniform kill of the susceptible check, it appears there is some shift in the reaction of the cultivars. This may be due to a possible race fluctuation of the pathogen population in the sick plot. Exact reason for this possible shift will be an area for future investigation in an effort to select for durable and stable resistance.

(C. Akem, R.S. Malhotra and S. Kemal)

3.4. Application of Molecular Techniques

3.4.1. Introduction

Sequence-tagged microsatellite markers (STMS) are the tools of choice for marker assisted selection (MAS) and breeding in many crops, because they are abundant and evenly distributed over the whole genome, are highly polymorphic also between closely related accessions, segregate in a codominant manner and - being based on PCR - are easy to use. The only drawbacks are the large efforts and expenses necessary for their generation. Marker-assisted selection in breeding and pyramiding of *Ascochyta* resistance genes in chickpea aims at generating a set of highly informative STMS, which will allow to tag every gene at a maximum distance of 10 cM, equivalent to a maximum number of 10 % false positives in marker-selected offspring.

Development of STMS markers normally starts with the generation of small-insert DNA libraries cloned into multicopy vectors of *E. coli*. These clones have inserts of 350 to 650 bp to enable the instant sequencing of their inserts. They are then hybridized to short, synthetic microsatellite motifs such as $(CT)_8$, $(GAA)_5$ or $(TAA)_5$. Clones that give rise to strong hybridization signals contain large microsatellites and are singled out in recurrent screening steps. Plasmid DNA is then isolated and the arrangement of nucleotides in the microsatellite-flanking regions determined by DNA sequencing. This knowledge allows the design of pairs of specific flanking primers, from which the microsatellite in between can be amplified from minute amounts of genomic DNA. Products derived from different accessions or species in most cases are of different length because of varying repeat unit numbers of the respective microsatellite (Fig. 3.5). A microsatellite with, for example, 20 repetitions of the (TAA) motif in one accession may contain 25 in another. The resulting length differences of amplification products are then detected by separating them on high-resolution agarose or polyacrylamide gels. Though being less abundant than dinucleotide motifs such as $(GA)_n$, it is intended to identify long tri- and tetranucleotide microsatellites with repeat unit numbers > 10, because these are more polymorphic than shorter ones and length differences are more easily detected than those in dinucleotide repeats.

Information about microsatellites in chickpea is available from our previous studies, in which highly polymorphic microsatellites were detected by hybridization of digested genomic DNA to synthetic di-, tri- and tetranucleotide motifs. Nevertheless, this technique detects only a small, distinct subset of extremly long microsatellites (ELMS) which is not representative for the overall microsatellite content and its polymorphism in the chickpea genome. Therefore, it was necessary to determine - in a pilot study - the abundance and polymorphism of microsatellites contained in small-insert libraries from chickpea and to find out, which restriction enzymes were best suited for their generation before starting the isolation of microsatellites on a large scale.

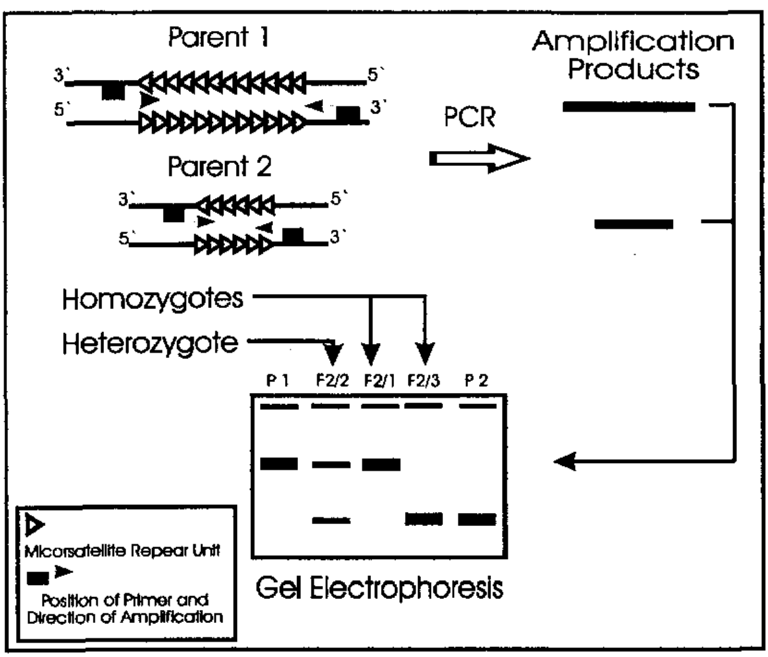

Fig. 3.5. Schematic presentation of varying number of tandem repeats as a cause for microsatellite locus polymorphism and the procedure used for its detection.

3.4.2. Abundance and Polymorphism of Several Microsatellite Motifs in Small-Insert Libraries: The Pilot Study.

3.4.2.1. Experimental Set-up

3.4.2.1.1. Library Construction and Screening for Microsatellite-Containing Clones

Following the scheme outlined above, two small-insert libraries were generated from genomic DNA of chickpea accession ILC 3279 digested with either a combination of the sticky-end isoschizomers Sau 3AI and Mbo I, or with combinations of enzymes that give rise to DNA fragments with blunt ends such as Alu I, Dra I, Eco RV, Hae III, Hpa I, Rsa I, and Sma I. Blunt-ended DNA fragments were pooled and - like the sticky-end fragments - separated on preparative agarose gels. Fragments in the range of 350 to 650 bp were isolated from the gels and ligated either into the Bam HI site (Sau 3A/ Mbo I-fragments) or the Sma I site (blunt-end fragments) of the pBluescript SK-vector. Ligated DNA was electroporated into the *E. coli* "Sure" strain as a host. Around 18.000 to 30.000 colonies from each ligation were plated on selective "master" agar plates. Two replicas of master plates were made on nylon membranes, colonies denatured and their DNA fixed onto nylon membranes. These membranes were subsequently hybridized to radioactively labeled di- and trinucleotide repeat motifs (e.g $(GT)_8$, $(GA)_8$, $(TAA)_5$)and mixtures of G/C-rich or T/A-rich trinucleotide motifs, respectively.

As shown in Table 3.36 number of positive signals varied considerably between microsatellite motifs, indicating the differing representation of the respective microsatellite at least in the libraries if not in the chickpea genome. Not only the number of signals obtained from different microsatellite motifs but also the strength of signals derived from one and the same motif varied largely, indicating large size differences between cloned microsatellites of the same class. Only colonies that gave very strong signals were selected for subsequent steps. Because hybridization had been performed on unordered, high-density libraries, microsatellite-bearing clones had first to be singled-out to get rid of contaminating neighbouring clones, before their inserts could be sequenced. This was achieved by scraping bacteria from the region of the "master" plate that produced strong hybridization signals on the respective membrane, plating them again at low densities on new plates and rescreening them with the respective synthetic oligonucleotide as described. Roughly half of the clones that were positive in the first screen could be reisolated in this second step.

3.4.2.1.2. DNA Sequencing and Primer Design

Well separated, positive clones were picked and their DNA isolated and sequenced using the M13 "reverse" sequencing primer in a single-reaction cycle sequencing protocol with fluorescent dye-labelled dideoxy nucleotides. Reaction products were separated on an ABI 371 sequenator. In several cases the sequences were ambiguous at the distal flank of the microsatellites so that no primers could be designed. This was especially true for G/C-rich microsatellites containing $(GA)_n$ or $(CA)_n$-motifs, indicating that insufficient melting of these sequences had occurred during cycle sequencing. In some cases one of the microsatellite flanking sequences was too short to permit the design of a primer. Where unambiguous sequences of sufficient length could be obtained, flanking primer pairs were designed via Internet using the "Primer 3"-software available at the Massachussets Institute of Technology (MIT). Primers with a maximum length of 27 nucleotides were constructed that had annealing temperatures above 50 °C. PCR was performed on 50 ng genomic chickpea DNA isolated using a modified CTAB protocol including a polysaccharide-precipitation step.

3.4.3. Results of the Pilot Study

3.4.3.1. Frequency and Composition of Microsatellite Motifs in the Chickpea Genome

The number of positive signals after the first hybridization varied considerably with $(TAA)_n$ being the most abundant motif as shown in Table 3.34. It occurred more frequently than all other tri-nucleotide repeats together. It was even more abundant than the two dinucleotide repeats. If an average spacing between repeats in the chickpea genome (738 Mbp) is assumed, it should occur every 130 kbp, whereas $(GA)_n$ and $(GT)_n$ should be present every 195 and 283 kbp, respectively. These 3 motifs together account for more than 12.000 loci so that the average distance between loci should be less than 65 kbp.

Of all motifs tested, $(TAA)_n$-microsatellites not only were most abundant but also contained the largest repeat unit numbers (in one case 54). Besides 31 perfect repeats the 41 unambiguous sequences contained 5 imperfect repeats with interruptions in the repeat motif, 4 repeats that were made up of several repeat motifs with interruptions and 1 compound repeat with only perfect repeats. Interrupted-compound and compound repeats often were of high complexity and length. Some contained no less than 6 different repeat motifs.

3.4.3.2. Polymorphism and Stability of STMS Markers in Chickpea Accessions and *C. reticulatum*

Variability of STMS loci was tested on chickpea accessions ILC 1272, ILC 3279, C104 and WR 315 and *C. reticulatum* ILWC 36, thereby assessing also if the length of the amplified fragment was similar to the length expected from sequencing data and whether the bands could be interpreted with sufficient accuracy. From the 28 loci tested, 2 did not give rise to bands of the expected size from ILC 3279 DNA, the accession used for sequencing. Three loci produced bands that could be hardly interpreted, whereas 25 gave sharp to very sharp bands. For a few loci two bands were generated, probably because of a second internal priming site within the amplified fragment. No polymorphism was found for 4 loci, 22 showed polymorphism between chickpea accessions and *C. reticulatum*, and 16 varied between the chickpea accessions.

The stability of an STMS locus was tested in 7 S_1 individuals each of accessions WR315 and C104, and 16 recombinant inbred lines (RILs) derived from a cross between the two. Except in one case, where one S_1 individual displayed a band that deviated in size from the respective parental bands, all loci were stable. As shown in Fig. 3.6., analysis of RILs demonstrated the power of STMS for the detection of heterozygote. One individual could be clearly identified as heterozygous for locus CaSTMS10.

Table 3.34. Frequency and composition of microsatellites in the chickpea genome.

Microsatellite Motif	Number of Positive Clones after 1. Screen	Number of Sequenced Clones	Range of Repeat Unit Numbers	Number/Average Distance in the Genome
$(GT)_8$	25	7	4-42	2600 / 283 kbp
$(GA)_8$	43	15	9-32	3780 / 195 kbp
$(TAA)_5$	75	16	5-54	5680 / 130 kbp
Tri1 (G/C) rich	22	2	4-9	n.d.
Tri2 (A/T) rich	40	4	4-5	n.d.
Total	205 (100 %)	44 (25 %)	4-54	>12000 / <65 kbp

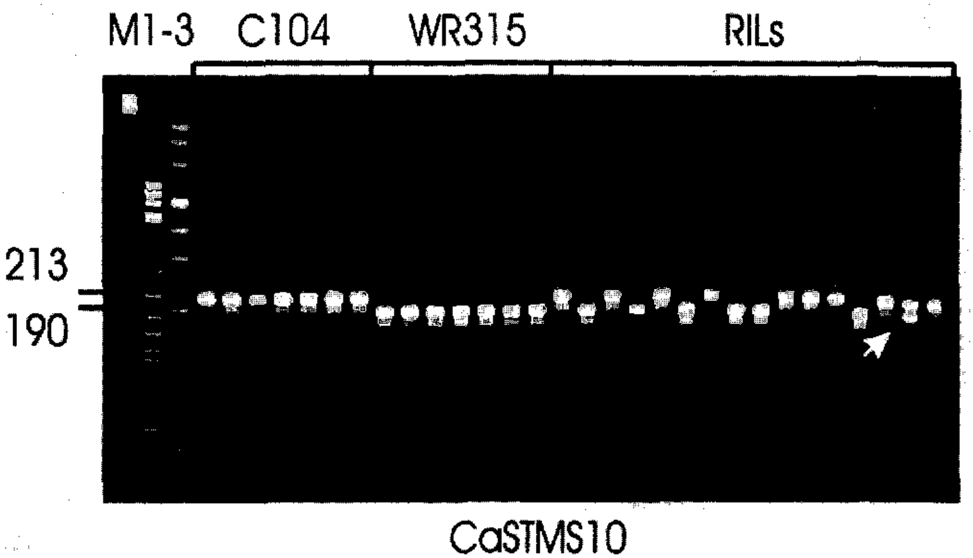

Fig. 3.6. Stability and segregation of CaSTMS10 in parental lines C104, WR315 and their offspring from F7 recombinant inbred lines (RILs). Molecular weights are given in bp at the left. M1-3 are 3 different molecular weight standards. Note the heterozygote locus indicated by the arrow.

3.4.3.3. Conclusions and Consequences for the Large-Scale Generation of STMS in Chickpea.

The pilot study proved that the experimental set-up principally was suitable for the generation of large amounts of STMS markers. With respect to their abundance, repeat unit number and polymorphism, $(TAA)_n$ microsatellites were obviously the best candidates for a large-scale approach. Due to their low melting temperature they could be much easier sequenced than high-melting $(GA)_n$ or $(CA)_n$ repeats, so that in many cases also sequences distal to the microsatellite motif could be read without ambiguity and used for primer design, which in many cases was impossible with other di- and trinucleotide motifs. The high repeat unit numbers found in $(TAA)_n$-microsatellites render them highly informative also in closely related chickpea accessions. Trinucleotide repeats additionally advantageous for an application in a breeding program. They are less prone to polymerase-stuttering during PCR and therefore give more clear-cut banding patterns than dinucleotide repeats.

In the pilot study the use of several blunt-end-generating restriction enzymes and co-separation of the respective restriction fragments for size selection in the same lane had turned out to be disadvantageous, because in cases where no amplification product could be obtained it was not clear if the sequences used for design of the flanking primers were derived from contiguous DNA or from co-ligated fragments generated by different restriction enzymes whose restriction sites could no longer be identified. As a consequence only single-enzyme digests were used for library construction in further experiments.

(S.M. Udupa and F. Weigand, ICARDA; Bruno Hüttel, Peter Winter and Günter Kahl, University of Frankfurt)

3.4.4. Large-Scale Generation of STMS Markers from $(TAA)_n$ Microsatellites

3.4.4.1. Cloning, Sequencing and Amplification of $(TAA)_n$-Microsatellite loci

For the large-scale production of $(TAA)_n$-microsatellites essentially the same successful protocol was used as in the pilot study. For the generation of libraries genomic DNA was digested with Alu I, Rsa I and Sau 3A/Mbo I, size-selected independently and ligated into suitable restriction sites of the vector. A total of 200.000 Alu I-, 60.000 Sau 3AI- and 40.00 Rsa I-clones were plated and screened with the $(TAA)_5$ probe. To avoid the sequencing of around 20 % false positives (as in the pilot study) another clone purification step was added and clones sequenced as above. The probe detected around 600 microsatellite-containing colonies, of which 242 were sequenced. As shown in Fig. 3.7, repeat unit numbers and

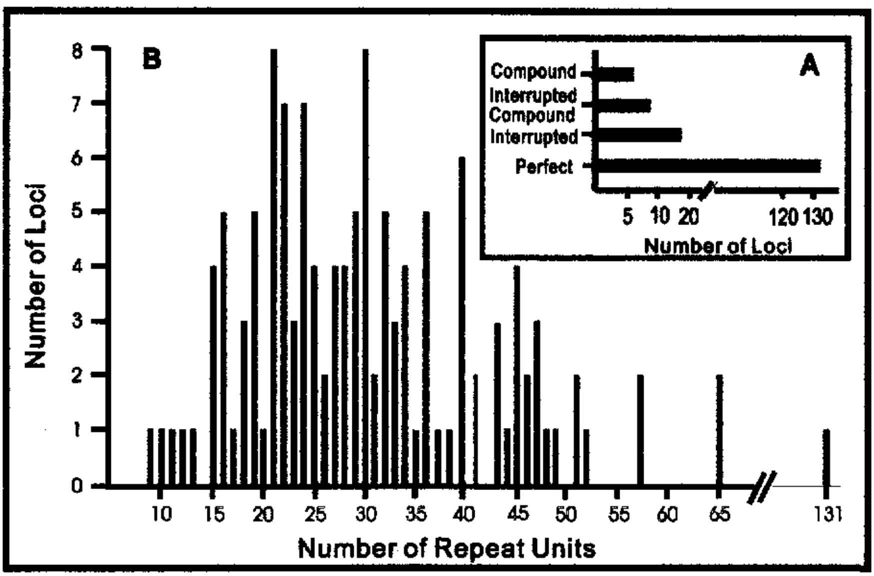

Fig. 3.7. Composition and length distribution of (TAA)n microsatellite loci.

A) Composition of microsatellite motifs.

B) Number of (TAA) repeat units in perfect repeats.

microsatellite type varied considerably between loci. Onehundredthirtyone perfect, 6 compound, 9 interrupted-compound, and 18 interrupted repeats were found with repeat unit numbers differing between 9 and a maximum of 131.

As a rough estimate, one third of the sequences were useless, because either the microsatellite was accompanied by insufficient flanking sequence to permit the design of primers, or the sequence was unreadable because of recombination events that occurred in *E. coli*. The second third required repeated sequencing, whereas the other third was suitable for primer design. Using all available sequence information from 242 sequences 122 primers with a length of 22 to 27 nucleotides and with melting temperatures between 55 °C and 60 °C were designed. Two PCR protocols were developed that alternatively allow the reproducible amplification of nearly all microsatellite loci. *The sequences of flanking* primer pairs together with the name of the respective loci, expected size of product in ILC 3279 and whether they detect polymorphisms is given in the appendix.

3.4.4.2. Variability of Microsatellite Loci

Inter- and intraspecific polymorphism of these loci was determined by separating the amplification products derived from chickpea accession ICC4958 and an accession of *C. reticulatum* on ethidium bromide-stained, native polyacrylamide gels. To date 122 loci were tested of which 90 gave interpretable amplification products. Of these 83 (92 %) showed interspecific variability (see appendix for more detail).

3.4.4.3. Distribution of $(TAA)_n$-Microsatellites in the Chickpea Genome

Locus TA76 was amplified from 70 individuals of a recombinant inbred line together with parental loci P_A and P_B and run on a native 8% PAA-gel. Molecular weights of products are given in bp. A 100bp ladder was used as a molecular weight marker.

A vital question and prerequisite for the use of molecular markers for gene tagging and breeding is their bona fide random distribution in the genome. As has been shown in tomato, especially $(TAA)_n$ microsatellite loci tend to be clustered at and around centromere. In order to test whether these loci also show a tendency for clustering in chickpea, genetic distances between 29 loci were calculated on the interspecific F_7 recombinant inbred line. In these experiments sometimes a multiplexing approach could be used in which amplification products of different length derived from 2 primers were subsequently loaded on one and the same polyacrylamide gel. A typical result of separation of amplification products on PAA-gels is shown in Fig. 3.8. After linkage analysis with MAPMAKER some loci appeared to be positioned close to each other in small clusters, whereas others showed no coupling at all. As shown in Fig 3.9, especially in LGIV. A vital question and prerequisite for the use of molecular markers for gene tagging and breeding is their bona fide random distribution in the genome. As has been shown in group IV a dense clustering of $(TAA)_n$ microsatellites occurs (within only 3.5 cM 5 markers are clustered). Two markers showed no recombination at all, indicating that they are derived from the same locus. Overall the 18 linked markers span a genetic distance of 90 cM with 11 markers remaining unlinked.

3.4.5. Conclusions and Consequences for Ongoing Work

The final aim of this work is to generate STMS markers from the chickpea genome that are closely linked to one or more genes responsible for *Aschochyta* blight resistance in chickpea. To that end it is intended to generate around 300 STMS markers and test them on populations segregating for resistance. Up to now around 150 primer pairs have already been designed and tested. Of these 109 pairs produced

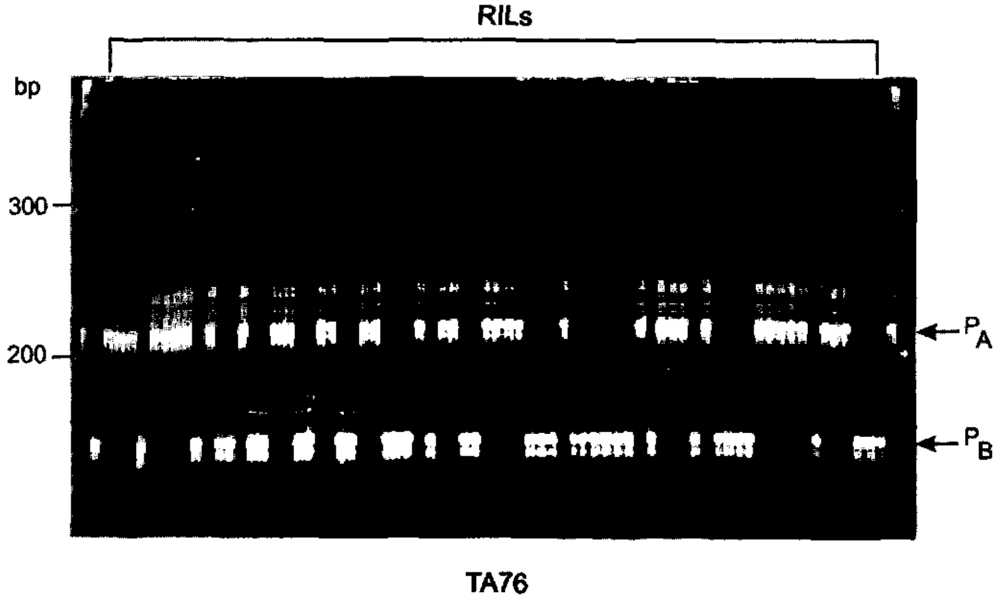

Fig. 3.8. Separation of amplified (TAA)-microsatellite loci on PAA-gels. Locus TA76 was amplified from 70 individuals of a recombinant inbred line together with parental loci P_A and P_B and run on a native 8% PAA-gel. Molecular weights of products are given in bp. A 100bp ladder was used as a molecular weight marker.

interpretable amplification products, though several of them had to be redesigned and tested a second time.Around 92 % of bands were polymorphic between C. arietinum and C. *reticulatum*. A detailed study of intraspecific polymorphism is still pending but preliminary results indicate that around two thirds of loci will also be polymorphic. All in all, generation and amplification of STMS was successful and, without doubt, the same protocol can be used for the generation of another 150 markers.

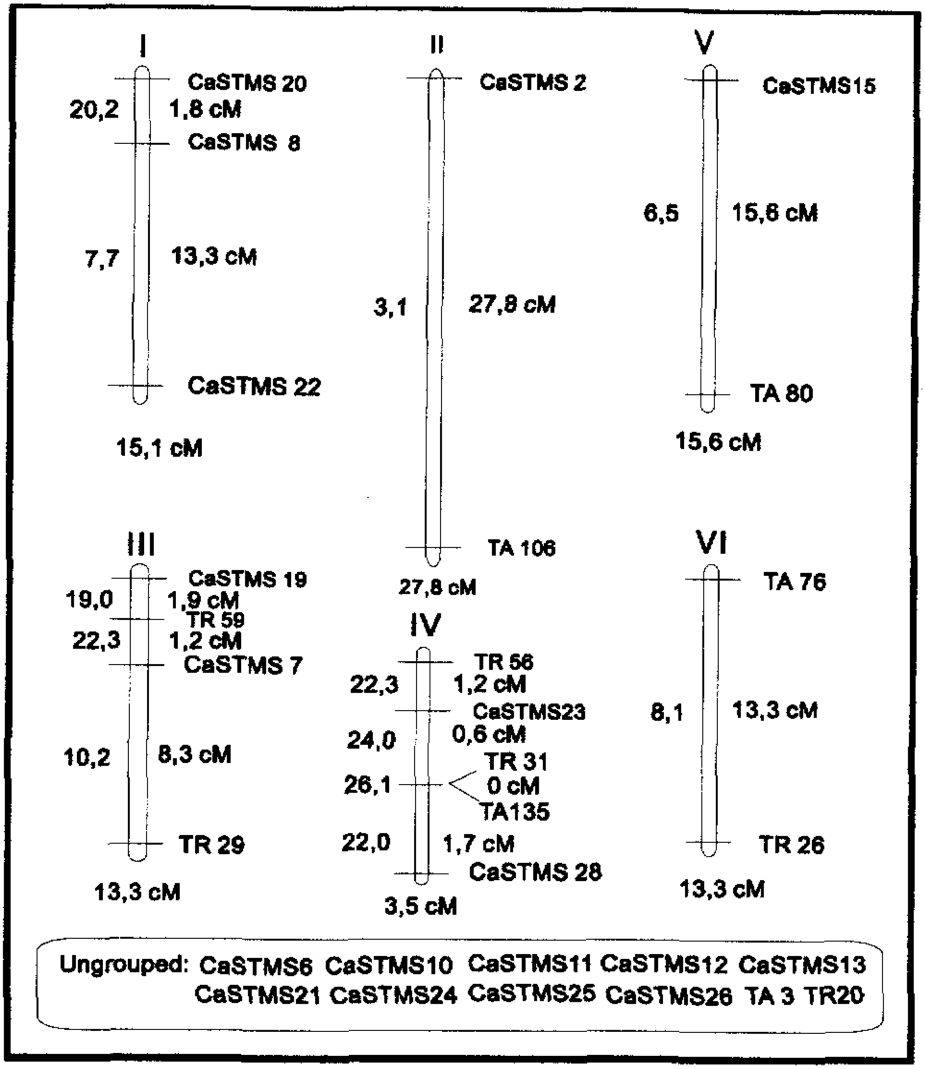

Fig. 3.9. Grouping of 18 microsatellite markers in the chickpea genome. Logarithm of ods in favour of grouping against the probability that no grouping is present is given on the left side of the bar. Genetic distances between markers is given on the right in centiMorgan (cM).Note that in group IV 5 markers are clustered at a distance of only 3.5 cM with no recombination found between markers TR 31 and TA 135.

The only reason of concern is the apparently dense clustering of (TAA)-microsatellites in some regions of the genome, as is demonstrated for those in group IV in Fig. 3.9 From a total of 29 tested loci 20 were of the $(TAA)_n$-type. Of these only 3 remained unlinked to other loci, whereas 9 of the $(GA)_n$-, $(GAA)_n$-,and $(GT)_n$-type were not grouped together. At this moment it is not clear whether the observed clustering is due to the high level of segregation distortion found in the interspecific population which is indicative of suppressed recombination in specific regions of the genome, or if this holds true also for other populations. Anyway, it will be necessary to test more markers on that and other populations, before a decision in favour of generating more STMS from otherwise extremely useful $(TAA)_n$ microsatellites can be made. On the other hand, markers in other groups are coupled at reasonable genetic distances, with markers containing different microsatellite motifs intermingled with each other. Therefore, it can be expected that if clustering of $(TAA)_n$-microsatellites is a reality, only a subset of these will be affected and the others will be highly useful for the tagging of resistance genes.

(S.M. Udupa and F. Weigand, ICARDA; Peter Winter, Theo Pfaff, Shabaz Sahi & Günter Kahl, University of Frankfurt)

3.4.6. Mating Types, Segregating Populations and Mutations in *Ascochyta rabiei*.

3.4.6.1. Mating Types and Segregation Analysis

For the first time the molecular characterization of A.rabiei (teleomorph *Didymella rabiei*) mating types was achieved. Two US-isolates (mat1-1 and mat1-2, respectively) were compared with virulent isolates from diverse countries by oligonucleotide fingerprinting. Greatest genetic variability was detected between the Syrian isolate AA6 and the mating type isolates from North America. Pairings were performed and AA6 was identified as a mat1-2 isolate. A segregating population was obtained from a cross between the compatible isolates AA6 and mat1-1 (ATCC76501). Independent cultures were grown from 62 single ascospores. These 62 F1 and the parental isolates were analyzed by oligonucleotide fingerprinting and results showed, that several tested microsatellite motifs were stably inherited to the F1 progeny and segregated according to Mendelian ratios. From 66 polymorphic fingerprint bands detected in this cross so far, 5% are significantly distorted in their Mendelian segregation. From the remaining 52 bands, 30 are linked at a Lod score of 4 on five different linkage groups.

On the parents and a subset of the F1 population 79 RAPD primers, 17 MP-PCR primers (microsatellite primed-PCR) and few primers used under DAF conditions have been tested. Polymorphic bands were amplified by every third primer in the case of RAPDs, and by less than every second primer in the case of MP-PCR. In DAF experiments, a rate of 0.5 polymorphisms detected per primer can be expected. These marker types are very useful: they can be applied quickly and easily be cloned after their isolation from stained agarose- or PAA-gels. Cloned markers, that are linked to a phenotype (e.g. pathotype or mating type) can be used to screen genomic libraries to isolate distinct loci and create locus-specific probes (primers) of epidemically important traits of the pathogen, which can be used to forecast epidemic outbreaks. More markers will in future be applied to achieve a high resolution genetic linkage map of the *A. rabiei* genome.

3.4.6.2. Discovery of Mutations at Microsatellite Loci

To investigate and develop multiple marker types, which is necessary for high density linkage mapping, cloned MP-PCR bands were used as hybridization probes upon Southern blots with restriction-digested genomic DNAs of *A. rabiei*. Single and duplex primer MP-PCR experiments were performed and low *molecular weight bands (400-600bp) were* cloned. One band obtained with $(GACA)_4$ as a single primer produced an inverted repeat fragment that carried an AT-rich sequence. This cloned fragment represents a genomic fingerprint probe which produces up to 40 different bands in Southern analysis. A similar fragment, that detects multicopy DNA sequences in Southern analysis, was isolated from a PCR reaction using $(GATA)_4$ as a single primer.

Single and low copy fragments were amplified and cloned from a duplex experiment using the primer combination $(GTTTGG)_3/(GGAT)_4$. One fragment (600bp) detected three loci in RFLP analysis and one band of these was polymorph in the investigated cross. Another cloned fragment (400bp) of the same PCR reaction displayed a single-locus RFLP, and furthermore revealed a molecular mechanism, how *A. rabiei* develops genetic variability during sexual ascospore production. By applying the cloned single-locus MP-PCR fragment as hybridization probe on a Southern blot, a locus polymorphic between the parents was detected. Using this probe on the F1 population revealed its 1:1 segregation and usefulness as a molecular marker. One F1 isolate (Fig. 3.10, lane 10) developed a new (larger) allele present in neither parent. A locus-specific primer pair (STMS-primers) was deduced from the sequence of the cloned MP-PCR fragment and applied to parents and progeny. STMS analysis confirmed the development of a new length allele in the isolate (Fig 3.10; lower half). A nested STMS-primer pair was used for direct sequencing of the parental alleles and the mutated F1 allele. The nucleotide sequence revealed the presence of a *hypervariable compound microsatellite composed of several* pentameric and decameric repeat units. The parental alleles differed by 130 nucleotides and displayed a VNTR-type (variable number of tandem repeat) of polymorphism. Several tandem repeat blocks displayed variable repeat numbers in the parents. Sequence data made also clear, that the mutated

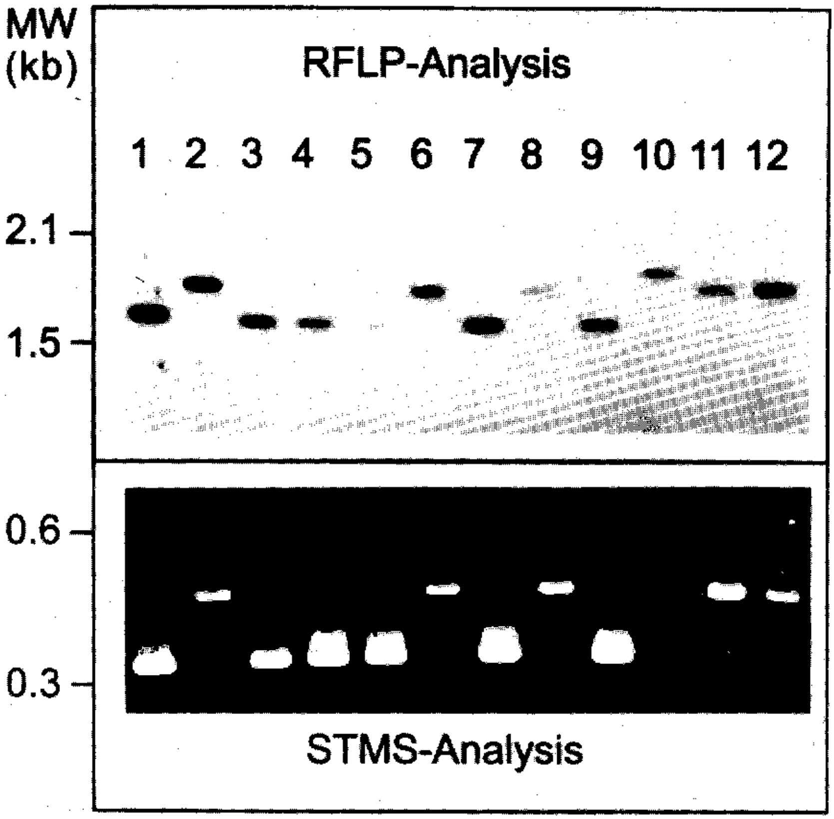

Fig. 3.10. RFLP and STMS analysis of a hypervariable compound microsatellite locus in a segregating F1 population of *A. rabiei* (lanes 3-12), resulting from a cross between the Syrian isolate AA6 (lane 1) and the North-American isolate ATCC76501 (lane 2). For RFLP analysis, the cloned MP-PCR fragment was used as hybridization probe on a Southern blot containing *Hinf*I-digested *A. rabiei* DNA. For STMS analysis, the locus was amplified from *A. rabiei* DNA using the flanking primers indicated in Fig. 3.11. Both RFLP and STMS analysis allow to trace the F1 bands back to the parents, and to detect the increased allele size in one of the F1 progeny (lane 10). Positions of molecular weight markers (MW) are given in kilobase pairs (kb).

(larger) F1 allele was donated by the US parent, and the cause for the +60 bp difference of the new length allele was the expansion of a TATTT-repeat: a $(TATTT)_{53}$ mutated to a $(TATTT)_{65}$ during sexual ascospore production. This is an excellent example for one of the mechanism leading to hypervariability at microsatellite loci of fungi (Fig. 3.11; upper half). In addition, three more alleles from isolates originating from different geographical regions were sequenced to compare their microsatellite composition at this locus. STMS and sequencing primers worked in isolates from around the world, and results confirmed the hypervariability and also uniqueness of the locus. Exceptional was the allele of isolate TAr328 (Fig. 3.11; lower half), in which a duplication of two pentameric units created a new decamere repeat which was not present in any of the other investigated alleles. Comparison of all sequenced alleles suggests, that variation at this locus results from the combined action of point mutations (C T-transitions, A T-transversions), VNTR-type mutations (polymerase slippage and/or unequal crossing over) as well as duplications and excisions.

(S.M. Udupa and F. Weigand, ICARDA; (J. Giestlinger, G. Kahl, University of Frankfurt))

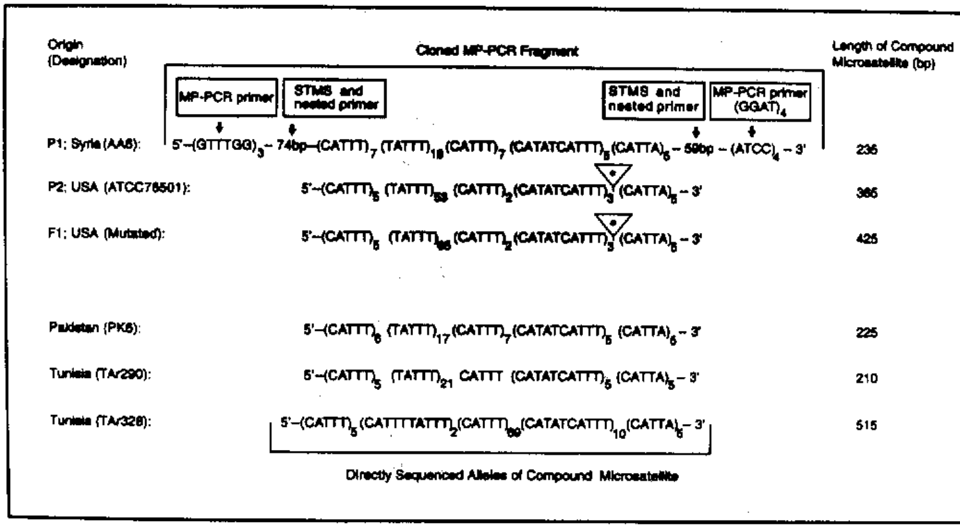

Fig 3.11. Allelic variation at a compound microsatellite locus of *A. rabiei*. The uppermost sequence (AA6) represents the originally cloned MP-PCR fragment and includes the microsatellite and its flanking regions. All other sequences were identified by direct sequencing of purified PCR products, using nested sequencing primers that bind within the 59 bp and 74 bp unique flanking regions, respectively. The upper three sequences were derived from the parents and one F1 mycelium of a cross between the Syrian isolate AA6 (P1) and the North-American isolate ATCC76501 (P2). The F1 allele is identical to the P2 allele except for an increased number of TATTT-repeats. The triangle symbolizes a unique 10 bp motif specific for the P2 allele. The lower three sequences were derived from three randomly chosen *A. rabiei* isolates from Tunisia and Pakistan. Binding sites for MP-PCR, STMS and nested sequencing primers are indicated on top.

3.5. Interspecific Crosses in Chickpeas

To obtain a hybrid between *Cicer arietinum* and *C. bijugum* and *C. pinnatifidum* different cross combinations were tried. Six cultivars and three accessions from each wild species have been taken (Table 3.35).

Table 3.35. Chickpea species used in crosses.

Cultivars (*Cicer arietinum*)	**Wild annual species**	
ILC 200 (Russian kabuli)	*Cicer bijugum*:	ILWC 32
		ILWC 62
ILC 482		ILWC 79
ILC 519 (Egyptian kabuli)	*Cicer judaicum*:	ILWC 46
		ILWC 61
ILC 6328 (Indian desi)		ILWC 95
FLIP 84-15C	*Cicer pinnatifidum*:	ILWC 171
		ILWC 236
AMDOUN-1 (Tunisian kabuli)		ILWC 250

These accessions have been chosen from wild annual chickpea species according to available resistances and tolerances for biotic and abiotic stresses. The wild species have been used as the pollen donor as these crosses are more easy to carry out. So far, all accessions except ILWC 61 and ILWC 95 have been crossed with all 6 cultivars. To enhance pollen tube growth and ovule development three hormone solutions have been applied. Also differences in duration of application are tested with all three solutions. So far 1764 crosses have been made from which 47 % has been successful (developing ovule). Differences between the different accessions (Table 3.36) are considerable between 32% and 79% for successful crosses. All the three accessions of *C. bijugum* give a better response than the accessions of *C. pinnatifidum*. The number and the quality of the ovules differed also between and within the different wild species. Flowers and seeds of *C.bijugum* are larger than the those of the other species, which makes the crosses with this species easier. Also the ovules that are rescued from crosses with this species are often larger.

Table 3.36. Results of crosses from 6 chickpea cultivars with different accesions of wild species.

ACCESSION	# CROSSES	# PODS	# OVULES	% SUCCESS
32	167	96	160	57
62	141	83	127	59
79	132	104	206	79
46	111	76	162	68
171	634	200	362	32
236	366	188	295	51
250	213	89	173	42
TOTAL	1764	836	1485	47

Differences between cultivars in crossability and successful fertilization are not substantial (Table 3.37). Only AMDOUN-1 shows in general a low response independent from the cross combination.

Hormones, cultivars and wild species all interact. For example ILC 519 shows a very different response when crossed with different accessions when hormones are applied. For some crosses ID is better than GA3 and reversed.

Table 3.37. Results of the crosses per cultivar.

CULTIVAR	# CROSSES	# PODS	# OVULES	% SUCCESS
ILC 200	228	106	203	46
ILC 482	425	216	371	51
ILC 519	273	149	266	55
ILC 6328	227	110	186	48
FLIP 84-15C	346	132	221	38
AMDOUN-1	265	123	238	46
TOTAL	1764	836	1485	47

The three hormone solutions tested are all published results on food legumes (Table 3.38). ID and GA3 have been more extensively used for crosses. IC has so far just been tested on crosses with ILWC 171. For ovule rescue and possible embryo rescue, ovules are needed that have developed normally. They should be dark green without being translucent and should have a size exceeding 3 mm. Although the rate of success with IC is considerable, the number of pods containing dark green ovules bigger than 3mm ovules are smaller as compared in crosses where GA3 is applied. So far GA3 seems in general the best hormone, but the differences in combination should be noted and used when further crosses are made.

A high number of successful crosses does not automatically imply a high quality of ovules. Apparently, a continuous high or low dose of hormones has a negative effect (Table 3.39).

Table 3.38. Accumulated data on the effect of the application of three hormone solutions on interspecific chickpea crosses.

Hormone Solution	# Crosses	# Pods	# Ovules	% Success	% Of Good vules
ID *	829	352	612	42	64
GA3 **	828	393	635	47	64
IC ***	142	91	173	64	30
TOTAL	1657	836	1420	AV= 50	39

* 8 mg gibberellic acid, 1 mg kinetin and 1 mg naphtalenic acid per liter

** 200 mg gibberellic acid per liter

*** 87.5 mg gibberellic acid, 5 mg kinetin and 25 mg naphtalenic acid.
This hormone was just applied on crosses with ILWC 171

Table 3.39. Results of different applications of hormones.

Treatment	# Crosses	# Successful	# Ovules	% Good Ovules
First day	66	25	27	29
Every day	66	32	55	37
Every other day	67	25	32	63
First 10 days	65	28	42	46
First 5 days	65	19	29	35
First 10 days every other day	65	26	31	31
TOTAL	394	155	216	-
AVERAGE	66	26	36	41

Ovules are cultured on different induction and regeneration media. 9 induction media and 17 regeneration media have been tested. It is possible to regenerate calli from good quality ovules which in some cases develop roots or shoots. A possible hybrid (with roots and shoots) was obtained but does not develop any further to maturity and roots have turned black.

So far one possible hybrid has been obtained of the combination ILC 519 * ILWC 236 with .

To assure that any obtained hybrid can be maintained, grafting techniques has been tested. It should be possible to graft shoots from sterile in vitro conditions on non-sterile cultivars. Tests have been made with very young shoots of different wild species (Table 3.40). Grafts from sterile shoot on non sterile cultivars have been tested. The connection between root stock and graft are protected using parafilm to tie the graft and to avoid contamination. As a way of cloning the hybrids, explant culture of plants grown in vitro on medium containing sugar are tested.

3.5.1. Chickpea Transformation

Although protocols on Agrobacterium-mediated gene-transfer have been reported, it has to be stated, that -at least for the time being- reliable protocols for the production of transgenic chickpea do not exist. Chickpea regeneration from protoplasts has not been reported so far. In particular for kabuli-type chickpea, a protoplast-system was not available.

3.5.2. Agrobacterium-Mediated Transformation

Two strategies can lead to transgenic plants following Agrobacterium inoculation: 1) De novo regeneration from selected callus tissue 2) Rapid shoot-proliferation from already existing meristems. General culture conditions were optimized suitable explants were selected and optimum coculture conditions were determined.

Table 3.40. Grafts of wild species on cultivars.

GRAFT	# GRAFTS	# SUCCESSFUL	# FLOWERS	# PODS
ILWC 61 + ILC 6328	1	1	1	
ILWC 61 + FLIP 84-15C	1	0		
ILWC 62 + ILC 6328	1	1	-	
ILWC 62 + AMDOUN-1	1	1	-	
ILWC 62 + FLIP 84-15C	2	2	4	2
ILWC 79 + ILC 519	1	1	-	
ILWC 250 + ILC 200	1	1	-	
ILWC 250 + ILC 6328	1	1	1	
ILWC 250 + * AMDOUN-1	1	1	3	2
ILWC 95 + * ILC 6328	3	1	-	-
ILWC 95 + * AMDOUN-1	2	0	-	-
ILWC 95 + FLIP 84-15C	5	1	-	-
TOTAL	20	11	9	4

* Grafts were made with sterile shoots of 10 days old seedlings on non-sterile cultivars.

(B. van Dorrestein and M. Baum)

3.5.3. General Tissue Culture Conditions

The tendency of chickpea tissue to browning and necrosis was reduced by using B5-medium as a basal medium. Comparably low levels of mineral nitrogen and explant preparation under non-oxidative conditions (submers) were sufficient. The use of absorbents like PVP or activated charcoal or antioxidants like ascorbic or citric acid was not found beneficial in tissue culture. Generally a better ventilation leads to improved germination. However, a germination experiment has shown, that neither temperature (22 C vs. 26 C) nor ventilation (open/closed jars) but year of harvest exhibited (1995>1996) a significant impact on percentage of germination and hook-necrosis.

3.5.4. Genotype Dependent Different Susceptibilities for Agrobacterium Tumefaciens Mediated Gene Transfer

By using the reporter genes a-glucuronidase (gus) and the green fluorescent protein (gfp) gene, different chickpea seedling-explants were analyzed for their respective susceptibility *Agrobacterium*. It was found that -as in other grain legumes- high number of transformation events were detected in the rapid proliferating cambial tissue. Only limited expression was found in shoot-proliferating tissue. From the transient expression assays it was concluded that the transformation process itself is not a limiting step. Examples for detection of transformation events are shown in figure 3.12. The experiments did not show significant difference in the number of transformation events between the three lines (ILC482, ILC 1929, ILC-3279).

3.5.5. Regeneration Capacity

Since general culture conditions, susceptibility for Agrobacterium-mediated transformation and vector construction do not represent insurmountable problems, the induction of multiple shoots on meristems appeared to be the most important and urgent constraint. As long as de novo regeneration is not achieved, inoculation and transformation of preexisting meristems followed by rapid multiplication is the only way to regenerate transgenic plants. The application of cytokinins like BAP or TDZ at fairly high concentrations (above 5 M) leads to necrosis in shoot tips, while this necrosis is more pronounced in kabuli-type than desi-type chickpea. BAP, toxic at high concentrations even in pea, was replaced by TDZ and CPPU, which are known for their high biological activity and less toxic effects in other grain legumes. However, both let to shoot-tip necrosis. In several sets of experiments the application of 3-5 uM Kinetin or 0.5-1 uM TDZ appeared to be beneficial. Although shoot tip necrosis could not be inhibited, multiple shoot or shoot bud formation was observed on more than 50% of the treated explants (apical thin layers).

In addition, prolonged incubation with low amounts of cytokinin resulted in the production of proliferating clones (Fig. 3.13). However, when using TDZ after 2-3 month in culture, more callusing was observed.

3.5.6. Vector Construction

The Bayer company provided the Stilbene-Synthase gene (Vst-1) from *Vitis ssp.*for direct DNA-transfer in a high-copy pUC19 plasmid and a *binary* plasmid for use in Agrobacterium-mediated transformation. Both plasmids have been successfully transformed into E.coli strain NM522 and the binary plasmid was introduced into hypervirulent strain EHA105. For EHA105/EHA101, the hypervirulent vir-donor strains, a binary plasmid can be used, which can be regarded as a universal binary plasmid, i.e. might be used also if additional constructs for fungus-resistance can be made available.

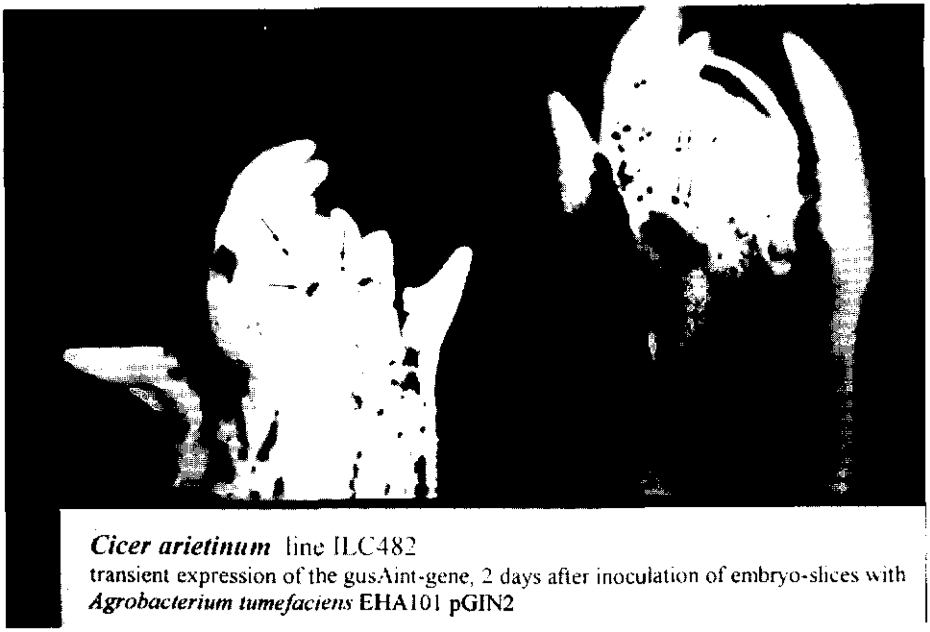

Fig. 3.12. Histochemical detection of transformation events on shootapical slices of *kabuli*-chickpea.

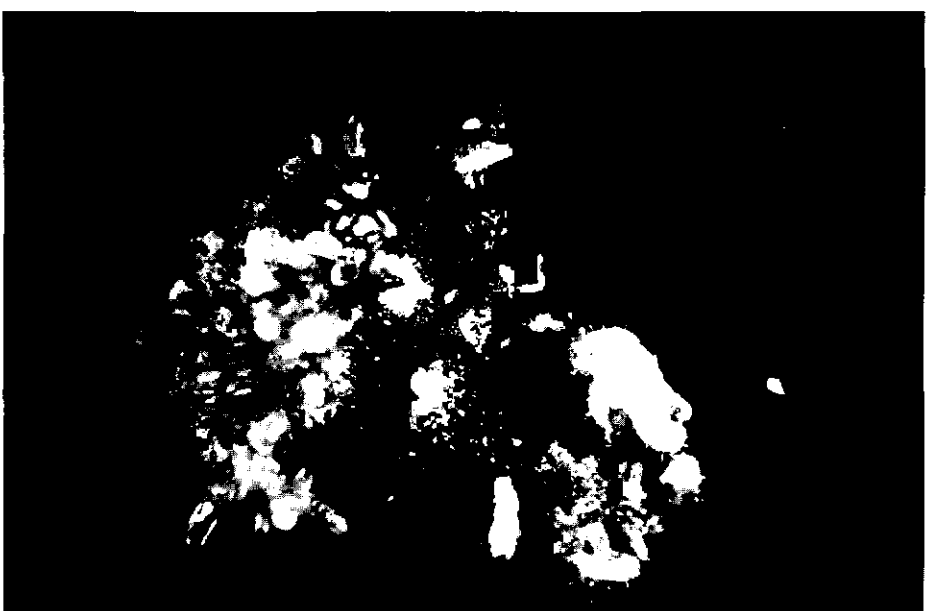

Fig. 3.13. Multiple shoot fomation on decapitated desi-chickpea upon application of 25 μM TDZ.

3.5.7. Protoplast Culture

A standardized protoplast isolation and kallus regeneration protocol for chickpea has been worked out. Routinely a high yield of viable protoplasts (5-8 x 106 from 50 explants) can be obtained, which will allow transformation of calli via PEG mediated DNA transfer.

3.5.8. Protoplast Transformation and Selection

For the first transformation experiments constructs harbouring the pat gene were used, which allowed to use phosphinotricin (10 mg/l) to select the positive clones during the cultivation in suspension culture in light at 22 C. Due to the herbicide action non transformed calli turned brown after one week. The transformed calli were placed on B5 solid medium with 10 uM/l TDZ and 10mg/l phosphinotricin.

(H. Kieseker, A. de Kathen, H.J. Jacobsen)

3.6. Chickpea Entomology

3.6.1. Leafminer and Pod Borer Management

Several treatments were included in this experiment for the control of Leafminer (*Liriomyza cicerina* Rond.) and pod borers (*Helicoverpa armigera* Hubner and *Heliothis viriplaca* Hufnagel): Neem oil (1ml/l), Neem powder(6g/l), Neem seed extract (50g/l), Hostathion (2.4l/ha), Thiodan(2cc/l), and Decis(0.5cc/l). Neem formulations were sprayed three times on winter-sown chickpea and four times on spring-sown chickpea starting from the insect appearance at ten days intervals. The other chemicals were sprayed twice starting from the insect appearance at three weeks intervals on both spring and winter-sown chickpea. This experiment was carried out at Tel Hadya and in a farmer field at Sheikh Youssef. At Tel Hadya station, two different dates were compared, winter and spring-sown Chickpea. Whereas at Sheikh Youssef location, we only had the spring-sown Chickpea.

In the two locations and for the two planting dates, the percent pod damage due to Pod borer was very low (about 2%), and thus the results are not discussed in this report.

The level of infestation by the leafminer was mild; the visual damage score of the control at Tel Hadya was 4 (scale 1-9) for winter chickpea and 5 for the spring-sown chickpea. The percent mining was also higher on spring-sown chickpea (21.6%) than on winter-sown chickpea (10.8%). Significant and similar levels of control were achieved by Decis, Thiodan and Hostathion. Neem seed extract also provided a significant level of control against leafminer. However, the differences in yield were not significant (Tables 3.41 and 3.42). Similar results were obtained at Sheik Youssef on spring-sown chickpea (Table 3.43). New Neem formulations are being tested this season against Leafminer and Pod borer.

(M. El Bouhssini, N. Sharaf Eldin, and A. Joubi)

Table 3.41. Effect of three Neem formulations, Hostathion, Thiodan, and Decis on Leafminer infestation and seed yield at Tel Hadya in winter-sown chickpea, 1995/96.

Treatment	Visual Damage Score (15 May)	%Mining (15 May)	Grain yield (Kg/ha)
NEEM OIL	3.25	9.15	1894.00
NEEM POWDER	2.75	8.95	2037.25
NEEM SEED EXTRACT	2.00	10.05	2169.25
HOSTATHION	1.00	1.53	1947.75
THIODAN	1.00	2.15	2124.00
DECIS	1.25	1.17	1950.75
CONTROL	4.00	10.83	1905.25
LSD AT 5%	0.98	6.14	365.20

Table 3.42. Effect of three Neem formulations, Hostathion, Thiodan, and Decis on Leafminer infestation and seed yield at Tel Hadya in spring-sown chickpea, 1995/96.

Treatment	Visual Damage Score (21 May)	%Mining (26 May)	Grain yield (Kg/ha)
NEEM OIL	4.25	41.33	924.25
NEEM POWDER	5.00	50.90	1141.75
NEEM SEED EXTRACT	3.25	44.23	1034.00
HOSTATHION	2.50	23.90	1154.75
THIODAN	2.25	35.92	1056.75
DECIS	3.00	39.80	1038.00
CONTROL	5.00	45.98	1179.25
LSD AT 5%	1.67	14.60	267.76

Table 3.43. Effect of three Neem formulations, Hostathion, Thiodan, and Decis on Leafminer infestation and seed yield at Sheikh Youssef in spring-sown chickpea, 1995/96.

Treatment	Visual Damage Score (23 May)	%Mining (23 May)	Grain yield (Kg/ha)
NEEM OIL	5.00	42.60	1683.25
NEEM POWDER	5.25	44.15	1597.25
NEEM SEED EXTRACT	4.00	40.40	1557.00
HOSTATHION	2.00	14.80	1603.75
THIODAN	2.00	17.43	1672.25
DECIS	2.00	16.90	1569.50
CONTROL	5.00	46.20	1567.50
LSD AT 5%	0.54	4.70	215.70

4. FORAGE LEGUMES IMPROVEMENT

Annual forage legume crops, such as vetches (*Vicia spp.*) and chicklings (*lathyrus spp.*) are recognized for their potential to produce extra feed from the fallow lands. They are one of the major options being considered either to interrupt barley monoculture or to replace the fallow in the fallow-barley rotations. These species are sown and harvested in a single year and can be used for direct grazing during winter, harvested for hay in spring either in pure stand or in mixtured with cereals (Oat, barley or triticale), or for grain and straw at full maturity). They differ from food legume crops only in the end use. They are mainly used to feed livestock, whereas food legume crops for human consumption. There is one exceptional case: that is *lathyrus sativus* (grasspea or chickling pea) which is a popular food and fodder crop in certain Asian and African countries such as Bangladesh, India, Pakistan, Nepal and Ethiopia, because of its resistance to drought, water-logging and moderate salinity and because of its low inputs. When other crops fail under adverse *climatic conditions L. Sativus can become* the only available food source for the poor section of the population and sometimes a survival food during times of drought-induced famine. Although seeds of *L. sativus* are tasty and protein-rich, over consumption can cause an upper motor neurone disease known as "neurolathyrism", an irreversible paraparesis of the lower limbs. The neurotoxic causal of this disease was identified as 3-N-oxalyl-L-2, 3-diaminopropanic acid (B-ODAP or its synonym BOAA: B-N-Oxalylamino-L-alanine). The level of this compound in the dry seeds varies widely depending on genetic factors and environmental conditions. Our efforts are being made to eliminate B-ODAP from *L. sativas* seeds by breeding using the available genetic resources from different origins.

Flexibility in forage legume crops to meet different types of utilization in different agroecological zones is always of great importance in developing new adapted cultivars. Each species tends to have an ecological niche., e.g. *L. sativus* is prominent in lower rainfall areas, between 250 to 300 mm, because of its great drought tolerance, *Vicia dasycarpa* and *V. panonica* are adapted to high elevation cold areas because of their rapid winter growth and cold tolerance.

The introduction of *Vicia* spp. and *Lathyrus* spp in the rotation also increases the production of feed resources and subsequently, the carrying capacity of the land in a sustainable manner. This is because of the maintenance of organic matter and nitrogen status of soil, improved soil physical condition and better control of diseases and pests as compared to continuous culture of cereals rotations.

Forage legumes production is also expected to have a positive effect on rangelands by: (a) reducing overgrazing problem and (b) allowing for adoption of proper grazing systems. At present, livestock move into range at the beginning of the rainy season, causing great damage to newly emerging vegetation through repeated trampling and defoliation.

4.1. Environmental Adaptation

Although there is a huge diversity of species of *Vicia* and *Lathyrus* in the Mediterranean region, only a few have been used as feed crops and these have received little attention in the past from plant breeders and agronomists.

In the region, there are at least three species of *Lathyrus* and nine species of *Vicia* of potential importance. We focus only on those species within the two *genera* which are annual and adapted to areas where rainfall is between a total of 250 and 400 mm per annum. In areas where rainfall is less than 300 mm *Lathyrus* spp. are common, whereas in higher rainfall areas *Vicia* spp. are better adapted. *V.*

narbonensis is adapted to drier sites, whereas *V. sativa* and *V. ervilia* perform better with a more assured moisture. *V. villosa* spp. *dasycarpa* and *V. panonica* are better adapted to cold environments in the highlands than the other *Vicia* species and those of *Lathyrus*. Underground vetch (*V. amphicarpa*) and underground chickling (*L. ciliolatus*) are adapted to areas with marginal lands, hilly rocky lands and low rainfall.

4.1.1. Germplasm Enhancement

The general objective of our breeding program is to develop and produce improved cultivars of feed legume crops, mainly vetches (*Vicia* spp.) and chicklings (*Lathyrus* spp.) and to target these crops to feed livestock in areas with less than 400 mm, rainfall, either in arable land or marginal non-arable lands. It is also highly desirable to have widely adapted cultivars that can be recommended for different locations with similar agro-ecological conditions. While attempting to improve yield potential and adaptation to environment, emphasis is laid on ensuring that the quality components such as palatability, nutritive value protein content, intake of herbage, hay, grain and straw are acceptable by animals. This work is being done in close collaboration with the Pasture, Forage and Livestock Program (PFLP).

To achieve this broad objective, two approaches are adopted to develop improved lines of *Vicia* spp. and *Lathyrus* spp. In one, selection is affected in the wild accessions to develop improved cultivated types. In the second, hybridization is done to introgress desirable traits using selections from wild accessions. The research is carried out by a multi disciplinary team involving breeder, pathologist, entomologist, marginal and rangelands specialist and animal nutritionist. All the research was done under rainfed conditions without any supplementary irrigation.

As international center with major responsibility to serve National Agricultural Research Systems (NARS), we aim to serve the national forage improvement programs through (1) assembling, classifying, evaluating, maintaining and distributing germplasm, (2) developing and supplying NARS with breeding populations with adequate diversity to be used in different environments for different end-uses and (3) coordinate international trials to facilitate multilocation testing and identification of adapted genotypes for specific environments.

(A. M. Abd El-Moneim)

4.1.1.1. Germplasm Evaluation of *Vicia* spp.

The appraisal was carried out of 712 accessions of *Vicia* spp. Collected from different origins, in nursery observation rows in collaboration with the Genetic Resources Unit (GRU). A total of 144 entries (genotypes) were selected on the basis of evaluation of seedling vigour, winter and spring growth, cold effect, leafiness, erect growth habit, pod-shattering (seed retention), and earliness to flowering and maturity.

Special attention will be given to these genotypes in further evaluation of their herbage and seed yields, reaction against major foliar and root diseases and suitability for different end-uses. Detailed results of this part are reported in 1996 annual report of the GRU.

(A. M. Abd El-Moneim and L. D. Robertson)

4.1.1.2. Evaluation of four *Vicia* spp. For adaptation

Four experiments were conducted at Tel-Hadya. One hundred and fourty-four accessions from different origins each of common vetch (*Viccia sativa*), narbon vetch (*V. narbonensis*), bitter vetch (*V. ervilia*), and broad-podded vetch (*V. hybrida*) were planted in a cubic lattice designs with three replicates (6 rows each) for each experiment. All experiments were fertilized with 40 kg P_2O_5/ha.

In these trials, the accessions were visually scored at 1-9 scale (1=poor; and 9=very good) for seedling vigour, winter growth, spring growth, leaf-retention and pod-shattering (seed-retention) at maturity. The two middle rows were harvested at full maturity to estimate grain yield. Time to start flowering, 100 % flowering and full maturity were recorded.

A broad variation was observed between the four species and within the same species for the character studied (Table 4.1). The results show that there is a wide range of adaptation which has been fully documented for reference and future exploitation. Accessions showing good adaptation were identified as promising genotypes and basic materials for future breeding program.

(A. M. Abd El-Moneim)

4.1.2. Preliminary Evaluation in Microplot Field Trials (MYT)

The availability of an appropriate genetic base is an indispensable prerequisite for our breeding program aiming at the development of improved cultivars. Therefore, special attention is given to the material evaluated in observation rows for adaptation for the following agronomically important characters: Cold tolerance, rapid winter growth, leafiness, erect types, early in flowering and maturity, high herbage and grain yields and resistance to biotic and abiotic stresses.

The study of variation in such agronomic characters is of significant practical value. It helps the breeder to

Table 4.1. Range, mean, standard error and coefficient of variation (cv%) for ten characters of 144 accessions of *Vicia sativa*, 49 accessions of *V. narbonensis*, 49 accessions of *V. ervilia* and 81 accessions of *V. hybrida*.

Character	*Vicia sativa*				*Vicia narbonensis*			
	Range	Mean	SEM±	CV%	Range	Mean	SEM±	CV%
Seedling Vigour +	3.9-9.0	7.0	0.97	17.5	4.0-9.0	6.95	1.11	22.67
Winter growth +	2.7-9.0	6.7	0.89	18.9	3.0-9.0	7.23	0.86	16.87
Spring growth +	3.0-9.0	6.8	0.99	20.5	1.0-9.0	6.03	0.84	19.67
Leaf-retention +	4.5-8.8	5.7	0.95	16.0	5.5-9.0	8.2	0.69	12.9
Pod-Shattering +	1.5-8.0	4.9	1.01	19.5	3.5-7.5	6.1	1.12	16.8
Plant+height+(cm)	70-107	90.8	7.98	12.4	45-115	84.2	8.47	14.3
Days to 1st flowering	115-135	127.0	1.64	1.8	105-114	109.0	1.23	1.58
Days to 100% flowering	128-145	137.0	1.96	2.1	115-126	120.0	1.36	1.61
Days to maturity	152-173	162.0	3.07	2.7	136-153	144.0	1.98	1.94
Grain yield (kg/ha)	521-2219	1562.0	171.30	15.5	53-3015	1406.0	187.0	1.00
	Vicia ervilia				*Vicia hybrida*			
Seedling vigour	3.8-9.5	6.92	0.96	19.5	4.6-9.3	6.24	0.67	15.0
Winter growth	3.5-9.5	6.22	0.89	20.0	3.0-9.0	6.16	1.02	23.50
Spring growth	3.0-9.0	7.45	1.16	22.0	3.0-9.0	7.0	0.78	15.70
Leaf-retention	4.3-7.0	5.11	0.67	15.0	-	-	-	-
Pod-shattering	4.0-8.0	6.70	1.90	20.0	1.0-5.0	3.2	0.49	16.0
Plant+height+(cm)	46-80	63.0	6.60	14.8	52-70	61.0	4.20	9.55
Days to 100% flowering	105-142	122.0	2.35	2.68	108-148	129.0	1.80	1.97
Days to maturity	117-154	135.0	2.14	2.36	123-159	142.0	1.78	1.76
Grain yield (kg/ha)	150-173	162.0	2.38	2.07	149-188	174.0	3.62	2.94
	186-2391	1440.0	270.00	26.60	64-1182	505.0	121.0	33.90

(+) On visual score where 1 = poor; 9 = very good establish a suitable breeding program to develop improved cultivars.

Objective selection for desirable traits begins in microplot yield trials in the year following nursery row evaluation for selected genotypes and continues through advanced yield trials at Tel Hadya, Breda (Syria), Terbol and Kfardan (Lebanon) locations before regional testing of selected promising lines by national programs.

In 1995/96 season, microplots trials of two *Vicia* spp. i.e., *Vicia panonica*, and *V. narbonensis* and two *Lathyrus* spp. i.e., *L. Sativus* and *L. cicera* were grown at Tel Hadya in 3.5 m^2 plots arranged in triple lattice design. Number of entries for each trial was 49, seed rate was 100 kg/ha and fertilizers were applied at 40 kg P_2O_5/ha.

These microplots were in two sets, one was harvested at 100% flowering to determine the herbage yield (DM) and the other was harvested at maturity to measure seed and straw yields and other agronomic traits.

4.1.2.1. Narbon Vetch (*Vicia narbonensis*)

A total of forty-nine selections were tested at Tel Hadya, and 12 selections which combined both high seed yield and herbage (DM) production were identified for more critical evaluation (Table 4.2). The total biological yield (grain & straw) at maturity varied from 1318 to 9675 kg/ha, grain yield from 148 to 2780 kg/ha, and days to full maturity from 149 to 161 days.

The moderate and high temperatures in winter and spring accompanied with high rainfall at Tel Hadya facilitated the development of foliar diseases such as downy mildew (*Peronospora viciae*), powdery mildew (*Erisiphi pisi*), and chocolate spot/blight (*Botrytis fabae*). Natural infection on leaves led to low biological and grain yields. Selected genotypes showed a relatively high level of natural resistance to the above mentioned diseases are shown in Table 4.2. These are identified for further tests under artificial infection in the disease nurseries in 1996/97.

Table 4.2. Winter growth (WG), biological (B) and grain (G) yields and days to flowering and maturity of the top 12 selections of Narbon vetch, in preliminary yield trials at Tel Hadya.

IFLVN	WG+	Yield (kg/ha)		HI%	Days to	
		B	G		Flower	Nature
2561	9	9676	2087	21.5	129	158
2379	8	5459	1722	31.5	124	150
2380	7	3691	1089	29.5	120	149
2386	6	4282	1472	34.4	124	155
2387	7	5207	1009	19.4	125	153
2389	8	9367	2781	29.7	124	155
2393	7	7642	2129	27.8	125	155
2464	6	3253	857	26.4	127	159
2468	7	5725	1691	29.5	129	159
2471	6	5169	1733	33.5	127	156
2634	6	3406	1036	30.4	126	157
2704	6	4004	1040	26.0	124	152
Mean++	6.79	4587	1249	26.5	126	155
SEM±	0.70	699	222	3.8	1.53	2.00
LSD(P=0.05)	2.00	1996	633	8.9	4.36	5.69
CV%	15.79	26.00	22.00	25.0	2.10	2.22

(+) on a scale 1 to 9, where 1 is slow; and 9 very rapid growth measured in mid-February, 1996.
(++) Mean for all 49 selections.

4.1.2.2. Hungarian vetch (*Vicia panonica*)

Forty-nine selections were assessed in microplot field trials at Tel Hadya. Herbage yield varied from 1500 to 3219 kg/ha, whereas, grain yield varied from 770 to 1502 kg/ha. Hungarian vetch showed some cold tolerance, had slow winter growth which was followed by rapid spring growth and long flowering period. The relatively high grain yield and harvest index is due to the moderate winter and spring temperatures. Table 4.3. shows the performance of the top 12 selections for both high herbage and grain yields combined with earliness in flowering and maturity.

Table 4.3. Winter growth (WG), herbage (H), biological (B) and grain (G) yields (kg/ha), harvest index (%) and days to flower and mature of the top 12 selections of Hungarian vetch in preliminary yield trials at Tel Hadya.

IFLVP	WG	Yield (kg/ha)			HI (%)	Days to	
		H	B	G		Flower	Mature
2677	8	3219	3747	1240	33.0	113	152
2653	7	2792	3670	1212	33.0	112	145
2655	7	2875	3750	1450	38.6	110	147
2656	9	2591	4320	1502	34.7	110	147
2659	8	2689	4160	1420	34.0	111	149
2660	6	3028	4280	1310	30.6	108	143
2664	8	2610	4960	1360	27.5	107	141
2667	9	2946	4320	1410	32.6	110	148
2671	8	2611	3930	1289	32.8	105	142
2674	7	2800	3748	1110	29.6	112	148
2675	8	2730	4038	1220	30.2	116	150
2676	7	2610	4645	1105	24.0	120	158
Grand mean+	7.45	2820	3990	1079	23.0	115	153
SEM±	0.61	206	195	75	4.5	0.7	1.2
LSD (P=0.05)	1.75	590	560	201	7.9	2.1	3.4
CV (%)	14.5	17.0	13.0	12.0	19	1.5	1.7

(+) Mean for all 49 entries.

4.1.2.3. Common Chickling or Grasspea (*Lathyrus sativus*)

Forty-nine selections were tested in microplot field trials at Tel Hadya. Results of the top 12 selections are show in Table 4.4. Herbage yield varied from 3167 to 5923 kg/ha, biological yield from 4148 to 7542 kg/ha, whereas, the grain yield from 1171 to 1940 kg/ha. The winter growth was relatively rapid, and the spring growth was fast. The late rains favored attack by powdery mildew (*Erisiphi pisi*) when pods were formed.

Table 4.4. Winter growth (WG), herbage (H), biological (B) and grain (G) yields (kg/ha) harvest index (%) and days to flower and mature of the top 12 selections of common chickling in preliminary yield trials at Tel Hadya.

IFLVS	WG	Yield (kg/ha)			HI(%)	Days to	
		H	B	G		Flower	Mature
593	8.0	4478	6430	1488	23.0	126	168
595	7.5	4599	6895	1545	22.5	130	168
596	7.8	4324	6925	1419	20.5	129	168
598	9.0	4562	6685	1514	22.6	130	164
600	7.6	4733	6944	1484	21.4	127	171
602	7.0	4949	6914	1514	22.0	126	163
604	7.6	4616	6312	1480	23.5	126	164
605	8.5	5629	7367	1651	22.4	124	160
609	6.9	5227	7436	1472	19.8	120	166
610	6.7	5923	7542	1941	25.7	115	170
614	8.1	4670	7382	1505	20.4	122	163
618	8.5	4500	7104	1601	22.5	119	167
Grand mean⁺	7.50	4211	6234	1348	21.3	123	163
SEM±	0.70	617	745	152	2.6	0.92	2.25
LSD(P=0.05)		1340	2101	42.8	5.7	2.60	6.39
CV(%)	17.5	22	23	18	24	1.08	2.09

(+) Mean for all 49 entries.

4.1.2.4. Dwarf Chickling (*lathyrus cicera*)

Forty-nine selections were tested in microplot field trials at Tel Hadya. Herbage yield varied from 5083 to 8182 kg/ha, whereas, grain yields ranged from 781 to 1610 kg/ha; and harvest index from 10.0 to 19.0%. Table 4.5 shows the performance of the top 12 selections. In contrast to *L. ochrus*, *L. cicera* is a cold and drought tolerance species. These results indicate the clear need to collect and evaluate the native ecotypes of *L. cicera* which might show desirable attributes of cold and drought tolerance, early winter and spring growth as well as high yield potential.

Table 4.5. Winter growth (WG), herbage (H), biological (B) and grain (G) yields (kg/ha) harvest index (%) and days to flower and mature of the top 12 selections of dwarf chickling in preliminary yield trials at Tel Hadya.

IFLLC	WG	Yield (kg/ha)			HI(%)	Days to	
		H	B	G		Flower	Mature
629	8	6212	7646	935	12.3	115	160
630	8	7133	7881	1328	16.7	116	155
631	8	6953	6524	1062	16.0	113	153
632	8	6836	7638	1206	15.3	116	155
633	8	6979	7604	1168	15.4	118	162
634	7	7075	7254	1150	16.0	118	161
635	8	9834	8118	1045	13.2	118	165
636	8	7052	7571	1184	16.3	117	162
638	7	7374	7888	1285	16.1	114	155
641	8	7327	7892	1243	15.8	113	159
644	8	7570	8749	1610	18.4	110	155
646	7	8182	7535	1340	17.8	115	159
Grand mean[+]	7.45	6788	7017	1046	15.10	116	161
SEM±	0.70	692	498	131	1.75	0.99	1.41
LSD(P=0.05)	2.00	1969	1429	376	5.01	2.82	3.30
CV(%)	16.20	18.00	12.00	22.00	20.00	1.77	1.40

(+) Mean for all 49 entries.

The study of variation in agronomic characters helps us to establish a suitable breeding program to develop improved cultivars for different niches and utilizations. Selection of desirable traits such as rapid winter growth for early grazing, cold tolerance, high herbage production and seed yield and early flowering begins in microplots in a year after nursery row evaluation. This leads to more critical evaluation in advanced yield trials before multilocation testing of selected promising lines.

As the forage legume species can be used for grazing during winter and early spring or harvested for grain and straw at maturity for winter feeding, one can see how the four studied species can fit into farming systems. The high harvest index and early maturity of *Vicia narbonensis* suggest that it can be used for straw and grain production, whereas, *Lathyrus sativus* would be recommended for hay, straw and grain production. *Vicia panonica* is more suitable for cold areas, because of its cold tolerance and may be for its requirement for vernalization. *Lathyrus Cicera* can be used for grazing in late winter or for grain and straw production at full maturity.

(A.M. Abd El-Moneim)

4.1.3. Advanced Field Trials (AYT)

Elite lines from our breeding program are tested over multiple environments (locations & years) for yield performance, utilization (grazing, hay, grain and straw) and consistency. Yield of these lines and their relative ranking or consistency in performance trials form the basis for recommendations to growers.

Experiments were carried out to test elite promising lines of *Vicia* spp (vetches) and *Lathyrus* spp. (chicklings) at Tel Hadya (TH), Breda (Br), Terbol (T) and Kfardan (Kfr). Materials used in these trials are either progenies of single plants (selections or pure lines) selected from the wild types or selected F3 and F4 families of intra-specific crosses. These lines are selected on the basis of their performance in microplot yield trials for two years. The trials were sown and managed as microplots but with larger plot size (28 m^2).

4.1.3.1. Advanced Yield Trials of Common Vetch (*Vicia sativa*)

Thirty six promising lines were tested at Tel Hadya, Breda, Kfardan and Terbol. The four sites were chosen to sample the environmental conditions of the cereal zone in Syria and Lebanon.

There were great variation between lines within the same location, and between location for winter and spring growth, herbage, biological and grain yields and days to flowering and maturity.

The large variations in both herbage, biological and grain yields (Table 4.6) were mainly due to the differences among tested lines in their winter and spring growth as indicated by the highly significant correlation between winter and spring growth and total biological yield of r = + 0.617, r = + 0.810, r = + -.510, and r = +0.501 at Tel Hadya, Breda, Kfardan and Terbol, respectively.

Table 4.6. Location means of winter growth (WG), biological (B) and grain (G) yields, harvest index (HI%) and days to flower and mature for 49 lines of common vetch in AYT.

Location	WG+	Yield (kg/ha)			HI%	Days to	
		H	B	G		Flower	Mature
Tel Hadya	7.4 ±0.90	4664 ±377	5943 ±442	1138 ±144	32.5 ±2.0	112 ±0.95	160 ±0.70
Breda	7.5 ±0.70	5582 ±552	5933 ±662	724 ±169	19.0 ±1.70	129 ±1.60	168 ±0.97
Kfardan	7.2 ±0.93	3200 ±310	4509 ±318	1240 ±140	27.5 ±2.70	101 ±1.01	145 ±1.9
Terbol	6.0 ±0.95	3969 ±413	8270 ±662	2340 ±210	28.30 ±2.10	113 ±0.93	161 ±1.2

(+) on 1 to 9 visual scale basis, where 1 = poor and 9 = excellent growth on Mid February, 1996.

Table 4.7. shows the most promising and adapted lines at each site. IFLVS 2483 and 2566 showed high adaptation and were the most promising across the four sites as indicated by their high herbage, grain and biological yields, that exceeds the overall average of all tested lines at each site. Their exploitation in future breeding program in case of high herbage and grain yields would be most desirable. IFLVS 2505 and 2485 are specifically adapted to Kfardan and Terbol. This is mainly due to their cold tolerance and resistance to chilling effects.

Table 4.7. The most promising and adapted lines of common vetch at Tel Hadya, Breda, Kfardan and Terbol.

Location	Promising lines (IFLVS#)
Tel Hadya	2483*, 2484, 2490, 2493, 2499, 2501, 2560, 2487, 2495, 2566*
Breda	2483*, 2566*, 2025, 2626, 2619, 2003, 2628, 2493
Kfardan	2483*, 2485, 2487**, 2490, 2493, 2497, 2499, 2502, 2505**, 2566*
Terbol	2458*, 2485, 2487**, 2488, 2494, 2503, 2504, 2505**, 2566*

(*) The most adapted line at the four locations.
(**) Adapted for Kfardan and Terbol.

4.1.3.2. Advanced Yield Trials of Narbon Vetch (*Vicia narbonensis*)

Experiments were carried out to assess twenty five promising lines of narbon vetch at Tel Hadya, Breda in Syria and Kfardan in Lebanon to determine the relative value of these locations for development of high and stable yielding narbon vetch cultivars. Seed yield was greater at Kfardan than Tel Hadya and Terbol, whereas, the total biological yield was greater at Breda than Tel Hadya and Kfardan (Table 4.8). The low grain and biological yields at Tel Hadya were mainly due to the relatively high rainfall, especially late season rains and mild winter and spring temperatures that favored the development of downy mildew caused by *Peronospera viciae*, which caused a severe damage to certain lines. Resistant lines were selected for more critical assessments under artificial conditions to confirm their reaction under natural conditions of 1996 season. Also, the top 5 lines were selected (Table 4.8) based on early maturity, rapid winter and spring growth, high biological and grain yields and resistance to downey mildew.

4.1.3.3. Advanced Yield Trials of Bitter Vetch (*Vicia ervilia*)

Twenty five elite lines were tested at Tel Hadya, Kfardan and Terbol. Herbage Yield at 100% flowering varied from 3327 to 6709 kg/ha at Tel Hadya, from 2093 to 3867 kg/ha at Kfardan and from 6304 to 11770 kg/ha at Terbol. Herbage and biological yields were greater at Tel Hadya than Terbol and Kfardan, whereas, grain yield was greater at Terbol (Table 4.9). Bitter vetch showed rapid winter and spring growth accompanied with early flowering. Its yield was relatively better than other vetches, because earliness in flowering and maturity resulted that the crop escape from the severe attack of broom-rape (*Orobanche crenate* Forsk). No symptoms of downy mildew or ascochyta blight appeared. Table 4.10 shows the most promising and adapted lines at each location IFLVE 2509, 2511, 2512, 2517 were promising and adapted at all location.

Table 4.8. Biological and grain yields (kg/ha), and days to mature for the top 5 lines of narbon vetch (*Vicia narbonensis*) in advanced yield trials at Tel Hadya (TH), Breda (Br) and Kfardan (Kfr).

IFLVN#	Biological yield (kg/ha)			Grain yield (kg/ha)			Days to mature		
	TH	Br	Kfr	TH	Br	Kfr	TH	Br	Kfr
2367	4604	6324	4762	1272	1476	2076	164	160	150
2377	5742	6533	5428	1638	1760	2095	166	156	146
2379	6822	8306	4895	2171	2223	2114	160	159	141
2398	5125	5462	5066	1325	1769	2133	163	158	140
2369	6870	7246	5352	2089	1465	2171	158	159	142
Mean+	4280	6861	4983	1138	1419	1853	162	162	143
SEM±	644	1100	522	228	290	193	1.90	1.06	0.65
LSD(0.05)	1633	3050	1484	649	872	550	4.50	3.01	1.84
CV%	24	28	19	26	28	19	1.82	1.13	0.90

(+) Mean for all 25 lines.

Table 4.9. Location means of winter growth (WG), herbage (H), biological (B) and grain (G) yields (kg/ha), harvest index (HI%) and days to flower and mature for 25 lines of bitter vetch in AYT.

Location	WG	Yield (kg/ha)			HI%	Days to	
		H	B	G		Flower	Mature
Tel Hadya	7.48 ±0.80	4871 ±608	5610 ±368	1200 ±122	21 ±1.75	121.0 ±1.2	165 1.01
Kfardan	8.40 ±0.46	3094 ±334	4990 ±494	1449 ±118	29 ±2.85	110 ±1.9	156 ±1.1
Terbol	6.08 ±0.60	3041 ±456	9586 ±1035	3184 ±270	33 ±2.10	108 ±3.0	163 ±2.0

Table 4.10. The most promising and adapted lines of bitter vetch at Tel Hadya, Kfardan and Terbol.

Location	Promising lines IFLVE#
Tel Hadya	2509, 2511, 2512, 2513, 2517, 2646, 2648
Kfardan	2509, 2511, 2512, 2513, 2517, 2518, 2546, 2650
Terbol	2508, 2509, 2510, 2511, 2512, 2514, 2517, 2518, 2646

4.1.3.4. Advanced Yield Trials of Wooly-pod Vetch (*Vicia villosa* ssp. *dasycarpa*)

Twenty five promising line were evaluated at Tel Hadya, Breda (Syria) and Kfardan (Lebanon). There were great differences in winter and spring growth, seedling vigour, phenotolgy, herbage, biological and grain yields, between lines within locations and also between locations (Table 4.11). Herbage and grain yields were greater at Tel Hadya than Breda and Kfardan. Generally, low grain yields was mainly due to delays in the first appearance of floral buds, especially at the onset of high temperature when bud development is inhibited by high temperature. In contrast to other vetches, wooly-pod vetch is characterized by a long flowering period, high herbage yield and low seed yield.

Table 4.11. Location means of herbage (H), biological (B) and grain (G) yields (kg/ha), harvest index (HI%), and days to flower and mature for 25 lines of wooly-pod vetch in AYT.

Location	Yield (kg/ha)			HI(%)	Days to	
	H	B	G		Flower	Mature
Tel Hadya	6218 ±661	6348 ±1104	458 ±106	7.4 ±1.7	139 ±1.5	167 ±2.4
Breda	4671 ±586	6501 ±995	294 ±70	4.5 ±0.6	140 ±0.74	182 ±0.85
Kfardan	4564 ±365	3036 ±330	307 ±56	10.0 ±1.6	126 ±0.9	162 ±1.2

These characters make it the most suitable for grazing or hay making. Early maturity types are needed as it is indicated by the significantly negative correlations between grain yields with days to flowering and maturity. Our results of 1996, when the natural conditions favored the severe attack of broomrape (*Orobanche crenate* Forsk), confirmed that wooly-pod vetch is resistant to broomrape.

Table 4.12 shows the most promising lines at the three locations. IFLVD 2432 showed promise at all three locations. More emphasis has to be give to reduce young pod abortion, pod shattering, high leaf retention and earliness to flowering and maturity to improve the productivity of wooly-pod vetch.

Table 4.12. The most promising and adapted lines of wooly-pod vetch at Tel Hadya, Breda and Kfardan.

Location	Promising Lines IFLVD#
Tel Hadya	2424, 2431, 2432+, 2438, 2439, 2443, 2454, 2456
Breda	2432+, 2433, 2435, 2437, 2438, 2439, 2441, 2452
Kfardan	2424, 2432+, 2434, 2440, 2450, 2457, 2452

(+) The most promising and adapted line over the three locations.

4.1.3.5. Advance Yield Trials of Palestine Vetch (*Vicia palaestina*)

Twenty five elite lines of palestine vetch were tested at Tel Hadya and Kfardan. The herbage yield varied from 1900 to 5484 kg/ha at Tel Hadya and from 799 to 1977 kg/ha at Kfardan. At maturity, the grain yield ranged from 1116 to 2083 kg/ha at Tel Hadya and from 419 to 1057 at Kfardan. Generally, herbage, biological and grain yields were greater at Tel Hadya than Kfardan (Table 4.13). This is mainly due to the sensitivity of palestine vetch to cold. Palestine vetch produces high straw yields due to its, high leaf-retention at maturity, and tall erect plant type.

4.1.3.6. Advanced Yield Trials of Three *Lathyrus* spp.

Twenty five promising lines each of common chickling or grasspea (*Lathyrus sativus*), dwarf chickling (*L. Cicera*) and ochrus chickling (*L. ochrus*) were tested at Tel Hadya, Breda and Kfardan. Table 4.14 shows herbage, biological and grain yields at the three locations. *Lathyrus ochrus* produced the highest yields at the three locations followed by *Lathyrus cicera* and *L. sativus*. This is mainly due to its highly

Table 4.13. Herbage, biological, and grain yields (kg/ha) for the 25 lines of palestine vetch (*Vicia paloestina*) in AYT at Tel Hadya (TH) and Kfardan (Kfr).

IFLVP	Herbage Yield (kg/ha)		Biological Yield (kg/ha)		Grain Yield (kg/ha)	
	TH	Kfr	TH	Kfr	TH	Kfr
2523	1900	935	5074	2683	1558	486
2524	3541	1413	5687	3526	1495	816
2525	5020	1581	5695	3653	1263	809
2526	5305	1972	5760	3599	1560	892
2527	5484	1958	5885	3294	1644	911
2528	3904	1366	5510	3756	1524	954
2529	4365	1597	5211	3392	1248	852
2530	4062	1667	5253	4089	1360	775
2531	5019	1577	4665	3618	1274	939
2532	4553	1371	5912	4168	1482	641
2533	3701	1388	6594	3998	1785	931
2534	4144	1339	5341	3545	1373	837
2535	5164	1816	5434	3737	1116	974
2536	4701	1459	5754	3439	2084	1057
2537	2871	799	4407	3166	1152	620
2538	4821	1754	5790	3472	1333	746
2689	4052	1244	5074	2487	1377	630
2690	3601	1421	5983	3358	1493	797
2691	3560	1336	5209	2742	1390	663
2692	3586	1127	5371	3190	1548	700
2693	2513	1744	4548	2470	1432	602
2694	3047	1878	5527	2908	1659	497
2695	3717	1611	6063	2900	1568	694
2696	2689	1624	5914	2686	1187	420
26970	3608	1398	5752	3377	1280	908
Mean	3945	1494	5496	3330	1447	766
SEM±	425	190	500	285	149	75
LSD(P=0.05)	1209	547	1423	817	423	218
CV%	18.70	22.00	16	15	18	17

resistance to the broomrape (*Orobanche crenate*). The low grain yields of *L. sativus* was mainly due to the severe effect of the broomrape during the pod formation and grain filling stages, especially at Tel Hadya. Great variability were observed between species and within the same species. The early maturing lines of *Lathyrus cicera* had high grain yields, and the high herbage yield of *L. cicera* and *L. ochrus* were due to their rapid winter growth.

Table 4.15 shows the most promising and adapted lines combining rapid winter and spring growth, early flowering and maturity, high herbage grain and straw yields at the three locations. IFLLS# 504 and 528, IFLLC#486 and 569, and IFLLO# 185, 539 and 540 were the widely adapted lines and were identified for more critical testing in the international nurseries program by NARS.

The results of the advanced yield trials of five *Vicia* species and three *Lathyrus* species show great differences between the three locations and among entries within the same location for the tested traits. Considerable genetic variation exists within each species for attributes indicative of yield and its components. This variation could be exploited by the appropriate breeding procedures to develop high yielding and quality cultivars. These cultivars can contribute significantly to animal production in rainfed agriculture. There use in rotation with cereals will increase yields and total biomass. Furthermore, they will increase the sustainability of farming systems by acting as a disease and *Orobanche* break, and by contributing to the nitrogen nutrition of cereals.

The characters required in *Vicia* spp. and *Lathyrus* spp. if they are to make a successful forage legume crops for different uses and agroecological zones vary from region to region and often within the same region, in accordance with utilizations, and other factors. As forage legumes can be used for direct grazing, hay making, strew and grain, we can begin to see how the various species will meet the farmers needs in the prevailing farming systems of WANA.

Table 4.14. Means and Ranges of Herbage, biological and grain yields (kg/ha) of three *Lathyrus* spp. In advanced yield trials at Tel Hadya (TH), Breda (Br) and Kfardan (Kfr).

Species	Herbage Yield (kg/ha)			Biological Yield (kg/ha)			Grain Yield (kg/ha)		
	TH	Br.	Kfr.	TH	Br.	Kfr.	TH	Br.	Kfr.
Lathyrus sativus									
Mean+	4216	3839	3384	5763	5889	3549	412	220	620
SEM±	292	411	391	452	717	358	83	24	83
Range	(3186-4834)	(3226-4775)	(2346-4162)	(3934-6906)	(4776-8155)	(2997-4193)	(218-536)	(173-275)	(305-933)
CV(%)	12	19	21	14	21	18	30	35	23
Lathyrus cicera									
Mean+	6534	3771	2575	6908	6950	5169	1235	764	3667
SEM±	611	256	281	505	930	315	150	148	246
Range	(4847-8519)	(2634-4950)	(2133-3440)	(1529-8458)	(3863-9818)	(4285-5809)	(799-1658)	(436-1180)	(3219-4152)
CV(%)	16	12	19	13	23	12	21	30	14
Lathyrus ochrus									
Mean+	6900	3017	2374	7831	9045	6215	2050	2993	1635
SEM±	615	296	241	867	523	518	271	212	196
Range	(5100-8800)	(2449-3962)	(1672-3087)	(6519-8706)	(7684-10049)	(5331-6997)	(1959-2725)	(2461-3518)	(1250-2170)
CV(%)	18	17	18	20	13	15	23	13	21

(+) Mean of the all 25 line

Table 4.15. The most promising and adapted lines of three *Lathyrus* spp at Tel Hadya (TH), Breda (Br) and Kfardan (Kfr).

Lathyrus sativus (IFLLS#)			*Lathyrus cicera* (IFLLC#)			*Lathyrus ochrus* (IFLLO#)		
TH	Br.	Kfr.	TH	Br.	Kfr.	TH	Br.	Kfr.
587	553	587	486+	486	486	185[+]	185	185
553	554	562	490	488	488	539[+]	537	537
504	564	565	491	489	491	540[+]	538	538
505	565	504	492	490	494	541	539	539
516	504	505	493	494	497	546	540	542
528[+]	522	533	496	495	499	548	541	543
529	528	528	497	497	569	551	543	540
533	531	535	569[+]	569	576	104	547	549

(+) The most promising and adapted lines over the three locations.

The long flowering period, prostrate or semi-erect growth habit, rapid winter and spring growth, cold tolerance and high herbage production are the most important attributes to make wooly-pod vetch the most suitable for grazing, especially at the high-elevation cold areas. The high grain and straw yields and early maturity of narbon vetch make it ideal feed legume for producing winter stocks of straw and grain for feeding sheep during the peak of feed demand in winter. It does not lose its leaves during harvest like many other feed legumes, and its seed contain around 28% crude protein. Palestine vetch can be used for hay making in spring or late grazing because of its rapid spring growth, high leaf-retention and vigorous growth habit. Common-vetch is a versatile feed legume crop. The rapid winter growth and cold tolerance types can be used for early grazing, the non-shattering erect types can be used to produce grain and straw, and the leafy and rapid spring growth types can be used for hay making either in pure stand or in mixture with barley or Oats. For farmers who require hay or grazing, dwarf chickling could be another option. The common chickling (grasspea) is susceptible to *Orobanche*, but in dry areas it can still be used for grain and straw, because of its tolerance to drought. Ochrus chickling is resistance to *Orobanche* and can be used for grazing or grain and straw production to reduce the build-up of seed bank of the parasite in areas where mainly food and forage legumes are grown and can be of value of developing integrated *Orobanche* control system.

Many selection criteria are considered important in the improvements of yield and quality of forage vetches and chicklings. It is also considered convenient to group the characters considered desirable in two categories (A) characters related to persistence, these included flowering time and period, resistance to trampling and ability to regrow after early grazing to produce seed for resowing, and tolerance to diseases and insects (b) character related to productivity, which include good winter and spring growth, ability to grow well in cold winters and high palatability and freedom from toxic properties that are injurious to animal health. However, it should be noted that there is some overlap between these two categories.

For instance, early winter production is largely influenced by seedling vigour and this in turn is closely related to seed production which is listed as persistence character.

Time of flowering and maturity are considered to be of major importance in determining the suitability of forage vetches and chicklings to particular environments. In dry areas with less than 300 mm rainfall, the essential requirement is that flowering must start early enough for adequate pods to be formed and matured by the end of the growing season. There are marked differences between species in their time of commencement of flowering and maturity and this facilitates the selection of suitable cultivars for a wide range in length of the growing season. In our breeding program we maintain genetic diversity for location specificity for yield, agronomic requirements for different end uses and quality factors in improved populations to be used by NARS, through our international nurseries program.

(A. M. El-Moneim)

4.1.4. Nutritional Quality

Improved forage quality is an important objective in our breeding program. Also, achieving high yield potential and adaptation to different niches and agroecological zones needs to be complemented by ensuring that the end products are acceptable by livestock. Therefore, quality parameters of hay, straw and grains are given great consideration.

4.1.4.1. Hay and Straw Quality of *Vicia spp.* and *Lathyrus spp.*

The quality parameters utilized in the forage breeding program are protein %, Neutral Detergent Fibers (NDF%), Acid Detergent Fibers (ADF%) and Dry Matter Organic Matter Digestibility (DOMD%).

Large differences were observed both between and within species. Generally, hays of vetches are more nutritious having higher protein contents and digestibility and lower fiber contents than the straws (Table 4.16a and b). Hay of *V. sativa* has the highest protein content within the *Vicia* spp., followed by *V. ervilia* and *V. villosa* ssp. *dasycarpa*, *Vicia palaestina* has relatively low protein content, high fibers and low digestibility. This is mainly due to its tiny leaves, tall stems and low leaf: stem natio. The fiber contents of *V.ervilia* are low, resulting high digestibility. Because of the high digestibility and palatability of *V.ervilia*, a great birds damage occurred at the seedling stage compared with other vetches.

Hays of *Lathyrus sativus* and *L. cicera* are high in protein content and digestibility. This is mainly due to the high leaf: stem ratio, and high degree of leaf retention. The same trend was found in the case of straws (Table 4.16b).

Table 4.16a. Mean and range of protein content%, NDF%, ADF%, and DOMD for hays and straw of *Vicia* spp. promising lines in advanced yield trials at Tel Hadya.

Species		Hay				Straw			
		Protein%	NDF%	ADF%	DOMD%	Protein%	NDF%	ADF%	DOMD%
Vicia sativa	Mean	16.2	31.0	22.0	69.0	11.3	38.0	30.0	51.0
	Range	14-17	24-36	16-26	60-81	9-14	33-44	28-33	46-57
V. ervilia	Mean	15.3	19.0	16	78.5	11.6	30.0	25	52
	Range	11-18	9-25	11-22	66-93	10-13	25-36	21-28	43-60
V. palaestina	Mean	12.0	40	25	64.0	7.9	43.0	32	32
	Range	9-16	36-45	22-26	56-73	6-13	37-48	30-34	23-45
V. villosa ssp. Dasycarpa	Mean	15.0	39.0	28.0	63.0	14.0	45.0	33.0	53.0
	Range	12-17	36-42	24-30	56-69	11-15	42-48	32-35	43-62
V. narbonensis	Mean	-	-	-	-	10.5	42	32	50
	Range	-	-	-	-	8-14	34-48	28-36	33-60

Table 4.16b. Mean and range of protein content%, NDF%, ADF%, and DOMD for hays and straws of three *Lathyrus* spp. promising lines in advanced yield trials at Tel Hadya.

Species		Hay				Straw			
		Protein%	NDF%	ADF%	DOMD%	Protein%	NDF%	ADF%	DOMD%
Lathyrus sativus	Mean	17.0	41.6	29	72	14	54	32	63
	Range	15-18	36-50	25-32	68-77	12-15	44-67	28-38	53-71
Lathyrus cicera	Mean	16.5	32.0	25.0	75	15	55	33	58
	Range	14-18	28-37	21-28	72-78	13-16	42-64	29-36	54-62
Lathyrus ochrus	Mean	-	-	-	-	13	60	29	57
	Range	-	-	-	-	12-15	50-68	30-39	51-64

4.1.4.2. Protein and Neurotoxine B-N-Oxalyl-L-∝-B-Diaminopropionic Acid (B-ODAP) Contents in Three *Lathyrus* spp. Grains, at Three Locations, Tel Hadya, Breda, and Kfardan.

Chicklings (*Lathyrus* spp.) have high yield potential in areas with less than 300mm rainfull. Grasspea (*Lathyrus sativus*) is particularly adapted to dry areas. It represents the major component of human diets in times of drought induced famine in Asia and East Africa. One of the drawbacks of grasspea, however, is that its excessive consumption causes "Lathyrism", a nervus disorder resulting in incurable paralysis of lower limbs. This disease in human beings and domestic animals is caused by the presence of a free amino acid known as B-N-Oxalyl-L-∝-B-Diaminopropionic acid in the seeds.

Protein and the neurotoxine B-ODAP for promising lines of *Lathyrus sativus*, *L. cicera* and *L. ochrus* grown at Tel Hadya, Breda and Kfardan were estimated with a Near-Infrared Reflectance (NIR) Model NYRSystems 5000, with a wave length setting between 1100 and 2500 mm. Every tenth sample was verified by Macro-Kjeldahl method for crude protein and classical spectrophotometric analysis for B-ODAP. It was possible to develop a good calibration which permitted a good correlation (r=0.96) for B-ODAP and crude protein, respectively. Table 4.17 summarizes the results of crude protein% and B-ODAP% for 25 promising lines each of *Lathyrus sativus* , *L. cicera* and *L. ochrus* grown at three locations. The results indicate that none of *Lathyrus spp.* lines were B-ODAP free, although some lines were very low, below 0.1% (the threshold is 0.2%). Large variation was found between species and between lines in the same species. *Lathyrus ochrus* had the highest protein and B-ODAP contents at the three locations, whereas, *L. cicera* had the lowest B-ODAP at Tel Hadya and Kfardan. The presence of such variation in protein and B-ODAP suggests that there is a good potential

Table 4.17. Mean and range of protein content%, and ODAP%, for grains of three *Lathyrus* spp. at Tel Hadya (TH), Breda (Br) and Kfardan (Kfr).

Species		Tel-Hadya		Breda		Kfardan	
		Protein%	B-ODAP%	Protein%	B-ODAP%	Protein%	B-ODAP%
Lathyrus sativus	Mean	31.0	0.19	31.2	0.15	30.2	0.15
	Range	30-38	0.15-0.27	29-33	0.08-0.23	28-31	0.11-0.18
Lathyrus cicera	Mean	32.2	0.15	32.7	0.22	31.0	0.12
	Range	31-34	0.11-0.20	30-35	0.15-0.32	30-33	0.08-0.16
Lathyrus ochrus	Mean	33.0	0.32	33.7	0.33	31.5	0.24
	Range	31-35	0.28-0.37	31-36	0.30-0.37	29-33	0.17-0.30

for developing lines of the three tested species with low B-ODAP and high protein contents.

4.1.4.3. Tanins and Protein Contents in *Vicia narbonensis* Seeds.

Tanins are polyphenolic compounds which form complexes with proteins, carbohydrates, alkaloids, vitamins and minerals. They adversely affect palatability, rate of digestion and nutritional value of the diet and may have a direct effect on animals.

Tanins and protein content of 42 line of narbon vetch indicate that none of the lines were tanin-free. Tanin content of the whole seed varied from 0.10 to 0.26% whereas, protein content varied from 23 to 31%. Lines with low tanin were susceptible to downy mildew (*Peronospora viciae*) aphis (*Aphis craccivora*), and pod borer (*Helicoverpa* sp.). The presence of moderate levels of tanins in narbon vetch may have beneficial effects on plants as defense against insects and diseases and on animals by reducing the risk of bloat.

In conclusion, the improvement of forage quality is of a paramount importance to the performance of ruminant animals (sheep and goats). Modest increase in protein and digestibility from the development of new promising lines can increase the performance of consuming animals. Both quantity and quality of the forage consumed contribute to animal response. Therefore, progress in breeding for high yield potential is always supplemented by improving the quality of the herbage, grain, and straw. Forage vetches and chicklings improved in quality are developed by breeding for (a) greater nutritional value, (b) increasing intake and digestibility and © lower content of toxic properties that are reduce the intake and injurious to animal health. The most useful selection criteria in our breeding program for voluntary intake is the leafiness. Thus, leafiness appears to be an important attribute at morphological level in the early stages of the breeding program and could help in the improvement of nutritive value of the herbage and straw. Leafiness has also been found to be positively correlated with protein and digestibility.

(A. M. Abd El Moneim)

4.1.4.4. Yield and Quality of Mixture of two Barley Varieties with Five Vetches and One Ochrus Chickling.

Adequate feed is essential for the rapidly growing livestock population of West Asia and North Africa. Hay production from adapted forage legumes such as vetches and chicklings and from forage cereals such as barley and oats suggested as one way of increasing feed from fallow land in traditional rainfed cropping systems. It is also a common practice in North African countries. However, growing either legumes or cereals in pure stand is not considered ideal for hay production. Legumes such as vetches and chicklings have a twining growth, resulting in difficulties in mechanical harvesting and in poor hay yield. Forage cereals, although high in dry matter, have rather, low feed quality especially when harvested late. A better alternative is to grow these species in mixtures.

Twelve forage mixtures and their pure stands components were sown using five vetches and one ochrus chickling with two barley varieties table 4.18. Each mixture was sown at 100 kg/ha^{-1} seed rate and 33:66 was cereal-legume ratio, in a randomized complete block design, with three replicates. Plot size was 28.0 m^2 and all plots were fertilized with 40 P_2O_5 kg/ha^{-1} at planting in 15 December 1996. Forages were harvested the following April, when the legume in each mixture was in full flowering and early podding stage and the barley at dough stage-using a sampling area of (6 x 2 m) in the middle of each plot. Mixtures were separated into their species components and samples oven-dried at 70°C to determine dry matter (DM) yield. Subsamples were ground and used for Kjeldahl nitrogen analysis and estimation of crude protein percentage. Samples from the various mixtures and pure stands were also used for *In vitro* Dry Matter Digestibility (IVDMD) and Neutral Detergent Fibers (NDF) estimations.

In all mixtures the herbage yield and hay quality were greater when barley variety Rihan is used in the mixture (Table 4.18). *Vicia dasycarpa* and *Lathyrus ochrus* gave the highest yield of hay in mixtures with both Rihan and Salmas barley varieties. Their herbage yields exceeded the yield of either one or both components of the mixture grown as pure stands. *Vicia sativa* and *V. palaestina* ranked the second and third. The percentage of crude protein in pure stand legumes and mixtures was approximately twice than in monoculture barley. Dry matter digestibility (%) showed a similar pattern in that described for crude protein (Table 4.18). The mixtures containing *V. dasycarpa* and *L. ochrus* had dry matter digestibility more than other mixtures. For quality and yield potential these two legumes appeared nutritionally better and genotypes of these two species can be used in breeding program to evolve varieties suitable for high yield and quality hay, in mixtures with different barley varieties with different phenologies, flowering time of both barley and forage legumes should be considered when the selected varieties are intended for use for hay production.

(A.M. Abd El-Moneim and S. Ceccarelli)

4.1.5. Genetic Improvements

4.1.5.1. Improving Seed Retention (Pod-shattering) in Common-vetch (*V. sativa*)

An essential character of a grain legume crop, and desirable

Table 4.18. Dry matter (herbage) yields (kg/ha) and quality of mixtures of two barley varieties with five vetches and one ochrus chickling at Tel Hadya.

Component		Dry Matter Yield (kg/ha)					Quality Parameters (%)		
		Barley	Legume	Mixture (Total)	Barley Pure stand	Legume Pure stand	Protein	NDF	IVDMD
B_1/*Vicia sativa*	2604	819	2285	3104	-	-	11.6	58	61
B_1/*V. sativa*	2556	934	2137	3071	-	-	11.2	50	64
B_1/*V. sativa*	2558	1039	1903	2924	-	-	10.5	51	68
B_1/*V. palaestina*	2532	1251	1405	2656	-	-	11.5	55	56
B_1/*V. dasycarpa*	683	1011	2724	3735	-	-	14.7	50	65
B_1/*Lathyrus ochrus*	185	1226	2233	3457	-	-	13.4	51	68
B_2/*V. sativa*	2604	795	2206	3001	-	-	11.0	53	62
B_2/*V. sativa*	2556	924	2058	2982	-	-	12.0	53	63
B_2/*V. sativa*	2558	703	1735	2437	-	-	12.0	53	67
B_2/*V. palaestina*	2532	1030	1286	2316	-	-	13.0	48	61
B_2/*V. dasycarpa*	683	1033	2759	3397	-	-	14.0	52	60
B_2/*L. ochrus*	185	887	2481	3368	-	-	14.0	50	66
Vicia sativa	2604	-	-	-	-	3398	16.5	47	72
V. sativa	2556	-	-	-	-	3182	17.9	44	75
V. sativa	2558	-	-	-	-	2251	13.9	47	70
V. palaestina	2532	-	-	-	-	2227	19.5	45	66
V. dasycarpa	683	-	-	-	-	3381	20.2	46	64*
L. ochrus	185	-	-	-	-	3438	18.7	50	76*
Barley var. Rihan (B_1)		-	-	-	1418	-	7.2	58	55
Barley var. Salmas (B_2)		-	-	-	1764	-	8.4	50	62*
Mean		884	2101	3005	-	-	13.7	51	64
SEM±		150	190	260	-	-	0.61	2.9	2.5
LSD (P=0.05)		390	561	730	-	-	1.73	8.4	7.0
CV (%)		25	16	15	-	-	8	10	6.5

one in a forage legume crop is the ability to retain its seed long enough to allow mechanical harvesting at full maturity. Pod shattering (seed retention) in common vetch reduces its popularity as forage legume crop for fallow replacement. The vetch seed germinating during the cereal phase of the rotation represent serious "weed" problem. Therefore, a breeding program to develop non-shattering cultivars suitable for mechanical harvesting was initiated using three natural wild non-shattering mutants with undesirable agronomic traits.

The genetics of pod-shattering was studied by using P_1, P_2, F_1, F_2, BC_1, BC_2, BC_3 and BC_4 generations obtained from crosses between non-shattering wild types and promising breeding lines with highly desirable agronomic traits but with high proportion of pod-shattering. The results revealed that non-shattering trait is conditioned by a single recessive gene. Incorporation of this gene into agronomically promising lines was achieved by backcrossing, selfing, and selection for non-shattering trait, in erect, soft-seeded, leafy and early matured types. Seven superior lines, IFLVS (NS) 709, 715, 1448, 2014, 2557, 2558, and 2565, were selected having 95-98% non-shattering pods as supposed to 20-30% in the original breeding lines.

Incorporation of non-shattering gene into our breeding lines of common vetch resulted in a range of non-shattering lines which are now grown in some countries on a large scale. IFLVS 715 was released as a variety in Jordan and it is used on a large scale in Iraq. IFLVS 709 was released in Morocco. It is a variety of high L/S ration, erect, relatively drought tolerance and resistant to Orobance. The reduction of pod-shattering was sufficient to allow normal header harvesting at maturity with minimal loss of seeds. Reduced pod shattering at maturity is the major agronomic advance achieved in common vetch, and it is continuing to include more lines with specific adaptation.

4.1.5.2. Improving Herbage Production of Underground vetch (*Vicia sativa* ssp. *amphicarpa*) and Cold and Drought Tolerance of Common vetch (*Vicia sativa* ssp. *sativa*)

Species and subspecies hybridization is an important aspect in feed legumes breeding, to incorporate useful genes carried out by parental species and also to increase variations for selection. Our studies on underground vetch (*V. sativa* ssp. *amphicarpa*) revealed that its ability to produce aerial and underground pods increases its winter hardiness, drought tolerance and persistence under heavy grazing. The disadvantages of underground podding habit, which may limit its utilization as a pasture plant are low rate of vegetative growth, shattering of above ground pods and the dependence of amphicarpy to edaphic conditions. In contrast, the common vetch grows well under favourable conditions but is not cold and drought tolerant, and there are some improved lines with non-shattering pods.

To enhance the herbage production of underground vetch and improve the drought and cold tolerance of common vetch, crosses between the two subspecies were made to develop a more agriculturally valuable forage crop from both subspecies.

The material was derived from the strait and reciprocal crosses of improved lines of common vetch IFLVS # 1416, 715, 713, 1448 and 1416 with two wild accessions of underground vetch IFLVA # 2416 and 2614 originated from Turkey and Syria, respectively. High vigour was observed in the F_1 plants carrying few underground pods near the soil surface.

The F_2 population released enormous variability transcending even the limits of the parents in some traits such as numbers of underground pods, drought tolerance and herbage production. Selection was done in F_3 from the plants selected in F_2. Through multiple trait selection in F_3, F_4 and F_5 selected families were classified according to the desirable traits to improve the underground vetch and common vetch. Families with 5-10 underground pods per plant and more 50% increase in herbage production were selected in F_4 and F_5 as improved lines of underground vetch. Also, families with cold and drought tolerance and maintaining the vigorous growth of common vetch were selected as improved lines of common vetch. Seeds of improved lines of underground vetch were multiplied and are being used to rehabilitate the marginal lands at Tel Hadya in collaboration with PFLP. Improved lines of common vetch were tested at Tel Hadya, Breda and Kfardan.

4.1.5.3. Improving Nutritional Quality of Grasspea (*Lathyrus sativus*)

Lathyrus sativus (grasspea or common chickling) is a popular food and feed crop in certain Asia and African countries such as India, Pakistan, Bangladesh and Ethiopia because of its resistance to drought, flood and moderate salinity. Unlike other legumes, it thrives very well under adverse climatic conditions, and requires very little if any, management inputs and attention during its growth cycle when other crops fail under adverse climatic conditions. *L. sativus* can become the only available source for food for the poor section of the population and sometimes a survival food during the time of drought-induced famine. Although seeds of *L.sativus* are tasty and protein rich (30g/100g edible seeds), and contain a large amount of free L-homoarginine, which can act as precurson of the aminoacid lysine in higher animals, overconsumption can cause an upper motor neurone disease known as "neurolathyrism", an irreversible paraparesis of the lower limbs. The neurotoxic casual agent of this disease was identified as 3-N-oxalyl-L-2,3 diaminopropanic acid (B-ODAP) or its synonym BOAA (B-N-oxalylamino-L-alanin).

One of our main objectives of *L.sativus* breeding is developing lines with low B-ODAP content, high yield and maintain adaptability to adverse climatic conditions. Screening program of available germplasm and landraces from different origins for agronomic characters, protein and B-ODAP content was initiated in 1991. Five lines with low B-ODAP content less than 0.1 were identified. Those lines unfortunately are late in flowering and maturity, and susceptible to downy mildew (*Prenonospora Viceae*) and powdery mildew (*Erysiphe martti* f.sp. *Lathyri*).

A breeding program was initiated and the base material consisted of thirty landraces of *L. sativus* representing the available variability for B-ODAP (more than 0.41%) and diversity in agroclimatic conditions among places of their orogin. These genotypes were crossed with the five lines with low B-ODAP as testers and the 150 hybrid combinations were obtained. Gene markers such as seed, flower, and stem colours were used to eliminate pods which might have developed from selfing. Due to transgressive segregation toward earliness an high B-ODAP content, a large portion of the F_2 population matured earlier than the parents. Selection from F_3 onwards was directed for early maturity, small and large seed size, white or cream seed colour and less than 0.08%B-ODAP. In 1996, 110 families with their parents were grown under rainfed at Tel Hadya and Breda to assess their yield potential and B-ODAP content. Ten F_5 families having low B-ODAP content, and high grain yield were selected at Tel Hadya and Breda (Table 4.19). The effect of location on B-ODAP content was not significant. These selected families were 15-20 days later in maturity than families with high B-ODAP at both locations, as indicated by the highly significant correlation between B-ODAP and days to maturity ($r=-0.34$ and -0.41, $P<0.01$) at Tel Hadya and Breda, respectively.

From these studies it is apparent that the neurotoxin B-ODAP content of the selections is much lower than most of the landraces. Further, it is interesting to note that the yield potential of these lines is quite comparable to and in cases even greater than the landraces. The results reported here appear to be highly encouraging towards the development of low neurotoxin varieties to overcome the problem of "Lathyrism" of this drought tolerance and hardy crop.

(A.M. Abd El-Moneim & H. Nakkoul)

4.1.5.4. Somaclonal variation in *Lathyrus sativus*

Recently protocols have been developed to obtain plants with a low concentration of B-ODAP. Different explants are cultured on medium where the dedifferentiate in to calli. From these calli plants are regenerated. Due to somaclonal variation a number of these plants may have a considerable lower concentration of B-ODAP than the original lines.

This technique was tried at ICARDA with five lines of *Lathyrus sativus*. Seed color of the seedlings relates to the amount of toxin in the seed. From 670 explants cultured 75% had dedifferentiated into calli (Table 4.20). Of these calli 43% has regenerated either roots, shoots or both on the first media they were cultured on. Some of the calli have been transferred to a regeneration media. After 2 months of culturing 19 explants have developed with roots and shoots. From which 10 have been transferred to soil. Two of the plants have flowered and have set pods. Differences in calli formation between the varieties are low. To enhance the somaclonal variation, it is important to have a good callogenic phase before the regeneration is induced. The medium B5L gives a high number of calli which show just rooting, an adjustment in the hormones will be tried to also induce shoot development. The calli with roots or shoots will be transferred to regeneration media to induce the development of roots and shoots.

(B. van Dorrestein, M. Baum and A. Abd El-Moneim)

Table 4.19. The B-ODAP Content (%) and grain yield of some promising low neurotoxine lines of *Lathyrus sativus* at Tel Hadya and Breda.

Tel Hadya			Breda		
IFLLS#	Yield (kg/ha)	B-ODAP (%)	IFLLS#	Yield (kg/ha)	B-ODAP (%)
672	1670	0.030	729	1475	0.073
434	1642	0.070	673	1480	0.092
735	1620	0.070	719	1314	0.078
483	1650	0.040	588	1550	0.078
712	1522	0.020	554	1406	0.059
702	1800	0.070	687	1800	0.067
654	1910	0.074	433	1950	0.060
776	2020	0.075	670	2010	0.060
555	1700	0.050	711	2040	0.054
692	1800	0.077	555	1900	0.054

4.1.6. Forage Legume Pathology

Host resistance to the major stem and leaf diseases is one of the selection criteria for developing productive forage legumes. The forage legume project has identified promising lines of *Vicia* spp. and *Lathyrus* spp., for yield and adaptation. The objectives of the pathology section of the project are to: 1) Evaluate the selected genotypes for resistance to the major diseases and obtain information on sources of resistance to individual and multiple diseases. 2) Monitor the relative importance of these diseases on the genotypes through annual disease surveys in the region.

Table 4.21 shows the reaction of three *Vicia species* and three *Lathyrus species* to the major diseases occurring on these crops, evaluated under artificial infections with infected debris and spore suspension sprays. None of the *Vicia* lines showed high levels of resistance to Ascochyta blight. Some of the entries showed moderate levels of resistance while most showed good tolerance. Most of the *Lathyrus* lines showed moderate levels of resistance to Ascochyta, with none showing high resistance. *L. cicera* had 17 lines with moderate levels of resistance and none rated as susceptible or highly susceptible. Most of the *Vicia* lines were tolerant to Downey mildew with very few showing good resistance. There were good levels of resistance to downey mildew in the *Lathyrus species* especially in *L. cicera* where 14 lines had a reaction rating of 2 and none of the entries was rated susceptible or highly susceptible. There were good levels of resistance to Botrytis stem blight in the *Vicia species*, with *V. narbonensis* and *V. sativa* having 5 and 7 entries respectively, with a rating of 2.

Table 4.20. Regeneration from calli of Lathyrus sativus explants

Explant source	IFLLS selection #	No. of explants	No. of callogenic explants	No. of undeveloped explants	Calli with roots	Calli with shoots	Calli with roots and shoots
Root	588	51	45	4	33	1	0
	85	22	2	11	0	0	0
	520	33	19	3	13	0	0
	482	41	29	2	20	0	0
	521	24	22	2	10	0	0
Internode	588	48	43	2	15	0	0
	85	19	11	1	5	0	0
	520	35	25	1	10	0	0
	482	46	40	2	13	1	1
	521	18	18	0	3	0	0
Shoot	588	54	43	7	1	17	4
	85	24	14	0	0	14	0
	520	34	21	3	0	8	11
	482	38	22	7	3	12	3
	521	24	24	0	0	4	0
Leave	588	33	27	0	3	0	0
	85	11	1	0	0	0	0
	520	41	30	1	4	0	0
	482	60	46	3	4	0	0
	521	14	14	0	0	0	0
Total		670	496	49	137	56	10

Table 4.21. Disease reaction (on 1-5 scale) of promising *Vicia* and *Lathyrus species* lines under field artificial conditions.

Legume spp and Disease	No. of lines in each disease category					
	1	2	3	4	5	Total
Vicia						
narbonencis	0	1	8	2	12	23
Ascocyta blight	0	1	1	6	5	13
Downey mildew	0	5	6	1	4	16
Botrytis						
V. sativa	0	3	10	4	0	17
Ascochyta blight	0	3	7	0	0	10
Downey mildew	0	9	10	3	0	22
Botrytis						
V. ervilia	0	3	4	6	2	15
Ascochyta blight	0	6	17	0	0	23
Downey mildew						
Lathyrus sativus	0	0	2	12	4	18
Ascochyta blight	0	0	15	0	0	15
Downey mildew	0	1	6	14	2	23
Botrytis						
L. cicera	0	17	8	0	0	25
Ascochyta blight	0	14	4	0	0	18
Downey mildew	0	9	9	2	0	20
Botrytis						
L. orchus	0	10	6	0	0	16
Ascochyta blight	0	6	10	0	0	16
Downey mildew	0	2	13	1	0	16
Botrytis						
Total	0	90	136	51	29	306

In Table 4.22 under natural field infection conditions, 8 lines of *V. sativa* in the AYT (2637, 2638, 2624, 1135, 2639, 2640, 1429 and 2023) were identified as highly resistant to downey mildew. Only a few lines in *V. narbonensis* had moderate resistance to downey mildew. Most were rated as susceptible. All 16 entries of L. ochrus evaluated in the AYT had high levels of resistance to downey mildew. Most *L. cicera* entries were intermediate in reaction with some showing high susceptibility to downey mildew.

Based on the field observations, some levels of resistance to downey mildew exist in the *Vicia species* evaluated but highly resistant sources are still unavailable. In the *Lathyrus species*, some resistance to Ascochyta blight exist but highly resistant sources are also not available. Resistance to downey mildew in the *Vicia species* and Ascochyta blight in the *Lathyrus species* will continue to receive high priority in the forage legume improvement project.

(C. Akem, A.Abd El-Moneim and M. Bellar)

Table 4.22. Disease reaction to Downey mildew in selected *Vicia* and *Lathyrus species* under natural field infection conditions.

Legume species	Trial	No. of lines each disease category					Total
		1	2	3	4	5	
Vicia narbonensis	Nursery	0	3	30	9	7	49
	Microplot	0	1	5	15	15	36
	AYT	0	2	4	18	1	25
Vicia sativa	AYT 1	0	2	9	14	0	25
	AYT 2	8	7	8	2	0	25
Lathyrus sativus	Nursery	0	51	13	0	0	64
	AYT	0	0	20	5	0	25
L. ochrus	AYT	16	0	0	0	0	16
L. cicera	AYT	0	7	18	0	0	25
Total		24	73	107	63	23	290

4.1.6.2. Survey of Forage Legume Diseases in Syria

Disease surveys were conducted in on-farm trials of Vicia species in different provinces and at different locations in Syria. Disease incidence and severity were recorded and samples collected at each location for confirmation of identification. The results are presented in Table 4.23.

Foliar diseases were prevalent in all the locations surveyed. The predominant ones were Ascochyta blight and Downey mildew. Phoma blight and Botrytis grey mold were also observed at some locations. The main stem disease was Sclerotinia stem blight which was prevalent at most locations and at high incidence and severity levels. Conditions were particularly favourable for the occurrence of this disease following heavy rains during the winter months which resulted in vigorous plant growth and dense canopy. At some locations, such as at Hama research station, about half of the trials were completely wiped out by Sclerotinia stem blight. Botrytis grey mold was also observed at locations where plant lodging from the vigorous growth occurred. Root knot nematodes were identified from some *Vicia* entries showing stunting and yellowing foliar symptoms with galled roots. The parasitic weed, Orobanche, was prevalent on entries at locations in Idleb and Aleppo provinces.

From the survey, it is clear that Downey mildew and Ascochyta blight continue to be the main forage legume diseases of concern while Sclerotinia blight also needs to receive some attention.

(C. Akem, M. Bellar and Syrian NARS Scientists)

Table 4.23. Disease Incidence (DI) and Disease Severity (DS) in *Vicia species* grown at different locations in Syria, 1995/96.

Province Location	Disease	DI(%)	DS (1-5)	*Pathogen isolated
Qunaytara				
Munshiyeh	Ascochyta blight	35	4	*Ascochyta sp.*
	Phoma blight	40	4	*Phoma sp.*
	Sclerotinia blight	65	4	*Sclerotinia sclerotiorum*
Hama				
Hama/Soran	Downey mildew	50	2.5	*Peronospora viciae*
	Scleretinia blight	60	5	*S. sclerotiorum*
	Root knot nemetode	15	4	*Meloidogyne sp.*
Idleb				
Aphes	Ascochyta blight	30	3	*Ascochyta sp.*
	Downey mildew	45	3	*P. viciae*
	Sclerotinia blight	85	4	*S. sclerotiorum*
	Orobanche	40	2	*Orobanche sp.*
Zouhur	Sclerotinia blight	55	3	*S. sclerotiorum*
	Ascochyta blight	30	3	*Ascochyta sp.*
	Root knot nematode	20	3.5	*Meloidogyne sp.*
	Orobanche	60	4	*Orobanche sp.*
Aleppo	Downey mildew	55	2.5	*P. viciae*
Tel Hadya	Ascochyta blight	50	2.5	*Ascochyta sp.*
	Botrytis gray mold	30	3.0	*Botrytis cinerea*
	Orobanche	85	4	*O. crenata*
Al-Hassakeh	Downy mildew	25	3	*P. Viciae*
Himo	Ascochyta blight	40	4	*Acochyta sp.*
	Sclerotinia blight	35	2.5	*S. sclerotiorum*
	Phoma blight	40	4	*Phoma sp.*
Hassoud	Phoma blight	45	2.5	*Phoma sp.*
	Sclerotinia blight	50	3	*S. sclerotiorum*
	Wilt/root rot	30	2	*Fusarium oxysporium*

* More than 50% identified from diseased sample.

4.1.6.3. Resistance of Forage Legumes to *Orobanche crenata* Forsk and *O. Egyptiaca Pers*.

Parasitic weeds of family *Orobanchacea* are devastating and threaten to many forage and food legumes in the Mediterranean basin. Greenhouse and field screening for six lines of wooly-pod vetch (*Vicia villosa ssp. Dasycarpa* Ten.), four lines of ochrus chickling (*Lathyrus ochrus L.* DC.), and one line each of common vetch (*Vicia sativa L.*) and narbon vetch (*Vicia narbonensis* L.) For resistance to *Orobanche crenata* Forsk, and *O. egyptiaca* Pers., revealed high interspecific and intraspecific variation. Lines of *L. Ochrus* were free from emerged both kinds of *Orobanche*, while *V. narbonensis* was highly susceptible to *O. crenata* and resistant to *O. egyptiaca*. Both kinds of *Orobanche* germinated underground and the haustorium did not emerge above ground in *V. villosa ssp. dasycarpa*, and no damage occurred to the plants. *V. sativa* #1448 was resistant to *O. crenata*, and moderately susceptible to *O. egyptiaca*.

Lines with resistance to *Orobanche* can reduce the build-up of a seed bank of the parasite in areas where mainly food and forage legumes are grown and can be of value of developing integrated Orobanche central system.

(A. Abd El-Moneim and M. Bellar)

4.1.7. Use of Forage Legumes by NARS

The Mashreq countries Syria, Lebanon, Iraq and Jordan, have an extensive work on farmers' fields to demonstrate the potential of forage legumes, as the best alternative in rotation with barley in the fallow-barley or continuous barley rotations. During the 1995/96 growing season several were conducted in farmers fields and gave promising results.

In Lebanon three species of legumes were evaluated under farmers' fields conditions at three sites (Table 4.24). Kasr location is the driest location which received only 252 mm of rain. It can be easily observed that *lathyrus cicera* is more adapted to the drier areas as compared to the other two species and therefore efforts in these areas should be focused on *lathyrus cicera*. Also it showed some elasticity and responded well when moisture conditions improved. **In Jordan**, several on-farm evaluation were conducted where *Vicia sativa* 715 (Bekia) gave the highest dry matter yield as compared to the local and to other *Vicia* species. In demonstrating Bekia grazing on six farmers' fields, farmers ewes and lambs start grazing the Bekia during March and April, the daily weight gain ranged from 135 to 289 g/head/day. Sheep owners were very pleased with this system and ready to adopt it.

In Syria twelve demonstrations of Bekia (common vetch) were planted for direct grazing in four governorates in zone two. The crop performance was good with an estimated dry matter yield ranged from 1.4 to 3.3 ton/ha. Sheep owners' flocks grazed the Bekia during March-April until early May. Some farmers left part of the field for seed and straw production. Weight gain ranged from 88 to 269 g/head/day with an average of 167 g/head/day for the twelve locations. Farmers who participated in the project are convinced about the benefit they are getting by including the Bekia in rotation with barley. They are able to produce high quality feed to their animals and improve their soils. Farmers in Syria who planted the Bekia in rotation with barley instead of the continuous barley noticed the significant decrease of infection with the cereal Mealy Bug insect. Measurements in the demonstrations showed that barley following Bekia have 5-10% incidence compared to 70-90% when barley followed barley in the rotation.

The future efforts in the Mashreq-Maghreb project will focus on expanding the on-farmer demonstrations of planting Bekia in rotation with barley, improve the management of the

Table 4.24. Dry matter, grain and straw yield (kg/ha) of three forage legumes species grown in three sites in Lebanon during the 1995/96 season.

Species	Saaydeh			Ain Esaoudah			Kasr		
	Dry matter	Grain	Straw	Dry	Grain	Straw	Dry	Grain	Straw
Vicia sativa 715/2556	3246	2238	5403	2727	970	2528	439	49	218
Vicia sativa 3030/2520	3115	1253	2517	3142	2691	3979	1703	335	826
Lathyrus Cicera 127/492	3183	2326	3812	3265	2004	5334	2386	682	1873

crop and give the farmer the choice, to harvest the Bekia as hay, or as straw and grain or to be directly grazed by the sheep. Moreover, efforts are being made for the identification of more adapted forage legumes species to specific environments (neches) in order to direct the species to its optimum environment where it can give the maximum sustainable yield.

(National Agricultural Research Systems)

4.1.8. International Testing Program

The international testing program on feed legumes is a vehicle for the dissemination of genetic materials and improved production practices, in the form of international nurseries and trials, to the national programs in and outside the WANA region. The forage legume international trials were initiated from 1991. The genetic materials in forage legume trials comprise elite lines with wide or specific adaptation, special morphological or quality traits, and resistance to common biotic and abiotic stresses. Nurseries are only sent on request and often include germplasm specifically developed for a particular region or a national program. A list of trials supplied in the 1995/96 season is given in Table 4.25.

The testing program helps in identification of genotypes with specific and wide adaptation. The performance data permit assessment of genotype x environment interaction and help in targeting breeding efforts for specific agro-ecological conditions.

We supplied 264 sets of 7 different types of trials and nurseries (Table 4.25) to various cooperating scientists in 54 countries for conduct during the 1996/97 season.

Several cooperators requested large quantities of seed of elite lines identified by them from the earlier international nurseries for multilocation yield testing and on-farm verification. The available seeds were supplied to the cooperators during the season.

The salient features of the 1994/95 international nursery results, received from cooperators are presented here. The stability analyses of some of the trials were done using Eberhart and Russell (1966) model.

Table 4.25. Distribution of Forage Legume International Nurseries to cooperators for the 1996/97 season.

International Trial/Nursery	No. of sets
Lathyrus Adaptation Trial (ILAT)	
- *Lathyrus sativus* (ILAT-LS-97)	47
- *Lathyrus cicera* (ILAT-LC-97)	28
- *Lathyrus ochrus* (ILAT-LO-97)	29
Vetch Adaptation Trial (IVAT)	
- *Vicia sativa* (IVAT-VS-97)	52
- *Vicia narbonensis* (IVAT-VN-97)	39
- *Vicia ervilia* (IVAT-VE-97)	32
- *Vicia villosa* ssp *dasycarpa* (IVAT-VD-97)	35
Total	264

For the second time three separate International *Lathyrus* Adaptation Trials (ILAT) namely, *Lathyrus sativus* (ILAT-LS), *Lathyrus cicera* (ILAT-LC), and *Lathyrus ochrus* (ILAT-LO); and four separate International Vetch Adaptation Trials (IVAT) namely, *Vicia sativa* (IVAT-VS), and *Vicia narbonensis* (IVAT-VN), *Vicia ervilia* (IVAT-VE), and *Vicia villosa* ssp *dasycarpa* (IVAT-VD) were supplied to cooperators during 1994/95. In each of these trials there were 15 test entries and one local check. The results of these trials are discussed separately as below:

In ILAT-LS, the mean seed yield of selections across locations varied between 1164 and 1614 kg/ha. The stability analysis indicated that the selections namely, Sel #529, #527, #530, #531, #510, and #508 with above average mean seed yield and non-significant deviations, were relatively stable and predictable across environments.

ILAT-LC was reported from 8 locations. The mean seed yield of entries across locations ranged between 1409 and 1711 kg/ha. Five selections namely, Sel #497, #494, #486, #496, and #493 gave higher seed yield than the overall mean (1525 Kg/ha) but only three of these selections, namely Sel #494, #486, and #493 showed predictable behavior across environments.

The results for ILAT-LO were reported from 8 locations from 4 countries. The mean seed yield of selections across locations ranged between 814 and 1142 kg/ha. The five heaviest yielding entries across locations included Sel #542, #185, #551, #537, and -538 with seed yield of 1142, 1108, 1054, 1043, and 1037 kg/ha respectively. The stability analysis of seed yield of the entries revealed that six selections namely, Sel -185, -538, -548, -543, -547 and -540 with above average seed yield (1002 Kg/ha) were adaptable across environments.

The results of IVAT-VS, IVAT-VN, IVAT-VE and IVAT-VD were reported from 16, 8, 10, and 11 locations, respectively. In IVAT-VS, the mean seed yield for entries across locations varied between 684 to 1591 kg/ha. Nine entries namely, Sel -2483, -2505, -2640, -2560, -2504, -2638, -2497, -2637, and -2642 exceeded the overall mean yield (1281 Kg/ha) but only four selections Sel -2483, -2560, -2638, and -2637, were stable and predictable.

In IVAT-VN, the mean seed yield of selections across environments varied between 2306 and 3144 kg/ha with average seed yield of 2696 kg/ha. Seven entries exceeded the overall mean, and among these, Sel -2561, -2393, and -2383 were stable across environments and were predictable in behavior.

In IVAT-VE, the mean seed yield of selections varied between 1310 and 1729 kg/ha. Seven selections exceeded the overall mean seed yield (1547 kg/ha) and six of these namely, Sel -2510, -2512, -2521, -2513, -2517, and -2515 were stable and predictable across environments.

In IVAT-VD, the mean seed yield of selections across locations varied between 885 and 1120 kg/ha. Seven selections namely, Sel -2456, -2439, -2562,-2438, -2431, -2454, and -2451 exceeded the overall mean in seed yield (964 kg/ha) and were stable and predictable across environments.

(National Program Scientists, R.S. Malhotra and A. Abd El Moneim)

4.1.9. Tissue Culture in *Lathyrus sativus*.

Consummation of seeds of *Lathyrus sativus* is restricted as they contain high concentration of the neurotoxin ODAP (§-N-oxalyl-L-, §-diaminopropionic acid) which causes paralyzation. Recently protocols have been developed to obtain plants with a low concentration of ODAP. Different explants are cultured on medium were they dedifferentiate in to calli. From these calli plants are regenerated. Due to somaclonal variation a number of these plants will have a considerable lower concentration of ODAP than the original seed/variety.

This technique was tried at ICARDA with four local varieties. Seed color of the seedlings relates to the amount of toxin in the seed. From 422 explants cultured 85 % had dedifferentiated into calli (Table 4.26). Of these calli, 43% had regenerated either roots, shoots or both on the first media they were cultured on. Some of the calli have been transferred to a regeneration media. After 2 months of culturing, 9 explants have developed with roots and shoots. Differences in calli formation between the varieties are low. To enhance the somaclonal variation, it is important to have a good callogenic phase before the regeneration is induced. The medium B5L gives a high number of calli which show just rooting, an adjustment in the hormones will be tried to also induce shoot development. The calli with roots or shoots will be transferred to regeneration media to induce the development of roots or shoots.

(B. van Dorrestein, M. Baum and A. Abd El Moneim)

Table 4.26. Regeneration from calli of Lathyrus explants.

Explant source	Variety	No. of explants	No. of callogenic explants	No. of undeveloped explants	Calli with roots	Calli with shoots	Calli with roots and shoots
Root	Sel 558 (P21)	33	28	3	13	0	0
	Fam 85	15	4	11	1	0	0
	Sel 520	33	28	2	13	0	0
	Sel 482	22	20	2	11	0	0
Internode	Sel 558	41	36	2	15	0	0
	Fam 85	6	5	1	0	0	0
	Sel 520	35	32	1	15	0	0
	Sel 482	26	22	2	9	1	0
Shoot	Sel 558	26	19	7	2	13	2
	Fam 85	4	4	0	0	1	0
	Sel 520	33	30	3	3	11	6
	Sel 482	27	20	7	2	17	1
Leave	Sel 558	22	19	0	4	0	0
	Fam 85	3	3	0	0	0	0
	Sel 520	39	36	1	2	0	0
	Sel 482	57	52	3	11	0	0
Total		422	358	45	101	43	9

5. DRY PEA IMPROVEMENT

Although peas have been cultivated in the ICARDA region for millennia, yields are low because of a lack of high yielding and stable genotypes, and poor crop management. To rectify this problem an integrated approach to pea improvement was initiated at ICARDA in 1986/87 following the receipt of grant from the Ministry for Economic Cooperation, Germany (BMZ). Since research has been extensive on improvement of dry pea at a number of institutions in the developed and some developing countries, it was envisaged that ICARDA would capitalize on existing research and identify dry pea varieties adapted to the farming systems of WANA. The work on pea improvement is therefore concentrated in the following area:

I. Assembling enhanced germplasm/cultivars from institutes working on dry pea in developed and developing countries and testing them at ICARDA sites to identify superior lines for evaluation by the national programs in WANA.

II. Developing suitable production technology and its transfer to the national programs for testing and adaptation.

5.1. Germplasm Collection and Evaluation

Two hundred and seventy four germplasm and improved cultivars were assembled during 1995/96, out of these 82 were from Australia, 178 from Romania, 1 from U.K., 10 from Russia and 3 from India. These were evaluated for yield performance and other traits in two genetic evaluation trials under Tel Hadya conditions.

5.1.1. Pea Genetic Evaluation Trials

The new accessions obtained from various institutions were evaluated at Tel Hadya in two different Genetic Evaluation Trials (PGEVT-1 and PGEVT-2) with 84 and 196 entries. The data were recorded on various phenological and morphological characters.

Pea Genetic Evaluation Trial 1: Days to flower ranged from 105 days (for Acc No. 675) to 124 days (for Acc No. 679), days to maturity ranged from 152 days (for Acc Nos. -224, -636, and -647) to 158 days (for Acc No. 679), and the harvest index ranged from 28% (for Acc No. 699) to 50% (for Acc No. 636), and seed yield ranged from 878 kg/ha (for Acc No. 704) to 3319 kg/ha (for Acc No. 712). Three of the test entries (Acc Nos. -677, -694 and -712) exceeded the improved check cultivar Acc No. 225, by a significant margin. The five highest seed yielding entries included Acc Nos. -712, -677, -694, -661 and -672 with seed yields of 3319, 3296, 3285, 3241 and 3030 kg/ha, respectively (Table 5.1).

Pea Genetic Evaluation Trial 2: Days to flowering ranged from 81 (Acc No. 887 and -888) to 132 days (Acc No. 828), days to maturity ranged from 129 (Acc No. 888) to 160 days (Acc Nos. 807, -828, -854, and -882); and seed yield ranged from 0 kg/ha (Acc No. 828) to 2840 kg/ha (Acc No. 834). Only one entry, Acc No. 834 exceeded the check by a significant margin. The five heaviest yielding entries included Acc No. -834, -884, -735, -825, and -837 with seed yields of 2840, 2574, 2484, 2457 and 2431 kg/ha, respectively (Table 5.2).

Table 5.1. Adjusted seed yield (SYLD=kg/ha) and rank (R), biological yield (BYLD=kg/ha), days to flower (DFLR), days to maturity (DMAT) and harvest index (HI) of some of the high yielding entries in Pea Genetic Evaluation Trial-1 (PGEVT-1) at Tel Hadya during 1995/96.

Acc. No.	Name	Origin	DFLR	DMAT	SYLD	Rank	BYLD	HI
631	889101-10-2	Australia	112	154	2537	20	6126	41.3
638	Spring Pea 3	Australia	109	154	2626	17	5978	44.2
661	P22LR 8	Australia	112	155	3241	4	8289	39.2
662	P22LR 10	Australia	111	155	2722	14	5730	47.6
664	PSSLR 11	Australia	110	155	3015	6	7096	42.5
665	P22LR 12	Australia	110	155	2833	12	7044	40.2
668	P22LR 15	Australia	111	154	2707	15	6974	38.9
670	P22LR 18	Australia	121	155	2852	10	7211	39.6
672	P22LR 20	Australia	109	154	3030	5	6711	45.3
674	P22LR 22	Australia	112	155	2467	23	6019	41.0
675	P22LR 23	Australia	105	154	2500	21	5837	42.8
677	P22LR 24	Australia	108	154	3296	2	7933	41.6
678	P22LR 25	Australia	107	154	2759	13	6611	41.6
680	P22LR 27	Australia	120	156	2833	11	7689	36.8
681	P22LR 28	Australia	112	154	2656	16	7511	35.9
687	P22LR 34	Australia	113	156	2600	18	5648	46.1
694	P22LR 42	Australia	112	157	3285	3	7941	41.3
697	P22LR 45	Australia	114	154	2456	24	5385	44.0
703	P22LR 51	Australia	108	154	2907	8	6433	45.1
705	P22LR 53	Australia	108	155	2426	25	5770	41.9
712	P22LR 60	Australia	111	154	3319	1	7941	41.8
713	P22LR 61	Australia	114	155	2593	19	7089	36.3
723	P22LR 71	Australia	108	155	2963	7	6511	45.6
725	P22LR 73	Australia	107	154	2856	9	5933	48.1
224	The Lincoln	Australia	109	152	2148	44	4567	46.8
225	S.L.Aleppo-2	Syria	113	154	2482	22	7496	33.1
Grand Mean			111	154	2230		5410	41.7
S.E. of Mean			0.6	0.5	279.1		594.5	3.0
LSD at P=0.05			1.8	1.3	784.7		1671.6	8.5
C.V.%			0.8	0.4	17.7		15.5	10.2

Table 5.2. Adjusted seed yield (SYLD=kg/ha) and rank (R), biological yield (BYLD=kg/ha), days to flower (DFLR), days to maturity (DMAT) and harvest index (HI) of some of the high yielding entries in Pea Genetic Evaluation Trial-2 (PGEVT-2) at Tel Hadya during 1995/96.

Acc. No.	Name	Origin	DFLR	DMAT	SYLD	Rank	BYLD	HI
517	88P022-6	Australia	102	138	2170	11	5413	38.7
520	88P037-5	Australia	99	138	2380	7	5688	42.7
735	Flagman	Former USSR	94	136	2484	3	5728	43.9
741	P66/87	Romania	99	138	1818	23	3922	45.9
756	P122/89	Romania	95	136	2055	14	4707	45.7
757	P22/74	Romania	95	137	1858	20	4927	36.7
774	P048/82	Romania	98	137	2003	15	5246	37.9
825	P3/95	Romania	96	134	2457	4	6135	41.4
827	P12/95	Romania	94	136	1926	19	6885	27.7
829	P15/95	Romania	96	137	2426	6	6447	38.2
832	P021/95	Romania	96	135	2365	10	6457	37.6
833	P024/95	Romania	116	137	2375	8	5877	41.3
834	P028/95	Romania	97	138	2840	1	6661	41.3
837	P032/95	Romania	96	137	2431	5	6171	40.0
838	P034/95	Romania	96	136	2370	9	6356	36.6
839	P035/95	Romania	98	137	1831	22	4398	40.3
841	P038/95	Romania	90	140	1975	16	5432	37.5
842	P045/95	Romania	94	136	2149	12	5925	34.8
847	P057/95	Romania	102	137	1847	21	4720	39.7
884	P84/95	Romania	93	136	2574	2	5794	42.5
886	P100/95	Romania	92	135	1816	24	4252	40.8
890	P112/95	Romania	87	131	1766	25	4043	43.9
894	P195/95	Romania	96	137	2111	13	5672	37.6
224	The Lincoln	Australia	96	135	1969	17	4350	45.0
225	S.L.Aleppo-2	Syria	106	138	1944	18	5112	36.8
Grand Mean			113	147	949		3758	23.8
S.E. of Mean			4.0	1.8	253.2		572.9	4.5
LSD at P=0.05			11.0	5.1	701.6		1587.8	12.4
C.V. %			5.0	1.8	37.7		21.6	26.5

5.1.2. Evaluation for Cold Tolerance

In cold tolerance nursery 210 dry pea accessions were evaluated during 1995/96 season. Visual cold tolerance ratings on 1-9 scale (where 1 = free from damage, 9 = killed) were assigned after the susceptible check was killed. The frequency distribution of the lines for cold tolerance reaction is given in Table 5.3. Seven and 34 accessions with 3 and 4 ratings were tolerant and moderately tolerant to cold and their details are given in Table 5.4.

Table 5.3. Frequency distribution of 210 pea lines evaluated for cold tolerance at Tel Hadya during 1995/96.

	Cold tolerance rating (1-9 scale)									
	1	2	3	4	6	8	7	8	9	Total
Number of lines	0	0	7	34	98	30	27	12	2	210

Table 5.4. Pea accessions with tolerance to cold at Tel Hadya during 1995/96.

Acc No.	Name	Origin	Acc No.	Name	Origin
		Rating 3			
195	D200-1-16	USA	337	PI 517922	USA
214	D175-2-3-1	USA	338	PI 517923	USA
243	Fenn	USA	342	PI 512065	USA
244	Glacier	USA			
		Rating 4			
83	NEP 24	Afghanistan	470	WIR-1878	Former USSR
89	NEP 37	Afghanistan	473	WIR-1925	Former USSR
92	10947	Finland	491	WIR-3611	Former USSR
93	51206	Finland	507	SU 88269	Australia
144	MG 101849	Ethiopia	552	89P 109-11	Australia
158	MG 102426	Italy	553	89P 109-12	Australia
186	D166-3-1	USA	557	89P 111-1	Australia
197	D200-4-3	USA	558	89P 123-3	Australia
211	D175-2-3-2	USA	559	89P 124-4	Australia
331	PI 512082	USA	560	89P 127-2	Australia
339	PI 517924	USA	611	88P038-10-18	Australia
340	PI 517925	USA	616	88P090-5-21	Australia
343	PI 512066	USA	619	88P101-10-1	Australia
346	PI 512069	USA	625	88P007-3-20	Australia
347	PI 512070	USA	626	88P022-2-22	Australia
352	PI 512075	USA	629	88P038-10-29	Australia
467	WIR-1441	Former USSR	632	88P022-3-9	Australia

5.2. Yield Trials

5.2.1. Preliminary Yield Trial (PYT)

Sixty four entries selected from the genetic evaluation and preliminary yield trials of the previous season were evaluated for yield performance in an 8 x 8 lattice design during 1995/96 at Tel Hadya and Terbol. Adjusted seed yield for the entries varied from 807 to 1495 kg/ha at Tel Hadya and 723 to 3093 kg/ha at Terbol. Location means at Tel Hadya and Terbol were 1203 and 2212 kg/ha, respectively. *None of* the test entries exceeded the improved check cultivar Acc No. 225. Based on the mean yield over locations, the five heaviest yielding lines included Acc Nos. -8, -605, -21, -603 and -549 with seed yields of 2225, 2163, 2121, 2076 and 2069 kg/ha, respectively (Table 5.5).

5.2.2. Pea International Adaptation Trial (PIAT)

Twenty three test entries were selected from PYT and PIAT-95 conducted during the previous season. These 23 entries alongwith a local check were tested in PIAT at Tel Hadya and Terbol during 1995/96. Sixteen test entries at Tel Hadya and 21 test entries at Terbol yielded significantly better than the local check. The five highest yielding entries at Tel Hadya included Acc Nos. -403 (NZL Acc No. 1670), -553 (89P 109-12), -501 (P 397-4), -504 (Local Ethiopian Cultivar) and -225 (Syrian Local Aleppo-2); and at Terbol included Acc Nos. 225 (Syrian Local Aleppo-2), -8 (Syrian Local Aleppo-1), -548 (WA 932), -448 (TARA), and -21 (Local Selection 1690). On the basis of mean seed yield over locations, Acc No. 225 (Syrian Local Aleppo-2) ranked first and was followed by Acc No. -8 (Syrian Local Aleppo-1), -553 (89P 109-12), -548 (WA 932), and -526 (88P 038-10) with seed yields of 3157, 2924, 2914, 2856 and 2806 kg/ha, respectively (Table 5.6). The Acc No. 569 (89P166-12) was the earliest to flower.

(R.S. Malhotra and M.C. Saxena)

Table 5.5. Adjusted seed yield (YLD=kg/ha) and rank (R), days to flower (DFLR) and days to maturity (DMAT) of some of the entries in Preliminary Yield Trial at Tel Hadya, and Terbol during 1995/96.

Acc. No.	Name	Tel Hadya		Terbol		Mean			
		YLD	R	YLD	R	YLD	R	DFLR	DMAT
62	Ballet	1495	1	1463	53	1479	52	128	181
173	MG 102703	1314	15	2640	13	1977	11	133	181
216	Collegian	1115	54	2956	3	2036	6	131	180
222	Early dun	1338	10	2567	21	1953	12	137	179
267	PS 210713	1379	6	1089	57	1234	55	129	181
496	M 152-8	1293	18	2611	18	1952	13	137	180
549	WA 933	1428	3	2709	9	2069	5	126	179
602	88P022-3-15	1288	19	2539	25	1913	19	131	180
603	88P034-3-13	1396	4	2757	7	2076	4	126	178
604	88P034-3-15	1286	20	2675	12	1981	10	123	178
605	88P034-3-24	1346	9	2980	2	2163	2	127	179
611	88P038-10-18	1434	2	2634	15	2034	7	133	182
618	88P090-5-26	1219	30	2634	14	1927	16	133	181
625	88P007-3-20	1132	49	2719	8	1925	17	128	180
627	88P035-4-4	1182	39	2682	11	1932	15	138	180
632	88P022-3-9	1379	5	2502	27	1941	14	135	181
634	88P022-6-22	1207	34	2616	17	1911	20	133	181
653	DMR-7	1208	33	2783	5	1996	9	135	181
655	DMR-20	1164	43	2683	10	1923	18	133	182
8	S.L. Aleppo-1	1356	8	3093	1	2225	1	136	181
21	S.L. 1690	1374	7	2867	4	2121	3	137	181
225	S.L. Aleppo-2	1255	23	2759	6	2007	8	138	180
Grand Mean		1203		2212		1707		132	181
S.E. of Mean		89.8		138.8					
LSD at P=0.05		251.6		389.0					
C.V. %		12.9		10.9					

Table 5.6. Mean seed yield (YLD=kg/ha) and rank (R), days to flower (DFLR), and days to maturity (DMAT) of entries at Tel Hadya and Terbol in PIAT-96.

Acc. No.	Entry Name	Tel Hadya		Terbol		Mean over locations			
		YLD	R	YLD	R	YLD	R	DFLR	DMAT
321	AMAC	917	24	2296	19	1606	22	138	182
380	DMR-8	2477	16	1269	23	1873	20	137	181
403	NZL Acc.No.1670	3231	1	1565	21	2398	19	137	182
448	TARA	2506	15	2954	4	2729	9	131	181
452	Praire No. 11	1653	23	1963	20	1808	21	127	183
498	P301	2412	17	2694	12	2553	15	129	180
499	P350-1	2139	20	2861	9	2500	17	140	181
501	P 397-4	2869	3	2583	17	2726	10	144	185
504	Ethiopian Loc	2856	4	2685	13	2771	6	127	181
507	SV88269	1697	22	1287	22	1492	23	137	182
517	88P022-6	2574	12	2583	16	2579	14	130	180
526	88P038-10	2713	6	2898	8	2806	5	140	182
545	88PX0034	2556	13	2926	7	2741	8	155	183
546	WA 930	2532	14	2500	18	2516	16	133	182
548	WA 932	2648	9	3065	3	2856	4	132	180
553	89P109-11	2639	10	2620	15	2630	13	127	181
553	89P109-12	2903	2	2926	6	2914	3	131	182
554	89P109-13	2679	7	2815	10	2747	7	128	180
557	89P111-1	2583	11	2704	11	2644	12	130	181
569	89P166-12	2181	19	2676	14	2428	18	124	181
8	S.L.Aleppo-1	2671	8	3176	2	2924	2	125	181
21	S.L.1690	2366	18	2926	5	2646	11	128	182
225	S.L.Aleppo-2	2843	5	3472	1	3157	1	135	182
Local	Check	2009	21	963	24			131	183
Location Mean		2444		2517		2419		133	182
S.E. of Mean		148.1		178.3					
LSD at P=0.05		421.7		507.6					
C.V. %		10.5		12.3					

5.3. International Testing Program

The international testing program on dry pea is a vehicle for the dissemination of genetic materials and improved production practices, in the form of international nurseries and trials, to the national programs in and outside the WANA region. The genetic materials comprise elite lines with wide or specific adaptation, and special morphological or quality traits. Nurseries are only sent on request and the pea germplasm has been assembled from other centers and then tested for adaptation at ICARDA. A total of 61 sets of the Pea International Adaptation Trial (PIAT) were dispatched for the 1996/97 season.

The testing program helps in identification of genotypes with specific and wide adaptation. The performance data permit assessment of genotype x environment interaction.

The results of Pea International Adaptation Trial-1995 (PIAT-95) were reported from 19 locations. At 11 locations, some of the test entries exceeded the local check in seed yield by a significant ($P \leq 0.05$) margin. The mean seed yield across environments varied from 541 kg/ha at Toshevo (Bulgaria) to 8515 kg/ha at Dirab (Saudi Arabia). The stability analysis for seed yield revealed that 8 entries namely, Syrian Local Aleppo-1, Local Sel. 1690, Syrian Local Aleppo-2, TARA, 88P038-10, P 301, WA 932 and A 149 with above average seed yield and non-significant deviations from regression, were adaptable across environments.

(NARSs Scientists and R.S. Malhotra)

6. PUBLICATIONS

6.1. Journal Articles

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7. LEGUME VARIETIES RELEASED BY NATIONAL PROGRAMS

Country	Variety	Year of release	Specific features
Lentil			
Algeria	Syrie 229	1987	Yield, seed quality
	Balkan 755	1988	Yield, seed quality
	ILL 4400	1988	Yield, seed quality
Argentina	Arbolito (ILL 4605x-4349)	1991	Yield, tall & early
Australia	Aldinga (FLIP84-80L)	1989	Yield
	Digger (FLIP84-51L)	1993	Yield, red cotyledon
	Cobber (FLIP84-58L)	1993	Yield, red cotyledon
	Matilda (FLIP84-154L)	1993	Yield, yellow cotyledon
	Northfield (78S 26013)	1995	High yield, Ascochyta blight res.
	Falguni - BARI Masur 2 (Sel.ILL4353xILL353)	1993	Rust res. & yield
Bangladesh	BARI Masur 4(Sel. L5 x FLIP84-112L)	1995	Rust and Stemphylium resistance
Canada	Indianhead (ILL 481)	1989	Green manure
	CDC Redwing (Eston x ILL 5588)	1994	Ascochyta res.
	CDC Matador (Indianhead (ILL 481) x (Eston x PI179310)	1994	Ascochyta res.
Chile	Centinela (74TA470)	1989	Rust res.& yield
China	FLIP87-53L	1988	Yield in Qinghai Province
Ecuador	INIAP-406 (FLIP 84-94L	1987	Rust res. & yield
Egypt	Precoz (ILL 4605)	1990	Early maturity
	Sinai 1 (sel ILL 4605)	1996	Early, high protein for N. Sinai
	Giza 51 (FLIP84-51L)	1996	Tol. high soil mositure
Ethiopia	R 186	1980	Yield
	Calew (ILL 358)	1984	Rust res. & yield
	Chikol (NEL 2704)	1994	Rust res. & yield
	FLIP84-7L	1994	Rust resistant, high yield
	Gudo (FLIP84-78L)	1995	Rust resistant, high yield
	Ada'a (FLIP86-41L)	1995	Rust resistant, high yield
Iraq	Baraka (78S26002)	1994	Yield, standing ability
Jordan	Jordan 3 (78S 26002)	1990	Yield, standing ability
Lebanon	Talya 2 (78S 26013)	1988	Yield, standing ability
	Toula (FLIP86-2L)	1995	High yield, large seeds
Libya	El Safsaf 3 (78S26002)	1993	Yield, st. ability in E. Libya
Morocco	Precoz (ILL 4605)	1990	Rust res. & yield, early maturity
Nepal	Sikhar (ILL 4402)	1989	Yield

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Country	Variety	Year of release	Specific features
Lentil			
N Zealand	Rajah (FLIP87-53L)	1992	Yield, red cotyledon
Pakistan	Manserha 89 (ILL 4605)	1990	Ascochyta & rust res.
	Masur-95 (18-12 x ILL 4400)	1995	Ascochyta & rust res.
	ShirAZ-96 (ILL 5865)	1996	Winter-hard
Sudan	Aribo 1 (ILL 818)	1993	Yield in Jebel Mara
Syria	Idleb 1 (78S 26002)	1987	Yield, reduced lodging
Tunisia	Nsir (ILL 4400)	1986	Large seeds & yield
	Nefza (ILL 4606)	1986	Large seeds & yield
Turkey	Firat 87 (75Kf 36062)	1987	Small seeds & yield
	Erzurum 89 (ILL 942)	1990	Spring sowing & yield
	Malazgirt89 (ILL 1384)	1990	Spring sowing & yield
	Sazak 91 (NEL 854)	1991	Winter sowing, red cotyledon
	Sayran 96 (ILL 1939)	1996	High yield & harvestability
U.S.A.	Crimson (ILL 784)	1991	Yield in dry areas
Kabuli Chickpea			
Algeria	ILC 482	1988	High yield, blight resistance
	ILC 3279	1988	Tall, blight resistance
	FLIP 84-79C	1991	Cold, blight resistance
	FLIP 84-92C	1991	Blight resistance
China	ILC 202	1988	High yield, for Ginghai pr.
	ILC 411	1988	High yield, for Ginghai pr.
	FLIP 81-71C	1993	High yield
	FLIP 81-40WC	1993	High yield
Cyprus	Yialousa (ILC 3279)	1984	Tall, blight resistance
	Kyrenia (ILC 464)	1987	Large seeds
Egypt	ILC 195	1993	Blight, wilt resistance
France	TS1009 (ILC 482)	1988	Blight resistance
	TS1502 (FLIP 81-293C)	1988	Blight resistance
	Roye Rene (F 84-188C)	1992	Cold, blight resistance
Iran	ILC 482	1995	High yield, blight resistance
	ILC 3279	1995	High yield, blight resistance
	FLIP 84-48C	1995	High yield, blight resistance
Iraq	Rafidain (ILC 482)	1991	Blight resistance, high yield
	Dijla (ILC 3279)	1991	Tall, blight resistance
Italy	Califfo (ILC 72)	1987	Tall, blight resistance
	Sultano (ILC 3279)	1987	Tall, blight resistance
	Pascia (FLIP 86-5C)	1995	Blight resistance, high yield
	Otello (ICC6306/NEC206)	1995	Blight resistance, desi, feed

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Country	Variety	Year of release	Specific features
Kabuli Chickpea			
Jordan	Jubeiha 2 (ILC 482)	1990	High yield, blight resistane
	Jubeiha 3 (ILC 3279)	1990	High yield, blight resistance
Lebanon	Janta 2 (ILC 482)	1989	High yield, wide adaptation
	Baleela (FLIP 85-5C)	1993	Green seed consumption
Libya	ILC 484	1993	High yield, blight resistance
Morocco	ILC 195	1987	Tall, blight resistance
	ILC 482	1987	High yield, blight resistance
	Rizki (FLIP 83-48C)	1992	Large seed, blight resistance
Morocco	Douyet (FLIP 84-92C)	1992	Large seed, blight resistance
	Farihane (FLIP 84-79C)	1995	Large seed, blight resistance
	Moubarak (FLIP 84-145C)	1995	Large seed, blight resistance
	Zahor (FLIP 84-182C)	1995	Large seed, blight resistance
Oman	ILC 237	1988	High yield, Irrigated conditions
	FLIP 87-45C	1995	High yield, blight resistance
	FLIP 89-130C	1995	High yield, blight resistance
Pakistan	Noor 91 (FLIP 81-293C)	1992	High yield, blight resistance
Portugal	*Elmo (ILC 5566)*	1989	Blight resistance
	Elvar (FLIP 85-17C)	1989	Blight resistance
Spain	Fardan (ILC 72)	1985	Tall, blight resistance
	Zegri (ILC 200)	1985	Mid-tall, blight resistance
	Almena (ILC 2548)	1985	Tall, blight resistance
	Alcazaba (ILC 2555)	1985	Tall, blight resistance
	Atalaya (ILC 200)	1985	Mid-tall, blight resistance
	Athenas (ILC72xCA2156)	1995	Large seed, blight resistance
	Bagda (ILC72xCA2156)	1995	Large seed, blight resistance
	Kairo (ILC72xCA2156)	1995	Large seed, blight resistance
Sudan	Shendi	1987	High yield, irrigated conditions
	Jebel Marra-1 (ILC 915)	1994	High yield, irrigated conditions
	Ghab 1 (ILC 482)	1986	High yield, blight resistance
Syria	*Ghab 2 (ILC 3279)*	1986	Tall, blight resistance
	Ghab 3 (FLIP 82-150C)	1991	High yield, cold & blight res.
Tunisia	Chetoui (ILC 3279)	1986	Tall, blight resistance
	Kassab (FLIP 83-46C)	1986	Large seeds, blight resistance
	Amdoun 1 (Be-sel-81-48)	1986	Large seeds, wilt resistance
	FLIP 84-79C	1991	Blight, cold resistance
	FLIP 84-92C	1991	Large seed, blight resistance
Turkey	ILC 195	1986	Tall, blight resistance
	Guney Sarisi 482	1986	High yield, blight resistance

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Country	Variety	Year of release	Specific features
Kabuli Chickpea			
	Damla (FLIP 85-7C)	1994	Blight resistance
	Aziziye (FLIP 84-15C)	1994	Blight resistance
	Akcin (87AK71115)	1991	Tall, blight resistance
	Aydin 92 (FLIP 82-259C)	1992	Large seed, blight resistance
	Menemen 92 (FLIP 85-14C)	1992	Large seed, blight resistance
	Izmir 92 (FLIP 85-60C)	1992	Large seed, blight resistance
USA	Dwelley (SurutatoxFLIP 85-58C)	1994	Blight resistance
	Sanford (SurutatoxFLIP 85-58C)	1994	Blight resistance
Forage Legumes			
Morocco	V. sativa (ILF-V-1812)	1990	Erect, tolerant to *Orobanche*, high yield, early
	V. narbonensis 573/2387	1994	High yield
	V. narbonensis	1994	High yield
	V. villosa (1812/2083)	1992	High yield
	V. sativa (709)	1994	High yield
Jordan	V. sativa 715	1994	
	L. ochrus 101	1994	*Orobanche* resistant
Pakistan	V. villosa spp.dasycarpa	1996	
	Kukak-96	1996	Cold tolerance, high yield

8. STAFF LIST

1.	Dr William Erskine	Lentil Breeder
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4.	Dr Ali Abdel Moneim	Forage/Legume Breeder
5.	Dr K.B. Singh*	Chickpea Breeder (ICRISAT)
6.	Dr Crysantus Akem	Food Legume Pathologist
7.	Dr Rajendra S. Malhotra	International Trials Scientist
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9.	Dr Seid A. Kemal	Post-Doctoral Fellow
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28.	Mr Khaled El-Dibl	Senior Research Technician
29.	Mr Riad Ammaneh	Senior Research Technician
30.	Mr Nidal Kadah	Senior Research Technician
31.	Mr Omar Labban	Senior Research Technician
32.	Mr Pierre Kiwan	Senior Research Technician (Terbol)
33.	Mr Ra'afat Azzo	Research Technician
34.	Mr Mohamed K.Issa	Research Technician
35.	Mr Bounian Abdel Karim	Research Technician
36.	Mr Diab Ali Raya	Research Technician
37.	Mr Mohamed El-Jasem	Research Technician
38.	Ms Setta Ungi	Research Technician
39.	Ms Nouran Attar	Research Technician

40. Ms Aida Naimeh	Assistant Research Technician (Terbol)
41. Mr Joseph Karaki	Assistant Research Technician (Terbol)
42. Mr Ghazi Khatib	Assistant Research Technician (Terbol)
43. Mr Noaman Ajanji	Driver/Store Keeper
44. Mr Fawaz El-Abdallah	Laborer
45. Mrs Hasna Boustani	Senior Secretary
46. Ms Tamar Varvarian	Secretary

Consultants

47. Dr B. Bayaa	Lentil Pathologist
48. Dr S. Khalil	Faba Bean Breeder

*** Left the Program during 1996**