NEW PERSPECTIVES FOR AN ANCIENT SPECIES

The chickpea in the economy and diet of Mediterranean peoples

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International Agricultural Research
European Service

ICARDA

International Center for Agricultural Research
in the Dry Areas

About ICARDA

Established in 1977, the International Center for Agricultural Research in the Dry Areas (ICARDA) is governed by an independent Board of Trustees. Based at Aleppo, Syria, it is one of the 16 centers supported by the Consultative Group on International Agricultural Research (CGIAR), which is an international group of representatives of donor agencies, eminent agricultural scientists, and institutional administrators from developed and developing countries who guide and support its work.

The mission of the CGIAR is to promote sustainable agriculture to alleviate poverty and hunger and achieve food security in developing countries. The CGIAR conducts strategic and applied research, with its products being international public goods, and focuses its research agenda on problem-solving through interdisciplinary programs implemented by one or more of its international centers, in collaboration with a full range of partners. Such programs concentrate on increasing productivity, protecting the environment, saving biodiversity, improving policies, and contributing to strengthening agricultural research in developing countries.

In the context of the challenges posed by the physical, social and economic environments of the dry areas, ICARDA's mission is to improve the welfare of people in the dry areas of the developing world by increasing the production and nutritional quality of food while preserving and enhancing the resource base. ICARDA meets this challenge through research, training, and dissemination of information in partnership with the national agricultural research and development systems.

ICARDA serves the entire developing world for the improvement of lentil, barley and faba bean; all dry-area developing countries for the improvement of on-farm water-use efficiency, rangeland and small-ruminant production in the West Asia and North Africa region for the improvement of bread and durum wheats, chickpea, and farming systems. ICARDA's research provides global benefits of poverty alleviation through productivity improvements integrated with sustainable natural resource management practices.

Much of ICARDA's research is carried out on a 948-hectare farm at its headquarters at Tel Hadya, about 35 km southwest of Aleppo. ICARDA also manages other sites where it tests material under a variety of agroecological conditions in Syria and Lebanon. However, the full scope of ICARDA's activities can be appreciated only when account is taken of the cooperative research carried out with many countries in West Asia and North Africa and elsewhere in the world.

The results of research are transferred through ICARDA's cooperation with national and regional research institutions, with universities and ministries of agriculture, and through the technical assistance and training that the Center provides. A range of training programs is offered extending from residential courses for groups to advanced research opportunities for individuals. These efforts are supported by seminars, publications, and specialized information services.

New Perspectives for an Ancient Species: The Chickpea in the Economy and Diet of Mediterranean Peoples

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Introduction

Chickpea was first cultivated about 5000 years BC. The species originates from the Mediterranean basin, where it is still widely grown. But 90 percent of the world chickpea growing area is now concentrated in Asia.

Along with other grain legume species (beans, lentil, faba bean), chickpea is a basic component in the diet of North African and West Asian countries. Its proteins effectively substitute those present in food of animal origin, such as, meat, milk, eggs and cheese.

The history of Italian chickpea cultivation shows a trend shared by other developed countries. Once widely cultivated (180,000 ha in the 1920s and 100,000 as late as the 1950s), by 1993 chickpea was grown on only 4000 ha in Italy. This decline resulted from an increasing consumption of animal instead of plant protein, and serious problems in crop mechanization.

Recently, however, a reversal in this trend has been seen. Diet excesses and high animal-protein consumption common to all developed countries, including Italy, are a frequent cause of nutritional imbalance and metabolic troubles. This, along with a better knowledge of the basic principles of nutrition, is encouraging many consumers to re-evaluate plant protein foods (mainly beans, chickpea and lentil), and reintroduce them into the everyday diet.

Italian agriculture may benefit from an expansion of national chickpea cultivation. Increased internal production could lead to a replacement of the sizeable quantities (85 percent of national needs) at present imported from Turkey and Mexico. Furthermore, due to its low water requirements, and capacity to fix atmospheric nitrogen, chickpea is particularly adapted to farming systems in central and southern Italy.

During the last 10 years, new chickpea cultivars have been bred and released in Italy. Their characteristics helped overcome most of the problems that had prevented profitable chickpea cultivation and production. These include resistance to cold stress and parasites (*Ascochyta rabiei* and *Fusarium oxysporum)*, which is necessary if the crop is to be planted in the fall, when it can use water reserves created in the soil by fall–winter rain. Germplasm characterized by an erect habit was also included in breeding programs, so that harvesting could be completely mechanized and cultivation costs lowered. The first two cultivars possessing such characteristics, Sultano and Califfo, were officially registered and released by ENEA (Italian Agency for Renewable Energy) in 1987. Five more cultivars are now waiting for official registration.

The use of modern cultivars has enabled increases in chickpea production in Italy of around 90 percent. Such increments could only be achieved with the introduction in the national breeding programs of qualified germplasm distributed by the International Center for Agricultural Research in Dry Areas (ICARDA) of Aleppo, Syria. Italian scientists from ENEA, the Experimental Institute for Plant Pathology and the Experimental Institute for Cereal Culture have cooperated in such programs with ICARDA scientists, also with funds from the cooperation department of the Italian Ministry for Foreign Affairs. More recently, the European Union has financed other chickpea breeding programs carried out by ENEA and the University of Tuscia in Italy, in association with Spanish and British institutions.

There would not, however, be sufficient grounds for an expansion of chickpea consumption in Italy and other developed countries if research had not highlighted some basic benefits related to the inclusion of such legumes in the human diet. The blood glucose content, for instance, is lower after a legume-based meal, although it will remain relatively high levels for longer. It is therefore possible to avoid both the early peaks and, later, the deficiencies of glucose, which are typical of the diabetes syndrome. For such reasons, chickpeas are among foods with lower glycemic index, which is a measure of the glucose absorption. Furthermore, the inclusion in the diet of dry, cooked legumes reduces the blood

cholesterol level, thus eliminating or alleviating some cardio-circulatory afflictions.

Further encouragement for an expansion of chickpea consumption may stem from a more innovative attitude on the part of the processing industry—output is currently confined to canned chickpeas. This could lead to the introduction of several new products with high added value, such as, biscuits, snacks, and pre-cooked flour, so far marketed only on a pilot scale. To that end, a first meaningful, cheap opening could be the production and sale of chickpea flour ready for cooking. In West Asian countries, it represents the raw material for the cooking of a purée-like meal called *humus*, which is unanimously appreciated by European travellers and local people.

A parallel strategy, more likely to yield results in a short period of time, may consist of the identification, development and large-scale diffusion of a series of chickpea-based regional dishes. The organizers of this conference have made an effort in that direction, by distributing a chickpea cookbook including West Asian, Spanish, Italian, biblical and Ancient Roman recipes, and by offering a chickpea-based meal.

During this conference, an effort was made to focus on the issues and problems of chickpea improvement, cultivation and marketing, through the contribution of some components instrumental to their solution: agriculture, agro-industry, and consumer research.

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International Genebanks and Crop Improvement in Developing Countries

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For practical crop-improvement purposes, genepools of interest to plant breeders may be classified into:

1. *Unrelated taxa* that can contribute simply inherited traits through genetic engineering, and which are generally best conserved *in situ*.
2. *Wild crop relatives* that are an important source of specific traits, but which may require special techniques to transfer them into the cultivated genepool. They may be conserved both *in situ* and *ex situ*.
3. *Landraces and farmer varieties* that hybridize readily, but which may also be associated with undesirable traits. They may be conserved both on farm and *ex situ*.
4. *Released modern cultivars,* which are often the gene source preferred by plant breeders and are generally best conserved *ex situ*.

Ex-situ conservation has the advantage that the material is readily available to users and can be well documented. However, it is only possible to conserve *ex situ* a relatively small proportion of potentially useful genetic diversity cost effectively, and the conserved material is unable to continue to evolve under natural or farmer selection pressure.

An effective *ex-situ* conservation program involves a number of linked activities, including the taxonomic, spatial and temporal assessment of genetic diversity; collection, characterization, evaluation, documentation and conservation of material, and the dissemination and exchange of material and information. *Ex-situ* conservation is not an end in itself, but a mechanism for ensuring genetic diversity is accessible to those who need to use it, today and in the future.

While the largest number of *ex-situ* accessions is maintained worldwide in seed genebanks, generally under low-temperature conditions, many species cannot be effectively conserved in this way. Species which have recalcitrant seeds (i.e. cannot withstand desiccation and die in low temperatures), or which are clonally propagated, have to be conserved in field genebanks, *in-vitro* under slow-growth conditions, or as cryopreserved tissues.

Many institutions are involved with the conservation of genetic resources worldwide. Within countries, these range from local communities and NGOs to national institutions such as genetic resources units, breeding programs, botanical gardens, herbaria, university departments and private seed and breeding companies. Many countries are developing national programs to promote co-ordination among the various groups.

In an attempt to rationalize and backstop national efforts, international and regional research institutes provide a range of services. These include making their genebank facilities available to national programs (e.g. for housing duplicate samples); providing training and information, and safeguarding and disseminating the genetic materials held within their genebanks.

The Food and Agriculture Organization (FAO) of the United Nations is organizing an international network of *ex-situ* collections in an attempt to provide an action framework for plant genetic resources globally. Guidelines have been prepared, or are under preparation, for genebank management, including the safe movement of germplasm. In June 1996, FAO will host the International Technical Conference for Plant Genetic Resources for Food and Agriculture. It is planned that this ministerial-level meeting, to be held in Leipzig, Germany, will adopt a Global Plan of Action for plant genetic resources, which will contain a new framework for action, and a set of costed activities that the international community considers to be of high priority.

The international policy framework for plant genetic resources is in a state of flux. New policies relating to access to genetic resources and the equitable sharing of the benefits arising from their use are being discussed in a number of different fora. The

original international instrument covering plant genetic resources, the International Undertaking on Plant Genetic Resources, is now being re-negotiated to bring it in line with the new, legally binding, Convention on Biological Diversity. The issue of how to implement farmers' rights is also under discussion within the context of the revision of the International Undertaking. From the very open, essentially unregulated system of the past, the Convention's recognition of national sovereign rights over genetic resources requires governments to accept responsibility to conserve genetic resources, and at the same time to adopt measures to ensure access to them on mutually agreed terms, including provisions for sharing benefits. At the same time, the Multilateral Trade Agreement requires that countries adopt measures to recognize intellectual property on plant varieties, either through patents or a *sui generis* system such as plant breeders' rights. It is expected that there will continue to be considerable debate on these issues before agreement is reached internationally.

The world's largest international effort to conserve and use agricultural biodiversity is being undertaken by the international agricultural research centers of the Consultative Group on International Agricultural Research (CGIAR). Of the 16 centers located around the world, 12 have genebanks that collectively house germplasm collections comprising a total of about 500,000 accessions, mainly of major food crops and their relatives. In 1994, the centers signed agreements with FAO recognizing that these collections are not center assets but that the cen-ters act as trustees of the material on behalf of the international community. By signing the agreements, the 12 centres became the first formal members of the FAO International Network.

Also in 1994, the CGIAR established the Systemwide Program on Genetic Resources (SGRP) to co-ordinate action among the centres and with national programs and international organizations outside the system. The SGRP promotes collaborative research, training and other activities on both *in-situ* and *ex-situ* conservation. It is sponsoring an in-depth external review of all its genebank operations and is establishing the Systemwide Information Network on Genetic Resources (SINGER), which will provide access to data on all the holdings via Internet and through other electronic means.

Plant breeders worldwide have traditionally looked to genebanks as a source of novel genetic variation to use in their crop improvement work. As repositories of major collections, international genebanks have played, and continue to play, a leading role in the supply of this diversity. Through the activities of genetic-resources programs, the genetic variation contained within landraces and farmers' varieties is being tapped for use in the development of new varieties.

Whereas modern varieties may be widely adopted by farmers, especially in the more favored environments, this is not uniformly the case throughout the world. The formal plant-breeding sector may be unable, for technical and economic reasons, to develop varieties of crops that are adapted to the often harsh and highly variable conditions that prevail in the more marginal environments, which are widespread in many developing countries. In order to provide greater support to farmers living under such conditions, genebanks should reassess their role. In addition to serving the needs of formal-sector breeders, genebanks could provide greater support directly to the farming communities through such actions as providing a safe repository for local landraces, under conditions that ensure continued access by local communities; through providing locally-adapted materials originating in similar agroecological conditions for direct testing and selection by farming communities; and through providing advice and information, both to the communities themselves and back to the plant breeders working in the formal sector. In this way, genebanks could become not just a system for conserving genetic diversity for use by present and future plant breeders, but also significant actors in support of communities' efforts to develop their own improved crops and agricultural systems.

Chickpea Breeding in Italy

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Summary

The first Italian chickpea-breeding program started in 1982, as a collaborative effort by ENEA (Italian Agency for Renewable Energy, Rome), the Experimental Station for Wheat Culture (Caltagirone, Catania) and the Experimental Institute for Plant Pathology (Rome). During the succeeding years, the activities expanded and were divided into two projects. One, involving ICARDA (International Center for Agricultural Research in the Dry Areas) and five Italian research institutions (ICARDA/Italy project 1988–1994), was funded by the Italian Foreign Ministry. The other, involving Spain, Italy and England (ENEA/ECLAIR project AGRE CT 90–0051/1991–1995), was funded by the European Union. These breeding programs aimed at developing cultivars and lines characterized by high-yield potential; satisfactory cold tolerance; resistance to *Ascochyta rabiei* for winter planting; drought tolerance, and resistance to *Fusarium oxysporum* f.sp. *ciceri* for spring sowing. The experiments also considered erect plant habit, which facilitates mechanical harvesting, and high quality grain.

Germplasm from ICARDA, ICRISAT, and the Spanish seed company KOIPESOL, as well as Italian ecotypes collected by ENEA and by the Germplasm Institute of Bari, were evaluated and used.

Two ideotypes suited for winter and spring sowing, were identified. Further genetic variability was generated through intra- and interspecific hybridizations. The segregating progenies were grown according to the pedigree method (ICARDA/Italy project), and by a modified bulk method (ENEA/ECLAIR project). In this last case, two generations per year, one in the southern hemisphere, were grown. Agronomic assessment of advanced lines was carried out with replicated field trials at different Italian locations. The incidence of ascochyta blight and fusarium wilt on all the genotypes at both the vegetative and reproductive stages was recorded, either in the open field or in the greenhouse. Germplasm accessions and advanced lines were also tested for cold tolerance by laboratory and field methodologies.

New lines of chickpea are being developed through the hybridization programs. Several interspecific crosses have also been tried, but fertile offspring were obtained only from the combinations *Cicer arietinum* x *C. reticulatum*; *C. arietinum* x *C. echinospermum*, and *C. echinospermum* x *C. reticulatum*. Karyotype studies of *Cicer* wild species and hystological investigations on pollinated flowers were also performed.

In 1990, four cultivars were released: Sultano and Califfo, adapted to winter sowing because of their resistance to ascochyta blight and cold tolerance; and Calia and Principe, suitable for spring sowing and drought tolerant. In particular, Sultano is characterized by good seed quality for industrial use, but its small grain size has reduced its market acceptance. Within the ICARDA/Italy and ENEA/ECLAIR projects, new selections from ICARDA nurseries produced the new cultivars Pascià, Otello, Emiro, Visir, and Alì, which were registered and added to Italy's variety list. These varieties are resistant to ascochyta blight, cold tolerant, and outyield Sultano and Califfo. Pascià and Visir have large grains and are potentially popular with the consumers, while the other varieties are of interest as animal feed.

Estimates of the economic returns at the farm and country level from the introduction of these new chickpea cultivars have been reported.

Studies are also needed to develop new chickpea food products through the application of appropriate processing technologies.

Introduction

Chickpea (*Cicer arietinum* L.) is a grain legume well adapted to the pedoclimatic conditions of central and southern Italy. It represents an alternative to the prevailing monoculture of durum wheat in these areas, because of its high nitrogen-fixing capability; high protein content, and variety of uses as food and feed. The last 50 years have seen a drastic reduction in chickpea cultivation in Italy, from about 140,000 ha to the present 4000 ha (FAO Yearbook 1993). Several factors have caused this decline, (1) the poor grain yield (1.1 t/ha; FAO Yearbook 1993) of the traditional spring-sown ecotypes; (2) the reduction in consumption of pulses as a result of changing economic and social standards; (3) the lack of varieties adapted to the climate in southern Italy; and (4) the cultivated material's high susceptibility to several pathogens, mainly *Ascochyta rabiei* (Pass.) Labr., which cause severe damage to the crop.

Compared to the traditionally spring-sown material, a significant increase in grain yield may be achieved by winter sowing (Saxena 1980, 1990). In this case, however, chickpea is seriously hampered by its high susceptibility to ascochyta blight (Singh 1993) and poor tolerance to cold (Singh et al. 1993). In many countries where the crop is traditionally spring-sown, new varieties adapted to winter sowing have been released (Singh, 1987; SAT, 1992; ICRISAT, 1994). The productivity of spring-sown chickpeas has also been improved by breeding for drought tolerance (Saxena et al. 1993; Rahangdale et al. 1994; Gowda et al. 1995).

Following the pioneer work of ICARDA (Singh et al. 1983), chickpea-breeding programs have been initiated through national

Table 1. Italian research programs on chickpea.

Project title	Partners	Funding institution	Years
New protein sources	Italian Institutions	CNR (IPRA)	1982–1986
Improvement of Italian chickpea crop.	ENEA; Experimental Institute of Plant Pathology, Rome; Experimental Station for Wheat Culture, Caltagirone, Catania	ENEA	1982–1987
Quantitative and qualitative improvement of food legumes (faba bean, chickpea and lupin).	Experimental institutes of Italian Agricultural Ministry; Italian universities, CNR; ENEA	Italian Agriculture Ministry	1987–1993
Development of chickpea germplasm with combined resistance to ascochyta blight and fusarium wilt using wild and cultivated species.	ICARDA; universities of Viterbo and Naples; ENEA; Experimental Institute of Plant Pathology; Experimental Station for the Wheat Culture	Italian Foreign Ministry	1988–1994
Development of chickpea germplasm, which is resistant to ascochyta blight and fusarium wilt, and is therefore suitable for winter-sowing in southern Europe as an alternative to cereal grains.	KOIPESOL, Semillas, Spain; ENEA, University of Cordoba, Spain; Centro de Investigaçion y Desarrollo Agrario, Spain; University of London, UK	European Union (ENEA/ECLAIR project AGRE CT 90-0051)	1991–1995

and international projects (Table 1), at several Italian research institutions, with the objectives of:

- evaluating germplasm and identifying ideotypes adapted to Italian conditions
- developing new genetic variability through intra- and interspecific hybridizations as well as mutagenesis and biotechnological approaches
- releasing new cultivars.

The activities referred to here were carried out within the ENEA, ICARDA/Italy and ENEA/ECLAIR projects. The ENEA project aimed at defining the ideotypes adapted to Italian conditions and at developing the first Italian varieties of chickpea. The strategy adopted (fig. 1) was based on the evaluation of exotic and local germplasm, the characterization of Italian isolates of *A. rabiei*, and the development of agronomic practices, such as, sowing time and density, weed control, *Rhizobium* inoculation, and rotations. Investigations of the technological characteristics of the grain have also been performed to differentiate the genetic material for human consumption from that suited to animal feeding.

The ICARDA/Italy project used eight wild annual species of *Cicer* to develop new germplasm with combined resistance *to A. rabiei, F. oxysporum* and cold. In addition, karyotype analyses and histological investigations were conducted to understand the bases of incompatibility barriers among the *Cicer* spp. Studies of the interaction between *A. rabiei* and chickpea were also undertaken to elucidate the mechanisms of resistance. The relative strategy is described in fig. 2.

The ENEA/ECLAIR project involved the identification of useful germplasm; the combination of genetic resources by intraspecific hybridizations; agronomic assessment of advanced lines, and development of winter varieties combining high yield, possibly large grain, and resistance to *A. rabiei*. In order to speed up the process of homozygosis, two generations per year were run: one in Italy and the other in the southern hemisphere. The strategy adopted is described in fig. 3.

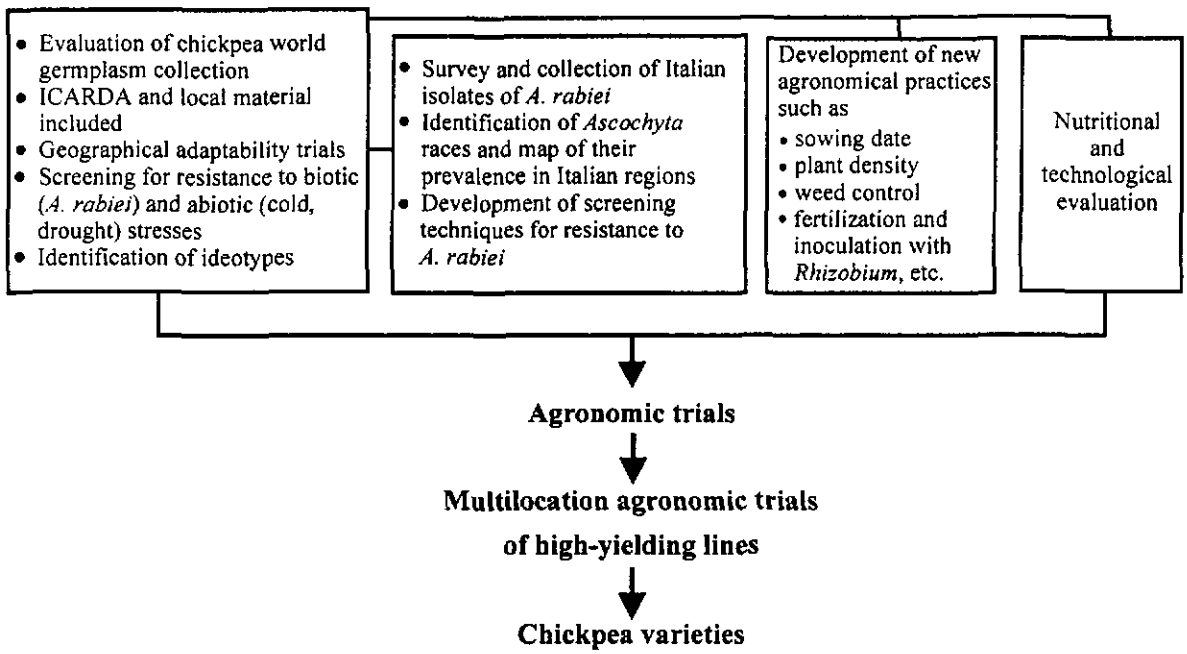

Fig. 1. Chickpea improvement in Italy: strategy adopted by ENEA project (1982–1987)

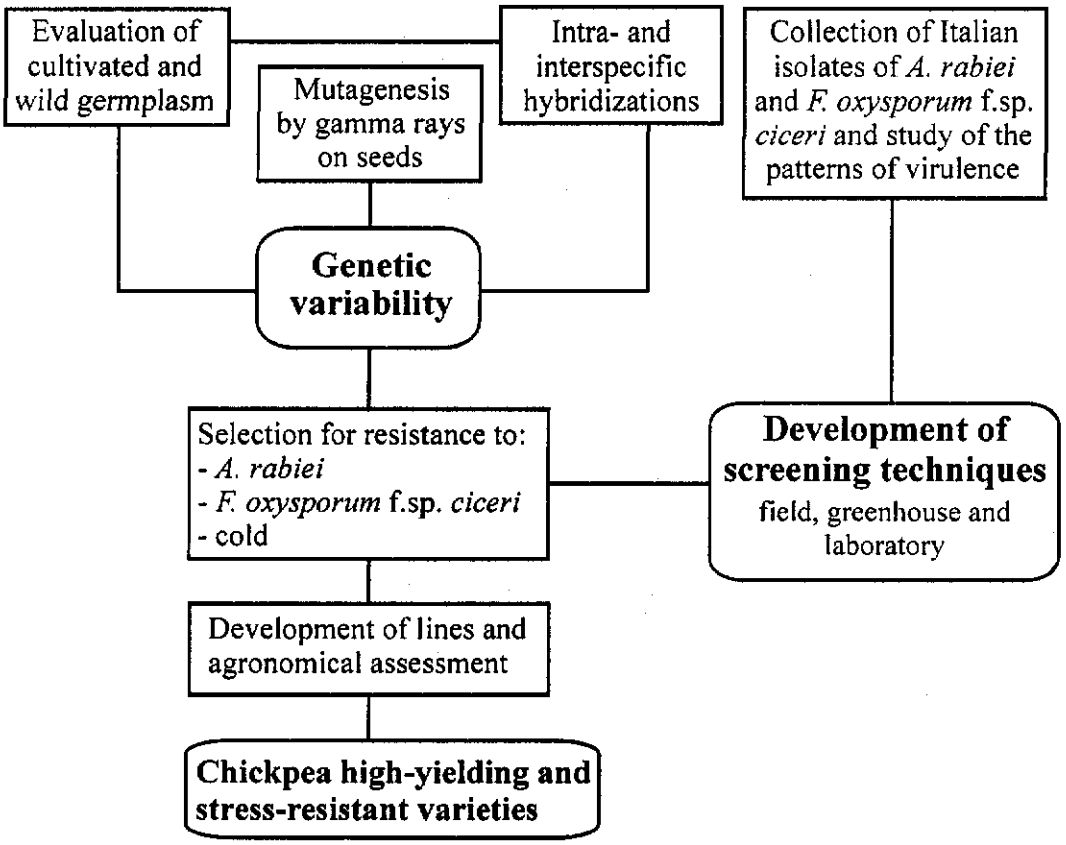

Fig. 2. Chickpea improvement in Italy: strategy adopted by the ICARDA/Italian Institutions project (1988–1994)

Germplasm evaluation and identification of ideotypes adapted to the Italian environment

ENEA project

In Italy, chickpea is almost exclusively cultivated as an early spring-sown crop (sowing at the end of February or at the beginning of March and harvesting in July), because in this period the temperature starts rise and the winter rain ceases. Sowing to chickpea in November or early December allows the physiological maturity of the plant to be reached in June/July, after a longer biological cycle. The plant thus profits from the moisture accumulated in the winter, which provides conditions, which favor high yields (Saxena 1980). In this respect, results obtained within the ENEA project showed that (1) chickpea genotypes can survive winter conditions even when the temperatures fall to -12°C, (2) winter-sown ascochyta-resistant materials on average yield twice as much as the susceptible and spring-sown genotypes; (3) under Italian conditions, the yield of winter-sown chickpea can reach 3 t/ha (Saccardo and Calcagno 1990; Crinò et al. 1992). Furthermore, a large variability for yield components and disease resistance was observed in a wide collection of native and foreign germplasm. A total of 2131 entries were assessed, under both winter and spring sowing, and at different locations in central and southern Italy. Germplasm resources included material from ICARDA and ICRISAT collections as well as Italian ecotypes. Twenty-one genotypes were identified as interesting for central and southern Italy. Some of these, characterized by high yield and erect plant standing, cold tolerance and resistance to ascochyta blight, were adapted to winter sowing and corresponded to a 'winter ideotype.' Ascochyta blight represented the most serious disease of winter-sown chickpea (Porta-Puglia and Crinò 1993).

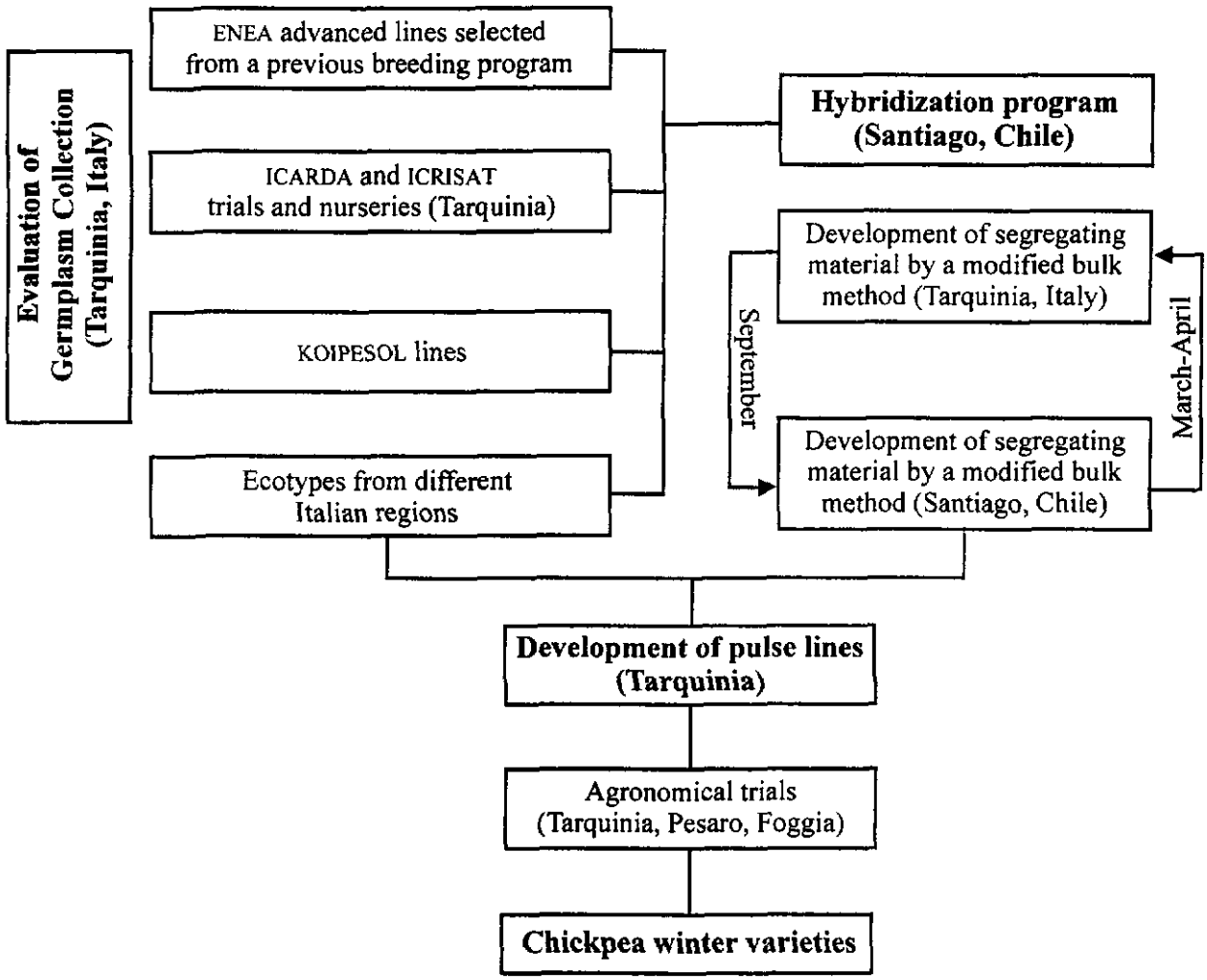

Fig. 3. Chickpea improvement in Italy: strategy adopted by the ENEA/ECLAIR AGRE CT 90–0051 project (1991–1995)

However, even in the presence of a devastating attack of the blight, high levels of resistance to *A. rabiei* were recorded. Italian ecotypes, analyzed in different locations and years, were highly susceptible to the blight.

Cold tolerance is also an important component of adaptation of the crop to winter-sowing conditions. Cold tolerance was identified in 27 selections from ICARDA accessions, after screening winter-sown material, in localities where, in January and February, the temperature normally falls below zero (-10 to -11°C) (Tucci et al. in these proceedings).

Other genotypes, including some Italian ecotypes, showed good adaptability to spring sowing and had all the prerequisites of a 'spring ideotype.' Resistance to *F. oxysporum* f.sp. *ciceri* and drought tolerance are important factors for this material. The spring ideotype presents a semi-prostrate habit with several lateral branches, a deep root system and a large number of seeds, all characteristics associated with drought tolerance. Spring-sown chickpeas usually present a high photo-respiration rate, because of the high temperatures occurring during vegetative growth, which negatively influences the crop yield. Nevertheless, it was possible to select genotypes adapted to spring planting, which maintained a high yield potential and presented distinct traits—like earliness, a high number of fertile branches in the basal part of the plant, and a good grain quality.

The main characteristics of both winter and spring ideotypes adapted to the Italian environment are summarized as follows:

Winter Sowing

- Long vegetative cycle: 150–180 days
- Resistance to *A. rabiei*
- Cold tolerance
- Erect plant habit
- Apical position of the pods

Spring Sowing

- Short vegetative cycle: 100–120 days
- Resistance to *A. rabiei* and *F. oxysporum*
- Drought tolerance
- High number of fertile branches in the basal part of the plant
- Good grain quality

Among the genetic resources evaluated within the ENEA project, five ICARDA accessions (ILC 72, ILC 482, ILC 484, ILC 515, and ILC 3279) were selected as particularly adapted to winter sowing. These lines present all the agronomic and physiological characteristics of the corresponding winter ideotype. They are resistant to *A. rabiei* and cold tolerant. Under conditions of ascochyta attack and cold, the lines ILC 3279 and ILC 72 gave acceptable yields (> 2 t/ha) and a plant survival rate of about 70%, which was higher than that observed for the Italian ecotypes (5–20%). Two high-yielding Sicilian ecotypes, Calia and Principe, corresponding to the spring ideotype, were identified.

Plant progenies were derived from ILC 72, ILC 3279, Calia and Principe and evaluated at different sowing dates. ILC 3279 selections, presenting the characters of the winter ideotype, gave the maximum grain yield when the sowing was advanced to December, while selections from Calia, with the traits of the spring ideotype, expressed the highest potential when sown in February (Saccardo and Calcagno 1990). Increasing the sowing density from 24 to 80 plants/m^2 improved seed yield up to a density of 40–45 plants/m^2. Furthermore, high plant density of the tall and erect plant types, typical of ILC 3279, allows them to successfully compete with weeds. The small, semi-prostrate growth habit of the plant progenies obtained from the ecotype Calia, showed an optimal plant density of 25–30 plants/m^2 (Saccardo and Calcagno 1990).

Development of new genetic variability

Intraspecific hybridizations

ICARDA/Italy Project, and ENEA/ECLAIR Project

Annually, beginning in 1983, F_3 and F_4 progenies from ICARDA nurseries were grown, analyzed and selected in central and southern Italy for agronomical traits and resistance to ascochyta blight and cold tolerance. In both locations, a high variation for plant height (35–78 cm), plant productivity (6–55 g) and harvest index (0.32–0.68) was observed (Saccardo et al. 1987). Some progenies, well adapted to winter-sowing conditions, proved to be high yielding and resistant to stresses. A few advanced F_9 and F_{10} lines, selected for resistance to *A. rabiei* and cold tolerance in central Italy, and to drought and sometimes to *A. rabiei* in southern Italy, outyielded the other experimental lines and controls. The different yield responses under dry and humid conditions (as found in Caltagirone and Foggia in south Italy, and Tarquinia in central Italy, respectively) emphasizes the availability of lines adapted to specific environments. A comparison of the grain yield showed that lines identified in Tarquinia realized their high productivity potential only in central Italy, where the selection was done, whereas the lines selected under the dry conditions of southern Italy, in particular C 145, C 149 and C 153, reacted well also to the humid conditions of Tarquinia (fig. 4). Within the ENEA/ECLAIR project, new genetic resources were acquired to widen the ENEA germplasm collection. The new cycle of selection confirmed the validity of ICARDA lines ILC 3279 and ILC 72, and allowed the identification of new lines, like 27117-6, FLIP 84-92C and ICC 3996, as sources of resistance to ascochyta blight in several cross-combinations. Moreover lines were identified for the character large grain, which is particularly requested for direct human consumption as well as for agro-industry several genotypes have also been identified among Italian ecotypes and ICARDA screening nurseries and have been used as parents in the hybridization programs.

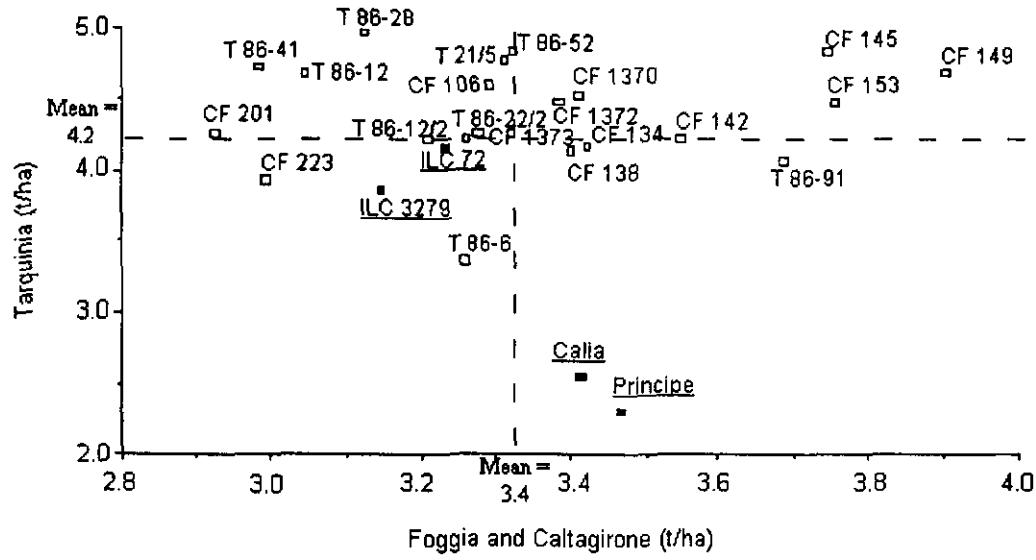

Fig. 4. Grain yield of chickpea lines selected under humid (Tarquinia, central Italy) and dry (Caltagirone and Foggia, southern Italy) conditions and agronomically assessed in both environments (T = Tarquinia; CF = Caltagirone and Foggia)

Among 208 Italian ecotypes evaluated, those coming from the regions of Marche and Abruzzo showed the highest grain yield and, together with the Sicilian ecotypes, revealed also the largest grain weight (100-seed weight > 50 g). Only four ecotypes survived a severe attack of ascochta blight in Pesaro during 1991–92.

A total of 203 cross-combinations were accomplished using parents resistant to ascochyta blight and others presenting interesting agronomical traits (Table 2). Particular care was taken to combine ascochyta resistance and large seed. In planning the hybridization program, advantage has also been taken of the large-seeded lines provided by KOIPESOL. Breeding material was handled according to a modified bulk in which, in each generation, seeds from the most promising plants were utilized to make the new bulk. Two generations per year were grown: one in Chile, where climatic conditions are not favorable to the development of *A. rabiei*, and the second in Italy, under late spring sowing.

Seed generation, number of bulks and selections of individual plant progenies harvested at the end of the ENEA/ECLAIR project are given in Table 3. Because of the two generations per year, F_6 and F_7 progenies originated from the hybridizations accomplished in 1991/92 and 1992/93, were raised and F_7 and F_8 seeds were harvested.

Table 2. Breeding objectives and successful cross-combinations accomplished during the four years of ENEA/ECLAIR project.

Objectives	Cross-combinations				
	1991/92	1992/93	1993/94	1994/95	Total
Resistance to both ascochyta blight and fusarium wilt	2	0	0	0	2
Large seed, resistance to ascochyta blight and erect plant architecture	1	19	4	10	45
High yield and resistance to ascochyta blight	41	14	9	14	78
New genetic potential for large seed	8	47	18	0	73
Combining genetic sources of resistance to ascochyta blight	0	5	0	0	5
Total	63	85	31	24	203

Table 3. Chickpea breeding material obtained within ENEA/ECLAIR PROJECT AGRE CT 90–0051.

Seed generation	Provenance	Bulks (No.)	Plant-progenies (No.)
F_2	ENEA hybridizations	10	-
F_4	ENEA hybridizations	27	-
F_5	ICARDA nursery CIF4N-SL-95	-	7
F_6	ICARDA nurseries CIF4N-MR-94	-	21
	and CISN-LA-95	-	21
	ENEA hybridizations	66	-
F_8	ICARDA nursery CIYT-LA-95	-	5
	ENEA hybridizations	57	-
		-	1,200
F_9	ENEA hybridizations	-	6
Total		160	1,260

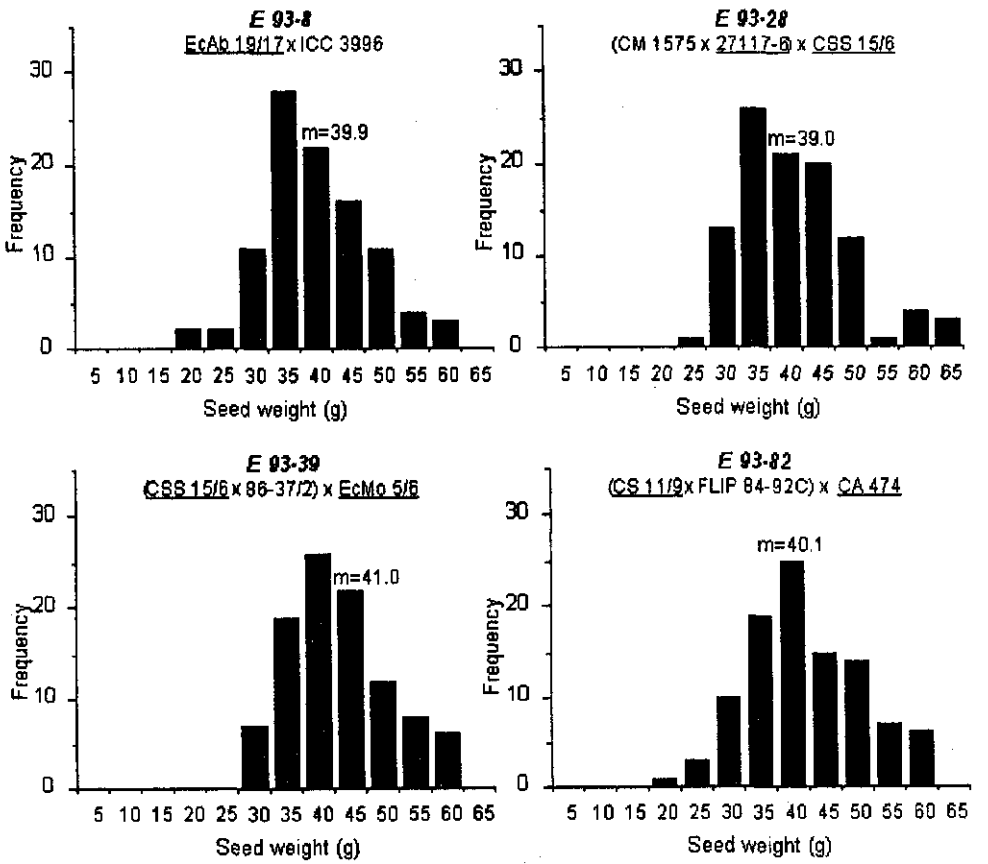

Fig. 5. Frequency distributions of 100 seed weight in some F_6 bulks harvested during 1994/95 within ENEA/ECLAIR project AGRE CT 90–0051 (parents with 100-seed weight > 45 g are underlined)

Table 4. Reaction to ascochyta blight, fusarium wilt and cold in wild accessions of annual *Cicer* spp. (ICARDA/Italy project).

Cicer sp.	No. accessions *A. rabiei* R	*A. rabiei* S	*F. oxysporum* R	*F. oxysporum* S	Cold R	Cold S	Accessions with resistance to multiple stresses†
C. reticulatum	3	16	7	16	7	12	ILWC 21-21, ILWC 21-31
C. echinospermum	2	2	1	3	0	0	-
C. bijugum	5	12	22	1	5	9	ILWC 7/7-12, ILWC 8/S-3 ILWC 34/8-1
C. judaicum	16	2	9	16	2	28	-
C. pinnatifidum	8	12	5	20	1	21	-
C. yamashitae	0	0	0	2	0	1	-

R=resistant; S=susceptible

† 2 to at least stresses

The analysis of the average seed weight of F_4, F_6 and F_8 bulks, harvested in Tarquinia in 1995, indicated that progenies with large seeds (> 45 g) have been obtained. An example of the variability observed for seed weight, within individual bulks, is given in fig. 5, where 100 seed weights as small as 20–25 g and higher than 60 g are present. These bulks have been derived from cross-combinations involving parents resistant to *A. rabiei* (ICC 3996, 27117-6, 86-37/2, FLIP 84-92C) and donors of large grain. The resistance to ascochyta blight of the large-seeded progenies remains to be tested.

Interspecific hybridization

ICARDA/Italy project

By artificial inoculations with *A. rabiei* and *F. oxysporum* f.sp. *ciceri* as well as by cold treatment, new sources of resistance/tolerance were identified in *C. reticulatum*, *C. judaicum*, *C. bijugum*, and *C. pinnatifidum*. Two accessions of *C. reticulatum* and three of *C. bijugum* also revealed resistance to multiple stresses, at least to *A. rabiei* and cold (Table 4). Some accessions of these species showed levels of resistance to ascochyta blight higher than those identified in the cultigen (Crinò et al. in these proceedings). Haware et al. (1992) and Kaiser et al. (1994) obtained similar results.

Screenings for resistance were performed in both greenhouse and field using Italian isolates of *A. rabiei* and *F. oxysporum* f.sp. *ciceri*. New screening techniques for resistance to ascochyta blight and fusarium wilt (Crinò et al. in these proceedings) and for tolerance to cold (Tucci et al. in these proceedings) were developed. Karyotype relationship among *Cicer* spp. were established by computerized image analysis (Venora et al. 1991). The eight wild annual species were divided into two non-intercrossable groups: one comprising *C. arietinum*, *C. reticulatum*, and *C. echinospermum*, and the other involving the remaining five species (Ocampo et al. 1992).

To combine resistance to different stresses

Table 5. Interspecific hybridizations performed among *C. arietinum* and wild annual species of *Cicer* (ICARDA/Italy project).

Cross combination	Pollinations	Seeds obtained	Hybrid plants
C. arietinum x *C. reticulatum*	160	50	22
C. reticulatum x *C. arietinum*	56	6	3
C. arietinum x *C. echinospermum*	130	43	23
C. echinospermum x *C. arietinum*	45	6	2
C. arietinum x *C. bijugum*	560	0	0

in the same genetic background of *C. arietinum*, several interspecific hybridizations were attempted (Table 5) but fertile offspring were obtained only from the cross-combinations *C. arietinum* x *C. reticulatum*, *C. arietinum* x *C. echinospermum* and reciprocals (Singh and Ocampo 1993; Mosconi et al. 1995), confirming the findings of karyotype analyses.

Post-zygotic incompatibility barriers between *C. arietinum* and *C. bijugum* have been observed by fluorimetric (Bergamini and Mulcehj 1987) and histological analyses of pollinated flowers. Foreign pollen, germinated on the stigma, grew down the style and reached the ovule. The hybrid embryos collapsed 6–8 days after fertilization (Mosconi et al. 1995).

Twenty-four and 48 fertilized ovaries from the cross-combinations *C. arietinum* x *C. bijugum* and *C. arietinum* x *C. judaicum* were cultured *in vitro* on the medium proposed by Agnihotri (1993). After 30 days of culture, the ovaries developed. This indicated that *in-vitro* culture may represent a promising technique to overcome incompatibility barriers among *Cicer* species (Mosconi et al. 1995).

The crosses *C. arietinum* x *C. reticulatum* were performed to transfer cold tolerance into the cultigen, and F_3 and F_4 progenies have already been obtained.

Mutagenesis and biotechnological approach (ICARDA/Italy project)

In order to induce sources of resistance against particularly aggressive Italian isolates of *A. rabiei* (Porta-Puglia et al. in these proceedings), dry seeds of the susceptible cultivar Calia were irradiated with 200 Gy of gamma rays. In M_2, the appearance of chlorophyll (albina, xantha, chlorina) and morphological mutants confirmed the effectiveness of gamma irradiation.

A total of 25,000 M_2 plants were artificially inoculated at the plantlet stage in the greenhouse. Repeated cycles of artificial inoculation were applied on M_3–M_5. Eighteen M_5 progenies could be selected because they showed mild disease severity (Saccardo et al. 1993). After a further inoculation performed in the greenhouse with $5x10^5$ spores/ml sprayed on M_6 adult plants (podding stage), two of these progenies showed resistance to ascochyta blight at both the plantlet and the podding stages.

As for other grain legumes, biotechnological methods seem promising (Udupa et al. 1993; Barn and Wakhlu 1994; Eapen and George 1994; Suhasini et al. 1994; Sharma et al. 1995) and are being used for genetic transformation (Islam et al. 1994; Chowrira et al. 1995). *In vitro* regeneration from mature embryos has been induced by thidiazuron. This technique has been applied in genetic transformation experiments with encouraging preliminary results (Mosconi et al. 1995; Santangelo et al. in these proceedings).

Release of New Varieties

ENEA Project

After five years of agronomic assessment and evaluation of ascochyta resistance and cold tolerance of chickpea germplasm under winter-sowing conditions in central and southern Italy, ILC 3279 (from the former USSR) and ILC 72 (from Spain) were selected as high yielding, cold tolerant, and ascochyta-resistant genotypes. The best plant progeny among those raised from these genotypes, was submitted to the official trials of the Italian Ministry for Agriculture, Food and Forest Resources and, in 1990, Sultano, from ILC 3279, and Califfo, from ILC 72, were jointly released by ENEA and the Experimental Station for Wheat Culture. Because of their encouraging yield levels, the good degree of resistance to ascochyta blight, and their adaptability to mechanical harvesting, these varieties are the first contribution to the improvement of chickpea in Italy (Calcagno et al. 1988; Crinò et al. 1992; Saccardo and Crinò 1993). The yield of Sultano and Califfo is not reduced by ascochyta attacks, even in the presence of high disease severity. The varieties Principe and Calia, susceptible to *A. rabiei* and cold and therefore recommended for spring sowing, were obtained as plant selections from the homonymous Sicilian ecotypes and released in 1990. The origin and the characteristics of the four varieties are summarized in Tables 6 and 7.

Table 6. Origin of ascochyta-resistant varieties of chickpea released or requested for release in Italy.

Varieties	ICARDA line of provenance	Origin of the germplasm	Pedigree	No. of patent
Sultano	ILC 3279	ex-USSR	-	70 NV/93
Califfo	ILC 72	Spain	-	69 NV/93
Principe	-	Italy	Sicilian ecotype	71 NV/93
Calia	-	Italy	Sicilian ecotype	68 NV/93
Pascià†	FLIP 86-5C	ICARDA CIYT-L	ILC 202 x ILC 3355	-
Otello†	FLIP 86-21C	ICARDA CIABN	ILC 136 x (ILC 202 x ILC 893)	-
Visir†	FLIP 83-7C	ICARDA CIABN	(ILC 480 x ILC 72) x ILC 263	-
Alì‡	ILC 6188	USSR	-	-
Emiro†	cross no. X81 TH85	ICARDA CIF3N	ILC 72 x ILC 191	-

†*Inscription to the National Variety List requested by* ENEA
‡Inscription to the National Variety List requested by University of Naples
CIYT-L: Chickpea International Yield Trial - Large Seed
CIABN: Chickpea International Ascochyta Blight Nursery

ICARDA/Italy project

The varieties Visir and Alì were obtained through selection, by the pedigree method, within the ICARDA accessions FLIP 83-7C and ILC 6188. Selection was applied for resistance to ascochyta blight and tolerance to cold in different Italian localities and years. Cold tolerance of Visir and Alì was also maintained in the field when the temperatures fell below zero (Tucci et al. in these proceedings). Laboratory tests demonstrated the occurrence of different mechanisms of cold tolerance in both varieties. Alì survives cold only if subjected to an acclimatization period of 15 days at +2°C; Visir shows cold tolerance without this pre-treatment. The level of cold tolerance of Visir is similar to that observed for cultivar Sultano (Tucci et al. in these proceedings). The grain yield of both varieties, in various environments and years, is not too different from that recorded for the control variety Sultano (+3% +13%).

Table 7. Main traits of Italian chickpea varieties released or requested for release (averages of four year agronomical trials in three localities).

Variety	Sowing time	Plant height (cm)	Plant habit	Reaction to *A. rabiei*	Plants survived to cold in the field* (%)	Grain yield (t/ha)	100-seed weight (g)	Seed type
ENEA and Stazione Sperimentale di Granicoltura per la Sicilia								
Sultano	Winter	75	Erect	Resistant	80	3.0	36	Light, smooth
Califfo	Winter	70	Erect	Resistant	N.D.	3.1	37	Light, smooth
Principe	Spring	52	Semi-prostrate	Susceptible	40	1.6	51	Light, rough
Calia	Spring	50	Semi-prostrate	Susceptible	N.D.	1.7	37	Light, smooth
ENEA								
Pascià	Winter	65	Semi-erect	Resistant	N.D.	2.8	58	Light, rough
Otello	Winter	56	Erect	Resistant	N.D.	3.5	32	Black, smooth
Emiro	Winter	63	Erect	Resistant	N.D.	3.9	33	Light, rough
University of Naples								
Visir	Winter	60	Semi-erect	Resistant	90	3.1	52	Light, rough
Alì	Winter	50	Erect	Resistant	70	3.4	36	Light, smooth

N.D.=not determined

However, a remarkable improvement of yield (+94%) was observed for the large-seeded (100-seed weight: 52 g) and ascochyta resistant Visir, in comparison to the ascochyta-susceptible and large-seeded cultivar Principe. The origin and agronomic performances of both varieties are reported in Tables 6 and 7.

ENEA/ECLAIR Project

A wide variation in yield performance has been observed among experimental lines agronomicaly assessed in different localities (Tarquinia, Pesaro and Foggia). The highest expressions of grain yield were associated with relatively small grains even though, compared to the high-yielding check varieties Sultano and Califfo (100-seed weight: 36–37 g), several lines were more productive and presented a 100-seed weight higher than 45 g. Some of these high-yielding lines proved to possess a broad adaptation in different environments and years.

A line selected within the ICARDA cross-combination ILC 202 x ILC 3355, and submitted for registration in January 1995, presented large grain (100 seed weight: 58 g) and good resistance to ascochyta blight together with an interesting yield potential similar to cultivar Sultano (-7%). This line is being registered as winter variety Pascià. Compared to the large-seeded variety Principe (100-seed weight: 51 g), Pascià represents a real breakthrough (yield +75%) because cultivar Principe is particularly susceptible to ascochyta blight, which prevents its cultivation under winter sowing. A second line, selected from the ICARDA cross-combination ILC 136 x (ILC 202 x ILC 893), was also submitted for registration in January 1995 and named Otello. This variety, characterized by high yield (+15% with respect to cultivar Sultano), good ascochyta resistance, relatively small and black grain, was developed as suitable for the feed industry. Otello is particularly adapted to southern Italy because it can be cultivated early.

A further line has been identified through many years of agronomic assessment. Compared to the cultivars Sultano and Califfo, it has a higher yield potential (+30% in respect to cultivar Sultano) and a somewhat higher resistance to *A. rabiei*. Because of its small grain, it is particularly suited for animal feed. This line has been obtained from an ICARDA CIF3SN and in January 1996 it was submitted for registration to the National List of Italian Ministry for Agriculture, Food and Forest Resources as a new variety named Emiro. The origin and characteristics of the new Italian varieties are reported in Tables 6 and 7.

Conclusions and Perspective

The existence of an Italian interdisciplinary group working since 1982 on chickpea has allowed the development of seven winter and two spring varieties, which could permit an increase in the area devoted to this crop, particularly if the traditional spring-sown materials are replaced by the new winter varieties. The new varieties, which are resistant to ascochyta blight and cold tolerant, have shown a yield potential higher than 2.5 t/ha, compared to the national average of 1.1 t/ha (FAO Year Book, 1993). The new varieties: the small grained Otello, Alì, Emiro, and the large-seeded Pascià and Visir, represent a real alternative to the cultivation of durum wheat in rainfed areas of central and southern Italy. In particular, the large grain of Pascià and Visir, by meeting the tastes of Italian consumers, might in a few years, replace the import of large-grained chickpea. Current Italian chickpea production only accounts for 15% of national consumption, the rest (22.391 t; FAO data 1993) comes from imports of large-seeded varieties, mainly from Turkey and Mexico.

Although the winter-sown varieties allow the doubling of grain yield compared to the spring-sown material, the latter might continue to be the only possible source for chickpea cultivation in some localities.

By intra- and interspecific hybridizations, a wide and new genetic variability for use in breeding programs has been obtained. Interesting sources of resistance to *A. rabiei*, *F. oxysporum* f.sp. *ciceri* and cold tolerance, sometimes combined in the same genotype for at least two stresses, have been found in accessions of wild *Cicer* ssp. The existence of particularly aggressive Italian isolates of *A. rabiei* emphasizes the need for the sources of resistance present in the wild species to be

transferred into *C. arietinum*, if incompatibility barriers can be overcome. The preliminary results of *in-vitro* regeneration and genetic transformation of chickpea open the possibility to transfer exogenous genes into the cultivated material.

Although in the last 15 years relevant results have been achieved in the development of genetic material that is cold tolerant and resistant to ascochyta blight, new efforts have to be devoted to developing methodologies for a thorough characterization of pathogen races with respect to all the traits of agronomic importance. Interdisciplinary approaches should also be strengthened in order to release cultivars capable of coping with biotic and abiotic stresses.

Demonstration activities are to be promoted to show farmers the potential of the new chickpea varieties and innovative agronomic practices including the introduction of chickpea in rotations with cereals. It is also important to encourage, by offering some incentives, the industries of central and southern Italy to use chickpea grain not only for human consumption but also for animal feed. Yield stability still remains one of the most important goals to be reached by chickpea breeding and agronomic research.

Estimates of economic return at farm and country level from the cultivation of newly-released chickpea cultivars

Farm value

The area under chickpea cultivation in central and southern Italy roughly overlaps that of durum wheat and other cereals. In rotations, chickpea's place is before or after cereals, which also represent the standard of reference when measuring crop competitiveness.

The comparative gross and net incomes from cultivation of durum wheat and modern and traditional chickpea varieties, respectively, are reported in Table 8.

To the extent that the above assumptions about productivity and prices will be true in the future, the introduction of modern chickpea cultivars in the agriculture of southern Italy may be regarded as a valid rotation alternative to durum wheat.

Country value

As reported in Table 9, Italian chickpea production only accounts for 15.3% of the country's needs, the rest being covered by imports, mainly from Turkey and Mexico.

Under the theoretical assumption of a complete replacement of imports by national production, and barring any increase in domestic consumption or export, chickpea cultivation could spread over a maximum of 10,480 ha, as shown in Table 10. The replacement of traditional chickpea varieties with the newly varieties will bring economic benefits to Italian agriculture. Only in this case, the income might reach 15.7 billion ITL, or 10 million USD per year, as shown in Table 11.

Acknowledgements

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Table 8. Comparative gross and net farm incomes from chickpea and durum wheat cultivation.

Crop	Average yield (t/ha)	Price (ITL/t x 1000)	EU Subsidy (ITL/ha x 1000)	Gross income (ITL/t x 1000)	Costs (ITL/t x 1000)	Net income (ITL/t x 1000)
Durum wheat	4.5	350	1,300	2,875	1,200	1,675
Chickpea (modern cultivars)	2.5	1,000	0	2,500	900*	1,600
Chickpea (traditional cultivars)	1.0	1,000	0	1,000	900*	100

*No utilization of herbicides or nitrogen fertilizers

Table 9. Quantities and values of chickpea production and trading in Italy (1994).

Type	Quantity (t x 1000)	Value (ECU x 1000)	Quantity percent of total
National production	4.0	(1,905)†	15.3
Import (Turkey, Mexico)	22.2	10,574	84.7
Total	26.2	(12,479)*	
Export	-	-	

Price/t = 12,479,000 ECU/26,200 t = 476,3 ECU/t = 1,024,045 ITL/t (2,150 ITL/ECU) †estimated

Table 10. Chickpea acreage to be grown to meet the national demand.

National demand	26,200 t
Productivity of newly-bred chickpea varieties	2.5 t/ha
Acreage needed to meet the demand	26,200/2.5 = 10,480 ha

Table 11. Potential country return from the introduction of new chickpea cultivars, assuming that the national demand may be entirely met by national production.

Potential chickpea national average	10,480 ha
Net income increase from newly-bred over traditional chickpea varieties (ITL)	1,500,000 ITL/ha
Value of increase in production: ha 10,480 x 1,500,000 ITL/ha	15,720,000,000 ITL or about 15.7 billions ITL/y

funded by the Government of Italy, and within the ENEA/ECLAIR Project AGRE CT 90-0051 (1991–1995) *Development of chickpea germplasm resistant to ascochyta blight and fusarium wilt as a winter planting alternative to southern European cereal grains,* funded by the European Union.

References

Agnihotri Abba. 1993. Hybrid embryo rescue. Pages 1–8 *in* Tissue Culture Manual (K. Lindsey, ed.). Kluwer Academic, Netherlands.

Barna, K.S and A.K. Wakhlu. 1994. Whole plant regeneration of *Cicer arietinum* from callus cultures via organogenesis. *Plant Cell Reports* 13 (9): 510–513.

Bergamini, G. and D.L. Mulcehj. 1987. *American Journal of Botany* 9: 1458–1459.

Calcagno, F., P. Crinò and F. Saccardo. 1988. Ritorna il cece con nuove varietà produttive e resistenti alla rabbia. *Il Giornale di Agricoltura* 30: 35–38.

Chowrira, G.M., V. Akella and P.F. Lurquin 1995. Electroporation-mediated gene transfer into intact nodal meristems in planta: generating transgenic plants without *in vitro* tissue culture. *Molecular Biotechnology* 3(1): 17–23.

Crinò, P., F. Saccardo, P. Vitale, C. Mosconi, A. Andolfi and F. Calcagno. 1992. Chickpea breeding for stress resistance in Italy. Pages 35–36 *in* Proceed-

ings of 1st European Conference on Grain Legumes. 1–3 June 1992, Angers, France. L'Association Euro-péenne des Proteagineaux (AEP), Paris, France.

Eapen, S. and L. George. 1994. Somatic embryogenesis in *Cicer arietinum* L.: influence of genotype and auxins. *Biologia Plantarum* 36(3): 343–349.

FAO Year Book. 1993. vol. 47. FAO.

Gowda, C.L.L., O. Singh, S.C. Sethi, K.B. Singh, B.V Rao, M.M Rahman, J. Kumar and M.A. Rahman. 1995. Registration of ICCV 10 chickpea. *Crop Science* 35(2): 589.

Haware, M.P., J.N. Rao and R.P.S. Pundir. 1992. Evaluation of wild *Cicer* species for resistance to four chickpea diseases. *International Chickpea Newsletter* 27: 16–18.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). ICRISAT cold tolerant chickpea varieties ICCV 88503, ICCV 88506, ICCV 88510. Plant Material Description 53. 4 pp.

Islam, R., T. Malik, T. Husnain and S. Riazuddin. 1994. Strain and cultivar specificity in the *Agrobacterium*-chickpea interaction. *Plant Cell Reports* 13(10): 561–563.

Kaiser, W.J., A.R. Alcalá-Jimenez, A. Hervas-Vargas, J.L. Trapero-Casas and R.M. Jimenez-Diaz. 1994. Screening of wild *Cicer* species for resistance to races 0 and 5 of *Fusarium oxysporum* f.sp. *ciceris*. *Plant Disease* 78(10): 962–967.

Mosconi, C., C. Coduti, P. Crinò and F. Saccardo. 1995. Resistance to *Ascochyta rabiei* by interspecific hybridizations in Italian chickpea. Page 243 *in* Proceedings of the L'Association Européenne des Proteagineaux (AEP) Conference on Improving Production and Utilization of Chickpea. 10–13 July, Copenhagen, Denmark. AEP, Paris, France.

Mosconi, C., E. Santangelo, P. Crinò and F. Saccardo. 1995. Multiple shoot production for *in vitro* genetic manipulation of chickpea. Page 434 *in* Proceedings of the AEP Conference on Improving Production and Utilization of Chickpea. 10–13 July, Copenhagen, Denmark. AEP, Paris, France.

Ocampo, B., G. Venora, A. Errico, K.B. Singh and F. Saccardo. 1992. Caryotype analysis in the genus *Cicer*. *Journal of Genetics & Breeding* 46: 229–240.

Porta-Puglia, A., and P. Crinò. 1993. *Ascochyta rabiei*—cece. Pages 350–370 *in* Miglioramento genetico delle piante per resistenza a patogeni e parassiti. (P. Crinò, A. Sonnino, F. Saccardo, M. Buiatti, A. Porta-Puglia and G. Surico Curatori, ed.). EDAGRICOLE, Edizioni Agricole, Bologna.

Rahangdale, S.L., A.M. Dhopte and K.B Wanjari. 1994. Evaluation of chickpea genotypes for yield stability under soil moisture deficit. *Annals of Plant Physiology* 8(2): 179–184.

Saccardo, F. and F. Calcagno. 1990. Consideration of chickpea plant ideotypes for spring and winter sowing. *Options Méditerranéennes, Séries Séminaires* 9: 34–41.

Saccardo, F. and P. Crinò 1993. Il cece: la soia del Centro-Sud. *Ortofrutta* 10: 26–31.

Saccardo, F., P. Crinò, P. Vitale, F. Calcagno and A. Porta-Puglia. 1987. Miglioramento genetico del cece in Italia: obiettivi perseguiti e primi risultati. *Terra e Sole* 534: 82–87.

Saccardo, F., A., Errico, P. Crinò, B. Ocampo and G. Venora. 1993. Mutagenesis and chromosome manipulation for stress resistance in cool-season legumes. Pages 343–358 *in* Breeding for Stress Tolerance in Cool-Season Legumes (K.B. Singh and M.C. Saxena, ed.). John Wiley & Sons, Chichester, UK.

SAT. 1992. New chickpea cultivars released in Morocco, Pakistan and Turkey. *SAT News* 10: 3.

Saxena, M.C. 1980. Recent advances in chickpea agronomy. Pages 96–98 *in* Proceedings of the International Workshop on Chickpea Improvement, ICRISAT, 28 Feb to 3 Mar 1979. Hyderabad, India. ICRISAT.

Saxena, M.C. 1990. Status of chickpea in the Mediterranean basin. *Options Méditerranénnes. Séries Séminaires* 9: 17–24.

Saxena, N.P., L. Krishnamurthy and C. Johansen. 1993. Registration of drought-resistant chickpea germplasm. *Crop Science* 33(6): 1424.

Sharma, P.C., P. Winter, T. Bunger, B. Huttel, F. Weigand, K. Weising and G. Kahl. 1995. Abundance and polymorphism of di-, tri-, and tetra-nucleotide tandem repeats in chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics* 90 (1): 90–96.

Singh, K.B. 1987. Chickpea breeding. Pages 127–162 *in* The Chickpea (M.C. Saxena and K.B. Singh, ed.). CAB International, Wallingford, UK.

Singh, K.B. 1993. Problems and prospects of stress resistance breeding in chickpea. Pages 17–35 *in* Breeding for Stress Tolerance in Cool-Season Food Legumes (K.B. Singh and M.C. Saxena, ed.). John Wiley & Sons, Chichester, UK.

Singh, K.B. and B. Ocampo. 1993. Interspecific hybridization in annual *Cicer* species. *Journal of Genetics and Breeding* 47: 199–204.

Singh, K.B., R.S. Malhotra. and M.C. Saxena. 1993. Relationship between cold severity and yield loss in chickpea (*Cicer arietinum* L.). *Journal of Agronomy and Crop Science* 170(2): 121–127.

Singh, K.B., M.V. Reddy and R.S. Malhotra 1983. Breeding kabuli chickpeas for high yield, stability and adaptation. Pages 71–90 *in* Proceedings of the International Workshop on Faba Beans, Kabuli Chickpeas, and Lentils in the 1980s (M.C. Saxena and S. Varma, ed.). ICARDA, 16–20 May 1983, Aleppo, Syria. ICARDA.

Suhasini, K., A.P. Sagare and K.V. Krishnamurthy. 1994. Direct embryogenesis from mature embryo axes in chickpea (*Cicer arietinum* L.). *Plant Science* 102(2): 189–194.

Udupa, S.M., A. Sharma, R.P. Sharma and Pai R.A. 1993. Narrow genetic variability in *Cicer arietinum* L. as revealed by RFLP analysis. *Journal of Plant Biochemistry and Biotechnology* 2(2): 83–86.

ICARDA–Italy Cooperation in the Development of Chickpea Cultivars

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Summary

The collaboration between ICARDA and Italian chickpea-breeding programs has resulted in the development of improved germplasm adapted to Italian chickpea-growing conditions and permitting the adoption of winter-chickpea technology. Another area in which Italy and ICARDA have made good progress is in the exploitation of wild species for chickpea improvement. Wild species are a valuable gene pool for resistance to several biotic and abiotic stresses. There is an indication that chickpea yield can be increased through the introgression of genes from wild relatives. These findings have great significance in further improvement of chickpea in the region.

Introduction

The genus *Cicer* belongs to the family Leguminoseae, subfamily Papilionaceae and tribe Cicereae. It comprises 43 species: 9 annuals, 33 perennials and one unspecified. The annual *Cicer* species are distributed from Turkey to central Asia, and in scattered pockets in Ethiopia, Sudan and Egypt. The cultigen is distributed from the Mediterranean basin to Myanmar, Australia, Ethiopia, Mexico, Chile, and the cooler parts of the tropics at elevations ranging from sea level up to 2400 m.

Chickpea (*C. arietinum* L.) is grown on 10.3 million hectares with a production of 7.0 million tonnes (FAO 1994). It occupies 15.1% of the world area devoted to pulses, providing 12.1% of the global pulse production. The world average yield of chickpea is low (about 0.7 t/ha). Although important in the past, chickpea is presently a minor crop in Italy, producing only 4000 tonnes during 1994. The interest in promoting this ancient crop in Italy has, however, been increasing.

ICARDA has been associated with the Italian chickpea-improvement program for nearly a decade as several Italian institutions have participated in ICARDA's International Legume Testing Network. The conference entitled *La Coltura Del Cece in Italia* organized by the Ente per le Nuove Tecnologie, l'Energia e l'Ambiente (ENEA), documented some of this collaboration (Crinò 1987). In 1990, Italy and ICARDA co-organized an international conference on *Breeding for stress tolerance in cool-season food* legumes and the proceedings were published (Singh and Saxena 1993). Our association with the Italian program has been mainly in the field of exploiting wild species. Here we will focus on this aspect and on varietal development.

Objectives of ICARDA's Chickpea Improvement Program

The overall goal of ICARDA's chickpea-improvement program is to increase and stabilize kabuli-chickpea production in the world, through collaborative research with national programs on the development of germplasm and production technology.

The immediate objectives of this program are to:

- enhance the level of resistance to ascochyta blight and cold.
- develop large-seeded and/or early maturing lines with resistance to ascochyta blight and high yields.
- develop lines with multiple resistance, especially to ascochyta blight, cold and fusarium wilt, and early maturing lines with resistance to soil-borne diseases.
- assist NARS to introduce winter sowing on a large scale.

In the long run we also aim to:

- upgrade the genetic yield potential through interspecific hybridization and increased biomass and harvest index;
- exploit wild species for the transfer of genes for resistance to biotic and abiotic stresses;

- prolong the reproductive phase through introduction of early flowering and podding at low temperature.

To achieve these objectives, a vigorous breeding program has been under way at ICARDA and diverse breeding material has been developed ranging from early segregating populations to genetically-fixed elite lines.

Distribution of international nursery

Using this material, the ICARDA–ICRISAT joint *Kabuli Chickpea Project* has been distributing 16 types of nursery (five yield, five screening, two segregating, and four stress). For the 1995/96 season, 403 sets of nurseries have been distributed to 100 collaborators in 46 countries (Fig. 1). This has helped strengthen NARS chickpea improvement programs. To date, 69 cultivars have been released in 21 countries (Table 1), and some of the best sources of resistance to various stresses have become available to NARS for use in their breeding programs (Table 2).

The cooperators in Italy have regularly received chickpea nurseries from ICARDA as per their needs. For the 1995/96 season, nine sets of nurseries were distributed to five cooperators. This collaboration has resulted in the release of chickpea cultivars in Italy. Our Italian colleagues have also identified good sources of resistance to various stresses in the ICARDA germplasm for use in their breeding programs.

Introduction of winter sowing of chickpea in the Mediterranean region

Chickpea is traditionally grown as a spring-sown crop in the Mediterranean region. Experiments conducted at ICARDA have shown that winter-sown chickpea produces 50–100% greater seed yield compared with spring-sown chickpea (Fig. 2). Increased yields are primarily from better use of available soil moisture and favorable temperatures during the winter and early spring growing season. Ascochyta blight, the most devastating disease of chickpea worldwide, is especially prevalent and severe in winter-sown chickpea crops. We have therefore developed cold- and ascochyta-blight-resistant cultivars and passed them to national programs in the region, which have released cultivars suitable for their own conditions from this material. There is no authoritative estimate of the area currently sown to winter chickpea, but there are indications that the area has been increasing. It is expected that winter chickpea will eventually be grown on over two million hectares, providing an additional income of over US$ 500 million annually.

This technology has also been of interest to Italy (details are given elsewhere).

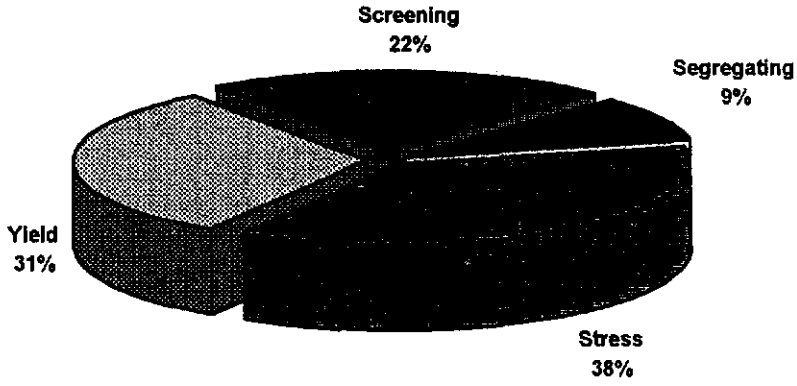

Fig. 1. Distribution of 1995 chickpea nursery

Table 1. Kabuli-chickpea cultivars developed at ICARDA and released by national programs.

Country	Cultivar(s)
Algeria	ILC 482, ILC 3279, FLIP 84-79C, FLIP 84-92C
China	ILC 202, ILC 411, FLIP 81-71C, FLIP 81-40C
Cyprus	Yialousa, Kyrenia
Egypt	ILC 195
France	TS1009, TS1502, Roye Rene
Iran	ILC 482, ILC 3279, FLIP 84-48C
Iraq	ILC 482, ILC 3279
Italy	Califfo, Sultano, Pascia, Otello
Jordan	Jubeiha 2, Jubeiha 3
Lebanon	Janta 2, FLIP 85-5C
Libya	ILC 484
Morocco	ILC 195, ILC 482, Douyet, Rizki, Farihane, Moubarak, Zahor
Oman	ILC 237, FLIP 87-45C, FLIP 89-130C
Pakistan	Noor 91
Portugal	Elmo, Elvar
Spain	Fardan, Zegri, Almena, Alcazaba, Atalaya, Athenas, Bagda, Kairo
Sudan	Shendi, Jeb el Mara 1
Syria	Ghab 1, Ghab 2, Ghab 3
Tunisia	Chetoui, Kassab, Amdoun 1, FLIP 84-79C, FLIP 84-92C
Turkey	ILC 195, Guney Sarisi 482, Damla 89, Aziziye, Akcin, Aydin 92, Menemen 92, Izmir 92
USA	Sanford, Dwelley

Table 2. Chickpea germplasm resistant to five biotic and two abiotic stresses developed at ICARDA, Syria and released to researchers for use in breeding programs.

Stress	Source of resistance
Ascochyta blight	ILC 200, ILC 6482, ICC 4475, ICC 6328, ICC 12004, FLIP 90-98C, FLIP 91-2C, FLIP 91-18C, FLIP 91-22C FLIP 91-24C, FLIP 91-46C, FLIP 91-50C, FLIP 91-54C
Fusarium wilt	ILC 267, ILC 1278, ILC 1300, FLIP 86-93C, FLIP 87-33C, FLIP 87-38C
Leaf miner	ILC 3800, ILC 5901, ILC 7738
Seed beetle	ILWC 39, ILWC 181
Cyst nematode	ILWC 292
Cold	ILC 8262, ILC 8617
Drought	FLIP 87-59C

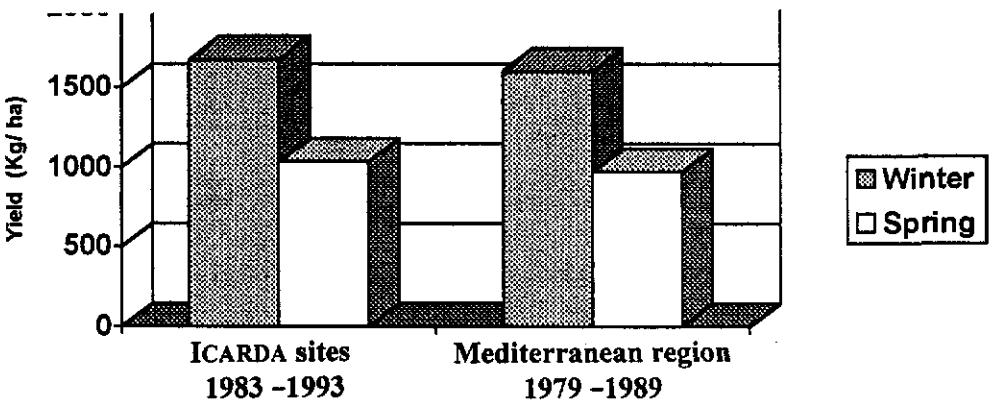

Fig. 2. Mean yield (kg/ha) of several chickpea lines grown in winter and spring at ICARDA sites and over 20 NARS sites in the Mediterranean environments

Evaluation of wild species for agronomic and morphological characters

Wild species have been exploited for the transfer of useful genes in most major crops, but little has been done to improve chickpea. Therefore, 228 accessions of eight annual wild *Cicer* species plus 20 kabuli chickpea lines were evaluated for 23 vegetative, flower, fruit and seed descriptors at ICARDA, Syria, during 1993/94 to identify useful variations (Table 3). Large differences between the cultivated and the annual wild taxa were found, suggesting that many traits underwent major changes during the domestication of *C. arietinum*. Overall, the annual wild *Cicer* species were of no advantage for direct genetic improvement of agronomic traits in chickpea. Nevertheless, interesting variability was found for a few descriptors: wide leaflets in *C. chorassanicum*; many branches in *C. bijugum* and *C. reticulatum*; and early flowering in *C. judaicum*. This variability in the annual wild *Cicer* taxa might be useful in distant hybridization to produce positive transgressive segregants. Because of this potential benefit, a major collaborative research program on exploiting wild *Cicer* spp. was developed by ICARDA and several Italian institutions with financial support from the Italian Government.

Table 3. Annual wild *cicer* species germplasm evaluated at ICARDA, Syria, 1993/94.

	Cicer sp.								
Origin	*ari*	*ret*	*ech*	*jud*	*pin*	*bij*	*cho*	*yam*	*cun*
Afghanistan	4			2			5	3	4
Ethiopia	2			2	1			4	
Jordan	33			8					
Lebanon	1			17	1				
Palestine	2			9	5				
Syria	7			22	8	4			
Turkey	1	51	11	7	34	33			
Total	20	51	11	67	49	37	5	7	4

1 *ari = arietinum* (check); *ret = reticulatum*; *ech = echinospermum*; *jud = judaicum*; *pin = pinnatifidum*; *bij = bijugum*; *cho = chorassanicum*; *yam = yamashitae*; *cun = cuneatum*.
Source: Robertson et al. (1995).

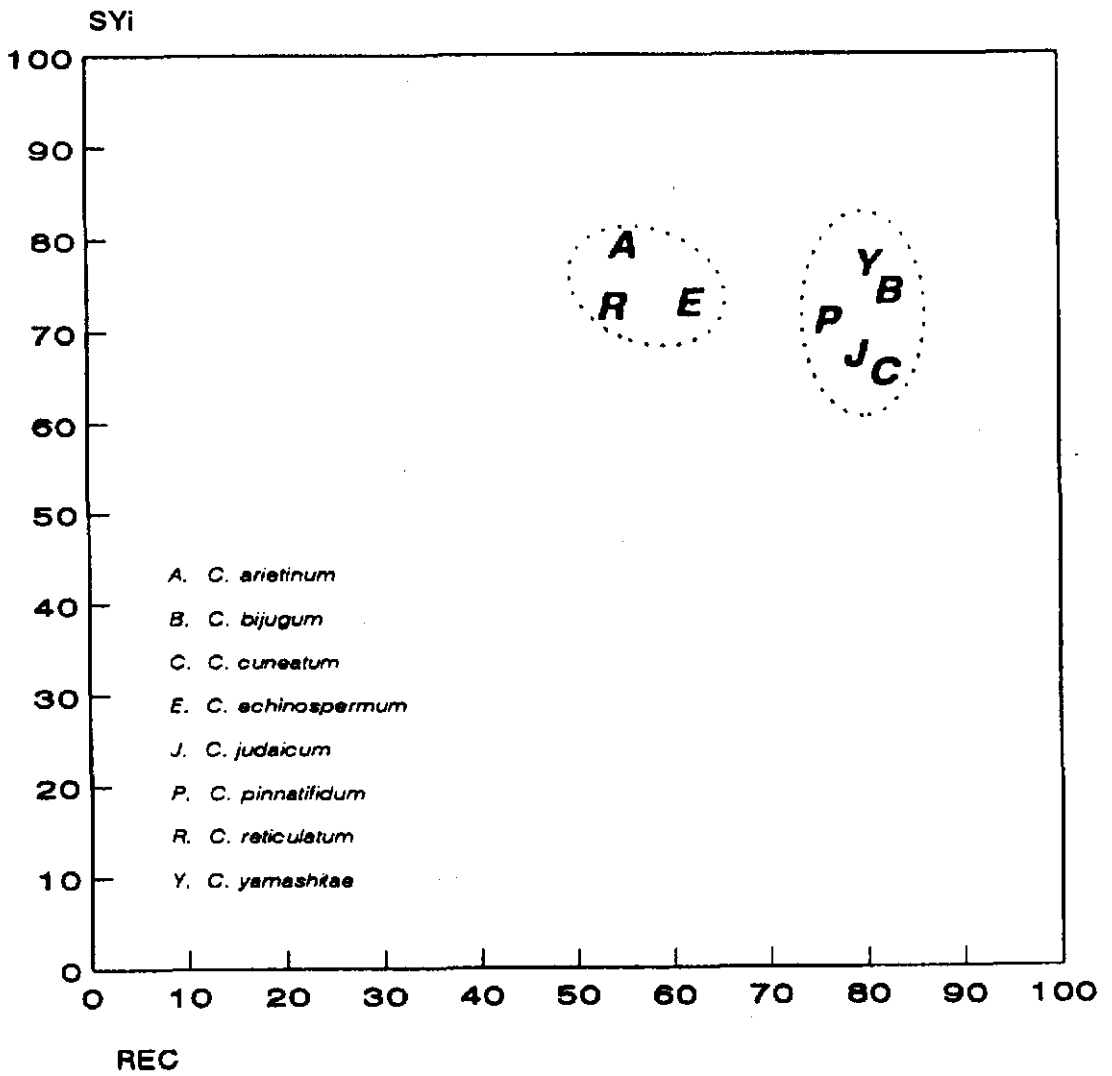

Fig. 3. Symmetry indices in annual *Cicer* species

Karyotype analysis in *Cicer*

In this collaborative program, detailed karyotype analysis of eight annual *Cicer* species, namely *C. arietinum, C. bijugum, C. cuneatum, C. echinospermuy, C. judaicum, C. pinnatifidum, C. reticulatum* and *C. yamashitae*, was carried out using the new computerized image-analysis system. The number of chromosomes in all the species was found to be 2n = 16. Based on the karyotype analysis, the eight species were sorted into two genes pools. *C. arietinum, C. reticulatum* and *C. echinospermum* (group one) and the remaining five species (group two) (Fig. 3). It is expected that interspecific hybridization will be easier within each group than between the groups.

Isozyme analysis of wild *cicer* species

ICARDA undertook a study to examine genetic variation within and between annual *Cicer* species. The nine species formed four phylogenetic groups based on genetic distance. The first group comprised *C. arietinum, C. reticulatum* and *C. echinospermum*; the second *C. bijugum, C. pinnatifidum* and *C. judaicum*; the third *C. chorassanicum* and *C. yamashitae*; and the fourth group consisted of one species only, *C. cuneatum* (Fig. 4). Genetic distance data supported a closer relationship of the cultigen to *C. reticulatum* than to *C. echinospermum*.

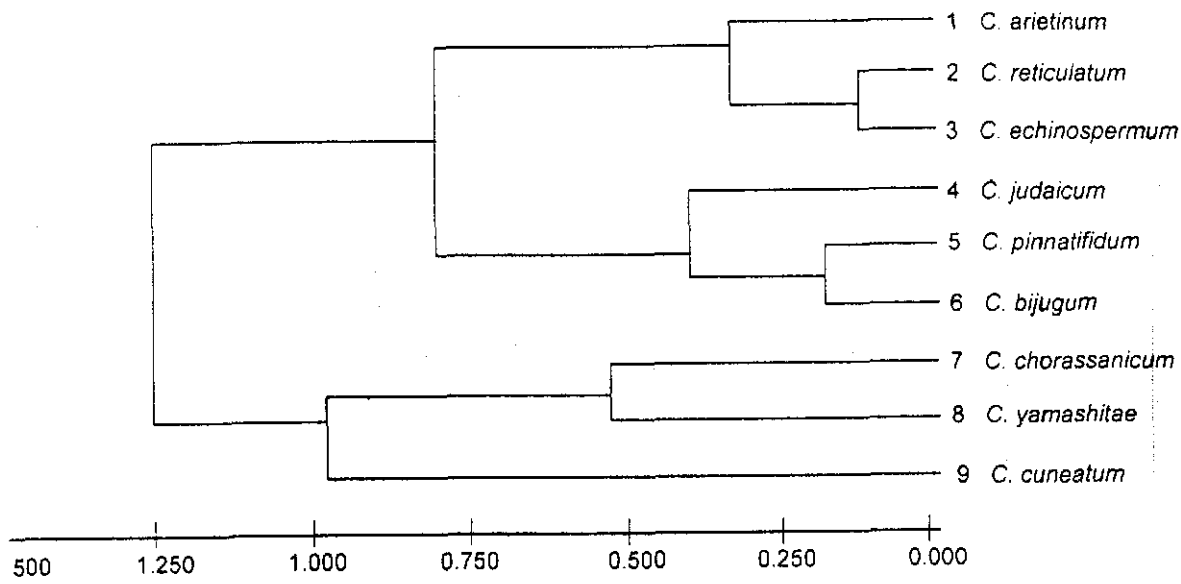

Fig. 4. Dendogram of relationships among annual *Cicer* species (Source: Labdi et al. 1995)

Screening wild *Cicer* species for resistance to fusarium wilt

Wilt caused by *Fusarium oxysporum* f. sp. *ciceri*, is a widespread soil-borne disease of chickpea. In an attempt to identify new sources of resistance to wilt, 102 accessions of six wild annual *Cicer* species were evaluated in the greenhouse at the Institute of Plant Pathology, Rome. The isolate used in this experiment was from central Italy. All accessions of *C. pinnatifidum* and *C. reticulatum* of *C. bijugum* and some of *C. echinospermum, C. judaicum* highly-resistant reaction to wilt showed (Table 4). Both accessions of *C. yamashitae* were susceptible. This evaluation has helped to identify new and diverse sources of resistance to wilt for use in chickpea breeding.

Table 4. Reaction of wild *Cicer* species accessions to *Fusarium oxysporum* f.sp. *ciceri* (isolate No. 526 II).

Cicer species	Number of accessions	Accessions with disease score [a]				
		0	>0–1	>1–2	>2–3	>3–4
C. bijugum	23	14	8	-	1	-
C. echinospermum	4	-	1	2	1	-
C. judaicum	25	1	8	10	6	-
C. pinnatifidum	25	-	5	10	10	-
C. reticulatum	23	2	5	7	9	-
C. yamashite	2	-	-	-	-	2
C. arietinum [b]	1	-	-	-	-	1

[a] Assessed on a 0-4 scale according to the percentage of wilting of plant canopy, 0 = 0%; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%.

[b] 'FLIP 85-88C'

Source: Infantino et al. (1995).

Effect of culture filtrate of *Ascochyta rabiei* on chickpea genotypes

Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Lab. is the major disease of chickpea in the Mediterranean basin including Italy. Resistant cultivars are the best way to control the disease. To accelerate cultivar development, a screening technique capable of evaluating germplasm for resistance to ascochyta blight in a short period was needed. In an attempt to develop such a technique, crude culture filtrate of *A. rabiei*, which induces phytotoxicity on chickpea cuttings, inhibits root elongation from the germinating seeds, and causes electrolyte leakage was tried. The reaction caused by culture filtrate on chickpea cuttings of susceptible genotypes resembled ascochyta blight. Resistant genotypes could be differentiated from the susceptible ones. Differential reaction was also observed when culture filtrate was produced by different pathogenic groups of Italian ascochyta isolates. While additional research is required, culture filtrate holds promise as a screening technique for resistance to ascochyta blight.

Exploitation of wild species for resistance to biotic and abiotic stresses

On evaluations of accessions of annual wild species at ICARDA, sources of resistance were identified for seven stress factors: ascochyta blight, fusarium wilt, leaf miner, seed beetle, cyst nematode, cold and drought (Table 5). The only sources of resistance for seed beetle and cyst nematode found so far are in wild species. Wild species also had higher levels of resistance than the cultivated species for fusarium wilt, leaf miner, and cold. Whereas no accession of the cultivated species was resistant to more than one stress, several accessions of wild species were resistant to four or five stresses. The most important species for resistance to different stress factors was *C. bijugum*, while *C. yamashitae* was the least important.

Improvement of yield through interspecific hybridization

One possible way to improve chickpea yield is through the incorporation of genes from the wild relatives. ICARDA has been following this line of approach since 1988. A 9 x 9 diallel cross involving eight annual wild *Cicer* species and the cultivated species was made in 1989. The results indicated that only two *Cicer* species, *C. echinospermum* and *C. reticulatum*, can be crossed with the cultigen. When F_1s were grown, heterosis up to 250% was observed against up to 75% in intraspecific crosses. This encouraged us to advance the material and select lines with high yields. During 1993/94, we tested over 100 F_6 lines and selected 22 lines, which produced

Table 5. Reaction of germplasm accessions of *Cicer* spp. to some biotic and abiotic stresses at Tel Hadya, Syria from 1987/88 to 1992/93.

Scale†	Blight	Wilt‡	LM	SB	CN	Cold	Drought
1	0	72	2	20	3	1	0
2	1	0	36	12	1	29	0
3	4	7	36	4	17	45	0
4	2	15	33	3	0	46	3
5	22	6	61	3	28	21	37
6	29	4	26	8	0	12	71
7	24	4	23	18	49	11	15
8	30	0	1	52	0	8	20
9	81	5	3	10	144	65	42
Total	193	113	231	130	241	238	188

† Scale: 1 = free; 5 = intermediate; 9 = killed.

‡ Evaluation for wilt was done at Istituto Sperimentale per la Patologia Vegetale, Rome.

Table 6. Some characters of five best F_7 lines derived from interspecific crosses at Tel Hadya, Syria, 1993/94.

Line	Cross[a]	Characters evaluated								
		DFL	DMA	HGT (cm)	SW (g)	GRH	UMAT	PDD	SCOL	SSH
F_7 derived lines										
53	RA	104	155	43.4	31.5	erect	u	nd	beige	owl
95	AE	105	159	44.1	19.4	erect	u	nd	beige	round
5	AE	102	159	44.6	26.9	semi-spreading	u	nd	orange	round
81	AE	115	159	55.4	33.2	semierect	u	nd	beige	round
55	AE	117	160	43.4	30.9	semi-spreading	u	d	beige	owl
Parents										
ILC 482 A		105	156	41.8	27.2	erect	u	nd	beige	owl
ILWC 124	R	119	158	23.4	11.6	spreading	nu	d	brown	angular
ILWC 179	E	121	158	11.1	11.7	spreading	nu	d	brown	owl
mean		112	160	39.0	25.2					
S.E.		1.89	1.98	5.13	2.152					
C.V.		1.48	1.14	11.99	7.52					

[a] A = *C. arietinum*, R = *C. reticulatum*, E = *C. echinospermum*, AE = *C. arietinum* (ILC 482) x *C. echinospermum* (ILWC 35); RA = *C. reticulatum* (ILWC 36) x *C. arietinum* (ILC 482),

DFL = days to 50% flowering; DMA = days to maturity; HGT = plant height; SW = 100-seed weight; GRH = growth habit; UMAT = Uniformity of maturity (u = uniform, nu = non-uniform) PDD = Pod dehiscence (d = dehiscence, nd = non-dehiscence); SCOL = seed colour; SSH = seed shape.

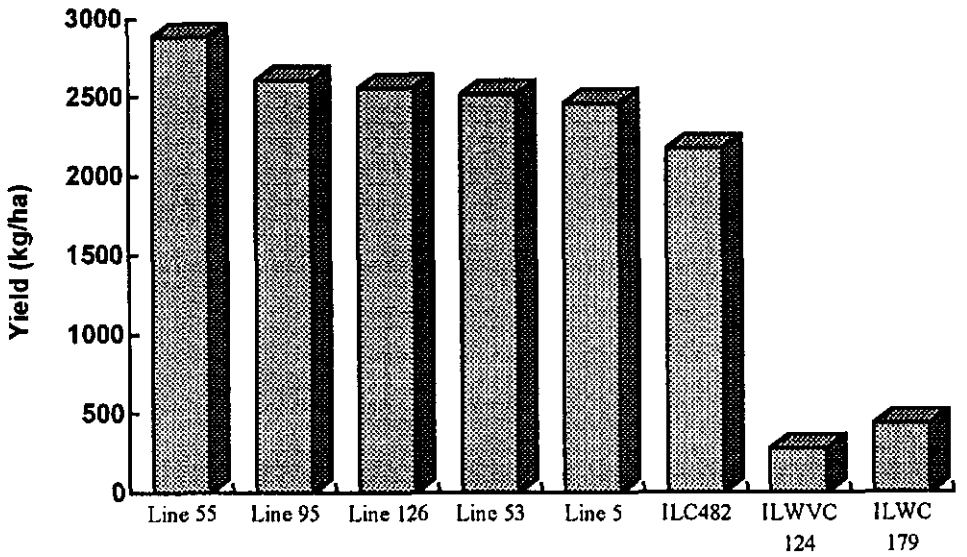

Fig. 5. Performance of five highest-yielding lines derived from interspecific hybridization

substantially higher yields than the check (ILC 482). These lines were evaluated in F_7 for seed yield and other characters. The performance of the five best lines is shown in Figure 5. The increase in seed yield over the cultivated check was up to 40%. Some of the undesirable characters associated with the wild species are given in Table 6. The derivatives from interspecific crosses were nearly free of undesirable genes from wild species.

Thus, we have succeeded in developing lines with substantially higher yield and with all observable characters similar to the cultigen parent. Increased seed size, above-ground shoot mass, and plant height seem to have contributed to increased yield in lines derived from interspecific crosses. Encouraged by these results, the research on this project has been expanded. Several crosses and backcrosses have been made and material is at different stages of development. Also, efforts are underway to add genes for resistance to biotic and abiotic stresses to develop cultivars with high and stable yields.

References

Crino, P. 1987. La Coltura del Cece in Italia. Ente per le Nuove Tecnologie, l'Energia e l'Ambiente (ENEA) and Societa Italiana Agraria Genetica (SIGA), Rome, Italy.

FAO. 1994. Production Yearbook. 48: 97.

Infantino, A., A. Porta-puglia and K.B. Singh. 1995. Screening wild *Cicer* species for resistance to fusarium wilt. *Plant Disease* 79: (In press).

Labdi, M., L.D. Robertson, K.B. Singh and A. Charrier. 1995. Genetic diversity and phylogenetic relationships among the annual *Cicer* species as revealed by isozyme polymorphism. *Euphytica* 70: (In press).

Mosconi, C., P. Crinò, F. Saccardo and K.B. Singh. 1995. Effect of culture filtrate of ascochyta rabiei on different chickpea genotypes. *Journal of Genetics and Breeding* 49: (In press).

Robertson, L.D., K.B. Singh, and B. Ocampo. 1995. A Catalog of Annual Wild *Cicer* Species. ICARDA, Aleppo, Syria.

Singh, K.B. and Saxena, M.C. (ed). 1993. Breeding for stress tolerance in cool-season food legumes. John Wiley & Sons, Chichester, UK.

Perspectives on Chickpea Use in Italy

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Summary

Given the productivity of new and recently developed cultivars, the demand for dry chickpea seeds in Italy can probably be satisfied by the cultivation of some 5000–6000 ha.

Unless the demand is increased, there is no scope for further applied-research efforts. To justify additional activities, it is necessary to promote chickpeas for human and livestock use.

It also seems clear that at present the agro-food industry is supposed to play the most important role in increasing the demand for chickpea and, as a consequence, increasing chickpea production.

Chickpea Production in Italy

In 1936, the area cultivated with chickpea in Italy was around 110,000 ha, mostly concentrated in central and south Italy, with a total production of about 40,000 tonnes and an average yield of 0.4 tonnes per ha (Fig. 1). In 1950, the same area produced 55,000 tonnes. Since then the area has continuously decreased to reach the present 3000–4000 ha.

Average yearly production over the last five years has been around 50,000 tonnes, with an average yield per ha equal to 1.2 tonnes per ha. In 60 years the average yield per ha tripled, starting, however, from a very low basis (0.4 t).

In 1993, chickpea production in Italy reached 48,000 tonnes, while the imported product was equal to 22,391 tonnes, involving an expenditure of around ITL 40 billion (Table 1).

Current use of chickpea in Italy is therefore equal to about 70,000 tonnes, an amount that can be produced on some 4,000–5,000 ha; particularly if we consider the usage of the newest high-yielding varieties, resistant to ascochyta blight, and adapted to fall seeding (Singh et al. 1984; Singh 1990; Saccardo and Calcagno 1990).

In conclusion, with the present level of chickpea use in Italy (about 1.3 kg per person per year very few thousand ha can satisfy human consumption needs.

Without an important increase in demand for human and livestock use further breeding and agronomic research and development are scarcely justified.

Hence the basic question, is it possible to increase the national and the international demand for this commodity, and if so how?

Analyzing the Demand for Chickpea

If we analyze the current pattern of demand, we can conclude that chickpeas are used as human food—especially a special type of soup (*pasta e ceci*) or boiled dressed with salt, vinegar, and olive oil, or stewed with tomato and some spices (garlic, sage, rosemary, etc.)

Moreover, like lentils, chickpeas are mainly used only in the winter period.

In rural areas people usually use dry seeds from their harvest, but in urban areas canned and frozen chickpeas are also used.

Processed chickpeas represent only some 25–30% of the total consumption.

Even with the increasing interest in grain legumes associated with the mediterranean diet, we cannot expect a significant increase in this kind of use.

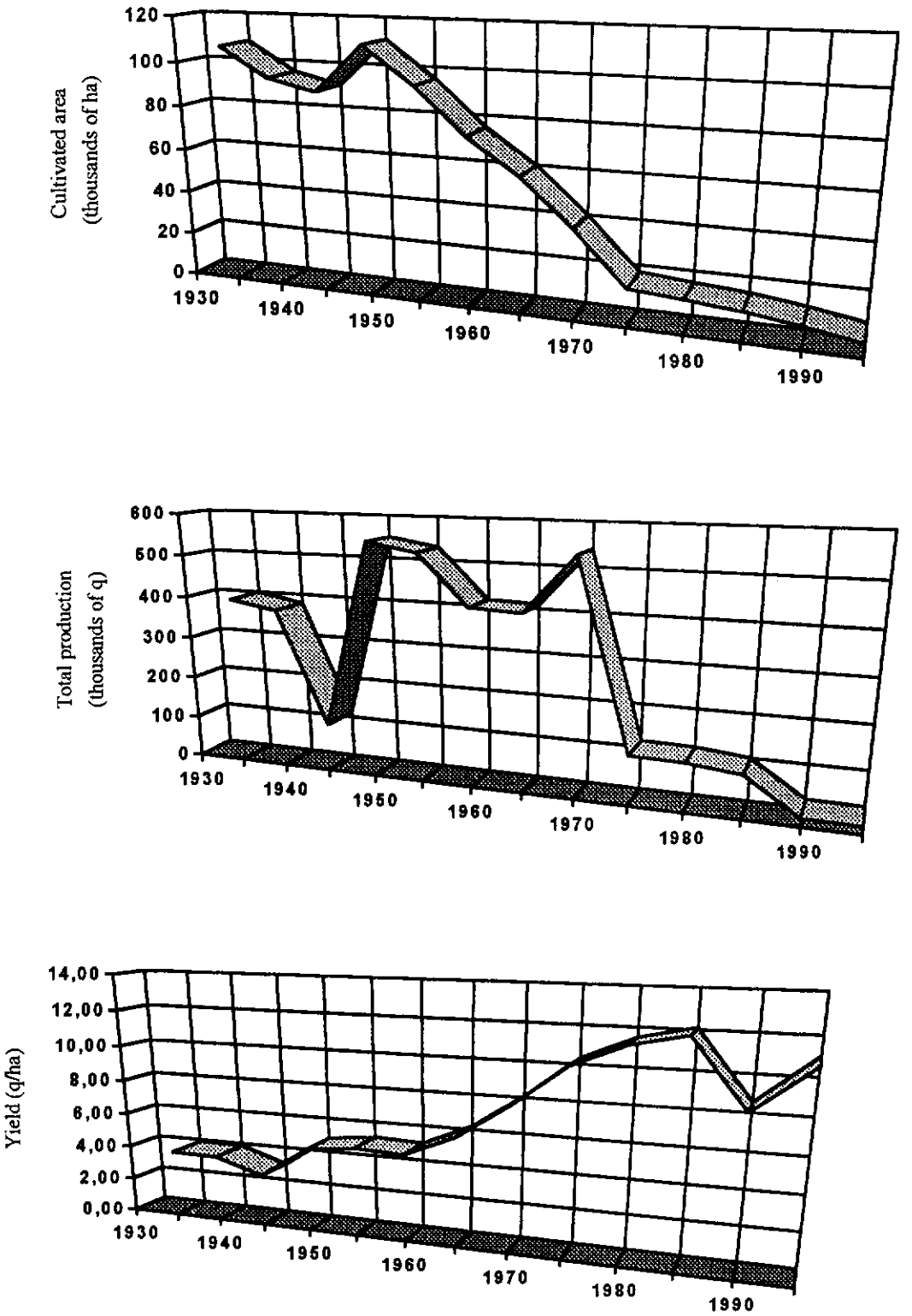

Fig. 1. Trends chickpea production from 1935 to present

Moreover, over the last few years, the food industry has hardly, if ever, advertised or promoted chickpea consumption in the Italian press or on television. a clear indication of the low expectation of the Italian food industry for this kind of commodity. Among food legumes, only peas and common beans enjoy some promotion and propaganda.

Table 1. Production and trade in Italy in 1993.

Commodity	Production (tonnes)	Imports (tonnes)	Imports (value)†	Exports (tonnes)	Exports (value)†
Faba bean	1,133,000	241,219	54,082	154	207
Common bean	374,000	67,350	2,590	1,929	2,993
Dry pea	291,000	22,944	7,513	641	668
Chickpea	48,000	22,391	2,454	238	231
Lentil	9,000	22,944	11,483	269	306
Total	1,855,000	374,948	138,122	3,231	4,405
Italian lira (billion)			221		7

† 000 US$.

In conclusion, prospects for an increase in chickpea demand in the near future do not appear to be brilliant.

The prospects for increased use of chickpea in the livestock feed industry are also bleak. This is because of the present rather low mean yield of the crop and the relatively low price the feed industry is likely to offer for this commodity, since it is possible to find similar products (soya bean flour, dry peas, dry faba beans) at rather low prices in the international market (for example soya bean from USA and Brazil; dry peas from eastern and North Europe; faba beans from China).

The only hope is that, with the new more productive and stable cultivars adapted to fall, spring and even summer planting (under irrigation) (Bozzini and Iannelli 1993), and with a demonstrated potential of producing 4–5, even 6 tonnes of dry seed per ha, the feed industry could finally express a real interest in chickpea.

At least a price of ITL 500,000 per tonne (some US$ 310–320) should be granted to producers as in the European Union financial support has been granted to lentils, chickpea and vetches equal to 130 ECU/ha (some ITL 277,000 US$ 170). This contribution covers about a quarter of the production costs.

In fact, to compete with durum-wheat revenue in the South, 4 tonnes of chickpea is needed, at the above-mentioned ITL 500,000 per tonne.

It is a pity that such a good product is to be fed to animals and not directly to humans!

How to Increase the Chickpea Demand?

At least three factors should be considered in the endeavor to increase chickpea production in Italy (and in the Mediterranean area), giving significance to the efforts of breeders, pathologists, and agronomists involved with this crop, at least in southern Europe.

(1) The need to provide Italians with better information on the benefits of a diet based on a higher consumption of grain legumes.

(2) The recognition that agricultural production and agroindustrial transformation are phases of the same technological and economical process; that they are strictly interconnected and complementary and, therefore, agroindustrial demand must be the lever or spring for increased agricultural production.

(3) The need to diversify chickpea use (Italians almost only use it as boiled seeds) and to advertise the quality and the advantages of new or uncommon chickpea products.

Here again the agroindustry is in a position to take the lead.

A number of temperate and tropical grain legumes, such as lentils, common beans, chickpeas, mung beans, adzuki beans, and faba beans, appear to have a beneficial effect on sugar metabolism and on the cardio-circulatory system, if consumed regularly (Bozzini 1986).

Research carried out in Europe and North America has shown that glucose remains in the blood for longer and at lower levels when a grain legume is included in the meal, thus

preventing first its excess and then its deficiency after each meal, in contrast to the diabetes syndrome. The use of grain legumes in the diet, particularly of older people, is therefore strongly recommended. Cooked dry legumes also seem to reduce cholesterol in the blood and are therefore beneficial in cardio-circulatory disorders.

Moreover, the high content of good-quality proteins present in most of domesticated grain legumes traditionally gave them a reputation as the 'poor people's meat.'

In the last decades, breeding efforts have also eliminated the presence of some residual antinutritional, unpleasant and even dangerous compounds, such as bitter and poisonous principles in lupins; vicine, and convicine responsible for favism in faba beans; a neurotoxic principle in chickling vetch, and tannins in faba beans and lentils.

None of these advances have found a fertile ground in the food industry, at least in Italy. The food industry prefers to play with conventional products, and is unwilling to take any risks with innovative products and initiatives.

When the technologists in the *Kellogg's* industry in the USA developed corn and oat flakes, they transformed a rather poor product into a much more palatable one with greatly increased value and fantastic profits!

More effort is needed to adopt and promote products that are already well known, at least in some parts of Italy, in the Mediterranean area, and even farther away, for example, in India.

The coastal area of Northern Tuscany and Liguria, up to the city of Genoa, is well known for a kind of pizza (*torta di ceci, cecìna*) made with chickpea flour. Its preparation is simple and its nutritional value, besides the good taste, is very high. If one in every 10 pizza houses in Italy cooked this pizza, it would be enough to multiply the chickpea demand by more then ten, maybe 50 times. The industry could easily prepare a 'ready to use' flour for both home and industrial use and properly advertise the good characteristics of this 'new' product.

In all Near Eastern countries, a kind of chickpea purée, *humus,* is eaten, particularly as a kind of appetizer dressed with lemon juice and with good quality olive oil and consumed with *pita* the flat Arabic bread. Now in most of these countries, it is possible to find canned, industrially prepared, humus ready for use. All of my Italian friends, who have visited these countries, have without exception, liked humus very much, finding it very tasty and pleasant. It is likely, that with very little risk humus could be introduced in Italy, with appropriate promotion.

In India, a thin sheet of a chickpea (and lentil) pastry is fried crisp, flavored with local spices and served as an appetizer. Similar dishes, starting from ready-made industrial preparations, can be found in Britain, The Netherlands, even in Australia, where Indian restaurants are common.

Many other possible dishes can be found, even in the regional Italian kitchen, some of which deserve proper attention and marketing (see the booklet distributed on this occasion).

The main conclusion of this paper is that the key to the future development of chickpea production in Italy is now mainly in the hands of the food industry. Although, it will certainly help if plant scientists will be able to provide farmers with higher and more stable yields, in the order of 4–5 tonnes per ha.

References

Bozzini, A. 1986. Le proteine vegetali fanno bene all'economia e alla salute. *Giornale di Agricoltura* 9: 13–15.

Bozzini, A. and P. Iannelli. 1993. Development of summer chickpea cultivation under irrigation in South Italy. *Agric. Medit.* 123: 1–6.

Saccardo F. and F. Calcagno. 1990. Consideration of chickpea plant ideotypes for spring and winter sowing. *Options Méditeranénnes. Séries Séminaire* 9: 35–41.

Singh, K.B. 1990. Winter chickpea: problems and potential in the Mediterranean region. *Options Méditeranénnes. Séries Séminaire* 9: 25–34.

Singh, K.B., H.E. Gridley and G.C. Hawtin. 1984. Strategy for breeding ascochyta blight resistant cultivars. Pages 95–110 *in* Ascochyta Blight and Winter Sowing of Chickpea (M.C. Saxena and K.B. Singh, ed.) Martinus Nijhoff Publishers, The Hague, Netherlands.

Plant Proteins in Human Nutrition: the Case of Chickpea

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Consumption

A study carried out among 10,000 Italian families by the Istituto Nazionale della Nutrizione (INN; INN 1992) produced the most (and probably the only) comprehensive information on legume (including chickpea) consumption in Italy today (Turrini et al. 1991). The national consumption of legumes, divided into geographical areas, is given in Table 1. The table shows a high consumption of fresh legumes, thanks, to a large extent, to the distribution of frozen foods. On average, total legume consumption was 26 g per capita/day (equivalent to an intake of once or twice a week); this figure varies slightly depending on the geographical region. Chickpeas, which come under the dry-legumes category, are the third most consumed legumes, after beans and lentils.

Recommendations

The Italian guidelines (INN 1992) analogous to those of other industrialized countries, where one of the main guidelines is to increase the variety of foods consumed, includes a specific recommendation regarding the consumption of vegetable foods (cereals and legumes, vegetables and fruit). The reasons being that cereals and legumes are high in polysaccharides (both starch and fibre), and have a positive effect on carbohydrate and lipid metabolisms; vegetables and fruit are high in vitamins—especially those having an antioxidising and protective effect, such as, vitamin C and carotene—and have a high fibre content. As these two food groups are low in fat, they contribute to reducing fats in the diet.

Table 1. Food intake in different geographical areas (mean, g/capita).

Food-legumes	Italy	North West	North East	Center	South
Fresh	10,0	10,2	5,6	8,4	12,9
Preserved	6,1	7,2	7,1	5,4	5,9
Dry and flours	5,0	1,6	2,3	4,3	7,4
Frozen	3,9	2,4	5,4	4,9	3,0
Dishes	1,0	1,0	0,7	1,8	0,6
Total	26,0	22,4	21,1	24,8	29,8

From Turrini et al. 1991

INN's current study on legume consumption aims to provide an up-to-date picture of how consumption has changed over the last 10 years and to assess the effects of the nutritional recommendation to increase legume consumption (reinforced in the last few years).

The recent Dietary Guidelines for Americans (US Department of Agriculture, and US Department of Health and Human Services 1995) give even greater importance to this recommendation, 'Choose a diet with plenty of grain products, vegetables and fruit.' In previous guidelines, the main

emphasis was on the consumption of these foods as a source of fibre and starch. The new guidelines emphasize how these foods contribute to total nutrient intake: their importance as a source of folates, potassium, calcium and magnesium.

Nutrient Composition

The nutrient composition of legumes, in particular the mean composition of chickpeas (Carnovale et al. 1994), certainly meets these guidelines (Table 3). They have 20% mean protein content, a balanced composition of essential amino acids (even though there is a certain deficiency in sulphur amino acids), a chemical score of 0.80 (refer to the FAO pattern, 1985); satisfactory thiamin, riboflavin and niacin content (according to recent data, they are also a good source of folates: 0.20 mcg/100 g), and are a good source of iron, zinc and calcium. Recently, attention has been drawn to magnesium and potassium content. It should be borne in mind that even though the mineral content is high, in that it is a good dietary source, their bioavailability is rather low due to the presence of chelating compounds. One of the particular characteristics of chickpeas, compared with other legumes, is their moderate lipid content, comprised mainly of polyunsaturated fatty acids (Table 4), which, although being a positive factor in nutritional terms, means that chickpea products, especially chickpea flour, go rancid more easily.

Table 2. Consumption frequencies within legume food groups.

Food	Italy %	North West %	North East %	Center %	South %
Legumes, fresh					
Bean, snap green	47,25	60,10	48,78	44,69	44,52
Pea, green	22,35	20,70	21,95	16,62	25,93
Bean, white, mottled	16,33	15,71	27,37	14,71	14,46
Broad bean	13,55	2,49	1,36	23,30	14,81
Other legumes	0,52	1,00	0,54	0,68	0,28
Legumes, frozen					
Pea	75,49	60,00	42,91	83,77	90,00
Beans	12,22	12,38	35,81	6,46	4,00
Bean, snap green	11,03	26,27	20,27	8,73	4,22
Broad bean	1,26	0,95	1,01	1,05	1,78
Legumes, dry and flours					
Beans	45,94	37,17	74,00	50,78	40,87
Lentil	34,15	36,28	9,00	29,75	39,16
Chickpea	16,07	17,70	7,00	17,13	16,69
Broad bean	1,53	1,77	0,50	0,47	2,10
Pea, green	1,05	1,77	7,00	0,16	0,59
Lupin	0,52	0,88	1,00	1,09	0,20
Other legumes and flours	0,36	1,77	1,00	0,00	0,33
Soybean	0,36	2,65	0,50	0,62	0,07

From Turrini et al. (1991)

Table 3. Nutrient composition of chickpea (% fresh weight).

	Raw	Cooked
Water	10,33	62,55
Protein	19,72	7,02
Lipid	6,29	2,36
Sugar	6,82	2,05
Starch	40,42	14,52
Soluble fiber	0,78	0,33
Insoluble fiber	12,45	5,29
Total fiber	13,23	5,62
Calcium, mg	142	58
Iron, mg	6,40	2,20
Zinc, mg	3,20	1,70
Phosphorus, mg	415	148
Natrium, mg	6,20	5,20
Potassium, mg	881	302
Magnesium, mg	140	-
Niacin, mg	2,51	0,90
Thiamin, mg	0,28	0,16
Riboflavin, mg	0,10	0,03

Table 4. Fatty acid composition and phytosterol content in chickpea.

Saturated fatty acids†	11,6
Monounsaturated fatty acids†	25,5
Polyunsaturated fatty acids†	62,5
Sytosterol‡	98
Phytosterol	
Stygmasterol‡	7

† % total fatty acid
‡ mg/100 f.w.
From Carnovale et al. (1994)

Polysaccharides are particularly interesting compounds. Comprising starch (40% mean content) and fibre (13% mean content), their composition, structure, and changes caused by technological treatments, (both in the home and industry), are the subject of more in-depth studies (Lintas et al. 1992; Lintas et al. 1995). The physiological and metabolic effects of polysaccharides in legumes (as well as those from other sources) have recently been reviewed (British Nutrition Foundation 1990).

As regards antinutritional factors, such as protease inhibitors, lectines, phytic acid and tannin, that interact with proteins and minerals and reduce their use, chickpeas contain similar moderate amounts to those found in lentils (Carnovale et al. 1989). Values found in the new cultivars, recently introduced into Italy (Principe, Calia, Sultano, and Califfo) also fall within the average values found for chickpeas (Carnovale et al. 1989).

Use of Chickpeas in the Production of New Foods

An increase in the consumption of legumes, which would be desirable, would certainly be helped by having new interesting products on the market to include along with the traditional ones.

The feasibility of using chickpea or broad bean flour to prepare new products obtained by extrusion cooking was studied in collaboration with Mapimpianti. Both salted and sweet products were prepared (biscuits, toasted and fried snacks and pre-cooked flours), using either solely a legume flour or mixing it with corn, rice or wheat flours. Their nutritional characteristics (composition, protein quality, negative nutritional factors) and organoleptic characteristics were assessed (Carnovale et al. 1990). The sweet products were not that palatable but the salted products, particularly those made mainly of chickpea flour, were very palatable. As the changes in nutritional quality caused by the cooking techniques used were limited, and the technological characteristics were positive, the global assessment of some of these products was satisfactory and therefore the continuation of experiments in this direction would be desirable.

Broad bean and chickpea precooked flour products are already marketed in some countries. The FAO/WHO supports their use in food preparations for infants and have already defined international regulations to market them. There is no reason why they should not also become widely used in adult diets.

Study of Hydration and Cooking Characteristics

A wider and more rational use of chickpeas, as well as of other legumes, in the food industry requires more in-depth knowledge of certain characteristics. For example, the firmness and water-absorbing capacity of the grains are of great importance as they have an effect on the processes used to transform chickpeas, both in home cooking and in industry.

The study on the effects of cooking, a parameter also of considerable interest to the industrial transformation of legumes, is hindered, among other things, by the absence of objective, quantifiable parameters required for its evaluation.

A study was therefore carried out on legumes, particularly chickpeas, with the aim of defining the hydration and firmness characteristics and the cooking properties of the grains and to find correlations between these processes and their probable causes. The first step was to define an analysis protocol based on objective parameters. Traditional gravimetric methods were used to study hydration and instruments such as Nuclear Magnetic Resonance were also used. The latter was found to be a new and interesting method for checking and quantifying water-absorption processes in grains and enabled various types of water (free and bound) to be assessed separately. Therefore, the hydration process in four new chickpea cultivars (Principe, Califfo, Sultano, and Calia) was assessed (Carnovale et al. 1992).

A study of the hydration and cooking characteristics of chickpea samples, which were from Turkey and Mexico, but were marketed in Italy, showed significant differences connected with the country of origin and the years in which they were produced (Carnovale et al. 1992).

The cooking properties of 15 tinned chickpea products available on the market in Italy were studied using the back extrusion test (INSTRON). A firmness range was identified, with a statistically significant difference ranging from 1.13 KN to 2.78 KN. The latter, the extreme value where extrusion is perceptible, can be taken as the maximum acceptable level (Carnovale et al. 1995). These experiments enabled the definition of an experimentation protocol—based on data taken from instruments under standardized conditions—to evaluate the technological characteristics of chickpeas, which can also be applied to other legumes.

In conclusion, chickpeas clearly meet the present nutritional recommendations, however consumption is still rather low. An increase in consumption can only be brought about by making the guidelines for a healthy diet better

known, and by producing and marketing new products, as well as traditional products which are nutritional and tasty.

References

British Nutrition Foundation. 1990. Complex Carbohydrates in Foods. Chapman and Hall.

Carnovale E., G. Lombardi Boccia, L. Marletta. 1989. *Riv. Soc. It. Scienza Alim.* 18: 321–326.

Carnovale, E., M. Cappelloni, C. Mosconi, L. Marletta and C. Pollini. 1990. Effect of extrusion cooking on protein availability and sulphur amino-acids of faba bean and chickpea products. Page 241 *in* Proceedings of the 7th World Congress Food Science Technology—Trends in Food Processing I.

Carnovale, E., E. Marconi, F. Paoletti, E. Brosio. 1992. Page 431 *in* Proceedings I European Conference on Grain Legumes, Angers.

Carnovale, E. et al. 1994. *Agricoltura Ricerca* 155: 135–144.

Carnovale, E., E. Marconi, F. Paoletti, M.C. Squasi. 1995. Page 344 *in* Proceedings II European Conference on Improving Production and Utilization L'Association Europeene des Proteagineaux, Paris, France of Grain Legumes, Copenhagen, Denmark.

INN (Instituto Nazionale Della Nutrizione). 1992. Linee guida per una sana alimentazione Italiana.

Lintas, C. and M Cappelloni. 1992. *Eur. J. Clin. Nutr.* 46, S103–104.

Lintas, C., M. Cappelloni, S. Montalbano, L. Gambelli. 1995. *Eur. J. Clin. Nutr.* 49, S298–302.

Turrini, A., A. Saba and C. Lintas. 1991. *Nutrition Research* 11: 861–873.

US Department of Agriculture and US Department of Health and Human Services. 1995. Dietary Guidelines for Americans. USA Government.

Agronomic Context and Problems of Chickpea Cultivation in Central and Southern Italy

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Abstract

The area of chickpea (*Cicer arietinum* L.) cultivation in Italy has shrunk progressively from 120,000 acres in the 1940s to 10,000 acres at the end of the 1980s.

This decrease is mainly due to backward cultivation techniques and to a lack of improved varieties.

The difficult Italian (especially Sicilian) pedoclimatic conditions–scarce and irregular spring rainfalls, sudden heat peaks in May and June, hot scirocco winds blowing during the spring chickpea sowing season–and the low cultivation densities adopted (20–40 plants/m^2), used to represent insurmountable limits to the productivity potential of this leguminous species.

Low and inconsistent grain yields, and the amount of labor needed for some agricultural practices have made chickpea cultivation progressively less competitive and destined to disappear.

Physiological and adaptation studies of new foreign germplasm, carried out through interdisciplinary research and cooperation between ICARDA and Italian institutions, have led to the release of new cultivars and the adoption of modern agrotechnologies. Of particular importance has been the availability of genotypes resistant to *Ascochyta rabiei*, which may be planted in fall, and the adoption of chemical weed control. Furthermore, the morphology of the new cultivars (erect habit, apical production) has permitted higher planting densities and mechanized harvesting.

Evaluation and Breeding of Chickpea for Cold Tolerance

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Summary

A technique for cold tolerance evaluation in controlled environments was developed. A good correlation between the laboratory test and field response was found only when plants acclimated at 2°C for 15 days and at seven-leaf stage were subjected to a temperature decrease of 2°C/hour from +15°C to -14°C followed by an increase back to +15°C at the same rate. This temperature cycle was repeated three times. Acclimation was necessary because it was found that while some accessions had a cold response independent of acclimation, others displayed their tolerance potential only after a period of acclimation. Plantlets survival after this temperature cycle showed a good correlation with cold tolerance in the field, as evaluated in terms of leaf and crown damage following frost.

Using this screening technique, we have evaluated several accessions of *Cicer arietinum* and of six wild *Cicer* species from ICARDA's collection. Selection for cold tolerance resulted in the identification of very interesting lines that are now being registered as cultivars Visir and Alí.

As far as the other *Cicer* species, it was found that the most tolerant wild relative of *C. arietinum* is *C. reticulatum*. We have crossed the two species and obtained F_1, F_2 and F_3 progenies. The latter are presently being evaluated for cold tolerance.

Introduction

Crop susceptibility to environmental stresses is one of the major causes of reduced productivity and yield fluctuations between cropping seasons all over the world. Most agronomic practices are in fact devoted to minimizing the negative effects of environmental constraints on crops, which alternatively could be overcome by using tolerant cultivars. The latter would be particularly effective in countries with low-input, mainly rainfed agriculture, where traditional agricultural systems are often not sufficient to contrast the effects of adverse environmental conditions on crop production.

The relative importance of the various abiotic stresses for chickpea varies with the production region. While drought is the most widespread abiotic stress throughout the cultivation area (Saxena 1993), in areas with a temperate, Mediterranean climate, cold stress is of major importance.

Due to its susceptibility to cold, chickpea is traditionally sown in early spring in areas where frost might cause severe crop damage, and eventually complete yield loss. Nevertheless, spring-sown chickpea has a lower yield potential than the winter-sown crop (Singh et al. 1993a). Autumn or early winter sowing is limited by the lack of cultivars that are tolerant to ascochyta blight and cold; able to nodulate well, and to compete with weeds at lower temperatures. Cultivars with those characteristics could be sown earlier (Summerfield et al. 1990) thus ensuring a better exploitation of available soil moisture and a longer crop duration from sowing to reproductive maturity, which produces in a dramatic increase in yield (Singh et al. 1989; Saxena 1990). In the last few years, cultivars adapted to winter sowing have been released in various countries (ICARDA/ICRISAT 1992; Singh et al. 1993b, c; ICRISAT 1994), but there is still a strong need for cultivars specifically suited to the different environments.

Breeding large populations for winter survival in the field is hindered by the fact that frost events vary in their occurrence and intensity. Thus, we developed a technique for evaluation of cold tolerance in controlled environments.

Materials and Methods

About 300 ICARDA accessions of *Cicer arietinum* of different origin and 90 accessions of six wild *Cicer* species (Table 1) were evaluated for tolerance to freezing and survival in winter.

A laboratory test for evaluating tolerance to freezing was set up using a growth chamber with temperature control. Non-acclimated (NA) greenhouse-grown plants, or plants cold acclimated (CA) for 15 days at 2°C, 14 h light, were kept at 15°C in the dark for three hours before the temperature was decreased to -7°C at a rate of 2°C/h. After three hours at -7°C, the temperature was increased back to 15°C at the same rate. This temperature cycle was repeated two more times, then plants were put in a growth chamber at 20°C, 14 h light. Chickpea plants tolerance to freezing was evaluated 7 days after the test and expressed as % survived plants compared to the total plant number. Genotypes with more than 50% plant survival were considered 'freezing tolerant.' For each experiment, 20 plants per genotype were tested, with three replications.

The optimal conditions for the controlled freezing test were set up using the sample genotypes ILC3279 and RF55, freezing tolerant and susceptible, respectively. The two genotypes were compared at four different stages: 2-leaf stage, 4-leaf stage, 7/8-leaf stage, 12/13-leaf stage.

For a preliminary evaluation of cold tolerance, about 300 *C. arietinum* genotypes were sown during the second half of December 1990 and 1991 in S. Angelo dei Lombardi, Benevento, Italy, in plots of 20 plants for each genotype and three replications in a randomized complete design. Trials were repeated on selected accessions in 1991/92 (about 80 accessions) and 1992/93 (about 60 accessions) on a mountain location (BN) at 700 m above sea level. Only during 1990/91 and 1992/93 was it possible to select for cold tolerance under field conditions, because frost events with temperatures as low as -7°C were recorded when plants had 8/10 leaves.

The effects of subzero temperatures were evaluated 15 days after each frost event. Genotypes showing more than 50% plant survival were considered to be freezing tolerant.

Selected genotypes were also evaluated for agronomic traits (growth habit, crop duration, productivity, seed size, harvest index, yield stability) during 1992/93 (about 60 accessions) in four locations in Italy: Benevento (concomitantly with the evaluation of frost tolerance); Bellizzi (Salerno); Viterbo and Tarquinia (Rome) and during 1993/94 and 1994/95 in Bellizzi (about 20 accessions) using the described experimental design.

Results

In order to determine the optimal stage for imposing freezing stress, plants of the genotypes ILC3279 and RF55 at 2,4, 7/8 and 12/13 leaf stages, with different levels of frost tolerance were subjected to the freezing cycle described in Materials and Methods. Seedlings (two-leaf) were found to be completely tolerant to the applied freezing conditions (Fig. 1). At the 4 leaf stage the two genotypes could be easily distinguished, but the difference was most striking at the 7/8 leaf stage, when the susceptible genotype did not survive, while the tolerant genotype had more than 60% survival. Older plants of both genotypes (12/13 leaves) were unable to survive the applied freezing conditions. Therefore, the 7/8 leaf stage was found to be optimal for freezing tolerance evaluation and chosen for further experiments. When the chickpea genotypes 1004, 1017, and ILC533, were evaluated for tolerance to the controlled freezing cycle, no correlation was found between data on NA plants and data on winter survival in the field (Table 1). In fact, NA 1004 and 1010 were highly susceptible in the growth chamber but were tolerant in the field.

Nevertheless, when plants were acclimated to cold prior to exposure to freezing stress in the laboratory, genotypes 1004 and 1010 were also found to be tolerant (Table 1). When we compared the freezing stress tolerance in the field and in the laboratory for a larger number of genotypes, we found a good correlation between the two parameters only when laboratory evaluated plants were cold acclimated before the onset of the controlled freezing cycle (Fig. 2).

During 1990/91, about 80 accessions were selected in the field, which showed frost tolerance. All those accessions were

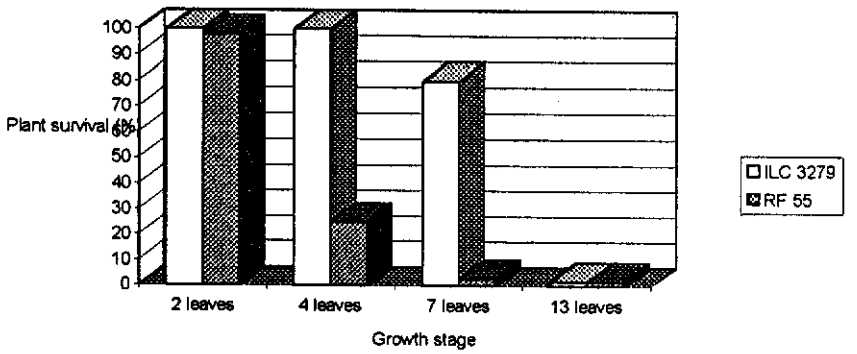


Fig. 1. Laboratory freezing test tolerance of two chickpea genotypes at different growth stages

Table 1. Freezing stress tolerance and yield of chickpea genotypes with different acclimation ability.

Genotype	Yield† (t/ha)	Plant survival to freezing stress‡ (%)		
		field[1]	laboratory test§ NA	CA
1017 (Visir)	2.43	90 a	80 a	99 a
1004 (Alí)	2.92	70 b	0 b	94 b
ILC 533	1.62	40 c	0 b	0 c

† Plants grown in Benevento during 1993, lowest recorded temperature -7°C;

‡ Values followed by different letters are different at P=0.05;

§ NA = greenhouse-grown, non-acclimated plants; CA = plants acclimated for 15 days at 2°C, 14h photoperiod.

evaluated under laboratory conditions and about 60 confirmed the tolerance found in the field. During 1991/92 temperatures in the field were too high at all locations to screen for frost tolerance. The next year, a frost event in Benevento allowed evaluation of freezing tolerance. Twenty-two lines were selected for their tolerance and good agronomic characteristics and tested again during 1993/94 and 1994/95 in Bellizzi.

Among the selected genotypes, two were found extremely interesting and are now in the process of being registered as cultivars Visir and Alí.

The controlled freezing test was also used for the preliminary screening of 90 accessions of six wild *Cicer* species. The same good correlation between laboratory and field results was found in terms of survival to freezing temperatures (Fig. 3) of CA plants. The most tolerant species is *C. reticulatum*, which showed an average plant survival of 51% at -7°C in laboratory tests and 46% survival at the same temperature under field con-ditions. On the contrary, *Cicer yamaschitae* is highly susceptible, and does not survive freezing conditions in either the laboratory or the field. Since it is possible to cross *C. reticulatum* with *C. arietinum*, a breeding program is now being developed to introgress the freezing tolerance of *C. reticulatum* into *arietinum* form.

Fig. 2a.

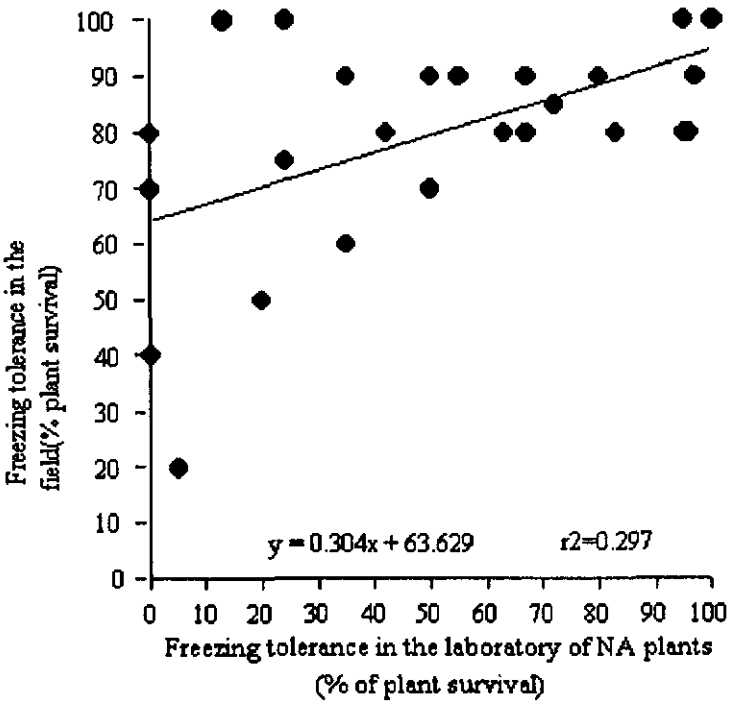
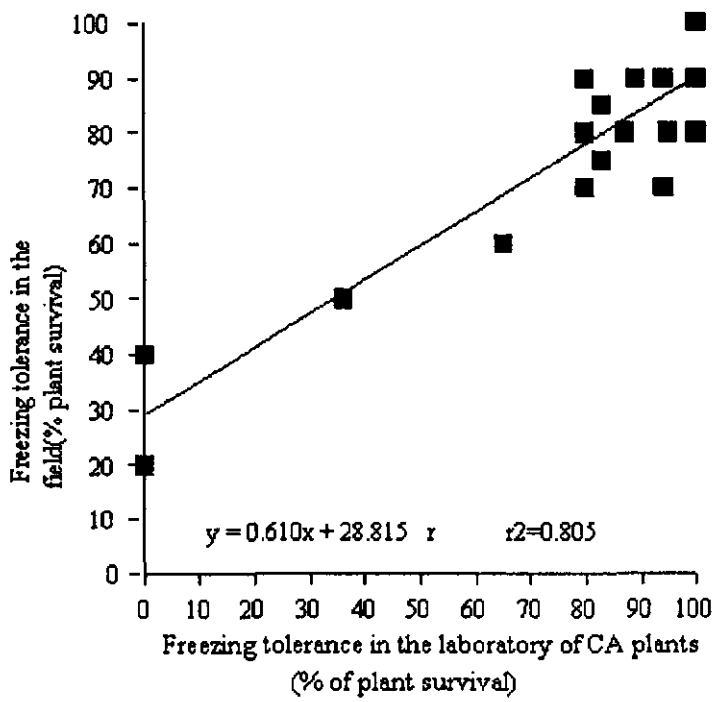


Fig. 2b.



2b Plants were cold acclimated prior to laboratory test.

Fig 2. Correlation between freezing tolerance of chickpea genotypes under field and laboratory conditions.

Discussion

One of the most important goals in chickpea breeding for adaptation to temperate climates is to obtain cultivars with tolerance to freezing stress. Field evaluation of large breeding populations during the winter is not always possible, since test winters when frosts occur with the appropriate intensity and at an optimal stage of plant development are very rare. Besides, genotype scoring can vary among seasons due to the non-repeatability of frost conditions. Therefore, the use of a test for the evaluation of freezing tolerance under controlled conditions is necessary. Several tests have been proposed for estimating freezing-tolerance in different crops, including measurements of electrolyte leakage from injured cells, chlorophyll fluorescence, and reduction of tetrazolium chloride. We have developed a screening technique based on visual evaluation of plant damage after a freezing cycle, which is easy and can be used with a large number of samples. The cooling rate for the freezing test was chosen in order to approach that naturally found under field conditions (Steffen et al. 1989). The optimal stage for imposing the freezing stress was identified in the 7/8 leaf stage. In accordance with common knowledge, that the post-emergence seedling stage is the most cold tolerant stage in the chickpea plant's growth cycle, the younger plants were found to be frost tolerant even in the most susceptible genotypes (Singh et al. 1982; Saccardo and Calcagno 1990; Wery 1990). Also, as expected, older plants were always dead after the freezing test, since frost tolerance decreases during the growth cycle of chickpea, with a minimum tolerance during pre-flowering and flowering, when temperatures below 15°C result in abortion of early flowers and buds (Sedgley et al. 1990) due to failure of pollen-tube development (Savithri et al. 1980).

When greenhouse-grown chickpea plants were abruptly subjected to the freezing test, some accessions, which were tolerant in the field, were completely killed. It is known that many plant species subjected to low temperatures undergo metabolic and physiologic changes, which increase their ability to resist frost damage (Levitt 1980). We investigated whether the behaviour of these accessions could be due to acclimation processes naturally occurring in the field during the winter. Therefore, the genotypes 1004, 1017 and ILC533 were grown for 15 days under environmental conditions (2°C, 14 h photoperiod) known to trigger acclimation in other herbaceous species (Chen et al. 1979) prior to imposing freezing stress. Cold-hardened plants subjected to the freezing test always showed a tolerance level comparable to that found in the field, thus demonstrating that some chickpea accessions are indeed able to undergo cold acclimation. On the basis of the results obtained, it was possible to classify chickpea accessions in three groups: (1) tolerant but unable to harden to the cold; (2) susceptible but able to harden to the cold; (3) susceptible.

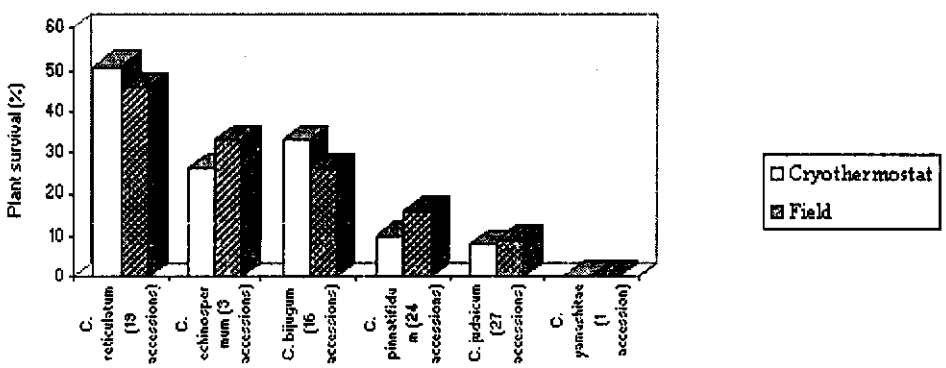


Fig. 3. Average freezing tolerance of different accessions of wild *Cicer* spp. under laboratory and field conditions. Laboratory tests were conducted on cold-acclimated plants

The laboratory evaluation allowed selection to continue even in 1992/93 when field evaluation was not possible as the temperatures were too mild. Therefore, selection was accelerated and cold-tolerant lines were identified among the 300 *C. arietinum* accessions under evaluation in only five years. Some of these accessions have a very high level of tolerance, with a frost-killing temperature as low as -12°C (results not shown). Among the selected lines, two with very favourable agronomic characteristics are now being registered in Italy as cultivars Alí and Visir.

The combined laboratory and field evaluations made it possible to readily identify tolerant accessions among the wild *Cicer* germplasm. Variability for cold tolerance is not very wide in *C. arietinum*. Among 5672 accessions examined, only a small number showed moderate tolerance to cold (Singh and Jana 1993). Therefore, sources of resistance to be found among the wild *Cicer* germplasm are of great interest. The possibility of crossing *C. arietinum* with the wild annual *Cicer* spp. has already been investigated by ourselves and other authors (Singh and Ocampo 1993), and it is possible to cross the cultigen to *C. reticulatum* and *C. echinospermum* Interspecific crosses between interesting accessions of *C. reticulatum* and *C. arietinum* have been included in a breeding program aimed to enlarge the variability available in *Cicer* for cold tolerance and to obtain tolerant varieties.

References

Chen, H.H, P. Gavinlertvatana and P.H. Li. 1979. Cold acclimation of stem-cultured plants and leaf callus of *Solanum* species. *Botanical Gazette* 140(2): 142–147.

ICARDA/ICRISAT. 1992. New chickpea cultivars released in Morocco, Pakistan and Turkey. *SAT News* 10: 3.

ICRISAT. 1994. Cold-tolerant chickpea varieties ICCV 88503, ICCV 88506, ICCV 88510. *Plant Material Description* 53: 4. ICRISAT, Patancheru, India.

Levitt, J. 1980. Responses of Plants to Environmental Stresses. 2nd edn. vol. 1. Academic Press, New York.

Saccardo, F. and F. Calcagno. 1990. Consideration of chickpea plant ideotypes for spring and winter sowing. *In* Present Status and Future Prospect of Chickpea Crop Production and Improvement in the Mediterranean Countries (M.C. Saxena, J.I. Cubero and J. Wery, ed.). *Options Méditerraneenes. Serie A: Seminaires Méditerraneenes* No 9. CIHEAM, Zaragoza, Spain.

Savithri, K.S., P.S. Ganapathy and S.K. Sinha. 1980. Sensitivity to low temperature in pollen germination and fruit-set in *Cicer arietinum* L. *Journal of Experimental Botany* 31(121): 475–481.

Saxena, M.C. 1990. Problems and potential of chickpea production in the nineties Pages 13–25 *in* Chickpea in the Nineties: Proceedings of the Second International Workshop on Chickpea Improvement, 4–8 Dec 1989, ICRISAT, Patancheru, India. ICRISAT.

Saxena, M.C. 1993. The challenge of developing biotic and abiotic stress resistance in cool-season food legumes. *In* Breeding for Stress Tolerance in Cool Season Food Legumes (K.B. Singh and M.C. Saxena, ed.). John Wiley & Sons, Chichester, UK.

Sedgley, R.H., K.H.M. Siddique and G.H. Walton. 1990. Chickpea ideotypes for Mediterranean environments. Pages 87–91 *in* Chickpea in the Nineties: Proceedings of the Second International Workshop on Chickpea Improvement, 4–8 Dec 1989, ICRISAT, Patancheru, India. ICRISAT.

Singh, K.B. and B. Ocampo. 1993. Interspecific hybridization in annual *Cicer* species. *Journal of Genetics and Breeding* 47(3): 199–204.

Singh, K.B. and S. Jana. 1993a. Diversity for responses to some biotic and abiotic stresses and multivariate associations in kabuli chickpea (*Cicer arietinum* L.). *Euphytica* 68: 1–10.

Singh, D.P., H.M. Rawson and N.C. Tuner. 1982. Effect of radiation, temperature and humidity on photosynthesis, transpiration and water-use efficiency of chickpea (*Cicer arietinum* L.). *Indian Journal of Plant Physiology* 1: 32–39.

Singh, K.B., R.S. Malhotra and M.C. Saxena. 1989. Chickpea evaluation for cold tolerance under field conditions. *Crop Science* 29: 282–285.

Singh, K.B., R.S. Malhotra and M.C. Saxena.

1993a. Relationship between cold severity and yield loss in chickpea (*Cicer arietinum* L.). *Journal of Agronomy and Crop Science* 170(2):121–127.

Singh, K.B., R.S. Malhotra and M.C. Saxena. 1993b. Registration of ILC 72 chickpea. *Crop Science* 33(6): 1409.

Singh, K.B., R.S Malhotra. and M.C. Saxena. 1993c. Registration of ILC 195 chickpea. *Crop Science* 33(6): 1409–1410.

Steffen K.L., R. Arora and J.P. Palta. 1989. Relative sensitivity of photosynthesis and respiration to freeze-thaw stress in herbaceous species—Importance of realistic freeze-thaw protocols. *Plant Physiology* 89: 1372–1379.

Summerfield, R.J., S.M. Virmani, E.H. Roberts and R.H. Ellis. 1990. Adaptation of chickpea to agroclimatic constraints. Pages 61–72 *in* Chickpea in the Nineties: proceedings of the Second International Workshop on Chickpea Improvement, 4–8 Dec 1989, ICRISAT, Patancheru, India. ICRISAT.

Wery, J. 1990. Adaption to frost and drought stress in chickpea and implication in plant breeding. *In* Present Status and Future Prospect of Chickpea Crop Production and Improvement in the Mediterranean Countries (M.C. Saxena, J.I. Cubero and J. Wery, ed.). Options Mediterraneenes. Serie A: *Seminaires Mediterraneenes* No. 9. CIHEAM, Zaragoza, Spain.

Ascochyta Blight and Fusarium Wilt Diseases in Chickpea: Host–Pathogen Interaction

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Summary

Biotic stresses are important constraints affecting the yield of grain legume crops. In chickpea (*Cicer arietinum* L.) ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Labr. and fusarium wilt, caused by *Fusarium oxysporum* Schlect. f. sp. *ciceri* (Padwick) Matuo & Sato, can cause severe losses in the field. The use of resistant varieties is the most reliable way to control these diseases.

In order to obtain chickpea varieties with durable resistance to diseases, research focused on the knowledge of host-pathogen interaction in chickpea was started by Italian research institutions in cooperation with the International Center for Agricultural Research in the Dry Areas (ICARDA, Aleppo, Syria).

Forty-one isolates of *A. rabiei* were collected in several chickpea-growing areas and were inoculated on 13 desi and kabuli chickpea lines. A high variability in disease virulence was observed and the isolates, according to the results of cluster analysis, were grouped into three main clusters corresponding to different degrees of virulence.

The effect of culture filtrates of *A. rabiei* isolates was investigated. When tested on susceptible chickpea cultivars, they induced wilting and reduction in root growth of stem cuttings and ion leakage of leaf discs. Purification and characterization of a toxin from culture filtrate of the fungus are in progress.

Image analyses were performed on the epidermis and the second outer-stem cell layers of uninoculated susceptible and resistant cultivars. The resistant cultivar had a thicker cell wall of epidermal cells and bigger cells in the subepidermal layer, along with a lower number of xylem elements and xylem parenchyma cells.

After inoculation with *A. rabiei*, the activity of two oxidative enzymes (peroxidase [POD] and diaminoxidase [DAO]) increased markedly in the resistant cultivar in comparison with the susceptible one. Lignosuberized barriers, set up in response to pathogen invasion, were thicker and wider in resistant plants and showed apparent hystochemical activities of both POD and DAO.

As regards *F. oxysporum* f. sp. *ciceri*, a collection of isolates was established. The isolate most virulent on the cultivar, Calia, was characterized on a set of chickpea differential lines. Then it was used in screening chickpea cultivars and accessions of *Cicer* wild species for resistance to the fungus.

Introduction

The major biotic stresses affecting chickpea (*Cicer arietinum* L.) in the Mediterranean region are ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Labr. and fusarium wilt, caused by *Fusarium oxysporum* Schlect. f. sp. *ciceri* (Padwick) Matuo & Sato. The incidence of the former is increasing with the shift from spring sowing to the agronomically more convenient autumn-winter sowing.

Fusarium wilt is also expected to become more important with the increase in chickpea growing areas, particularly in southern Italy, where the climatic conditions are more conducive to the disease.

Genetic resistance is the most desirable way to control both diseases. Thus, to provide the knowledge on which more efficient breeding programs could be based, several Italian

institutions, in cooperation with the International Center for Agricultural Research in the Dry Areas (ICARDA), have carried out research programs concerning the host-pathogen relationship. Several studies had pointed out the pathogenic variability of *A. rabiei*, for which both hypotheses of the existence of physiological races (Reddy and Kabbabeh, 1985) and of a continuous variation of virulence in the fungal populations (Gowen et al., 1989) have been put forward.

Research on the variability of Italian populations of *A. rabiei* were carried out to elucidate these aspects, to improve the knowledge of the local situation, and to develop methods of artificial inoculation and disease rating to be used in screening for resistance.

As concerns the variability of *F. oxysporum*, for which races have been described by several authors (Haware and Nene 1982; Jiménez-Díaz et al. 1989; Phillips 1988), a collection of isolates was established, and their behavior was tested and compared on many chickpea lines.

The mechanisms of resistance to fungal pathogens in food-legume species have been widely studied and many papers have been published in the world (see Barz et al. 1993). Both pre-formed and infection-induced defence mechanisms have been investigated in the chickpea–*A. rabiei* pair. These include hypersensitive response, and plant enzyme inhibitors, which counteract fungal enzymes (Tenhaken and Barz 1990; Vogeslang and Barz 1993; Nehra et al. 1994) and phytoalexins (Weigand et al. 1986).

Alam et al. (1989), Höhl et al. (1991), Strange and Alam (1992), hypothesized the involvement of exotoxins in the production of early symptoms of ascochyta blight on chickpea. These were identified as solanapyrones and, in particular, solanapyrone B, which was consistently the major toxin. Isolates of *A. rabiei* differ in the ability to form the solanapyrones (Strange and Alam 1992) but no correlation between the virulence of each isolate and toxin production has been established (Höhl et al. 1991). Recent studies of Chen and Strange (1994) suggested the presence of a new toxin, a polypeptide of relative weight of 7551. For these reasons the phytotoxic activity of culture filtrates of *A. rabiei* and their effects on resistant and susceptible lines were also investigated.

A role in the resistance of chickpea to ascochyta blight has been ascribed to pre-infectional secondary compounds and postinfectional pterocarpan phytoalexins. In chickpea, pterocarpan biosynthesis is elicited also by wounding. Moreover, it has been reported that peroxidase may have some role in chickpea resistance to ascochyta blight as the increase of this enzyme activity after inoculation was more pronounced and constant in leaves and stem of resistant cultivars then susceptible ones (Vir and Grewal 1974). The role of plant structure and oxidative enzymes present in the plant or induced/enhanced by stresses was investigated with the aim of contributing to the understanding of relatively less-studied aspects of the host-pathogen relationship.

Ascochyta rabiei

Morphological and virulence variability

The Italian isolates of the fungus showed a high morphological variability (Porta-Puglia et al. 1987), apparently not correlated with virulence in preliminary experiments. Therefore, morphological observations were abandoned and subsequent research focused on the study of variability in virulence.

A suitable method of inoculation and incubation was developed (Del Serrone et al. 1987; authors of this paper, unpublished data). A collection of single-spore isolates of the fungus from different locations in Italy was established.

Forty-one isolates were inoculated on a set of 11 ICARDA lines and two Italian landraces by spraying 15-day-old seedlings with a spore suspension (1.8×10^5 conidia/ml). The plants were incubated at 21±1°C in special plastic cabinets within a conditioned greenhouse, at RH>90% for the first five days. RH was then reduced by gradually opening the top of the cabinet after the fifth day. Fifteen days after inoculation, the disease severity was evaluated according to the following scale: 0 = no visible lesions; 1 = a few small (up to 5 mm^2) lesions on stem and/or foliage; 2 = superficial stem lesions exceeding 5mm^2 and absence of stem girdling; 3 = deep and extensive stem lesions, stem girdling that can cause breakage on no more than one branch; 4 = deep and extensive girdling stem lesions, causing breakage on more than one branch followed by extensive wilting;

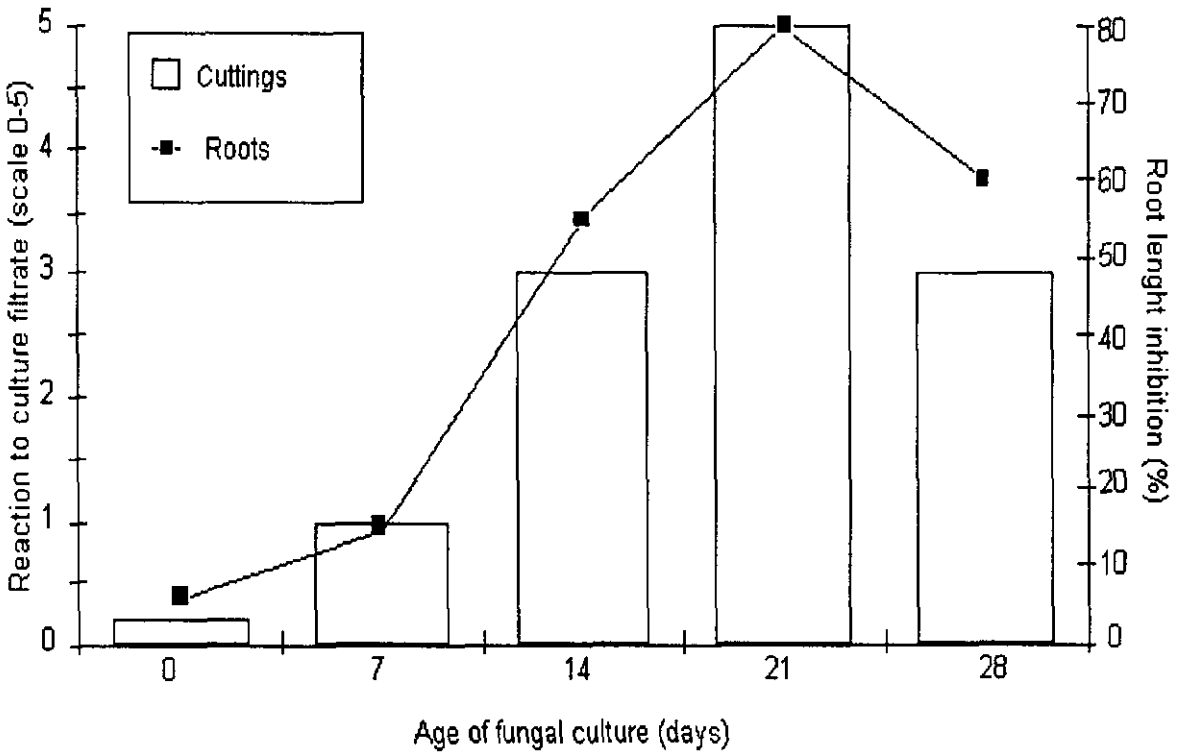

Fig. 1. Phytotoxic effects produced by filtrates of *A. rabiei* cultures of different age on chickpea cuttings and roots

5 = plant killed. Data submitted to ANOVA showed highly significant differences among both chickpea genotypes and ascochyta isolates. The interaction genotype × isolate was also highly significant (Porta-Puglia et al. 1996). The virulence rating of each isolate towards all the lines tested showed a large but continuous variability. Factor analysis showed that three factors describe 57.9, 7.9 and 7.1 % of the total variability, respectively. The chickpea lines that account for the major variability (rotated factor matrix) were: ILC 1929 and ILC 200 for factor 1, ILC 482 and ILC 484 for factor 2, ILC 191 and ILC 3279 for factor 3. The results of cluster analysis show that isolates can be grouped in three main clusters of 13, 11 and 17 isolates respectively.

A sub-set of the isolates used in this work has been analyzed by the random amplified polymorphic DNA analysis (RAPD) using three decamer primers. As a result, several amplification products common to all isolates were observed and any single isolates could be identified by an individual RAPD pattern. No correlation between RAPD pattern and the division of the isolates in pathogenic groups could be established. (Fisher et al. 1995).

Toxic metabolites

To produce culture filtrates of *A. rabiei* with the greatest expression of toxicity, the optimal conditions for fungal development were first investigated. Isolates of different virulence were grown for 14, 21 and 28 days (21±1°C) on Czapek-Dox broth and on that proposed by Nachmias et al. (1977). *In vitro* production of phytotoxic compounds by *A. rabiei* was demonstrated by comparing the activity of culture filtrates with that of uninoculated sterile broths. The immersion of the basal part of stem cuttings of the susceptible cultivar Calia in different dilutions of culture filtrates and broths showed that culture filtrates caused withering of the stem and inhibition of rooting. In order to produce culture filtrate, Nachmias broth was preferred with respect to Czapek-Dox in further testing because, when used as a control, it had only a slight effect on the cuttings. On the basis of damages scored on cuttings and reduction of root elongation, culture filtrates obtained after 21 days of fungal development on Nachmias broth showed a peak of phytotoxicity (Fig. 1); the effects on stem cuttings and root elongation were comparable. Culture filtrate of *A. rabiei* was also able to induce ion leakage from

Table 1. Outer epidermic cell wall thickness (μm) of stem of four chickpea cultivars, mean values and S.D. †

Calia	8.6 ± 0.7 B
Principe	9.1 ± 1.1 B
Califfo	11.0 ± 0.6 A
Sultano	12.0 ± 1.2 A

Means of 100 measurements.
† Values followed by the same letters are not significantly different according to Scott and Knott's cluster analysis (P=0.01).

chickpea leaflets of the susceptible cultivar Calia. When produced after 14, 21 and 28 days of fungal culture, it caused a great loss of electrolytes.

Similarly to the direct invasion of the fungus into the host tissues, culture filtrate of *A. rabiei* produced chlorosis, wilting and epinasty after 48 hrs of bioassay on stem cuttings of chickpea. Höhl et al. (1991) reported that solanopyrone fractions are also able to induce morphological changes in leaf structure as well as losses in viability and plasmolysis in cell suspension cultures. Besides, cell-wall degrading enzymes, such as cutinase, and other hydrolytic exoenzymes may be involved in the penetration process of the fungus (Höhl et al. 1990) and were also identified in our culture filtrate of *A. rabiei*. In the present experiments, the phytotoxicity was maintained in the dialysate fraction, thus suggesting the synthesis of toxic compounds with a molecular weight >10,000 by the pathogen. Contrary to the results of Alam et al. (1989), these compounds were also produced in the absence of inducing chickpea substances, considered as essential factors for toxin production by Chen and Strange (1991). These findings imply the need for more studies on the isolation and purification of toxic compounds from culture filtrates of the fungus in order to establish if other toxins, different from solanopyrones, are also synthesized by *A. rabiei*. In preliminary studies, in progress at the University of Naples, an 18,000 molecular weight protein and other molecules with high molecular weight seem to be associated with the toxic activity of *A. rabiei* culture filtrate (V. Fogliano, personal communication).

The ability to produce toxins consistently differs among the *A. rabiei* isolates. The less virulent isolate caused inhibition of root elongation quantitatively different from the most virulent one and similar observations were also obtained by ion-leakage tests, in which the highly virulent isolate did not express phytotoxicity and reacted similarly to the uninoculated broth. As already reported by Höhl et al. (1991), the formation of toxic compounds does not seem to be associated with the development of the fungus but it is influenced by the composition of the nutrient medium and by the growth conditions. A strong correlation between sporulation and the amount of toxic compounds was described by the same authors.

The effect of culture filtrates of *A. rabiei* on stem cuttings of other legumes seems to imply some specificity of action on the chickpea. The different reactions of the species were not evident with undiluted culture filtrate that caused too strong effects. A discrimination of the host was obtained with a lower concentration causing severe damage only on chickpea cuttings. A positive correlation was found between the reaction of resistant and susceptible lines to the culture filtrate and to inoculation using the same isolate of the fungus (Moscow et al. in press).

Pre-infectional defence mechanisms

The pathogen hyphae penetrate through the cuticle of stem, leaves and pods at cell junctions (Höhl et al. 1990), but not through the stomata. Recently, Köhler et al. (1995), working with GUS-transformed strains of the fungus, demonstrated that penetration can take place also through hydathodes. The fungus spreads and kills the host tissues and only the lignified tissue is not attacked (Pandey et al. 1987). In many plant species, preformed physical barriers such as cuticle, epidermis, and other outer structures, have been supposed to slow down or hinder penetration by pathogens (Akai 1959; Van den Ende and Linsken 1972; Royle 1976; Akai and Fukutomi 1980; Campbell et al. 1980). In order to determine whether the wall thickness and cytoplasm size of the epidermal cells and second outer-cell

Table 2. Area of cell lumen (μm^2) of first and second outer cell layers in chickpea stems, mean values and S.D.†

	First layer	Second layer
Calia	202.2 ± 47.2 A	764. ± 218.2 B
Principe	188.6 ± 87.5 A	528. ± 154.5 C
Califfo	230.0 ± 73.0 A	843. ± 79.3 B
Sultano	273.1 ± 86.2 A	1188. ± 250.3 A

Means of 80 measurements.
† Values followed by the same letters are not significantly different according to Scott and Knott's cluster analysis (P=0.01).

layer of the stem were related to resistance to *A. rabiei*, young seedlings of four kabuli chickpea cultivars (Sultano, Califfo, Calia, and Principe), with different morphological and bio-agronomical characteristics and various reactions to *A. rabiei*, were collected in the field and studied.

The cultivars examined showed different epidermic and subepidermic structure both with regard to the thickness of the cell wall and cell area. The outer cell wall of the stem epidermal cells was significantly thicker in the resistant cultivars compared to the susceptible ones (Table 1). Sultano had both the thickest cell walls of the epidermal cell and the largest mean area of the parenchymal cell of the outer layer (Table 2). This cultivar was one of the most resistant to ascochyta blight among the germplasm recently tested in Italy (Calcagno et al. 1987; Saccardo et al. 1987; Calcagno et al. 1992). Since the epidermal layer is also the site of storage of the isoflavones biochanin A and formononetin, precursors of phytoalexins in *C. arietinum* (Barz et al. 1993), it could be assumed that in this layer the protection assured by a thick wall can be completed by chemical mechanisms of resistance. Also in the cultivar, Califfo, the resistance to the pathogen seems to be associated with both wall thickness of the epidermal cells and size of the first parenchyma cell layer. The susceptible cultivars, Principe and Calia, do not differ significantly in outer cell wall thickness of the epidermal layer, while the cell area of the first parenchima layers is significantly different between the two cultivars (Venora and Porta-Puglia 1993).

In another investigation (Angelini et al. 1993), 15-day-old chickpea plants of the cultivars Calia and Sultano were submitted to histological analysis in order to ascertain further differences in tissue organization. Sultano showed a higher number of xylem cells and xylem parenchyma cells produced by the vascular cambium in the interfascicular regions both in the first and fourth internode, as compared to Calia.

These results need to be confirmed on a larger number of varieties. Notwithstanding, they may contribute to the understanding of the defence mechanisms acting against penetration of *A. rabiei* in the host. Whether *A. rabiei* penetrate by mechanical force or with the aid of cell-wall degrading enzymes, a thick epidermis is in itself a desirable trait that retards penetration.

Post-infectional defence mechanisms

Previous studies dealt with the involvement of peroxidase (POD) in the wound-healing process occurring in chickpea stems as a result of mechanical injury. POD activity is induced after the stem is wounded in parallel to the activity of diamine oxidase (DAO) (Angelini et al. 1990; Scalet et al. 1991). The latter enzyme, involved in polyamine catabolism, is supposed to be the source of H_2O_2 essential for POD activity in the lignosuberization process occurring in the walls adjacent to the wound (Angelini et al.1990).

As chickpea-ascochyta interaction may result in physiological responses similar to those observed in the wound-healing process, the time course of biochemical and histochemical POD and DAO activities after inoculation of the susceptible cultivar Calia and resistant cultivar Sultano with *A. rabiei* conidia was studied. Structural differences between the two cultivars as well as wall autofluorescence and lignosuberized depositions during fungal infection were also investigated. The isolate of *A. rabiei* used in this study, when tested on a set

of chickpea lines, induced a susceptible reaction on Calia and a resistant one on Sultano (Porta-Puglia et al. 1987). Calia and Sultano 15-day-old chickpea seedlings were inoculated by placing the inoculum lengthwise the fourth (first in some experiments) internode with a paint-brush.

After inoculation, the plants were incubated under conditions suitable for infection. Internodes with necroses comparable in extension were excised and used to obtain crude homogenates for determining enzyme activity or sections for histochemical studies. DAO activity was estimated polarographically using an oxigraph equipped with a Clark electrode. POD activity was analyzed spectrophotometrically following tetraguaiacol formation. Lignin/suberin depositions were detected by the phloroglucinol/HCl method and by autofluorescence analysis. Samples for scanning electron microscope (SEM) analysis were dehydrated in graded alcohol, dried with the critical point method and sputtered with gold (approx. 40 nm). No differences in conidia germination were observed in the two cultivars by SEM. Non-inoculated fourth internodes of the resistant cultivar Sultano showed a greater POD and DAO activity as compared to the susceptible cultivar Calia. After inoculation, both enzyme activities were significantly higher in Sultano as compared to inoculated Calia (Table 3). Tissue injury in inoculated resistant and susceptible seedlings was followed daily through autofluorescence and light microscopy analysis of autofluorescence and wall lignosuberization in sections obtained from the infected internodes. A crucial difference between the two cultivars is that invasion of pith parenchyma cells in the fourth internodes was never observed in the resistant cultivar while massive lysis of these cells occurred in Calia. Infection areas in both cultivars were surrounded by a barrier made by lignosuberization of cell walls of cortical parenchyma as shown by phloroglucinol/HCl incubation of internode sections. A greater extent of lignosuberized barriers appeared in the resistant cultivar Sultano as compared with cultivar Calia. Apparent histochemical POD and DAO activities were detected in the lignosuberized barriers. Similar findings were obtained from infected first internodes. These results suggest that both the structural organisation of xylem tissues, and the higher enzymatic activities of DAO and POD, and their induction after inoculation may have a key role in the resistance of the cultivar Sultano to *A. rabiei*.

Fusarium oxysporum* f.sp. *ciceri

Field surveys in the main chickpea cultivation areas were carried out to collect samples of

Table 3. DAO and POD activities in crude homogenates obtained from the fourth internodes of chickpea susceptible cultivar Calia and resistant cultivar Sultano, 12 dpi with *A. rabiei* conidia.

	DAO nKat\g FW†	POD μKat\g FW†
Calia	2.66	0.11
Calia inoculated	3.00	0.14
Calia inoculated§	3.75	0.74
Sultano	5.00	0.12
Sultano inoculated‡	7.83	0.28
Sultano inoculated§	10.54	1.54

† Internodes with necroses comparable in extension were respectively selected for two groups of infection: ‡necroses with approx. 2 mm longitudinal extension; §necroses with approx. 1 cm longitudinal extension. Similar results were obtained when enzyme activities were expressed on a protein basis. SD less than 10% of each value.

wilted chickpea plants and 17 *F. oxysporum* isolates were been obtained. Pathogenicity tests were carried out on the susceptible chickpea cultivar, Calia. A water-culture technique (Nene and Haware 1980), with slight modifications was used. The fungus was multiplied on potato-dextrose broth on an orbital shaker. A concentration of $1x10^6$ spores\ml was used. Seeds were surface disinfested with sodium hypochlorite and germinated in moistened sterile perlite. After 15 days, the seedlings were transferred into plastic glasses containing the inoculum. Thirty days after inoculation, the plants were evaluated using a 0–4 scale, according to the percentage of the plant canopy damaged by the acropetal progression of wilting (0 = 0%; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = >76–100%).

In comparative inoculations on plants belonging to several species of the Leguminosae, the isolates were pathogenic only to *C. arietinum*. They were identified as *F. oxysporum* f.sp. *ciceri*. The most virulent isolate of this collection (No. 526 II) was used onwards in all the screening tests (see Crinò et al., in this proceedings). In order to characterize this isolate, the following set of differential chickpea lines was used: JG 62, C 104, JG 74, CPS 1, BG 212, WR 315, Annigeri, Chafa, L 550 and K 850. Calia and FLIP 85-88-C were used as susceptible checks. The reaction of the differential lines (Table 4) did not fit in with the pattern of other races previously described.

The need for standardization of the techniques in order to obtain more comparable results from different countries is particularly noteworthy for pathotype classification of *F. oxysporum*.

Conclusions

The overall results obtained demonstrate that chickpea resistance to *A. rabiei* is an integrated process in which many morphological and physiological factors cooperate. Reinforcement of plant cell walls represents a major plant defense mechanism in response to the pathogen's attack. The occurrence of a polyamine-oxidizing, H_2O-generating system in the apoplast and its induction by fungal infection represents an important machinery never studied before in the defense response of chickpea to *A. rabiei*. Work is in progress to exploit this system as a tool for rapid screening of breeding material.

Further progress is expected in the understanding of *A. rabiei*–chickpea interaction through the use of molecular techniques. Recently, an important advance has been achieved in this field by the development of a transformation system for the fungus by Weltring et al. (1995).

The discovery of specific band patterns in Sultano and Calia, (respectively resistant and susceptible to *A. rabiei*) by using several arbitrary oligonucleotides, (Sonnante et al.

Table 4. Reaction of differential lines of chickpea inoculated with the Italian isolate of *F. oxysporum* No. 526 II used to evaluate wild *Cicer* accessions.

Genotype	Reaction	Disease score†
JG-62	2.9	S
C-104	1.6	M
JG-74	2.3	M
CPS-1	3.8	S
BG-212	0.0	R
WR-315	0.0	R
Annigeri	3.6	S
Chafa	1.3	M
L-550	1.3	M
K 850-3/27	0.5	R
FLIP 85-88C	4.0	S
Calia	4.0	S

† assessed on a 0-4 scale according to the percentage of wilting of plant canopy. Score 0= 0%; 1= 1–25%; 2= 26–50%; 3= 51–75%; 4= >75–100%. Scores of <1 = resistant (R); >1–3 = moderately susceptible (M); >3 = susceptible (S).

1995), represents a promising step in the marker-assisted breeding in chickpea.

At present, the status of knowledge of plant-fungus interactions in chickpea is more advanced in ascochyta blight than in fusarium wilt. For the latter, international cooperation is particularly needed in order to establish standard procedures in the study of the variability in the virulence of *F. oxysporum*.

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References

Akai, S., 1959. Histology of defense in plants. Pages 391–434 *in* Plant Pathology. An Advanced Treatise Vol. 1 (J.G. Horsfall and A.E. Dimond, ed.). Academic Press, New York.

Akai, S. and M. Fukutomi. 1980. Preformed internal physical defences. Pages 139–159 *in* Plant Disease. An Advanced Treatise, Vol. V. (J.G. Horsfall, and E.B. Cowling, ed.). Academic Press, New York.

Alam, S., J.N. Bilton, A.M.Z. Slawin, R.N. Williams, R.N. Sheppard and R.N. Strange. 1989. Chickpea blight: production of solanapyrones A and C by *Ascochyta rabiei*. *Phytochemistry* 28: 2627–2631.

Angelini, R., F. Manes and R. Federico. 1990. Spatial and functional correlation between diamine oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chickpea stems. *Planta* 182: 89–96.

Angelini, R., M. Bragaloni, R. Federico, A. Infantino and A. Porta-Puglia. 1993. Involvement of polyamines, diamine oxidase and peroxidase in resistance of chickpea to *Ascochyta rabiei*. *Journal of Plant Physiology* 142: 704–09.

Barz, W., W. Bless, W. Gunia, B. Höhl, U. Mackenbrock, D. Meyer, R. Tenhaken and R. Vogeslang. 1993. Mechanism of resistance to fungal pathogens in cool-season food legumes. Pages 193–210 *in* Breeding for stress tolerance in cool-season food legumes (K.B. Singh and M.C. Saxena, ed.). John Wiley & Sons, Chichester, UK.

Calcagno, F., I. Raimondo M. Iaiani, D. Mastrovitoe and G. Rivoira. 1987. Risultati di prove di confronto varietale di *Cicer arietinum* L. in diversi ambienti dell'Italia centro-meridionale ed insulare condotte nel 1984/85. *Terra e Sole* 42: 123–127.

Calcagno, F., P. Crinò, and F. Saccardo. 1992. Sultano and Califfo, the first Italian chickpea cultivars resistant to Ascochyta blight. Page 40 *in* 2nd International Food Legume Research Conference Abstracts 12–16 April 1992, Cairo.

Campbell, C.L., J. Huang and G.A. Payne. 1980. Defense at perimeter : the outer walls and the gates. Pages 103–120 *in* Plant Disease. An Advanced Treatise, Vol. V. (J.G. Horsfall and E.B. Cowling, ed.) Academic Press, New York.

Chen, Y.M. and R.N. Strange. 1991. Synthesis of the solanopyrone phytotoxins by *Ascochyta rabiei* in response to metal cations and development of a defined medium for toxin production. *Plant Pathology* 40: 401–407.

Chen, Y.M. and R.N. Strange. 1994. Production of a proteinaceous phytotoxin by *Ascochyta rabiei* grown in expressed chickpea sap. *Plant Pathology* 43: 321–327.

Del Serrone, P., A. Porta-Puglia and P. Crinò. 1987. Comportamento del cece verso *Ascochyta rabiei*: effetto dell'età e della concentrazione di inoculo. *Informatore fitopatologico* 37(6): 59–61.

Fisher, D., A. Porta-Puglia and W. Barz. 1995. RAPD analysis of pathogenic variability in *Ascochyta rabiei*. *Journal of Phytopathology* 143: 601–607.

Gowen, S.R., M. Orton, B. Thurley and A. White. 1989. Variation in pathogenicity of *Ascochyta rabiei* on chickpea. *Tropical Pest Management* 35(2): 180–186.

Haware, M.P. and Y.L. Nene. 1982. Races of *Fusarium oxysporum* f.sp. *ciceri*. *Plant Disease* 66: 809–810.

Höhl, B., M. Pfautsch and W. Barz. 1990. Histology of disease development in resistant and susceptible cultivars of chickpea (*Cicer arietinum* L.) inoculated with spores of *Ascochyta rabiei*. *Journal of*

Phytopathology 129: 31–45.

Höhl, B., C. Weidemann, U. Höhl and W. Barz 1991. Isolation of solanapyrones A, B and C from culture filtrates and spore germination fluids of *Ascochyta rabiei* and aspects of phytotoxin action. *Journal of Phytopathology* 132: 193–206.

Jiménez-Díaz, R.M., A. Trapero-Casas and J. Cabrera de la Colina. 1989. Races of *Fusarium oxysporum* f. sp. *ciceri* infecting chickpea in southern Spain. *In* Vascular Wilt Diseases of Plants, NATO ASI Series, Vol H28 (E.C. Tjamos and C. Beckman, ed.) Springer-Verlag, Berlin, Germany.

Köhler, G., C. Linkert and W. Barz. 1995. Infection studies of *Cicer arietinum* (L.) with GUS-(*E. coli* β-glucuronidase) transformed *Ascochyta rabiei* strains. *Journal of Phytopathology* 143: 589–595.

Mosconi, C., Crinò, P. Saccardo F. and K.B. Singh. Effect of culture filtrate of *Ascochyta rabiei* on chickpea genotypes. *Petria.* (In press.)

Nachmias, A., I. Barash, Z. Solel and G. Strobel. 1977. Purification and characterization of phytotoxins produced by *Phoma tracheiphila*, the causal agent of mal secco of *Citrus*. *Physiological Plant Pathology* 10: 147–157.

Nehra, K.S., L.K. Chugh, S. Dhillon and R. Singh. 1994. Induction, purification and characterization of chitinases from chickpea (*Cicer arietinum* L.) leaves and pods infected with *Ascochyta rabiei*. *Journal of Plant Physiology* 144: 7–11.

Nene, Y.L. and M.P. Haware. 1980. Screening chickpea for resistance to wilt. *Plant Disease* 64: 379–380.

Pandey, B.K., U.S. Singh and H.S. Chaube. 1987. Mode of infection of Ascochyta blight of chickpea caused by *Ascochyta rabiei*. *Journal of Phytopathology* 119: 88–93.

Phillips, J.C. 1988. A distinct race of chickpea wilt in California. *International Chickpea Newsletter* 18: 19–21.

Porta-Puglia, A., G. Di Giambattista, P. Crinò, G. Cecchini and N. La Gioia. 1987. Variabilità di *Ascochyta rabiei* in Italia. *Terra e Sole* 42(534): 114–116.

Porta-Puglia A., P. Crinò and C. Mosconi. 1996. Variability in virulence to chickpea of an Italian population of *Ascochyta rabiei*. *Plant Disease* 80: 39–41.

Reddy, M.V. and S. Kabbabeh. 1985. Pathogenic variability of *Ascochyta rabiei* (Pass.) Lab. in Syria and Lebanon. *Phytopathologia Mediterranea* 24: 265–266.

Royle, D.J. 1976. Structural features of resistance to plant diseases. Pages 161–193 *in* Biochemical Aspects of Plant-Parasite Relationships (J. Friend and D.R. Trelfall, ed.). Academic Press, New York.

Saccardo, F., P. Crinò, P. Vitale, F. Calcagno, and A. Porta-Puglia. 1987. Miglioramento genetico del Cece in Italia: obiettivi perseguiti e primi risultati. *Terra e Sole* 42: 82–88.

Scalet, M., R. Federico and R. Angelini. 1991. Time course of diamine oxidase and peroxidase activities, and polyamine changes after mechanical injury of chickpea seedlings. *Journal of Plant Physiology* 137: 571–575.

Sonnante, G., A. Marangi and G. Venora. 1995. Applicazione di marcatori molecolari RAPDs alla identificazione varietale in *Cicer arietinum* L. Pages 51–52 *in* Atti del XXXIX Convegno Annuale della Società Italiana di Genetica Agraria (SIGA), Vasto Marina (CH), 27–30 September 1995.

Strange, R.N. and S.S. Alam. 1992. Toxins of *Ascochyta rabiei* and their putative role in screening chickpea for blight resistance. Pages 170–180 *in* Disease resistance breeding in chickpea (K.B. Singh and M.C. Saxena, ed.). ICARDA, Aleppo, Syria.

Tenhaken, R. and W. Barz. 1990. Characterization of pectic enzymes from the chickpea pathogen *Ascochyta rabiei*. *Zeitschtift für Naturforschung* 46C: 51–57.

Van den Ende, G. and H.F. Linskens. 1972. Cutinolytic enzymes in relation to pathogenesis. *Annual Review of Phytopathology* 12: 247–258.

Venora, G. and A. Porta-Puglia, 1993. Observations on outer cell layers of stem in chickpea cultivars susceptible and resistant to ascochyta blight. *Petria* 3: 177–182.

Vir, S. and J.S. Greval. 1974. Peroxidase activity associated with ascochyta blight of gram (*Cicer arietinum* L.). *Phytopathologia Mediterranea* 15: 174–175.

Vogeslang, R. and W. Barz. 1993. Purification, characterization and differential hormonal regulation of a β-1,3-glucanase and two chitinases from chickpea (*Cicer arietinum* L.). *Planta* 189: 60–69.

Weigand, F., J. Köster, C. Weltzien and W. Barz. 1986. Accumulation of phytoalexins and isoflavone glucosides in a resistant and a susceptible cultivar of *Cicer arietinum* during infection with *Ascochyta rabiei*. *Journal of Phytopathology* 115: 214–221.

Weltring, K.M., H.P. Schaub and W. Barz. 1995. Metabolism of pisatin stereoisomers by *Ascochyta rabiei* strains transformed by the pisatin demethylase gene of *Nectria haematococca* MP VI. *Molecular Plant Microbe Interaction* 4: 499–500.

Resistance to Ascochyta Blight and Fusarium Wilt for Chickpea Cultivation in Italy

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Summary

In the last 13 years, intensive work on resistance to *Ascochyta rabiei* has been done in Italy. Until 1987, this work was carried out within a project coordinated by ENEA (Italian Agency for Renewable Energy, Rome), and then one funded by the Italian Foreign Ministry 1988–1994 and one by the European Union 1991–1995. Targets of the research activity were (1) the development of screening methodologies, (2) the identification of sources of resistance to ascochyta blight and fusarium wilt in cultivated and wild germplasm of *Cicer*, the utilization and the induction of sources of resistance to *A. rabiei*, (3) the development of agronomically valuable lines and cultivars of chickpea resistant to *A. rabiei*.

Efficient and reliable field and greenhouse methodologies were used to screen chickpea for resistance to both pathogens; laboratory-screening techniques, based on the use of ascochyta culture filtrate, have also been investigated on chickpea genotypes susceptible and resistant to the fungus and might be considered as screening techniques additional to artificial inoculations. A fusarium wilt sick plot and a water-culture technique were developed to screen chickpea material in the field and greenhouse.

After evaluation of many accessions coming from different germplasm collections, considerable progress has been made in identifying genotypes resistant to ascochyta blight. Most lines selected for resistance to ascochyta blight, under very severe conditions of fungal infection, originated from ICARDA nurseries and trials. A total of 130 cross-combinations have been accomplished using parents resistant to ascochyta blight and with other interesting agronomical traits. Two mutants resistant to particularly aggressive Italian isolates of *A. rabiei* have been induced by gamma-ray treatment on seeds of the susceptible ecotype Calia.

Interesting levels of resistance to ascochyta blight have been found in wild annual species of *Cicer* such as *Cicer judaicum*, *C. pinnatifidum* and *C. bijugum*. A highly resistant reaction to fusarium wilt was shown in *C. bijugum*, *C. echinospermum*, *C. judaicum*, *C. pinnatifidum*, and *C. reticulatum*. These wild species seem particularly interesting candidates for interspecific hybridizations with the cultigen, when innovative techniques will be developed to overcome the interspecific incompatibility barriers.

Through plant-selections from ICARDA lines ILC 3279 and ILC 72, in 1990, the first two Italian winter cultivars, high yielding and resistant to *A. rabiei*, were released with the names of Sultano and Califfo. Requests for the release of new cultivars (Pascià, Visir, Otello, Alì, Emiro) were recently submitted to the Italian Ministry for Agriculture, Food and Forest Resources. These cultivars present levels of grain yield and ascochyta resistance similar or superior to the resistant varieties Sultano and Califfo; Pascià and Visir combine reasonable grain yields, large grain and resistance to *A. rabiei*.

Introduction

Among the major constraints to chickpea (*Cicer arietinum* L.) production, *Ascochyta rabiei* (Pass.) Labr. represents the most devastating pathogen in countries with a Mediterranean climate, Italy included, where yield losses of more than 40% with winter-sown varieties are frequent. *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans f.sp. *ciceri* (Padwick) Mateo & Sato is not

yet widespread in Italy; attacks are limited to spring-sown chickpea in southern regions. Genetic resistance represents the most effective control measure for both pathogens (Singh, 1993).

In spite of the development of chickpea lines and cultivars resistant to ascochyta blight in different countries (Ponz-Ascaso et al. 1992; Reddy and Singh 1992; SAT News 1992; Açikgoz et al. 1993; Singh et al. 1993a, b; Singh and Reddy 1994), their use is sometimes ephemeral and geographically circumscribed due to the breakdown of resistance caused by the appearance of new fungal pathotypes. Resistance to ascochyta blight in chickpea seems to be controlled by single dominant (*Rar1* in ILC 195, *Rar2* in ILC 200, *Rar3* in ILC 182, *Rar4* in ILC 191, *Rar5* in ILC 482) or recessive genes (e.g. in BRG 8), depending on the genotype (Pieters and Tahiri 1986; Tewari and Pandey 1986; Singh and Reddy 1989; Halila et al. 1989) and probably influenced by other genes (Singh and Reddy 1990). Two dominant complementary genes are present in the lines GLG 84038 (Arc_1, Arc_2) and GL 84099 (Arc_3, Arc_4), whereas the resistance of the black-seeded genotype ICC 1468 is controlled by both a dominant ($Arc_{5(3,4)}$) or one recessive independent gene (Arc_1) (Dey and Singh 1993). Rheenen and Haware (1994) referred to a quantitative control of the resistance with a significant 'vertical' component and proposed that a strategy based on gene pyramiding should be used to obtain resistant lines. More studies are still needed to better understand the inheritance of resistance to ascochyta blight because of its important implications in breeding strategies.

Good progress has been made in developing chickpea material resistant to fusarium wilt in many countries but success has been limited by the location-specific races of the pathogen (Singh and Reddy 1991) and no data exist on the maintenance of resistance across locations (Jiménez-Diaz et al. 1991). Resistance has been found in chickpea germplasm and transferred into high yielding desi and kabuli backgrounds at ICRISAT (Kumar et al. 1985; Pawar et al. 1992; ICRISAT 1994 a, b; c; Gowda et al. 1995), and in India (Zote et al. 1993), Mexico (Morales 1986), California (Buddenhagen and Workneh 1988), and Tunisia (Halila and Harrabi 1990). At least two recessive and one dominant independent loci confer resistance to race 1 of *F. oxysporum* f.sp. *ciceris* but studies are still required on the control of resistance to other races of the fungus and on their possible effect against each of them (Jiménez-Diaz et al. 1993).

More research is also needed to increase the effectiveness and stability of both ascochyta and fusarium resistance, which are essential to chickpea cultivation in different pedoclimatic areas in Italy. Future efforts could be directed towards the development of cultivars with multiple stress resistance (Singh et al. 1994). Some authors referred that wild *Cicer* species represent promising sources of resistance to *A. rabiei* (Singh Gurdip et al. 1991; Haware et al. 1992; Dey et al. 1993; Singh and Reddy 1993) and to *F. oxysporum* f.sp. *ciceri* (Nene and Haware 1980; Haware et al. 1992; Kaiser et al. 1994).

This paper deals with research in Italy on resistance to both pathogens. Intensive work on resistance to *A. rabiei* has been undertaken in the last 13 years. Until 1987, these activities were carried out within the framework of a project co-ordinated by ENEA, with the collaboration of Experimental Institute of Plant Pathology in Rome and the Experimental Station for the Wheat Culture in Caltagirone (Catania). This was divided into two new projects: one funded by the Italian Foreign Ministry and carried out by ICARDA and five Italian institutions (1988–1994), and the other, AGRE CT 90-0051, financed within the program by the European Union (1991–1995), and carried out by Spain, Italy and England, ECLAIR. The activities on resistance to fusarium wilt are more recent and were included in the project with ICARDA and five Italian institutions.

Targets of the whole research activity on disease resistance in chickpea were (1) the development of screening methodologies, (2) the identification of sources of resistance to ascochyta blight and fusarium wilt in cultivated and wild germplasm of *Cicer*, (3) the use and the induction of sources of resistance to *A. rabiei*, (4) the development of agronomically valuable lines and cultivars of chickpea resistant to *A. rabiei*. This last point has been largely discussed by Saccardo et al. in these proceedings, and will not be reported in detail here.

Ascochyta blight

Development of screening techniques

(ENEA Project, 1982–1987 and ICARDA and the Five Italian Institutions Project, 1988–1994)

Field

The screening in the field represents a cheap method to test, under natural infection conditions and during the whole plant cycle, a large number of individuals. However, the environmental conditions as well as the nature of the inoculum available in the area and the interaction with other organisms, can complicate this type of screening and affect the expression of resistance; risks of confusing escape with resistance also exist (Porta-Puglia et al. 1994; Haware et al. 1995). Epidemics can be enhanced either by spraying a spore suspension on young plants or by interplanting some rows of a susceptible genotype (spreaders) in the lines. In case environmental conditions are not favorable to the disease development, sprinkler irrigation may facilitate the blight buildup. The presence of inoculum may also be assured by intercropping the material with some seeds of a susceptible genotype infected by immersion in a conidial suspension of the fungus.

A 1–9 scale, based on the visual evaluation of ascochyta damages in the plot (Singh et al. 1981), is used in different moments of the plant cycle.

Greenhouse

With respect to the large-scale field screening methodologies, those developed for the greenhouse may reproduce environmental factors such as relative humidity and temperature that are more favorable to the infection and disease development. In this way, lines with resistant reaction to *A. rabiei* may be re-tested by single fungal isolates. However, the space could represent a limiting constraint and, in addition, the greenhouse results always need to be validated in the field.

Inoculations are performed by spraying chickpea plantlets with a spore suspension of *A. rabiei*, inside a plastic cabinet with a thermostat set at 21±2°C). The inoculum is prepared by gently rubbing with a glass rod the surface of a mature colony sub-cultured (22±1°C; alternating 12h light/12h darkness) on Potato Dextrose Agar (PDA) and then soaked with sterile water. The conidial suspension was obtained after filtration through a double cheesecloth layer. During the incubation period, a high relative humidity (>90%) is assured by keeping the cabinet closed for the first five days; then, the cabinet top is opened gradually keeping wet the perlite layer on the bottom.

According to a 0–5 scale where 0=no visible lesions and 5=plant killed (Porta-Puglia et al., in press), ascochyta severity is recorded 15 days after inoculation on each individual plant. The average of the individual records corresponds to a resistant reaction for the scores <2.5 and to a susceptible reaction for the others. The value of 2.5 is considered a discriminate of reaction to *A. rabiei*, because it results from scores in which the appearance of girdling lesions on the stem is represented.

In order to identify the optimal conditions (plant age, concentration of the fungal suspension, incubation temperatures) for developing the greenhouse screening technique, preliminary tests were necessary. Different concentrations (2×10^4, 2×10^5, 2×10^6 condole/ml) of condole suspensions were sprayed on chickpea plants of both the susceptible ecotype Call and the resistant ICARDA line ILC 191 at different plant ages (15, 30, 45 days).

A clear discrimination between the genotypes was noticed using the first two concentrations of inoculum, preferably 2×10^5 condole/ml, sprayed on 15-day-old plants (Del Serrone et al. 1987). Besides, the plant age was better optimized by artificially inoculating 8-13-20 day old plantlets of susceptible (ILC 1929, ecotypes Principe and Calia) and resistant (ILC 202, ILC 191, ILC 3279) lines and ecotypes. Eight-day old infected seedlings were severely damaged by ascochyta blight without any possibility of differentiating the behavior of the genotypes. A clear-cut answer was obtained using 13 and 20 day-old plantlets, both preferred to screen the genetic material in the greenhouse (Fig. 1a). After artificial inoculations of chickpea lines resistant (ILC 72, ILC 3279, ILC 191, ICC 3996) and susceptible (ecotype Calia, ICC 5127, ILC 484) to the fungus using incubation temperatures 10, 15, 20, 25, 30°C, the genotypes developed high levels of ascochyta incidence at 15–25°C, when it was also possible to differentiate their reactions to ascochyta blight (Fig. 1b).

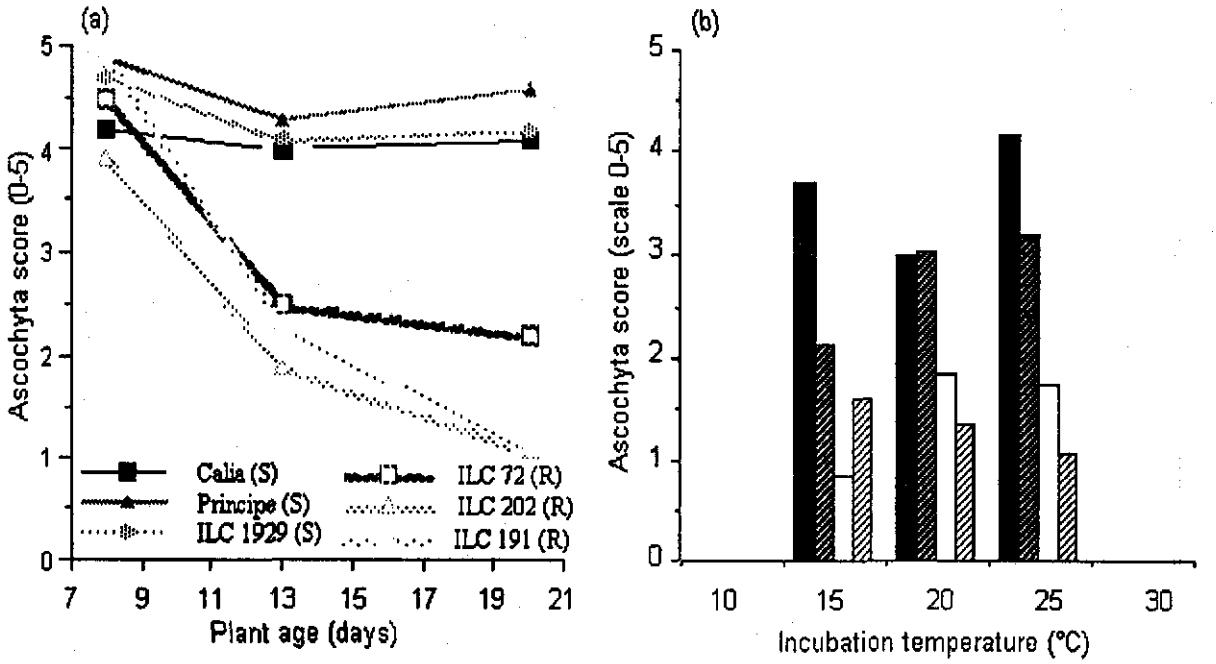

Fig. 1. Effect of different (a) plant ages and (b) incubation temperatures on the discrimination of resistant and susceptible genotypes of chickpea artificially inoculated with *A. rabiei* (Italian isolate Porano) in the greenhouse

Laboratory

This kind of screening can be very important when field conditions are not favorable to the infection and to the disease development or when it is necessary to apply a fast screening methodology to differentiate between plants/lines which are resistant and susceptible to *A. rabiei*.

As referred by Porta-Puglia et al. in these proceedings, culture filtrate of *A. rabiei* is able to reproduce on chickpea cuttings some symptoms similar to ascochyta blight. In addition to the spore suspension sprayed on the plantlets in the greenhouse, it could be used as a screening agent assuring the repeatability of the results under strongly controlled environmental conditions. The discriminating effect of ascochyta filtrate among chickpea genotypes was confirmed by different bioassays (Table 1). On chickpea cuttings, the filtrate concentration of 60% was the most suitable to show a clear

Table 1. Effect of Ascochyta culture filtrate on chickpea genotypes resistant and susceptible to the fungus.

Bioassay	Effect on chickpea genotypes S	R
Damages on stem cuttings	+	-
Inhibition of root elongation	+	-
Ion leakage	+	-
Inhibition of pollen germination	+	-
Protoplast mortality	+	-
Inhibition of *in vitro* multiple shoot formation	+	-
Induction of POD activity[†]	-	+

[†] The level of POD activity is normally increased in the resistant genotypes respect to the susceptible ones

R = resistant to fungus

S = susceptible to fungus

discrimination of the genotypes (Mosconi et al., in press); this reaction and the response of 15-day-old plantlets sprayed with a conidial suspension of the fungus in the greenhouse were significantly correlated for two Italian isolates of *A. rabiei*, Porano (r=0.80; P=0.01) and Racale (r=0.95; P=0.001).

Culture filtrate (isolate Porano) also induced low ion-leakage and high peroxidase (POD) activity after treatment of leaflets and cuttings of a chickpea genotype resistant to ascochyta blight. On the basis of pollen germination percentage and tube length, the filtrate concentrations of 5–20 µl ml^{-1} was able to discriminate the different reactions of genotypes to ascochyta blight (Svetleva and Mosconi, unpublished data). Both these parameters, in particular the germination percentage, were well correlated with the responses to fungal infection (r=0,929; P=0,01). By flow cytometry, the protoplasts of the susceptible and the resistant lines showed a differential response when challenged for 4 hrs by 6% diluted culture filtrate of *A. rabiei*; protoplasts of the susceptible genotype showing severe metabolic alterations and a high mortality (Lucretti et al. 1991). Besides, when challenged *in vitro* with 40% diluted culture filtrate of the isolate Porano, 75% of multiple shoot formation was observed from mature embryos of an ascochyta-resistant genotype in contrast to 30% recorded from those of a susceptible line (Mosconi, unpublished data).

Isolate selectivity of culture filtrate from Porano and Racale on chickpea genotypes was also observed. By the cutting bioassay, for example, the line ILC 3279, resistant to the blight when artificially inoculated with the isolate Porano and susceptible with the isolate Racale, maintained insensitivity and sensitivity to the corresponding toxic filtrates (Mosconi et al. in press). The same differential responses were confirmed by ion leakage test.

Identification, use and induction of sources of resistance

(ENEA Project, 1982–1987, ICARDA-five Italian institutions, 1988–1994, ENEA/ECLAIR Project AGRE CT 90-0051, 1991–1995)

A wide collection of local and foreign germplasm, involving 1527 entries of *C. arietinum* and 73 accessions of *Cicer* wild species (Table 2), was evaluated for resistance to ascochyta blight, in winter sowing and under natural infection conditions. This was done in experimental fields of central and southern Italy as well as in the greenhouse.

The analysis of the genotypes assessed during 1982–1995 showed that only 26% of them were resistant to *A. rabiei*, some of which also presented good yield potentials and interesting plant architecture with erect plant standing. Although material derived from ICRISAT, India, France, Iran, Spain, Pakistan, the ex-USSR and Turkey revealed some resistant entries, the most interesting and the highest percentage of sources of resistance to the fungus came from ICARDA nurseries and trials. Out of 133 ICARDA entries tested during 1995 in the experimental field of Tarquinia, where an extremely aggressive attack of *A. rabiei* has taken place since the early spring until June/July, fourteen accessions (FLIP 90-89C, FLIP 93-2C, FLIP 93-4C, FLIP 93-5C, FLIP 93-7C, FLIP 93-11C, FLIP 93-61C, FLIP 93-66C, FLIP 93-68C, FLIP 93-74C, FLIP 93-81C, FLIP 93-82C, FLIP 93-85C, FLIP 93-96C) presented a high level of resistance to ascochyta blight. The majority of Italian ecotypes were practically destroyed by the blight.

Table 2. Chickpea entries of different origins assessed for resistance to *A. rabiei* during 1982–1995.

Origin	Entries analyzed (no.)	Resistant entries (no.)
Afghanistan	3	-
Algeria	4	-
Bulgaria	6	-
Egypt	4	-
Ethiopia	1	-
France	15	6
ICARDA	1031	332
ICRISAT	55	6
India	62	5
Iran	19	2
Iraq	3	-
Israel	3	-
Italy	176	30
Jordan	3	-
Yugoslavia	3	-
Mexico	2	-
Morocco	10	-
Pakistan	15	1
Spain	2	-
Spain - KOIPESOL	21	11
USA	12	-
ex-USSR	35	7
Tunisia	6	-
Turkey	31	2
Unknown	5	-
Total	1527	402

Among 73 accessions of wild annual *Cicer* spp (15 of *C. reticulatum*, 5 of *C. echinospermum*, 15 of *C. bijugum*, 18 of *C. judaicum*, 20 of *C. pinnatifidum*), screened under controlled conditions at the plant age of four weeks, high levels of resistance to Italian aggressive isolates of *A. rabiei* (Porta-Puglia et al. in these proceedings) were found respectively in 16 (89%) accessions of *C. judaicum*, 7 (35%) of *C. pinnatifidum* and 2 (13%) of *C. bijugum*. The severity of the blight, scored only on the resistant wild accessions and on some lines of *C. arietinum* used as differentials of Italian pathogenic groups of *A. rabiei* (Porta-Puglia et al., in these proceedings), is reported in Fig. 2. The particularly aggressive isolates of *A. rabiei* are able to cause the breakdown of the resistance on the resistant genotypes ILC 72, ILC 3279, ICC 3996 which, along with the susceptible ecotype Calia and the line ICC 5127, presented high ascochyta scores. In particular, the most interesting level of resistance was found in the accessions No. 42 (ILWC 20/S-4) and 105 (ILWC 38/S-2) of *C. judaicum* as well as in No. 73 (ILWC 29/S-7) of *C. pinnatifidum* (Mosconi et al.1995).

Within the four-year ENEA/ECLAIR Project AGRE CT 90-0051, a total of 130 cross-combinations has been accomplished using parents resistant to ascochyta blight and others with interesting agronomical traits.

The successful hybridizations combined resistance to ascochyta blight with high yield, large seed, erect plant architecture, and resistance to fusarium wilt. Parents resistant to *A. rabiei*, such as the lines ILC 3279, ICC 3996, FLIP 84-92C along with ENEA lines were used.

As referred to by Saccardo et al. in these proceedings, 1237 interspecific hybridizations among *C. arietinum* and the resistant accessions of *C. judaicum*, *C. pinnatifidum*, *C. bijugum* were carried out unsuccessfully.

In order to obtain new sources of durable resistance to Ascochyta blight, also valuable against the particularly aggressive Italian isolates of *A. rabiei*, a mutagenesis program was performed (Saccardo et al. in these proceedings). After treatment with gamma rays (200 Gy) on dry seeds of the susceptible ecotype Calia and artificial inoculation of M_2 bulk and M_3-M_6 plant progenies, two mutants maintained resistance to an Italian aggressive isolate of *A. rabiei* at both the plantlet and the podding stage (Saccardo et al. 1993).

Genetic control of resistance
(ICARDA-five Italian Institutions, 1988–1994)

In order to study the genetic control of resistance to Ascochyta blight, artificial inoculations were carried out, under controlled conditions, on F_2 and BC_1 segregating material coming from the cross-combinations Calia x ILC 72, Calia x ILC 482 and Calia x ILC 3279 as well as on the parental genotypes (Table 3). The 282 F_2 plants of the cross Calia x ILC 72 showed a 3:1 segregation ratio, with a non-significant chi-square value, thus corresponding to a one dominant gene model for resistance character which was confirmed by 1:1 ratio of BC_1 material. For the other F_2 populations (275 plants from Calia x ILC 482 and 152 from Calia x ILC 3279), a 9:7 segregation ratio indicated the occurrence of two complementary dominant genes; non-significant values of X^2 were obtained. Their first backcrosses, with 1:3 segregation ratio confirmed these data.

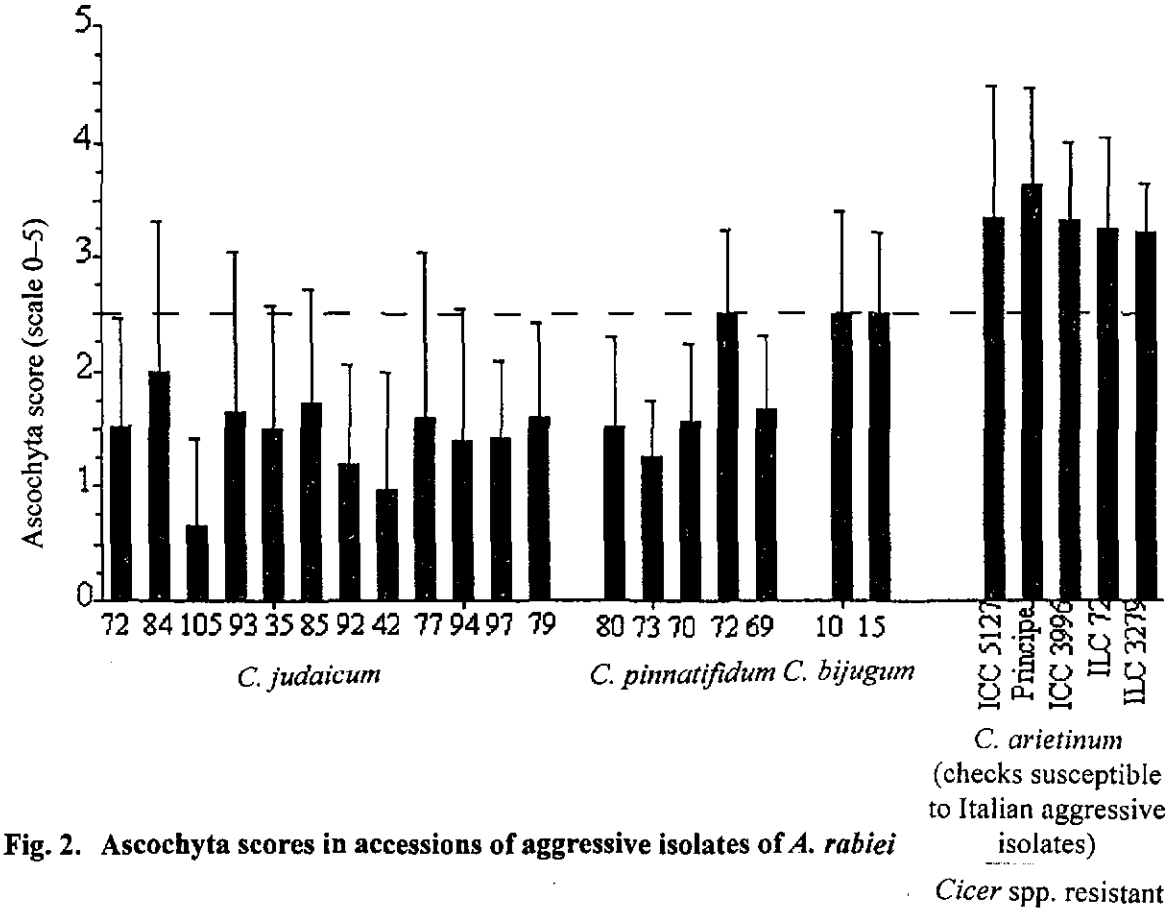

Fig. 2. Ascochyta scores in accessions of aggressive isolates of *A. rabiei*

Table 3. Reaction to infection with *A. rabiei* in chickpea F_2 and BC_1 material.

Cross-combinations and parents		No. of plants	Observed ratio Resistant	Observed ratio Susceptible	Segregation ratio	X^2
Calia x ILC 72	(F_2)	282	213	69	3:1	0.020 n.s.
(Calia x ILC 72) x Calia	(BC_1)	96	42	54	1:1	1.500 n.s.
Calia (S)		41	0	41		
ILC 72 (R)		13	13	0		
Calia x ILC 482	(F_2)	275	157	118	9:7	0.059 n.s.
(Calia x ILC 482) x Calia	(BC_1)	105	33	72	1:3	2.505 n.s.
Calia (S)		84	0	84		
ILC 482 (R)		64	64	0		
Calia x ILC 3279 (F_2)		152	89	63	9:7	0.241 n.s.
(Calia x ILC 3279) x Calia (BC_1)		88	27	61	1:3	1.515 n.s.
Calia (S)		41	0	41		
ILC 3279 (R)		37	37	0		

R=resistant; S=susceptible
n.s.=non significant

One dominant gene controlling resistance to ascochyta blight was already reported for the lines ILC 482 (Halila et al. 1989), ILC 72 and ILC 3279 (Singh and Reddy 1989). In addition to a common gene, other genes for resistance were supposed to be responsible for differences in the reaction of both ILC 72 and ILC 3279 to different pathogenic groups of ascochyta isolates and in different countries (Singh and Reddy 1989).

Development of lines and cultivars improved for resistance

(ENEA Project, 1982–1987, ICARDA-five Italian Institutions, 1988–1994, ENEA/ECLAIR Project AGRE CT 90-0051, 1991–1995)

Through plant-selections within ICARDA lines ILC 3279 (from ex-USSR) and ILC 72 (from Spain), in 1990, ENEA and Experimental Station for the Wheat Culture in Caltagirone (Catania) released the first two Italian winter and high-yielding varieties Sultano and Califfo, characterized by resistance to *A. rabiei* (Calcagno et al. 1988; Saccardo et al., in these proceedings). Resistance to ascochyta blight, confirmed in most Italian locations and by greenhouse tests, was effective against different Italian isolates (Crinò et al. 1992), except the most aggressive ones (3rd pathogenic group of Italian ascochyta isolates according to Porta-Puglia et al., in these proceedings). In January 1995, within the project ECLAIR AGRE CT 90-0051 (1991–1994) and that with the ICARDA five Italian Institutions (1988–1994), requests of inscription to the National List for new varieties, coming from hybridizations performed at ICARDA with genotypes resistant to *A. rabiei*, were submitted by ENEA (varieties Pascià, Otello) and the University of Naples (varieties Visir, Alì) to the Italian Ministry for Agriculture, Food and Forest Resources. Pascià and Visir combine reasonable grain yields with ascochyta resistance and a large size of the grain, while Alì and the desi-type, Otello, have a small seed. In 1996, within the ECLAIR Project AGRE CT 90-0051, ENEA requested the inscription to the National List for the high-yielding and small-seeded variety EMIRO, which is result a cross-combination between two resistant parents, ILC 72 and ILC 191. Also when rainy and cool spring seasons caused severe attacks of *A. rabiei*, the varieties Pasicà, Visir, Otello, Emiro, and Alì showed interesting levels of resistance to ascochyta blight in the field. A good

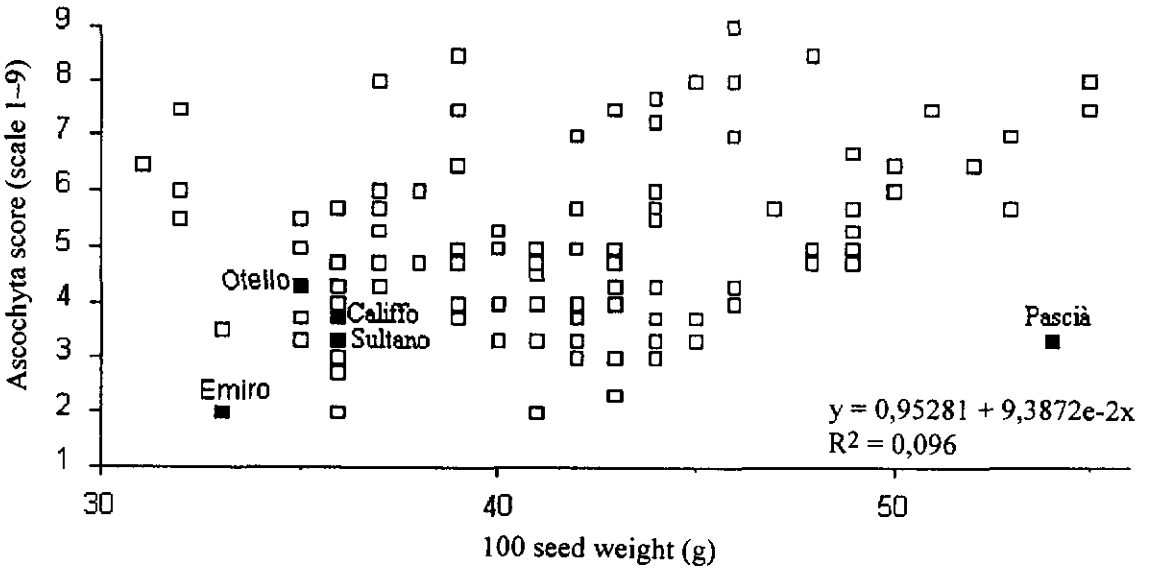

Fig. 3. Correlation between 100 seed weight and Ascochya score in chickpea lines agronomically assessed in Tarquinia during 1994–95

expression of resistance to ascochyta blight was observed for a further five lines, which outyielded the cultivars Sultano and Califfo by 17%. Four of these top-yielding lines, FLIP 82-126C, FLIP 84-182C, FLIP 91-58C, ENEA 88-20, also showed a 100 seed weight higher than 40 g, strongly indicating that a sensible progress in both the yield and the seed weight has been achieved on ascochyta-resistant material.

In the ENEA agronomical trial at Tarquinia (1994–95), no correlation was found between 100 seed weight and ascochyta score. Up to now, the most interesting large seed cultivar resistant to *A. rabiei* has been Pascià (Fig. 3).

Fusarium Wilt

(ICARDA-five Italian Institutions, 1988–1994)

Development of screening techniques

Field

In order to screen several chickpea lines for resistance to *F. oxysporum* f.sp. *ciceri*, since 1988, an artificially infested wilt sick-plot has been established in Monterotondo (Roma). According to the technique described by Fravel et al. (1985) for the inoculum production, the fungus was grown on Potato Dextrose Broth (PDB). After ten days, the fungal suspension was diluted at a known concentration with sterile water and mixed with a solution of sodium alginate and kaolin. Pellets incapsulating the fungus were produced by dipping the mix on a solution of calcium chloride. The pellets obtained were air dried at room temperature and incorporated into the soil (20 cm depth, 50–70 million spores/m^2).

The inoculations were repeated for four years. After each inoculation, a susceptible chickpea line was grown in order to check the establishment and the distribution of the fungus across the plots. After the first year, wilt symptoms were observed on 5% of the plants, 25% in the second, 50% in the third; and after the fourth year, the wilt sick-plot was considered suitable for screening activities.

Greenhouse

A water-culture technique (Nene and Haware 1980), with a few slight modifications was developed, and cultivated and wild chickpea germplasm were screened in the greenhouse at a temperature of 22±3°C under daylight conditions supplemented by artificial illumination to obtain a 12–h photoperiod.

Italian isolate no. 526 II of *Fusarium oxysporum* f.sp. *ciceris* was cultured for 15 days on an orbital shaker (160 rpm, 8 hrs per day, at room temperature) in 250 ml flasks containing 100 ml of PDB. Then, the liquid culture was filtered through a double layer of cheesecloth.

The seeds of each chickpea line or wild accession were surface disinfected by sodium hypochlorite (2% active Cl) for 5 min and then rinsed in sterile water; the seeds of wild *Cicer* species were scarified with a sterile lancet. The two-week-old plantlets were transferred from moistened perlite into 200 ml plastic glasses (five plants each held in place against the glass by a sterile styrofoam disk), containing 150 ml of either the inoculum suspension (1 x 10^6 spores ml^{-1}) or sterile water as control. Forty days after inoculation, 14 plants per accession were evaluated using a 0–4 scale (0= no symptoms; 4=plant dead), according to the percentage of the plant canopy damaged by the acropetal progression of wilting. Every week after inoculation, tap water and a nutritive solution were added to restore the initial level of liquid in the glasses.

Identification of sources of resistance

Lines of C. arietinum

In 1993, 576 chickpea lines from ICARDA's germplasm collection were evaluated in the wilt sick-plot where a susceptible line as control was sown every 20 rows. One-hundred and twelve lines (19.4%) reacted with a score ≤1.5. In 1995, 109 lines among those with a score ≤1,5 in the first evaluation, were tested again in the wilt sick-plot. The average score of the susceptible check was 3.2 whereas 87 out of 109 lines tested (80%) confirmed the score of the first experiment. Interestingly, seven lines scored as very resistant (score 0). These were the ICARDA lines ILC 195, ILC 2076, ILC 2081, ILC 2830, ILC 2959, FLIP 81-293C, FLIP 82-180C.

Wild Cicer species

One-hundred and two accessions of six annual wild *Cicer* species were evaluated for resistance to *F. oxysporum* in the greenhouse, using the water-culture technique (Falchetti et al. 1995). In a preliminary test, isolate no. 526 II was the most virulent of a number of isolates of *F. oxysporum* towards Italian chickpea landraces.

Good sources of resistance to *F. oxysporum* (score ≤1) have been mostly found in the accessions of *Cicer bijugum* (96%), but also in 25% of *C. echinospermum* accessions; 36% of *C. judaicum*; 30% of *C. reticulatum*, and 20% of *C. pinnatifidum* (Fig. 4). The accessions of *C. yamashitae* and *C. chorassanicum* were susceptible. The line FLIP 85-88C confirmed its susceptibility and died 40 days after inoculation.

The accessions of *C. bijugum* highly resistant to fusarium wilt (score=0) were: ILWC 64, ILWC 71, ILWC 73, ILCWC 76, ILWC 80, and ILWC 83; those resistant to *C. judaicum* and *C. reticulatum* were respectively ILWC 186, ILWC 126 and ILWC 130 (Infantino et al. 1996).

Twenty-two accessions of wild *Cicer* species, which were considered resistant to fusarium wilt according to the greenhouse method, were also tested in the wilt sick-plot. Several accessions confirmed their resistant reaction. In particular, all plants of the accession ILWC 257 (*C. reticulatum*) showed vigorous vegetative growth and a high degree of resistance to wilt in both experiments.

Discussion and Conclusions

The efficient techniques developed to screen chickpea lines for resistance to *A. rabiei* are based on (1) the artificial inoculations and on (2) the use of fungal culture filtrate. Fifteen-day-old plantlets can give a useful answer to differentiate, under controlled conditions, the susceptibility or resistance of chickpea genotypes also with low inoculum concentrations (2 x 10^5 conidia/ml). Considering the results obtained by different bioassays on chickpea, Ascochyta culture filtrate is able to differentiate between chickpea genotypes susceptible and resistant to the blight. Culture filtrate holds promise as a screening tool in addition to artificial inoculations in the greenhouse, where the environmental conditions are not always appropriate for the appearance of disease symptoms and for a clear selection of resistant plants. Procedures for large-scale field screenings, which were developed at ICARDA, were successfully applied in several countries, including Italy. Under field conditions, the genetic material may show variation in disease reaction, depending on both the season and the location. In fact, lines coming from Koipesol, Semillas (Spain), as resistant to *A. rabiei* and good yielders under Spanish environments, were heavily damaged

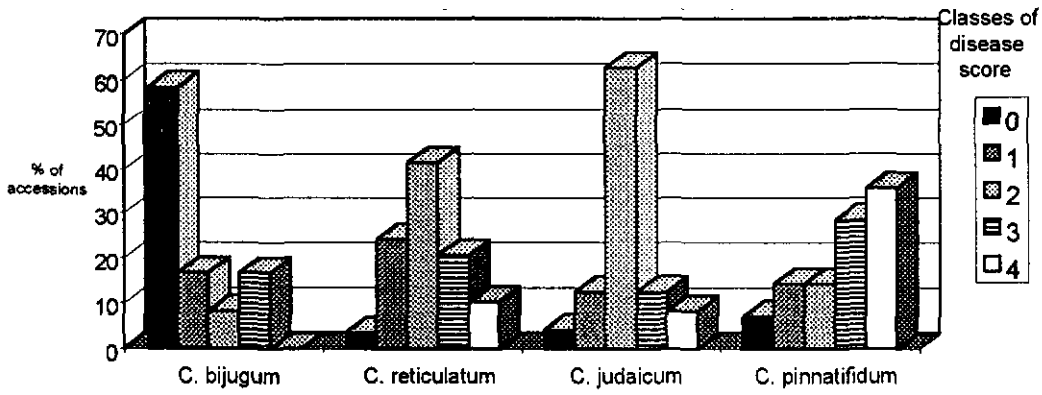

Fig. 4. Distribution of wild *Cicer* accessions in frequency classes according to disease score (0–4)

by severe fungal attack in Tarquinia (central Italy), in 1993–94 and 1994–95. This observation may represent a complication for the selection of ascochyta resistance in the open field because, if conditions are not favorable to the pathogen, the disease, even if initiated, does not develop further and the material can be considered as resistant. However, when conditions are favorable to ascochyta blight, there is a progressive exacerbation of the disease and many lines which otherwise would be considered as resistant could be indeed heavily attacked. So, it seems correct to conclude that effective screening for resistance to *A. rabiei* has to be carried out in environments which may ensure conditions favorable to the disease; greenhouse and laboratory tests are useful supplements to evaluate the lines and to confirm resistance identified in the field.

Field and greenhouse screening methodologies were also developed for resistance to *F. oxysporum* f.sp. *ciceri*, such as those performed respectively in an artificially infested wilt sick-plot and by water culture. Results obtained in the field agree with those of the greenhouse that, in general, are used to confirm the resistance of the most promising material identified in the field. In spite of the disadvantages of using the wilt-sick plot, like the occurrence and the uneven presence of other soil-borne pathogens of chickpea as well as of different Fusarium races in the plots, this screening procedure allows a large number of entries to be tested for resistance to fusarium and for other traits (Jiménez-Diaz et al. 1993). However, water culture allows challenging the host with selected isolates and reducing the risk of interaction from other organisms. This simple screening method requires little space and may assure reproducible results (Infantine et al. in press).

Chickpea lines and varieties with resistance to ascochyta blight have been developed in several countries as well as in Italy where, in spite of the existence of different pathogenic groups of the fungus, the new winter resistant cultivars are effective in several locations. Italian cultivars Pasciá and Visir are characterized by high yield, large seed, and resistance to *A. rabiei*, characters necessary for commercial varieties of chickpea. Several sources of resistance to fusarium wilt were also identified in Italy, either in *C. arietinum* or in wild *Cicer* species, but more research is needed to transfer this character into agronomically interesting lines or varieties. More tests are needed to validate these results in other locations and with other isolates. For their resistance to ascochyta blight and fusarium wilt, *C. judaicum*, *C. pinnatifidum* and *C. bijugum* seem to be particularly interesting candidates for interspecific hybridizations. Extensive efforts are still necessary to develop new germplasm with combined resistance to both ascochyta blight and fusarium wilt. In this respect, introgression of genes from wild *Cicer* species, such as *C. judaicum*, *C. pinnatifidum* and *C. bijugum* into *C. arietinum*, look promising. Tests on F_1 hybrids among *C. pinnatifidum*, *C. judaicum* and the cultigen had already been carried out in India. Hybrids screened for reaction to *A. rabiei* under artificial epidemic conditions were more resistant than their cultivated parents, suggesting that genes responsible for blight resistance are dominant in the wild species (Dey et al. 1993).

Innovative biotechnologies based on *in vitro* plant regeneration (Barna and Wakhlu, 1994; Eapen and George 1994) as well as on the identification of molecular markers (Udupa et al. 1993) and the achievement of transgenic plants (Chowrira et al. 1995; Islam et al. 1994) are being developed. Isolation of useful genes and their characterization as well as their *in vitro* manipulation could allow a further progress in plant breeding for disease resistance.

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References

Açikgoz, N., A. Kitiki, A.S. Cinsoy. 1993. Determination of resistance sources to Ascochyta blight (*Ascochyta rabiei* [Pass.] Labr.) of chickpea. *Anadolu* 3(2): 41–47.

Barna, K.S. and A.K. Wakhlu. 1994. Whole plant regeneration of *Cicer arietinum* from callus cultures via organogenesis. *Plant Cell Reports* 13(9): 510–513.

Buddenhagen, I.W. and F. Workneh. 1988. Fusarium wilt of chickpea in California. *Phytopathology* 78: 1563 (abstract).

Calcagno, F., P. Crinò and F. Saccardo. 1988. Ritorna il cece con nuove varietà produttive e resistenti alla rabbia. Il Giornale di Agricoltura no. 30: 35–38.

Chowrira, G.M., V. Akella and P.F. Lurquin. 1995. Electroporation-mediated gene transfer into intact nodal meristems *in planta*: generating transgenic plants without *in vitro* tissue culture. *Molecular Biotechnology* 3(1): 17–23.

Crinò, P., F. Saccardo, P. Vitale, C. Mosconi, A. Andolfi and F. Calcagno. 1992. Chickpea breeding for stress resistance in Italy. Page 35–36 *in* Proceedings of 1st European Conference on Improving Production and Utilization of Grain Legumes. L'Association Européenne des Proteagineaux (AEP) 1–3 June 1992, Angers, France. AEP, Paris, France.

Del Serrone, P., A. Porta-puglia and P. Crinò. 1987. Resistenza del cece ad *Ascochyta rabiei* (Pass.) Labr.: effetto dell'età della pianta e della concentrazione di inoculo. *Informatore Fitopatologico* 37(6): 59–61.

Dey, S.K. and G. Singh. 1993. Resistance to Ascochyta blight in chickpea—genetic basis. *Euphytica* 68(1/2): 147–153.

Dey, S.K., G. Singh, S.S. Gosal and M.M. Verma. 1993. Tissue culture response and resistance to Ascochyta blight in some interspecific crosses in chickpea. *Annals of Biology (Ludhiana)* 9 (2): 235–238.

Eapen, S. and L. George. 1994. Somatic embryogenesis in *Cicer arietinum* L.: influence of genotype and auxins. *Biologia Plantarum* 36(3): 343–349.

Falchetti, M.L., A. Infantino, A. Porta-puglia and K.B. Singh 1995. Evaluation of wild *Cicer* species for resistance to Fusarium wilt. Pages 94–95 *in* Proceedings 2nd AEP Conference Improving Production & Utilization of Chickpea, AEP, July 10–13, Copenhagen, Denmark. AEP, Paris, France.

Fravel, D.R., J.J. Marois, R.D. Lumdsen and W.J Connick Jr. 1985. Encapsulation of potential biocontrol agents in alginate clay matrix. *Phytopathology* 75: 774–777.

Gowda, C.L.L., O. Singh, S.C. Sethi, K.B. Singh, B.V. Rao, M.M. Rahman, J. Kumar and M.A. Rahman. 1995. Registration of 'ICCV 10' chickpea. *Crop Science* 35(2): 589.

Halila, H., M. Harrabi and A. Haddad. 1989. Genetics of resistance to *Ascochyta rabiei* in chickpeas. *Agr. Mediterranea* 119: 148–151.

Halila, H. and M.M. Harrabi. 1990. Breeding for dual resistance to ascochyta and wilt diseases in chickpea. *In* Present Status and Future Prospect of Chickpea Crop Production and Improvement in the Mediterranean Countries. Options Méditerranéennes. Série A: Séminaires Méditerranéennes: No. 9. CIHEAM, Zaragoza, Spain.

Haware, M.P., J.N. Rao and R.P.S. Pundir. 1992. Evaluation of wild *Cicer* species for resistance to four chickpea diseases. *International Chickpea Newsletter* 27: 16–18.

Haware, M.P., H.A. van Rheenen and S.S. Prasad 1995. Screening for ascochyta blight resistance in chickpea under controlled environment and field conditions. *Plant Disease* 79(2): 132–135.

Infantino, A., A. Porta-Puglia and K.B. Singh. 1996. Screening wild *Cicer* species for resistance to fusarium wilt. *Plant Disease* 80: 42–44.

International Crops Research Institute for the Semi-arid Tropics (ICRISAT). 1994a. Cold-tolerant chickpea varieties ICCV 88503, ICCV 88506, ICCV 88510. ICRISAT Plant Material Description 53.

International Crops Research Institute for the Semi-arid Tropics (ICRISAT). 1994b. Chickpea desi variety ICCV 82108. ICRISAT Plant Material Description 56.

ICRISAT. 1994c. Chickpea desi variety ICCV 10. ICRISAT Plant Material Description 57.

Islam, R., T. Malik, T. Husnain and S. Riazuddin. 1994. Strain and cultivar specificity in the *Agrobacterium*-chickpea interaction. *Plant Cell Reports* 13(10): 561–563.

Jiménez-Diaz, R.M., K.B. Singh, A. Trapero-Casas and J.L. Trapero-Casas. 1991. Resistance in kabuli chickpea to fusarium wilt. *Plant Disease* 75: 914–918.

Jiménez-Diaz, R.M., A. Alcalá, A. Hervás, and J.L. Trapero-Casas. 1993a. Pathogenic variability and host resistance in the *Fusarium oxysporum* f.sp. *ciceris/Cicer arietinum* pathosystem. *Hodowla Roslin, Aklimatyzacja i Nasiennictwo* 37(3): 87–94.

Jiménez-Diaz, R.M., P. Crinò, M.H. Halila, C. Mosconi and A. Trapero-Casas. 1993b. Screening for resistance to fusarium wilt and ascochyta blight in chickpea. Pages 77–96 *in* Breeding for Stress Tolerance in Cool-Season Legumes (K.B. Singh and M.C. Saxena, ed.). ICARDA and John Wiley & Sons, Chichester, UK.

Kaiser, W.J., A.R. Alcalá-Jimenez, A. Hervas-Vargas, J.L. Trapero-Casas and R.M. Jiménez-Diaz. 1994. Screening of wild *Cicer* species for resistance to races 0 and 5 of *Fusarium oxysporum* f.sp. *ciceri*. *Plant Disease* 78(10): 962–967.

Kumar, J., M.P. Haware and J.B. Smithson. 1985. Registration of four short-duration fusarium wilt-resistant kabuli (Garbanzo) chickpea germplasm. *Crop Science* 25: 576–577.

Lucretti, S., P. Lavermicocca, C. Mosconi, G. Giagnacovo, F. Moretti, P. Crinò, A. Lister and N. Iacobellis. 1991. Flow citometric analysis and sorting of plant protoplasts in the presence of phytotoxins. (Abstract). Pages 81–82 *in* Citometry, suppl. 5, abstracts of the XV Congress of the International Society for Analytical Cytology, Bergen, Norway, 25–30 August 1991.

Morales, G.J.A. 1986. Chickpea breeding program in Sonora. *International Chickpea Newsletter* 15: 11–12.

Mosconi, C., C. Coduti, P. Crinò and F. Saccardo. 1995. Resistance to *Ascochyta rabiei* by interspecific hybridizations in Italian chickpea. Page 243 *in* Proceedings 2nd AEP Conference on Improving Production & Utilization of Chickpea, AEP 10–13 July 1995, Copenhagen, Denmark. AEP, Paris, France.

Mosconi, C., P. Crinò, F. Saccardo and K.B. Singh. (In press). Effect of culture filtrate of *Ascochyta rabiei* on chickpea genotypes. *Petria*. (In press.)

Nene, Y.L. and M.P. Haware. 1980. Screening chickpea for resistance to wilt. *Plant Disease* 64: 379–380.

Pawar, K.B., N.J. Bendre, R.P. Aher and R.B. Deshmukh 1992. Wilt resistant chickpea lines in Maharashtra State, India. *International Chickpea Newsletter* 27: 16.

Pieters, R. and Tahiri A. 1986. Methods of screening resistance in chickpea to Ascochyta blight. *Euphytica* 35: 1007–1010.

Ponz Ascaso, R., L. De la Rosa Fernández and F. Varela Nieto. 1992. Evaluation of chickpea (*Cicer arietinum* L.) germplasm. Page 83 in *Monografías, Istituto Nacional de Investigacion y Technologia Agraria y Alimentaria* 83.

Porta-Puglia, A., C.C. Bernier, G.J. Jellis, W.J. Kaiser and M.V. Reddy 1994. Screening techniques and sources of resistance to foliar diseases caused by fungi and bacteria in cool-season food legumes. *Euphytica* 73: 11–25.

Porta-Puglia, A., P. Crinò and C. Mosconi. (In press). Variability in virulence of an Italian population of *Ascochyta rabiei* to chickpea. *Plant Disease*. (In press.)

Reddy, M.V. and K.B. Singh. 1992. Registration of five chickpea germplasm resistant to ascochyta blight. *Crop Science* 32(4): 1079–1080.

Rheenen, H.A van and M.P. Haware. 1994. Mode of inheritance of resistance to Ascochyta blight (*Ascochyta rabiei* [Pass.] Labr.) in chickpea (*Cicer arietinum* L.) and its consequences for resistance breeding. *International Journal of Pest Management* 40(2): 166–169.

Saccardo, F., A. Errico, P. Crinò, B. Ocampo and G. Venora. 1993. Mutagenesis and chromosome manipulation for stress resistance in cool-season legumes. Pages 343–358 *in* Breeding for Stress Tolerance in Cool-Season Legumes (K.B. Singh and M.C. Saxena, ed.). ICARDA and John Wiley & Sons, Chichester.

Singh Grump, Kauai Livened and Y.R. Sharma 1991. Ascochyta blight and gray mold resistance in wild species of *Cicer*. *Crop Improvement* 18(2): 150–151.

Singh, K.B. and M.V. Reddy. 1989. Genetics of resistance to Ascochyta blight in four chickpea lines. *Crop Science* 29: 657–659.

Singh, K.B. and M.V Reddy. 1990. Patterns of resistance and susceptibility to races of *Ascochyta rabiei* among germplasm accessions and breeding lines of chickpea. *Plant Disease* 74 (2): 127–129.

Singh, K.B. and M.V. Reddy. 1991. Advances in disease resistance breeding in chickpea. *Advances in Agronomy* 45: 191–222.

Singh, K.B. 1993. Experiences, difficulties and prospects of disease resistance breeding in chickpea. Pages 241–248 *in* Durability of Disease Resistance (T. Jacobs and J.E. Parlevliet, ed.). Kluwer Academic Publishers, Dordrecht, Netherlands.

Singh, K.B. and M.V. Reddy. 1993. Sources of resistance to Ascochyta blight in wild *Cicer* species. *Netherlands Journal of Plant Pathology* 99: 163–167.

Singh K.B. and M.V. Reddy. 1994. Registration of eight ascochyta-blight resistant, early maturing, large-seeded chickpea germplasms. *Crop Science* 34 (5): 1416–1417.

Singh, K.B., G.C. Hawtin, Y.L. Nene and M.V. Reddy. 1981. Resistance in chickpea to *Ascochyta rabiei*. *Plant Disease* 65: 586–587.

Singh K.B., R.S. Malhotra and M.C. Saxena. 1993a. Registration of 'ILC 72' chickpea. *Crop Science* 33(6): 1409.

Singh, K.B., R.S. Malhotra and M.C. Saxena 1993b. Registration of 'ILC 195' chickpea. *Crop Science* 33 (6): 1409–1410.

Singh, K.B., R.S. Malhotra, M.H. Halila, E.J. Knights and M.M. Verma. 1994. Current status and future strategy in breeding chickpea for resistance to biotic and abiotic stresses. *Euphytica* 73(1/2): 137–149.

Tewari, S.K. and M.P. Pandey 1986. Genetics of resistance to ascochyta blight in chickpea (*Cicer arietinum* L.). *Euphytica* 35: 211–215.

Udupa, S.M., A. Sharma, R.P. Sharma and R.A. Pai. 1993. Narrow genetic variability in *Cicer arietinum* L. as revealed by RFLP analysis. *Journal of Plant Bio-chemistry and Biotechnology* 2(2): 83–86.

Zote, K.K., N.B. Pawar, D.D. Zindurde, B.P. Dandnaik and S.P. Shirsikar. 1993. Source of resistance to Fusarium wilt of chickpea. *International Chickpea Newsletter* 29: 11–12.

Tolerance of Chickpea to Nematodes

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Summary

Nematodes are one of the major constraints affecting chickpea cultivation in many countries. The root-knot nematodes, *Meloidogyne artiellia*, *M. arenaria*, *M. incognita* and *M. javanica*, the cyst nematode, *Heterodera ciceri*, the root-lesion nematodes, mainly *Pratylenchus mediterraneus*, *P. penetrans* and *P. thornei*, and the reniform nematode, *Rotylenchulus reniformis* are the most noxious. *M. artiellia* is spread in North Syria, South Italy and Spain, while the other root-knot nematodes cause severe yield loss of chickpea in India, Nepal and Pakistan. *H. ciceri* is common in North Syria, in several areas of Turkey, and also in Jordan and Lebanon. Damage occurs whenever soil population densities of these nematodes at the time of sowing are above the tolerance limits of chickpea (0.14–0.015 second stage juvenile/cm^3 soil for *M. artiellia*, 0.2 for *M. incognita* and *M. javanica*, 1 egg/g soil for *H. ciceri*, 0.03–0.1 specimen/cm^3 soil for *P. thornei* and 0.5 for *R. reniformis*). Resistance to *H. ciceri* has not been found in lines of *Cicer arietinum*, but it is present in 49 lines of *C. bijugum*, six of *C. pinnatifidum* and one of *C. reticulatum*. Resistance in *C. reticulatum* is recessive and a breeding program is in progress to transfer resistance in cultigens. Resistance to *M. incognita*, *M. javanica* and *R. reniformis* was found, but not confirmed, in germplasm of *C. arietinum*. Few lines of cultivated and wild *Cicer* spp. were found resistant to *M. artiellia*, but this also needs confirmation. It is suggested that breeding for resistance to all major nematodes should be encouraged. Much work is needed to transfer genes resistant to wild *Cicer* spp. to *C. arietinum*, which is not compatible with them.

Introduction

Nematodes are major pests of chickpea (*Cicer arietinum* L.) in many countries (Sikora and Greco 1990; Greco et al. 1992; Di Vito et al. 1994a, b). Although more than 50 species of plant parasitic nematodes are associated with chickpea, only a few are very damaging. The most important are the root-knot nematodes, *Meloidogyne artiellia* (in the Mediterranean basin), *M. arenaria*, M. incognita and *M. javanica* (in the Indian sub continent), the chickpea cyst nematode, *Heterodera ciceri* (in Syria, Turkey, Jordan, and Lebanon), several species of root-lesion nematodes, *Pratylenchus mediterraneus*, *P. penetrans* and *P. thornei*, and the reniform nematode, *Rotylenchulus reniformis* (mainly in India). Other nematodes may have only local importance, among these *M. artiellia* and *Pratylenchus* spp. are present in southern Italy.

All these nematodes infect roots, thus impairing their efficiency, drought resistance, and *Rhizobium* nodulation (Ali et al. 1981). They may also interact with other soil borne pathogens, thus increasing disease severity (Goswami and Agarqwal 1978). Symptoms of nematode attack on aerial plant parts are not specific; affected plants look as if their root system is being hampered. Heavily-infested plants are stunted, yellowish, have an earlier senescence and yield poorly. These symptoms can be patchy or spread all over the field in case of heavy and uniform soil infestation.

Damage thresholds

The soil population density above which chickpea suffers from nematode attack is rather low, and varies according to nematode species. Investigations carried out under field and laboratory conditions have estimated tolerance limits of chickpea to the most important nematode species. In microplot experiments in Italy, the tolerance limit of chickpea to *M. artiellia* was 0.14 eggs/cm^3 soil for the winter-sown crop and 0.015 eggs/cm^3 for the spring sown one. A 50% yield loss and complete crop failure may occur at 2 and 8 eggs/cm^3 soil for winter

chickpea, and at 0.25 and 1 eggs/cm³ soil for spring chickpea (Di Vito and Greco 1988). According to plot experiments in India, the tolerance limit of chickpea to the other root-knot nematodes is less than 0.2 egg/cm³ (Nath et al. 1979; Srivastava et al. 1974). Microplot experiments in Syria demonstrated that the tolerance limit of this pulse to *H. ciceri* is about 1 egg/cm³ soil both for winter and spring chickpea and yield loss of 50% and 100% were estimated at 32 and 64 eggs/cm³, respectively (Greco et al. 1988). In a field experiment, also in Syria, the tolerance limit of chickpea to *P. thornei* was of 0.03 specimen/cm³ soil and a maximum yield loss of 50% was observed at 2 specimens/cm³ (Di Vito et al. 1992). Finally, in a pot experiment the tolerance limit of chickpea to *R. reniformis* was less than 0.5 nematode/g of soil (Mahapatra and Padhi 1986).

Control tactics

Because of severe yield loss suffered by chickpea, appropriate control measures are necessary. As chickpea is very often sown in marginal lands, these should be cheap, easy to apply, and environmentally friendly. Therefore, nematicide treatments and soil solarization, although very effective, are impractical. Crop rotation can be satisfactory for controlling nematodes with narrow host ranges, such as the chickpea cyst nematode, *H. ciceri*, but difficult for the control of those with rather large host ranges, such as root-knot, root-lesion and reniform nematodes.

Resistance in chickpea

The most convenient way to control plant parasitic nematode would be by using resistant cultivars. Unfortunately, there is currently no chickpea cultivar that is resistant to nematodes and has good agronomic characteristics. However, screening of several hundred chickpea lines has identified resistance to *M. artiellia* in *C. arietinum*, *C. judaicum*, *C. chorassanicum*, *C. cuneatum*, and *C. pinnatifidum* (Greco and Di Vito 1993). Resistance to other root-knot nematodes has also been reported (Sharma et al. 1994) but it has not been confirmed. A few chickpea lines showed resistance also to the reniform nematode, but further investigations are necessary to confirm this resistance.

In 1983, a research project was undertaken in cooperation with ICARDA (International Center for Agricultural Research in the Dry Areas) to obtain more insight on nematode problems of chickpea in Middle East and North African Countries and on the most appropriate method of control. Therefore, in 1986 it was decided to screen the chickpea germplasm, available at ICARDA to identify sources of resistance to *H. ciceri*, a very noxious nematode. Unfortunately, out of the about 10,000 *C. arietinum* germplasm lines tested the great majority was very susceptible to the nematode; only a few lines were moderately susceptible, and none were resistant (Di Vito et al. 1988). The screening proceeded then to the evaluation of wild germplasm. A total of 227 lines belonging to the wild species *C. bijugum* Rech, *C. chorassanicum* (Bge) M.Pop., *C. cuneatum* Hocst, *C. echinospermum* P.H.Davis, *C. judaicum* Bois, *C. pinnatifidum* Jaub & Sp., *C. reticulatum* Ladiz., and *C. yamashitae* Kitamura were tested. Of these 49 lines (67%) of *C. bijugum*, six (14%) of *C. pinnatifidum* and one (2%) of *C. reticulatum* were resistant to *H. ciceri* (Di Vito et al. 1988; Singh et al. 1989).

The resistance to *H. ciceri* was later confirmed with further screening, pathogenicity tests and histopathology studies. Although the tolerance limit of the resistant lines was the same as that of the two susceptible *C. arietinum* lines (1.34 eggs/cm³ soil), nevertheless at larger population densities of the nematode the relative yields of the resistant lines were higher than those of the susceptible lines. The maximum reproduction rate of the nematode was 52–75 on the susceptible lines, while for the resistant lines it was 2.4 on *C. bijugum* and *C. reticulatum* and less than one on *C. pinnatifidum* (Greco et al. 1993). The histopathology studies demonstrated that second stage juveniles of the nematode were able to penetrate the roots of the resistant lines but not to complete their development, because the cells around the cephalic region of the nematode soon necrotized, thus preventing the formation of syncytial cells which are necessary for nematode feeding (Di Vito and Vovlas 1990).

Analysis of *C. arietinum* x *C. reticulatum* F_1 and F_2 to *H. ciceri* showed that resistance in *C. reticulatum* is recessive (Di Vito et al.

1996 in press). Later the resistant *C. reticulatum* line has been purified and is now being registered as ILWC 292 (Singh et al. 1996 in press). Although resistance in *C. bijugum* and *C. pinnatifidum* is of no use, because these two species are not compatible with *C. arietinum*, resistance in *C. reticulatum* is interesting, because this species can be crossed easily with *C. arietinum*. Therefore, a breeding program is in progress, in cooperation with ICARDA to transfer this resistance to kabuli chickpea cultivars of agronomic interest.

Perspectives and conclusions

Unfortunately, besides the breeding program mentioned, no other work has been undertaken to obtain chickpea cultivars resistant to the other nematodes. However, although resistance to the other nematode may not occur in *C. arietinum*, screening of the germplasm of wild *Cicer* spp. appears promising (Di Vito et al. 1995). Moreover, lines of wild *Cicer* spp. also possess resistance and/or tolerance to other biotic and abiotic stresses. The presence in the same wild *Cicer* spp. line of resistance to more nematode species should not be excluded. Therefore, it is suggested that specialists in different agriculture disciplines cooperate to breed for multiple resistance. Although most of the annual *Cicer* spp. cannot be crossed easily with *C. arietinum*, cytogenetic studies and advanced genetic techniques could help to overcome the existing barriers for transferring resistant genes from wild *Cicer* spp. to chickpea cultigens.

References

Ali, M.A., I.Y. Trabulsi and M.E. Abd-Elsamea. 1981. Antagonistic interaction between *Meloidogyne incognita* and *Rhizobium leguminosarum* on cowpea. *Plant Disease* 65: 432–435.

Di Vito, M. and N. Greco. 1988. The relationship between initial population densities of *Meloidogyne artielita* and yield of winter and spring chickpea. *Nematologia Mediterranea* 16: 163–166.

Di Vito, M. and N. Vovlas. 1990. Differential histological response of selected *Cicer bijugum* and *C. arietinum* lines to *Heterodera ciceri*. *Nematologia Mediterranea* 18: 165–165.

Di Vito, M., N. Greco and M.C. Saxena. 1992. Pathogenicity of *Pratylenchus thornei* on chickpea in Syria. *Nematologia Mediterranea* 20: 71–73.

Di Vito, M., N. Greco, K.B. Singh and M.C. Saxena. 1988. Response of chickpea germplasm to *Heterodera ciceri*. *Nematologia Mediterranea* 16:17–18.

Di Vito, M., N. Greco, H.M. Halila, L. Mabsoute, M. Labdi, S.P.S. Beniwal, M.C. Saxena, K.B. Singh and M.B. Solh 1994a. Nematodes of cool-season food legumes in North Africa. *Nematologia Mediterranea* 22: 3–10.

Di Vito, M., N. Greco, G. Oreste, M.C. Saxena, K.B. Singh and I. Kusmenoglu. 1994b. Plant parasitic nematodes of legumes in Turkey. *Nematologia Mediterranea* 22: 245–251.

Di Vito, M., G. Zaccheo and F. Catalano. 1995. Risposta di alcune linee di cece agli attacchi di *Meloidogyne artiellia* e *Pratylenchus thornei* in serra. Pages 27–28 *in* Abstract V Congresso della Società Italiana di Nematologia. 19–21 October 1995. Martina Franca (TA), Italy.

Di Vito, M., K.B. Singh, N. Greco and M.C. Saxena. 1996. Sources of resistance to cyst nematode in cultivated and wild *Cicer* species. *Journal of Genetic Resources and Crop Evolution.* (In press.)

Goswami, B.K. and D.K. Agarqwal. 1978. Interrelationship between *Fusarium* and root-knot nematodes, *Meloidogyne incognita* in soybean. *Nematologia Mediterranea* 6:125–128.

Greco, N., M. Di Vito, M.C. Saxena and M.V. Reddy. 1988. Effect of *Heterodera ciceri* on yield of chickpea and lentil and development of this nematode on chick-pea in Syria. *Nematologica* 34: 98–114.

Greco, N. and M. Di Vito. 1993. Selection for nematode resistance in cool-season food legumes. Pages 157–166 *in* Breeding for Stress Tolerance in Cool-Season Food Legumes (K.B. Singh and M.C. Saxena, ed.). John Wiley & Sons, Chichester, UK.

Greco, N., M. Di Vito and M.C. Saxena 1992. Plant parasitic nematodes of cool season food legumes in Syria. *Nematologia Mediterranea* 20: 37–46.

Greco, N., M. Di Vito, K.B. Singh and M.C. Saxena. 1993. Effect of *Heterodera ciceri* on the growth of selected lines of *Cicer*

species. *Nematologia Mediterranea* 21: 111–116.

Mahapatra, B.C. and N.N. Pahdi. 1986. Pathogenicity and control of *Rotylenchulus reniformis* on *Cicer arietinum*. *Nematologia Mediterranea* 14: 287–290.

Nath, R.P., A.K. Banerjee, M.G.I Haider and B.K. Singh. 1979. Studies on the nematodes of pulse crops in India. I. Pathogenicity of *Meloidogyne incognita* on gram. *Indian Phytopathology* 32: 28–31.

Sharma, S.B., R.A. Sikora, N. Greco, M. Di Vito and G. Caubel. 1994. Screenings techniques and source of resistance to nematodes in cool-season food legumes. Pages 346–358 *in* Expanding the Production and Use of Cool Season Food Legumes (F.J. Muehlbauer and W.J. Kaiser, ed.). Kluwer Academic, Dordrecht, Netherlands.

Sikora, R.A. and N. Greco. 1990. Nematode parasite of food legume. Pages 181–235 *in* Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (M. Luc, R.A. Sikora and J. Bridge, ed.). CAB International, Wallingford, UK.

Singh, K.B., Di Vito Greco N. and M.C. Saxena. 1989. Reaction of wild *Cicer* spp. lines to *Heterodera ciceri*. *Nematologia* 17: 113–114.

Singh, K.B., M. Di Vito, N. Greco and M.V Saxena. 1996. Registration of ILWC 292, a chickpea cyst nematode resistant *Cicer reticulatum* Ladiz. parental line. *Crop Science* 36 (In press.)

Srivastava, A.S., K.D. Upadhyay and G. Singh. 1974. Effect of root-knot nematode, *Meloidogyne javanica*, on gram crop. *Indian Journal of Nematology* 4: 248–251.

In Vitro Culture Methods for Genetic Manipulation of Chickpea

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Introduction

The chickpea (*Cicer arietinum* L.), like other legumes, is recalcitrant to *in vitro* culture conditions. Although attempts to culture it have been carried out, results are rather scarce, and do not yet allow the use of biotechnological approaches for improvement of the crop.

Rao and Chopra (1989) induced embryogenic callus and somatic embryos, without obtaining rooted plants. Barna and Wakhlu (1993) have obtained somatic embryogenesis and plant regeneration getting, in any case, a less than 3% success rate. Shri and Davis (1992) observed the *ex-novo* formation of Cotiledon Like Structures (CLS) when immature cotiledons were placed on a medium supplemented with zeatin and 3-indolyl-acetic acid (IAA), but they did not report the result of rooted plants. On the contrary, Sagare et al. (1993) succeeded in having rooted plants using immature cotiledons regenerating on media containing in the first step 2,4,5-T and then zeatin. Nevertheless, among five used genotypes, only one gave a positive response.

An interesting solution, an alternative to the classic regeneration, might be the induction of multiple shoots. The formation of wide meristematic areas in the target tissue would be the most appropriate solution for the transfer of genetic material. In order to reach such results, it might be possible to exploit cytochinins capacity of inducing multiple shoots.

In one of the first papers, Kartha et al. (1981) observed that low benzyladenine (BA) concentrations (1mM/l) allowed an abundant branching in the main stem of the main legumes. In recent years, great interest has been focused on the thidiazuron (TDZ) effects. This compound (N-fenil-N' 1,2,3,-thiadiazol-5-ylurea) belongs to the phenilureas family, which has a biological activity similar to that of the cytokinins. Interesting experiments were carried out on geranium (Visser et al. 1992, Gill et al. 1992), peanut (Gill and Saxena 1992), bean (Malik and Saxena 1992a, b), pea, lentil and chickpea (Malik and Saxena, 1992c). In *Phaseolus* ssp. Malik and Saxena (1992a, b) demonstrated the *ex novo* origin of adventitious shoots: their formation was observed starting from a few cells (2 or 3) of epidermic tissue without callus production and visible connection with vascular tissue. In chickpea, Malik and Saxena (1992c), obtained about 50 shoots per seed using TDZ (5 mm/l), after 3–5 weeks of culture, starting from dry seeds. The same authors proved that regeneration occurred just after one week of culture in nodal and basal regions of the primary shoots.

The aim of the present work was the definition of a simple, rapid and efficient protocol for the *in vitro* proliferation of shoots that could be an alternative to the classic regeneration, which is more difficult and complex to reach in legumes. This protocol was at the same time applied in order to perform a preliminary study of genetic transformation using agronomically important cultivars.

Material

The plant material was composed of two cultivated genotypes and three ICARDA accessions, with the following characteristics:

- Sultano: Italian cultivar, well adapted to Mediterranean pedoclimatic conditions, and characterized by erect plant, resistance to *Ascochyta rabiei* and good productivity. The seed is smooth and kabuli type;
- Elmo: Portuguese cultivar, desi type, with semi-erect plant and highly productive;

- ICC 640, ICC 14877 and ICC 3996: ICARDA accessions with semi-prostate plant, rough seed and desi type; the first two accessions were successfully used in previous experiments of *in vitro* culture.

Once the protocol was identified, further ICARDA accessions of kabuli type (Flip 91-149 C, Flip 86-6 C, ILC 7974, Flip 92-67C, Flip 81-62C, Flip 91-59C, Flip 91-107 C, Flip 82-6, ILC 992, Flip 84-182 C, ILC 1071), and the Italian ecotype Bari 80 were chosen for their agronomic performance. All these genotypes were evaluated and screened to find the most adapted genotype. Sultano and Califfo were used as controls.

Methods

Before *in-vitro* culture, seeds were surface sterilized by immersion in pure ethanol for 30 seconds and, after three rinsings in distilled sterile water, dipped in sodium hypochlorite (1.2%) for twelve minutes; finally they were washed three times with distilled sterile water. After a soaking of 24 hours, the embryo was aseptically removed cutting the seed by scalpel just over the major diameter. Gentle opening of the two halves of the cotiledon attached to the embryos allowed the whole embryo to be isolated and it was then incubated at proper conditions. The sterile magenta or Petri dishes were kept in the growing chamber (24±1°C with 16 hours of daylight and 8 hours of darkness).

Comparison among cytokinins (BAP and kinetin) and TDZ for their efficacy on induction of multiple shoots

On the basis of bibliographic references TDZ, BAP and Kinetin are judged the most active compounds for the induction of adventitious shoots. Four concentrations per each hormone (0.01, 0.1, 1 and 10 mg/l) with three replicates (seven embryos each) were tested. The explants were collected from dry seeds of cultivars Sultano and Elmo.

Mature embryos were transferred to MS (Murashige and Skoog 1962) basal medium supplemented with Gamborg B_5 (1968) vitamins, sucrose 3%, appropriate amount of hormone and agar 0.8%. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 minutes. After 20 days, number of shoots, length and weight of proliferating part were recorded. Shoots were arbitrarily classified in two types, according to their length: *short* shoots, those that did not grow anymore, and *long* shoots, those that were not inhibited in their development. The different treatments were evaluated according to a randomized complete block design and the data were analyzed according to a factorial scheme.

Individuation of the most efficient TDZ concentration

Cultivar Sultano and two ICARDA accessions (ICC 640 and ICC 14877) were used. TDZ concentrations of 0.01, 0.05, 0.1, 0.3, 0.5, 1, 3, 5 mg/L were compared. For each dose, 60 embryos were tested dividing them in four replicates. Data (number of *long* and *short* shoots per embryo) were collected after 30 days of culture.

Determination of the required incubation time in TDZ

Sultano, ICC 640 and ICC 3996 were employed as plant material source. Chickpea embryos were incubated for 1, 3 and 7 days on basal medium supplemented with TDZ 0.3 mg/L. After the established time, the explants were transferred to a hormone-free basal medium. Each thesis (genotype and incubation time) was constituted of three replicates (15 embryos each). Data (number of *long* and *short* shoots) were collected after 30 days of culture.

Influence of different basal medium composition on TDZ activity

The comparison was carried out between MS and Gamborg B_5 media to which had been added sucrose 3%, TDZ 0.3 mg/l and agar 0.8%, adjusting pH to 5.8. The accession ICC 640 was used. The number of long and short shoots were recorded after 30 days of culture.

A completely randomized block design, with three replicates (15 embryos per replicate) was used; data were analyzed according to a factorial scheme.

Individuation of the most suitable genotype for the induction of multiple shoots

Considering that not all the genotype responded positively to the *in vitro* conditions, a screening among 14 of them was performed.

Sterilized embryos were placed on a Gamborg B_5 medium, supplemented with sucrose 3%, TDZ 0.1 mg/L and agar 0.8%, adjusting pH to 5.8. Thirty-six embryos per genotype, divided in three duplicates, were cultured. *Long* and *short* shoots were counted after one month.

In order to obtain a more normal distribution of the data the values of the number and length of the shoots were tranformed into root square, while weight data were converted in $\log_{10}$ (Compton, 1994). ANOVA was performed by the SYSTAT program; separation mean was achieved by Duncan test accomplished with the MSTAT-C programmed.

Approaches to genetic tranformation and assessment of transient expression

For transformation approaches, two *Agrobacterium tumefaciens* strains, kindly supplied by Prof. Van Montagu (Gent, Belgium), were used, both disarmed. The first one C58 (pGV2260) contained p35S-gusintron-3' 35S and pnos-npt-II-3'ocs chimaeric genes between the T-borders; the second, EHA 105, contained the same marker and reporter genes. Bacteria were maintained and grown for 48 hours on LB (Luria Bertrami) medium: once they reached the exponential phase, colonies were used to graze needles by which cultivar Sultano embryos were mechanically wounded. In order to maintain the tissue organization integrity, the wounding was carried out by puncturing four different areas:

(a) the middle of the embryos
(b) the two axillary meristems
(c) the apical meristem
(d) a, b and c at the same time.

Immediately after the *A. tumefaciens* inoculation, the embryos were placed on a B_5 medium supplemented with sucrose 3%, agar 0.8 %, TDZ 0.3 mg/l (pH 5.8 before autoclaving 121°C). Five petri dishes (considered as duplicates) were used for each thesis (twelve embryos/petri dishes). Co-cultivation was performed for 48 hours, then embryos were placed in a hormone-free medium containing cefotaxime 250 mg/L. The first half of the embryos were analyzed after two days for β-glucuronidase (GUS) activity, determined according to Jefferson (1987), while those remaining were maintained in a medium containing kanamycin 50 mg/L and after four more days were transferred to the same medium supplemented with kanamycin 200 mg/L.

Two days following the mechanical wounding, the number of embryos showing root and stem elongation were recorded. After X-Gluc assay, embryos with blue spots were counted considering the position and the extension of the spots, too. The extension was evaluated according to the following scale:

(1) one or a few small and disperse spots
(2) less than 1/3 of the embryo with spots
(3) from 1/3 to 2/3 of the embryo with spots
(4) more than 2/3 of the embryo colored
(5) embryo completely colored.

In order to reach a uniform wounding without excessive damage of the tissues, enzymatic digestion by macerozyme (0.025%) and cellulase (0.2%) was also investigated. In order to achieve the best digestion time, sterile embryos were incubated in this solution for 2, 4, 8, 16, 30, 60 and 120 minutes. Once this was determined, the following methods of wounding were compared:

- firstly mechanical and then enzymatic
- firstly enzymatic and then mechanical
- only mechanical
- only enzymatic.

For all the experiments, after wounding, embryos were co-cultivated with the strain EHA 105 for 48 hours on a hormone free medium and then the GUS activity was evaluated. After the assay, the following data were collected: (a) percentage of transformed embryos and spot color intensity (arbitrary scale 1–3; 1=slightly colored, 3=deeply colored); (b) number of germinating, deformed and spotted embryos, spot intensity color, and number of spot per embryo.

Results

Comparison among cytokinins (BAP and kinetin) and TDZ for their efficacy on induction of multiple shoots (Fig. 1)

- Explants grown on the medium containing kinetin reacted immediately

with a fast growth rate. However at the end of the experiment the stem was very thin.

- Embryos placed on media supplemented with TDZ and BAP showed a strong reduction of stem length and an enlargement of the rooting apparatus.
- The ANOVA put in evidence statistically significant differences for the interaction hormone x dose, relative to the average number of *long* and total shoots.
- TDZ showed the best induction of *long* shoots at lower concentrations (0.01 and 0.1 mg/L). At higher concentrations, TDZ increased the formation of *short* shoots.
- BAP 1 mg/L had a positive effect on proliferation of *long* shoots.
- The effect of kinetin on the induction of multiple shoots has been observed only at the highest concentration. In any case, the effect is not comparable to those of TDZ and BAP.

Individuation of the efficient TDZ concentration (Fig. 2)

- 0.3 mg/L appears as the most suitable TDZ concentration for the production of *long* shoots.
- Cultivar Sultano also produced a high number of *long* shoots at 0.1 mg/L, showing together with ICC 14877 a strong sensibility to TDZ concentrations higher than 0.5 mg/L.
- ICC 640 maintained a good rate of proliferation in a range between 0.3 mg/L and 5 mg/L of TDZ.
- The number of total shoots obtained exhibited the same trend: it increased from 0.01 to 0.3 mg/L, but decreased in a more or less sensitive manner at higher TDZ concentration.
- Limited to ICC 14877, at TDZ 1 and 5 mg/L, abundant callus formation on rooting apparatus was observed; in some cases the development of shoots was noted.

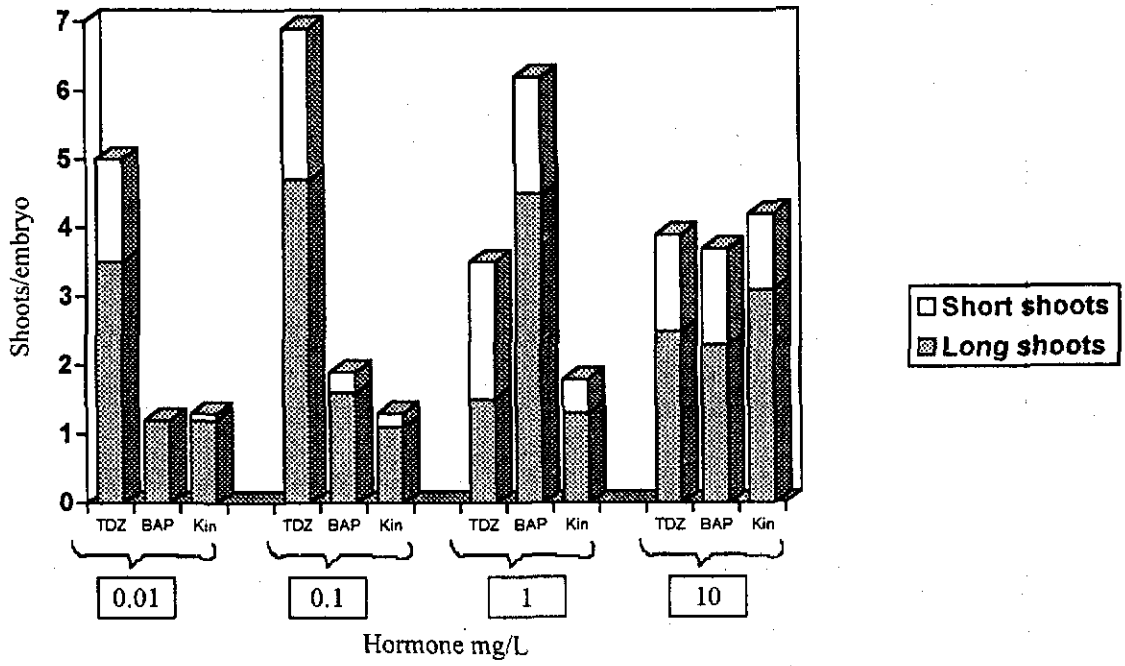

Fig. 1. Average number of short and long shoots observed after 20 days of culture, for embryos of cultivars Sultano and Elmo on a medium containing various doses of TDZ, BAP and Kinetin.
Data regard the source of variation hormone x dose. The differences among the values are statistically significant for the number of long and total shoots at $P<0.01$ level.

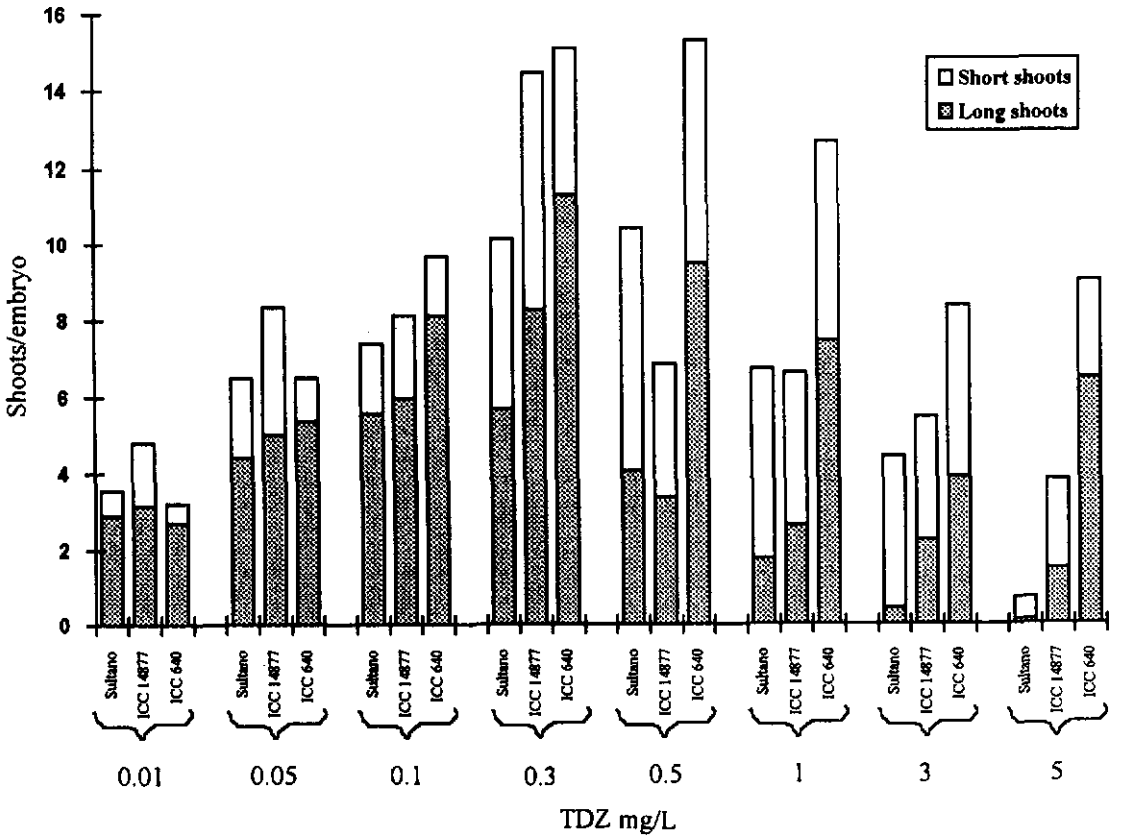

Fig. 2. Average number of short and long shoots observed after 30 days for embryos of cultivar Sultano, ICC 640 and ICC 14877 cultured on media containing various doses of TDZ.
Data regard the source of variation genotype x dose. The differences among the values are statistically significant for the number of short shoots at $P<0.01$ level.

Determination of the required incubation time in TDZ (Fig. 3)
The incubation of explants for 24 hours on a medium containing 0.3 mg/L is sufficient in order to obtain a number of shoots comparable to that recorded for a longer time of exposure.

4.4 Influence of different basal medium composition on TDZ activity (Fig. 4)

- The medium added with micro and macro salts of Gamborg B_5 allowed a rescue of a higher number of shoots than MS medium.

Detection of the suitable genotype for the induction of multiple shoots (Fig. 5)

- Clear influence of the genotype on the obtaining of multiple shoots.
- The induction of *long* shoots ranged from 0.6 in FLIP 86-6C to 7.2 in FLIP 96-67C.
- *Short* shoots fluctuated between 0.4 in FLIP 86-6C to 2.8 in FLIP 91-149C.
- The most appropriate genotype identified was FLIP 92-67C with a total of 9.5 shoots, followed by FLIP 91-149C; the worst ones were FLIP 86-6C and the Italian cultivar Sultano.

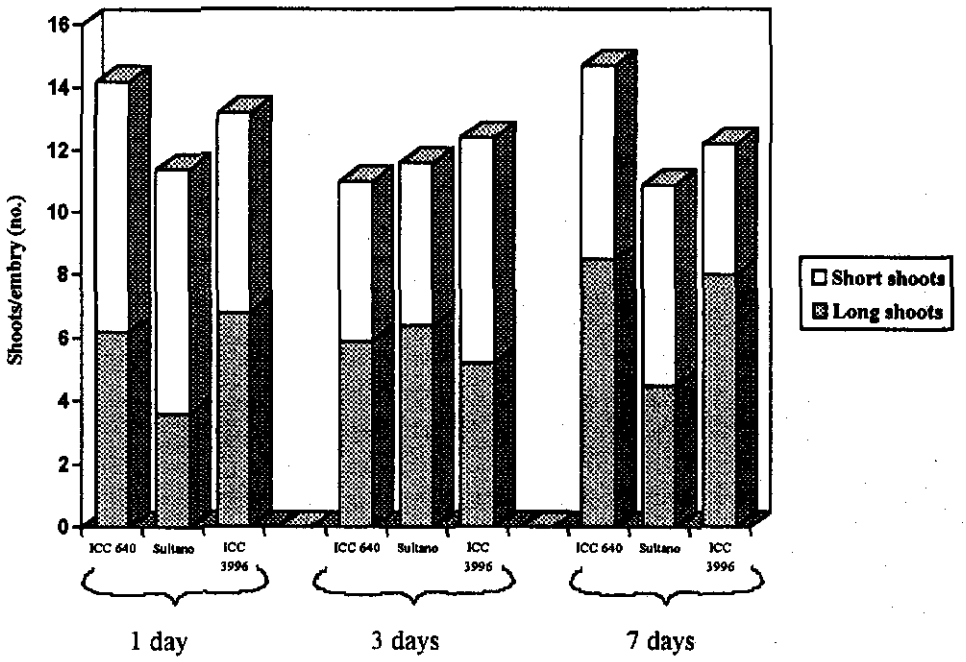

Fig. 3. Average number of short and long shoots observed after, 30 days of culture, for embryos of cultivar Sultano, ICC 640 and ICC 3996 grown for 1, 3 or 7 days on a medium supplemented with TDZ 0.3 mg/L.
Data regard the source of variation genotype x day. The differences among the values are not significant.

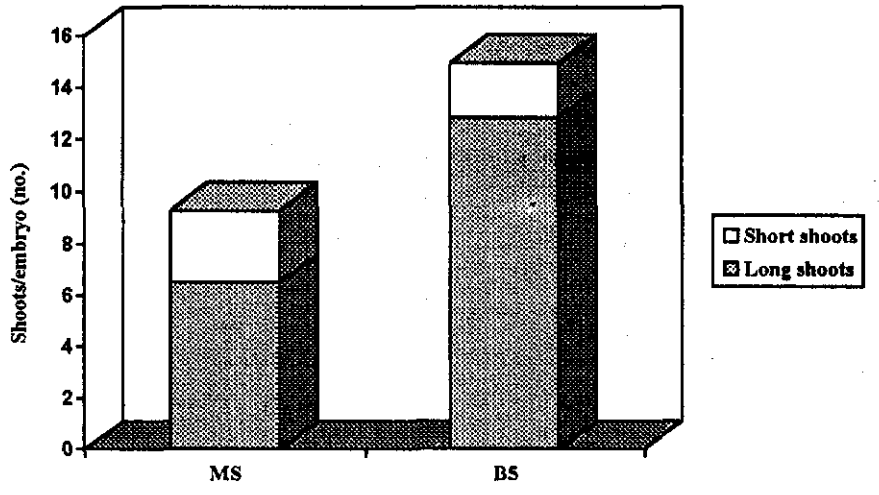

Fig. 4. Average number of short and long shoots observed after, 30 days of culture, for embryos of ICC 640 grown on media with micro and macro B_5 or MS salts, both supplemented with TDZ 0.3 mg/L.
The differences among the values are significant at $P<0.05$.

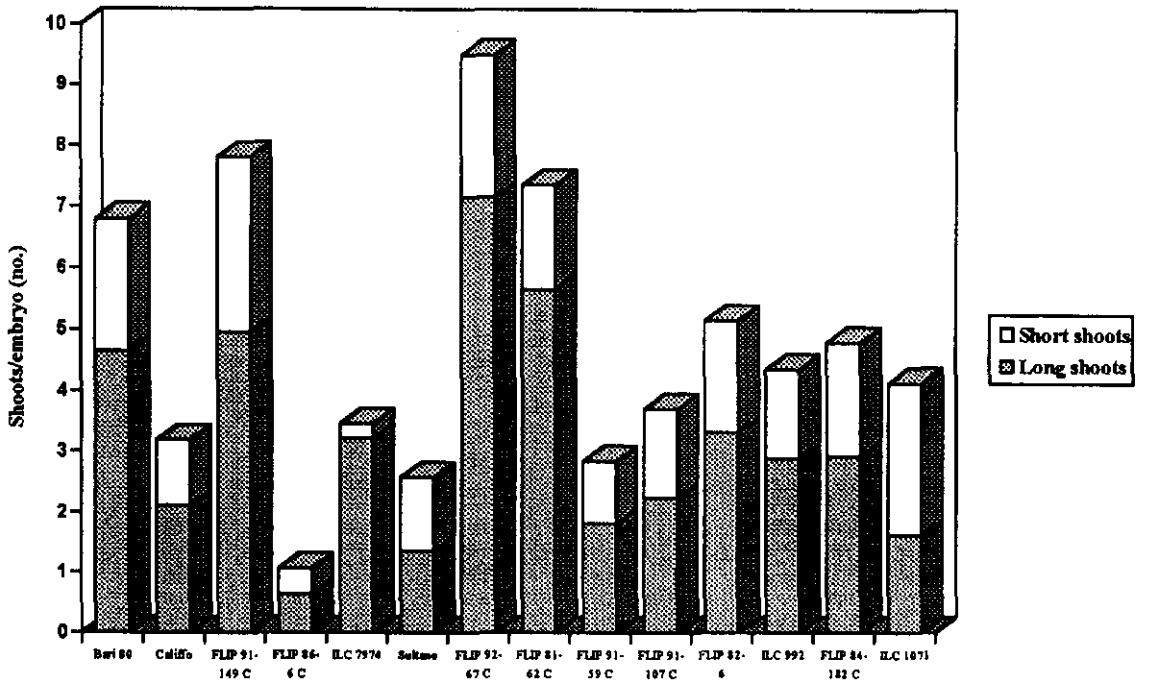

Fig. 5. Average number of short and long shoots observed, after 30 days of culture, from embryos of different accessions of chickpea on medium supplemented with B_5 salts and TDZ 0.3 mg/L.

Approaches to genetic transformation and assessment of transient expression

The assessment of transient expression put in evidence differences among the strains, procedure and methods of inoculation.

Number and percentage of embryos showing blue spots after the X-Gluc test were higher for EHA 105, reaching 50% of transient expression when the apex was punctured. The extension of the spots was rather restricted: the average index did not reach the value 2, indicating the presence of less than one-third of embryos with spots (Table 1).

After five days of incubation on a medium without TDZ and supplemented with kanamycin (50 mg/L), the embryos were transferred to the same medium with 200 mg/l of antibiotic. Fifteen days later, the number and percentage of vital and rooting

Table 1. GUS expression after inoculation with two *A. tumefaciens* strains with the help of a microneedle. The index of spot size is reported.

Strain	Wounding localization	Embryos with transient expression		Spot size index[†]
		N°	%	
EHA105	Central (C)	10	33.3	2.3
	Axillary meristem (Ax)	12	40.0	1.8
	Apical meristem (Ap)	15	50.0	1.3
	C, Ax, Ap	6	20.0	1.9
pGV2260	Central (C)	5	16.7	1.0
	Axillary meristem (Ax)	3	10.0	1.3
	Apical meristem (Ap)	3	10.0	1.0
	C, Ax, Ap	3	10.0	1.3

[†] Scale 1–5 (1=few disperse spots; 5= completely colored)

embryos and the average number of produced shoots were recorded (Table 2). The results evidenced a good percentage of surviving explants, underlying a substantial resistance for both inoculation and incubation to various concentrations of antibiotics. With few exceptions, all the inoculation procedures adopted permitted more than 50% of surviving embryos, with a maximum of 87% for the explants inoculated with pGV2260 at the apical meristem. Among the surviving embryos, those treated with EHA 105 showed a rooting percentage higher than the embryos inoculated with pGV2260. Root formation seemed stimulated by cefotaxime, beginning the development during the control phase of the bacterium and continuing after the transfer to a medium lacking this antibiotic. Table 2 shows that the *Agrobacterium* strains and inoculation procedures (wounding localization) followed by exposure to various doses of antibiotics in the medium differently influenced the proliferation of multiple shoots. The effect of enzymatic digestion, investigated by comparing different digestion times, showed that exposure of embryos for 2 minutes at a chosen concentration of enzymes was best in terms of spot color intensity and percentage of positive embryos to GUS assay.

Mechanical wounding proved a better inoculation procedure when comparing with enzymatic digestion with the combination of mechanical wounding and enzymatic digestion (Tables 3 and 4). Considering the different ways of inoculation, puncturing resulted in a higher percentage of embryo explants 'germination' with poor embryo deformation.

The number of explants showing transient expression was considerably higher when the inoculation was performed by mechanical wounding than for other methods, reaching a value (50% of treated embryos) similar to that recorded for transformed leaves of tobacco. The number of spots per embryo was elevated too, but interestingly the inoculation with *Agrobacterium tumefaciens* alone (without any kind of wounding) showed a good level of transient expression. It is evident that the procedures used for embryo extraction are rather traumatic and cause injuries that allow the bacterium to penetrate.

Discussion

The main goal of the research was to get ready suitable *in vitro* culture conditions aimed at the delivery of DNA via *Agrobacterium tumefaciens*. As already outlined, the individuation of a simple, fast, and efficient protocol of regeneration is of primary importance for the transformation techniques. The success obtained for soyabean and pea had not yet been repeated for bean, faba bean, chickpea or lentil (Christou 1994). For these leguminous plants, all the research efforts gave results limited to specific genotypes and not always easily repeatable (f.i. somatic embryogenesis).

Table 2. Reaction of cultivator Sultano embryos recorded after 30 days after inoculation with two *A. tumefaciens* strains with the help of a microneedle.

Strain	Wounding localization	Survived embryos		Rooting embryos		No. shoots per embryos
		N°	%	N°	%	
EHA105	Central (C)	12	40	6	50	2.6
	Axillary meristem (Ax)	19	63	17	89	3.0
	Apical meristem (Ap)	21	70	14	67	2.7
	C, Ax, Ap	20	67	17	85	2.8
pGV2260	Central (C)	20	67	11	55	2.1
	Axillary meristem (Ax)	11	37	8	73	1.8
	Apical meristem (Ap)	26	87	13	50	2.3
	C, Ax, Ap	16	53	9	56	2.6

Table 3. Number and percentage of germinating and deformed embryos resulting from different ways of inoculation cultivar Sultano (data collected three days after inoculation).

Treatment†	Embryos				
	Total (n)	Germinating		Deformed	
		(n)	(%)	(n)	(%)
E+M+At	49	14	29	4	29
M+E+At	48	21	44	14	67
M+At	44	23	52	6	26
E+At	50	15	30	5	30
At	48	21	44	6	29
Untreated	50	34	68	5	15

† E+M+At = Enzymatic digestion (2') + Mechanical injury + *Agrobacterium tumefaciens*; M+E+At = Mechanical injury + Enzymatic digestion (2') + *A. tumefaciens*; M+At = Mechanical injury + *A. tumefaciens*; E+At = Enzymatic digestion (2') + *A. tumefaciens*; At = *A. tumefaciens* only

Table 4. Number and percentage of spotted embryos, spot intensity and number of spot per embryos in cultivar Sultano after different methods of inoculation (data collected three days after inoculation).

Treatment†	Embryos			Spot intensity	Number of spot/ embryos
	Total	Spotted			
	(n)	(n)	(%)		
E+M+At	49	13	27	1.7	2.5
M+E+At	48	10	21	1.0	2.2
M+At	44	22	50	2.1	4.8
E+At	50	3	6	2.3	3.0
At	48	13	27	2.3	6.4
Untreated	50	0	0		
Tobacco	10	6	60	2.5	8.7

† E+M+At = Enzymatic digestion (2') + Mechanical injury + *Agrobacterium tumefaciens*; M+E+At = Mechanical injury + Enzymatic digestion (2') + *A. tumefaciens*; M+At = Mechanical injury + *A. tumefaciens*; E+At = Enzymatic digestion (2') + *A. tumefaciens*; At = *A. tumefaciens* only

For the improvement of genetic transfer of exogenous DNA a determinant condition could be the formation of wider meristematic areas into the target tissue. Cytochinins are phytohormons able to induce this phenomenon. Recent works have demonstrated a greater efficiency of TDZ in respect to the cytochinins routinely used for the proliferation of multiple shoots. These ones are *de novo* formed starting from the subepidermic tissues of different zones of embryo axes without a callus phase and without a connection with vascular tissue. In peanut, Gill and Saxena (1992) observed preferential areas of cell divisions originating from scattered zones of parenchimatic cells. The authors suppose a key role of TDZ in the interaction with endogenous hormones, both cytochinins or auxins: the capacity of TDZ to induce a kind of regeneration could be related to a release, synthesis or protection of auxinic compounds.

The use of TDZ to multiply meristematic areas coupled with gene transfer mediated by physic (microprojectile) or biologic (*Agrobacterium tumefaciens*) vectors, can be considered an innovative approach for recalcitrant species like chickpea.

The individuation of suitable *in vitro* culture conditions aimed at the optimization of multiple shoot induction was the first step for the definition of a protocol for delivering DNA in chickpea. The obtained results confirm the capacity of TDZ to produce a higher number of shoots than BAP and kinetin, at concentrations 100 and 1000 times lower respectively. According to other authors, this could be ascribed to the superior stability of TDZ, insensible to the degradative action of cytochinin-oxidases.

The evalutation of a range of TDZ concentrations on three important genotypes proved the genotype-dependence of the response and evidenced the best dose (0.3 mg/L) for shoot regeneration from embryo. Due to the TDZ strong action and slow metabolization inside the tissues, it was important to define how much time is required to induce the proliferation process. As observed in geranium (Visser et al. 1992), in chickpea it was sufficient only one day on a medium containing TDZ (0.3 mg/L) to obtain a number of shoots similar to that achieved by a longer exposition of embryos. Our data present positive implications with regard to genetic transformation, allowing a reduction of contact among explant and hormone, with a lower number of anomalous shoots and work saving.

The protocol tested for the transformation approach using *Agrobacterium tumefaciens* appeared highly promising. Other authors too (Srinivasan et al. 1988; Fontana et al. 1992; Islam et al. 1994) have demonstrated the capacity of *A. tumefaciens* strains to infect chickpea and show transient expression. With our technique, the bacterium, inoculated by an infected needle into the embryo apex meristem, was able to deliver the DNA inside the tissue, probably stimulated by inoculation injury. On the basis of these results, the rapid transfer of the embryos inoculated and maintained for 1–2 days on a medium containing TDZ, will promote an active cell proliferation, including those with integrated DNA, able to organize various meristems. Adventitious shoots obtained, resistant to the selective agent, could probably consent the rescue of transformed plants of chickpea. Considering the exogenous genes now available, an interesting perspective for this species improvement is now open.

Conclusions

The experiments focused on the possible use of advanced methodologies for genetic improvement in chickpea to emphasize innovative aspects until now scarcely developed.

Multiple shoots induction

- Thidiazuron appeared more efficient than other cytochinins.
- TDZ allowed the best shoot proliferation at the concentration of 0.3 mg/l.
- Good results were achieved by incubating mature embryos for 24 hours in Gamborg B_5 basal medium.
- The settled protocol can be easily, rapidly and efficiently applied.

***Experiment of transformation by* Agrobacterium tumefaciens**

- Enzymatic digestion and mechanical injury facilitated bacterium penetration.
- Mechanical injury allowed a transient expression of about 40% of treated embryos.
- Under our experimental conditions, enzymatic digestion gave less positive results than mechanical injury in terms of transient expression.
- Following the transformation technique, a lower shoot proliferation was observed.
- Such promising techniques require further study in order to support conventional schemes of breeding.

Acknowledgments

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References

Barna, K.S. and A.K. Wakhlu. 1993. Somatic embryogenesis and plant regeneration from callus cultures of chickpea (*Cicer arietinum* L.). *Plant Cell Reports* 12: 521–524.

Christou, P. 1994. The biotechnology of crop legumes. *Euphytica* 74: 165–185.

Compton, M.E. 1994. Statistical methods suitable for the analysis of plant tissue culture data. *Plant Cell, Tissue and Organ Culture* 37: 217–242.

Fontana, G.S., L. Santini, S. Caretto, G. Frugis and D. Mariotti. 1992. Genetic transformation in the grain legume *Cicer arietinum* L. *Plant Cell Reports* 12: 194–198.

Gamborg, O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research* 50: 151–158.

Gill, R., J.M. Gerrath and P.K. Saxena. 1992. High-frequency direct somatic embryogenesis in thin-layer cultures of hybrid seed geranium (*Pelargonium* x *hortorum*). *Canadian Journal of Botany* 71: 408–413.

Gill, R. and P.K. Saxena. 1992. Direct somatic embryogenesis and regeneration of plants from seedling explants of peanut (*Arachis hypogaea*): promotive role of thidiazuron. *Canadian Journal of Botany* 70: 1186–1192.

Islam, R., T. Malik, T. Husnain and S. Riazuddin. 1994. Strain and cultivar specificity in the *Agrobacterium*-chickpea interaction. *Plant Cell Reports* 13: 561–563.

Jefferson, R.A. 1987. Assaying chimeric genes in plants: the GUS gene fusion system. *Plant Mol. Biol. Reporter* 5: 387–405.

Kartha, K.K., K. Pahl, N.L. Leung and L.A. Mroginski. 1981. Plant regeneration from meristems of grain legumes: soybean, cowpea, peanut, chickpea and bean *Canadian Journal of Botany* 59: 1671–1679.

Malik, K.A. and P.K. Saxena. 1992a. Regeneration in *Phaseolus vulgaris* L.: high-frequency induction of direct shoot formation in intact seedligs by N^6-benzylaminopurine and thidiazuron. *Planta* 186: 384–389.

Malik, K.A. and P.K. Saxena. 1992b. Somatic embryogenesis and shoot regeneration from intact seedlings of *Phaseolus acutifolius* A., *P. aureus* (L.) Wilczek, *P. coccineus* L., and *P. wrightii* L. *Plant Cell Reports* 11: 163–168.

Malik, K.A. and P.K. Saxena. 1992c. Thidiazuron induces high-frequency shoot regeneration in intact seedlings of Pea (*Pisum sativum*), Chickpea (*Cicer arietinum*) and Lentil (*Lens culinaris*). *Australian Journal of Plant Physiology* 19: 731–740.

Mok, M.C. and D.W.S. Mok. 1985. The metabolism of [^{14}C]-thidiazuron in callus tissues of *Phaseolus lunatus*. *Physiologia Plantarum* 65: 427–432.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures *Physiologia Plantarum* 15: 473–497.

Rao, B.G. and V.L. Chopra. 1989. Regeneration in chickpea (*Cicer arietinum* L.) through somatic embryogenesis. *Journal of Plant Physiology* 134: 637–638.

Sagare, A.P., K. Suhasini and K.V. Krishnamurthy. 1993. Plant regeneration via somatic embryogenesis in chickpea (*Cicer arietinum* L.). *Plant Cell Reports*, 12: 652–655.

Shri, P.V. and T.M. Davis. 1992. Zeatin-induced shoot regeneration from immature chickpea (*Cicer arietinum* L.) cotyledons. *Plant Cell, Tissue and Organ Culture* 28: 45–51.

Srinivasan, M., N. Gupta and V.L. Chopra. 1988. *Agrobacterium*-mediated transformation of chickpea. *International Chickpea Newsletter* 19: 2–3.

Visser, C., J.A. Qureshi, R. Gill and P.K. Saxena. 1992. Substitution of auxin and cytochinin requirement for the induction of somatic embryogenesis in geranium hypocotyl cultures. *Plant Physiology* 99: 1704–1707.

The Last Decade of Cytogenetic Studies on Wild and Cultivated *Cicer* Species

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Summary

The results of international scientific research during the last decade are discussed with particular regard to the work carried out under the joint project between Italian institutions and ICARDA: *Development of chickpea germplasm with combined resistance to ascochyta blight and fusarium wilt using wild and cultivated species*. The application of technologies such as differential of the chromosome staining (C-banding, fluorochrome), the hybridization of rDNA probes directly on the metaphasic chromosomes (F.I.S.H.), as well as image analysis for the construction of karyotypes, have allowed a detailed knowledge to be acquired of the species and accessions examined until today. So it has been possible to mark out the phylogenetic relationships of the genus *Cicer* and, in some cases, give indications for crossability on the basis of the cytologic affinities. The development of cytologic knowledge combined with the possibilities given by molecular biology and genetic engineering will allow targeted genetic improvement of the species in question.

Introduction

Knowledge of chromosome morphology and structure is necessary in transferring genes from different taxa (Giorgi and Ceoloni 1988). This is of great significance in chickpea (*Cicer arietinum* L.) because of the presence of a wealth of genes for resistance to biotic and abiotic stresses in its annual wild relatives (van der Maesen and Pundir 1984; Malhotra et al. 1987; Singh et al. 1989, 1990; Singh and Reddy 1993), which could contribute to broaden its adaptation (Muehlbauer et al. 1994). However, the introgression of valuable traits from the wild to the cultivated taxa is hampered by barriers to interspecific hybridization (Bassiri et al 1987; Ahmad et al. 1988; Singh and Ocampo 1993).

For a comprehensive review of studies prior to 1986, see Bahl (1987) who summarized the cytologic knowledge of the genus *Cicer*. This paper will discuss the results of international scientific research during the last decade (1986–95) on the cytology of the genus *Cicer*, with special mention of the studies conducted within the framework of joint collaboration between Italian institutions and ICARDA on wild and cropped annual chickpea species.

Karyotype morphometry

Table 1 summarizes the results achieved from the various authors according to year and type of analysis conducted. In Table 2 the salient data of the nine annual species are reported, relative to the length (amount of DNA) of genomes, chromosomal range and number of satellited pairs. Only two papers refer to data obtained by meiotic analysis (pachytene), all the others report measurements achieved on mitotic chromosomes. Sharma and Gupta (1986) studied four species including *C. arietinum*, while Ahmad and Hymowitz (1993) report data only for the cultivated species. The data in the above two papers relative to this species are discordant. The analysis conducted by Ahmad and Hymowitz (1993) allowed the authors to carry out a detailed karyotype. This is not comparable with the other data in the literature that were obtained in mitotic chromosomes at metaphases.

Table 1. Cytogenetic studies on the genus *Cicer*.

Year			Karyotype	DNA		Differential staining			
	Meiosis	Mitosis	Morphology	amount	C-banding	Fluorochromes	FISH	Ag-NOR	Authors
1986	x	x	x						Sharma and Gupta
1987	x	x	x						Mukherjee and Sharma
1987	x								Ahmad et al.
1988	x	x	x						Ahmad
1989		x			x				Kabir and Singh
1991		x	x	x					Ohri and Pal
1992		x			x	x			Galasso and Pignone
1992		x	x						Ocampo et al.
1993	x		x						Ahmad and Hymowitz
1994		x	x		x				Tayyar et al.
1994		x					x		Abbo et. al.
1995		x		x	x				Venora et al. (a)
1995		x	x						Venora et al. (b)
1995		x		x	x	x	x	x	Galasso et al.

Table 2. Karyotype morphometry *in annual Cicer* species calculated using conventional method and 2C Nuclear DNA (pg)[†].and Pachytene analysis (μm)[‡] techniques

	Total haploid chromatin length (μm)							Chromosome length range (μm)				Satellited chromosome pair				
C. arietinum	16.9 419.6[‡]	17.62	2.4[†]	3.3–3.6[†]	14.38	335.3[‡]	3.29[†]	1.8–3.5 31.2–74.5[‡]	1.32–3.69	1.0–3.00	30.5–58.1[‡]	1°	1°	1°	1°	3°[‡]
C. bijugum	205.4[‡]	17.31	2.3[†]	2.54[†]	11.85			15.2–42.6[‡]	1.87–2.51	1.2–1.8			6°	2°	none	
C. chorassanicum	195.0[‡]	15.44						15.6–42.0[‡]	1.50–2.52				6°			
C. cuneatum	16.3	14.92	2.3[†]	2.50[†]	14.36			1.8–2.6	1.42–2.23	1.6–2.1		1°		1°	8°	
C. echinospermum		20.65		2.70[†]	15.3		2.61[†]		1.56–3.77	1.2–2.9			1°	1°	1°	
C. judaicum	11.4	15.78	2.8[†]	1.83[†]	12.73			1.3–1.6	1.52–2.39	1.2–1.9		none		2°	none	
C. pinnatifidum	12.9	16.50	2.8[†]	2.56[†]	13.38			1.3–2.1	1.58–2.63	1.3–2.1		1°	5°	8°	1°–2°	
C. reticulatum	20.7 311.6[‡]	18.85	2.6[†]	2.65[†]	16.37		2.65[†]	2.0–4.7 18.0–67.4[‡]	1.46–3.63	1.1–3.4		1°	1°	1°–2°	1°–2°	
C. yamashitae		15.11			12.33				1.47–2.31	1.2–1.9			?		3°	
Source	A	B	C	D	E	F	G	A	B	E	F	A	B	D	E	F

Source: A= Sharma and Gupta 1986; B=Ahmad 1988; C=Kabir and Singh 1989; D=Ohri and Pal 1991; E=Ocampo et. al. 1992; F=Ahmad and Hymowitz 1993; G=Galasso et al. 1995.

Table 3. Stebbins' categories of annual *Cicer* species.

	Categories			
C. arietinum	1B	1B	1B	1B
C. bijugum		1A	2A	1A
C. chorassanicum		1A		
C. cuneatum	1A	1A	2B	1A
C. echinospermum		1B	1B	1B
C. judaicum	1A	1A	3B	2A
C. pinnatifidum	1A	1A	2A	2A
C. reticulatum	1B	1B	1B	1B
C. yamashitae		1A		2A
Source	A	B	C	D

Source: A=Sharma and Gupta 1986; B=Ahmad 1988; C=Ohri and Pal 1991; D=Ocampo et. al. 1992.

Indeed, the relative length of the chromosomes differs because of the different condensation at meiosis compared with the mitotic metaphases. Moreover, Sharma and Gupta (1986) confirm that it is preferable not to compare different species in pachytene, because of differences in condensation specific to each species. Only one chromosome was observed attached to the nucleolus, the third in order of length. The percentage of chromocentrics was 28% of the total genome. Chromosome 3 was the most heterochromatic while the 5th was the most euchromatic.

As regards the amount of DNA (Kabir and Singh 1989; Ohri and Pal 1991; Galasso et al. 1995) all the authors agree on *C. reticulatum* and partly on *C. arietinum*, while for the other species the results are dissimilar. Also for the range of the chromosomal length the results are not concordant, with the exception of *C. arietinum*, *reticulatum*, *cuneatum* and *pinnatifidum*, the data for which are fairly similar for several authors, but never in total agreement. Comparisons as regards the satellited pairs are even more discordant. Only for *C. echinospermum* do all the authors agree that it possesses the first satellited pair.

In general, the heterogeneity of the results regarding *Cicer* from different authors may be ascribed to the use of different accessions and techniques. At present, it is feasible to avoid at least some problems in part due to the accuracy of the measurements. With the diffusion in cytologic laboratories of computerized images analysis, it is easy to obtain measurements of plant chromosomes which are objective and free from operator error, in a relatively short time (Venora et al. 1991), also on difficult chromosomes like those of *Cicer* (Ocampo et al. 1992; Venora et al. 1995b). Moreover, it is possible to effect quantitative determinations also of parameters valued only qualitatively (Venora et al. 1995a).

By means of the results obtained by karyotyping, the karyotypic symmetry may be calculated. This, in turn, allows classification of the single species, applying the Stebbins categories method (1971). In Table 3 the classifications obtained by different authors are reported. All agree in classifying the species *C. arietinum*, *echinospermum* and *reticulatum* in the category 1B. It may be noted that the classification achieved by symmetry reduces the variability due to the measurements of the individual parameters, and it gives a global assessment of the karyotype in question. In the general appraisal of chromosomal morphology, a further step forward is due to the use of special REC and SYi indices (Greilhuber and Speta 1976) that also allow a graphical representation. Figure 1 shows the relationships among the species achieved by means of the calculation of such indices (Ocampo et al. 1992). It is worth noting that, as already highlighted by other parameters, the annual species fall into two distinct clusters. In the genus *Cicer* this has a physiological correspondence, that is, between the species within each cluster it is possible to obtain hybrids, while this is not

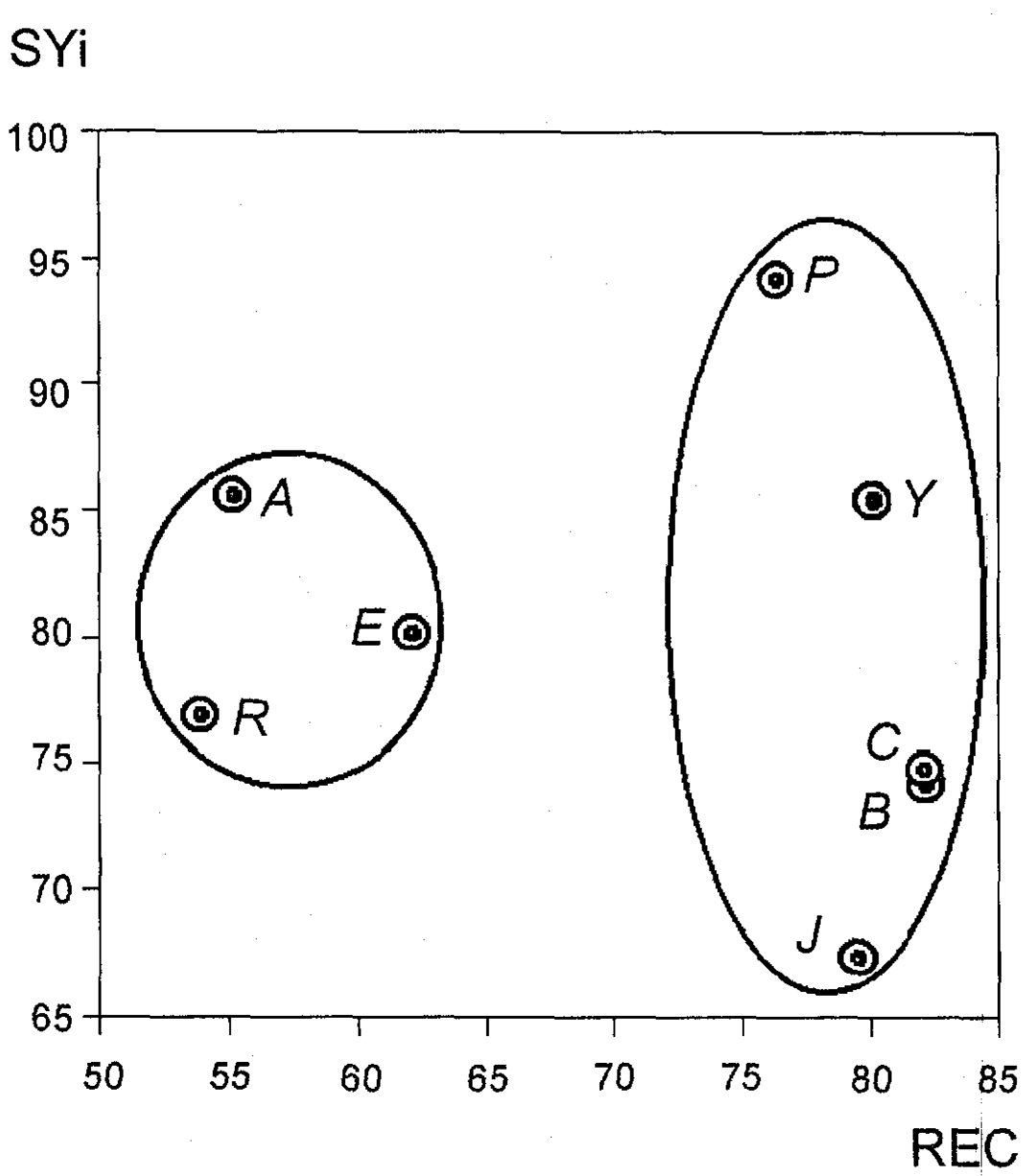

Fig. 1. Karyotype symmetry of annual Cicer species with REC and Syi indics (from Ocampo et al. 1992)

A = *C. arietinum;* E = *C. echinospermum;* R = *C. reticulatum;* P = *C. pinnatifidum;* Y = *C. yamashitae;* C = *C. cuneatum;* B = *C. bijugum;* J = *C. judaicum*

feasible between species of different clusters (Ocampo et al. 1992; Singh and Ocampo 1993). Therefore, the shape of the chromosomes, particularly the symmetry, is somehow in relationship to the possibility of obtaining hybrids between dissimilar species of the genus *Cicer*.

Chromosome Differential Staining and Molecular Cytogenetics

Table 4 summarizes the results concerning the application of different staining techniques allowing the analysis of the structure and the chromosomal organization. Coloring the heterochromatin with Giemsa

Table 4. Application of different staining techniques on chromosomes of *Cicer* species.

	C-banded karyotype			No. of satellited chromosomes					Fluoro-chrome DAPI CMA H33258		Ag- NOR	F.I.S.H.		% C-band heterochromatin content meristematic† and differentiated‡ cells				
C. arietinum	x	x	x	1	1	2/3	1	1	x	x	x	x	x	43.6	43.0	43	9.4†	9.0‡
C. bijugum		x			1									57.7			10.1†	9.6‡
C. chorassanicum		x			1									38.4				
C. cuneatum		x			1									61.3			9.9†	8.7‡
C. echinospermum		x	x		1			1		x	x		x	38.8		39		
C. judaicum		x			1									43.7			9.7†	7.5‡
C. pinnatifidum		x			1									46.0			10.5†	9.3‡
C. reticulatum		x	x		2	2		2		x	x	x	x	41.3		42	10.1†	9.4‡
C. yamashitae		x			1									39.9				
Source	B	C	F	B	C	D	E	F	B	F	F	D	F	C	E	F	A	A

Source: A=Kabir and Singh 1989; B=Galasso and Pignone 1992; C=Tayyar et al. 1994; D=Abbo et al. 1994; E=Venora et al. 1995a; F=Galasso et al. 1995.

produces evident bands that are intensely stained compared to others, which are almost colorless. Kabir and Singh (1989) studied the organization of the interphasic nuclei in six species of the genus *Cicer* by means of acid and alkaline treatments and subsequent staining with Giemsa. All the species displayed a chromocentric, not reticulate, organization, both in meristematic cells and differentiated ones. In these species the two acid and alkalin treatments, evidenced different amounts of heterochromatin, showing the presence of different classes of heterochromatin. The authors conclude that, on the basis of their results, the species *C. reticulatum* is to be considered the most primitive.

Galasso and Pignone (1992) described in detail the karyotype of *C. arietinum* on mitotic metaphases. Each individual chromosome is recognizable by means of a specific Giemsa banding technique. The heterochromatin is distributed preferentially in the centromeric region of all the chromosomes. The satellited chromosome and the second in order of length, seem to possess the main amount of heterochromatin. The application of fluorochromes, moreover, allowed the distinction of two classes of heterochromatin. Most of the heterochromatin that reacts positively to C-banding displays positiveness even when stained with the fluorochromes H33258 (Bis-benzimide derivate Hoechst 33258) or DAPI (4'-6-Diamino-2-phenylindole), while it is neutral to CMA (Chromomycin A3). The only exception is due to some heterochromatic blocks (chromosome 1 and 2) that react negatively both to DAPI and to H33258 but positively to CMA.

Tayyar et al. (1994) with the same method of C-banding, report the results of all nine annual species. Also in this case, as reported already for *C. arietinum* by Galasso and Pignone (1992), all the chromosomes of all the species in question exhibit bands essentially in the centromeric area and some intercalary bands on the longer chromosomes. Together with the chromosomal morphology data, the banding technique allowed pairing of the chromosomal pairs in all the species. As regards the relative amount of heterochromatin studied in this paper (Tayyar et al. 1994), *C. chorassanicum* has the lowest (38.4%) and the *C. cuneatum* the highest (63.1%) content. However, the authors conclude that banding pattern alone does not give adequate information concerning the interrelations among the annual species of the genus *Cicer*.

Abbo et al. (1994) were the first to study, in *Cicer* the chromosomal localization of repetitive DNA probes (F.I.S.H.), evidencing three hybridization sites in *C. arietinum* and two in *C. reticulatum*. This conflicts with all the cytologic evidence that reports only a nucleolar organizer in the cultivated species (only one satellited pair).

Merely qualitative estimation of the heterochromatin is often not adequate when comparisons are required in different species or diverse accessions of the same species. Therefore, objective and replicable quantification becomes necessary. An easy method that allows the accurate estimate of the heterochromatin amount stained with Giemsa was set up by means of image analysis (Venora et al. 1995). The data obtained in this way in the cultivated species are in agreement with the results of the literature. Moreover, it is possible to conduct sequential measurements with different kinds of dyes, which highlight diverse classes of heterochromatin (Galasso and Pignone, 1992), and to evaluate the amount of each class exactly.

Recently Galasso et al. (1995) studied three species of *Cicer* with narrow phylogenetic relationships among them, *C. arietinum*, *C. reticulatum* and *C. echinospermum*, applying many different methods of staining and also molecular cytogenetic techniques. The C-banding pattern, generally like the karyotype, is rather similar in the three species, but there are some evident differences that concern the first two pairs of chromosomes. *C. reticulatum* possesses an extra satellite on the B chromosome, *C. echinospermum* lacks a heterochromatic block on the satellited arm of chromosome A and shows a different centromeric location of the B chromosome as regards the other two species. The application of fluorochromes allowed different classes of heterochromatin to be distinguished. *C. reticulatum* and *C. echinospermum*, as noted already by Galasso and Pignone (1992), possess two distinct classes of heterochromatin. The first is

positive to CMA and negative to DAPI and to H33258, while the second is neutral to CMA and positive to the other two dyes. All three species possess two pairs of chromosomes positive to CMA, whose sites are located in the secondary constriction of chromosomes A and B of *C. reticulatum*, and subterminal of the B chromosome in *C. echinospermum* and *C. arietinum*.

As regards the hybridization sites of the probes used (F.I.S.H.) the results were similar for all three species analyzed : the probe pTa71 hybridizes in the same sites as CMA (two sites per haploid genome), while the two signals of the probe pTa794 are sited with one adjoining the probe pTa71 on the B chromosome, the other on a chromosome of intermediate length.

The silver staining of the organizer regions of the nucleolus (Ag-NOR), in agreement with the other observations, highlighted in *C. reticulatum* two sites of different intensity at the two secondary constrictions (chromosomes A and B). In the other two species, it was possible to detect only a positive area at the secondary constriction of chromosome A.

The authors conclude that all the data agree in defining the narrow phylogenetic relationship of the three species. Moreover, in agreement with the literature, they assume that the species *C. reticulatum* is the common ancestor.

Intraspecific variability

The possibility of discovering a certain karyologic variability within cultivated species has always been duly considered by the authors, who have studied *C. arietinum* from the cytologic point of view. In the last decade (1986–96), many papers report data obtained on accessions, lines and cultivars of different type, desi and kabuli, and morphophysiological characteristics. All the papers unanimously affirm that the chromosomal diploid number of this species is 2n=16. In Table 5, the more significant karyomorphological data are summarized according to the author.

Sharma and Gupta (1986) studied six desi cultivars, finding small differences with regard to the length and arm ratio of single pairs. Nevertheless, in general the karyotype is always constituted by a pair of long chromosomes with an evident satellite and submetacentric primary constriction, six pairs of intermediate length metacentrics or submetacentrics and finally a pair of very small chromosomes. Only one satellited pair was always observed both in mitosis and in meiosis. The small differences found are therefore attributable to structural changes that permit varietietal differentiation within a species.

Mukherjee and Sharma (1987) analyzed 12 varieties of which one was of the kabuli type, invariably finding eight chromosomal pairs. On the contrary, a wide variability was observed for chromosomal length and for the number of satellited chromosomes, up to three pairs. The authors finally assert that it is possible to distinguish each of the 12 varieties on the basis of the karyotype and of specific chromosome markers.

Ahmad (1988) reports the results obtained on five cultivated accession: 2n=16 chromosomes and only one satellited pair were constantly observed. The average karyotype of the five cultivars is characterized by a satellited submetacentric pair of long chromosomes, six pairs of intermediate length (2.75–1.62 μm) of which four metacentrics and two submetacentrics, and finally a very small metacentric pair (1.32 μm). No variability was reported between different accessions.

Ohri and Pal (1991) also studied five different cultivars. Only one satellited pair is recorded in all the cultivars. In general, the karyomorphological parameters were substantially identical, except for small structural rearrangements deducible from the karyotypic formulas of each cultivar.

Venora et al. (1995b) studied four cultivars, which were all of the kabuli type, the one least studied. They discovered low variability, both in chromosomal length and number of satellited pairs, always only one pair, which was the first in order of length. However, on the basis of the karyotype, it is possible to distinguish each cultivar from the others by means of the identification of chromosome markers, significantly different and specific for each cultivar. The authors propose as reference karyotype for the kabuli type, the cultivar Sultano, that represents karyologically 75% of the analyzed plates. While discussing the results of the literature concerning intraspecific variability, the authors note that the desi type is more

Table 5. Intraspecific variability of cultigen karyomorphometry calculated using the conventional method and 2C Nuclear DNA (pg)[†] technique

Total haploid chromatin length (μm)					Chromosome length range (μm)				Satellited chromosome pair				
15.6	12.98	17.62[§]	3.30[†]	14.38[†]	1.1–3.3	1.1–2.6	1.32–3.69[§]	1.03–2.97[†]	1	1	1[§]	1	1[†]
17.1	15.57		3.34[†‡]	16.82[‡]	1.3–3.2	1.1–3.2		1.22–3.29[‡]	1	1		1[‡]	1[‡]
14.3	15.67		3.39[†]	15.87[‡]	1.1–3.4	1.3–3.2		1.15–3.30[†]	1	1		1	1[‡]
16.1	15.99		3.47[†]	15.81[‡]	1.2–3.5	1.2–2.7		1.30–3.20[†]	1	1		1	1[‡]
15.2	16.09		3.57[†]		1.3–3.1	1.0–3.4			1	1–2		1	
16.9	17.20				1.3–3.5	1.2–4.0			1	1–2			
	17.38[‡]					1.3–3.9[‡]				1[‡]			
	18.32					1.2–4.0				1–2			
	18.82					1.5–3.9				1–2			
	19.56					1.3–4.5				1–2–3			
	20.22					1.7–4.5				1			
	21.51					1.7–5.0				1–2			
A	B	C	D	E	A	B	C	E	A	B	C	D	E

[‡]Kabuli type; [§]mean value of five measurements

Source: A=Sharma and Gupta 1986; B=Mukherjee and Sharma 1987; C=Ahmad 1988; D=Ohri and Pal 1991; E=Venora et. al. 1995b.

variable than kabuli. This cannot be explained by any specific reason, but it could be due to the broader adaptability of the desi type compared with the kabuli one. Moreover, it is to be considered that the desi type is more ancestral than kabuli, which originated from it by means of mutation.

Conclusions

In the genus *Cicer* in the determination of the karyotype, there are greatly conflicting results. As already recalled, this is attributable, above all, to the use of different accessions and, particularly, to the use of the most disparate techniques of pretreatment of the somatic cells, for the accumulation of useful metaphases for the measurements. Finally, but not less important, measurement techniques frequently do not allow error-free evaluations in very small chromosomes and of insufficient quality in general.

Despite such evident differences, it is unanimously held that the karyotype of the genus *Cicer* is rather symmetrical, little evolved according to the classification of Stebbins (1971). Indeed in this genus, karyotypic symmetry seems to play a fundamental role in determining the outcome of the crosses between different species, thus impeding the flow of useful genes that the breeders seek.

The adoption of objective measurement methods, such as the image analysis of the metaphasic chromosomes, efficient pretreatment techniques for *Cicer* (e.g. the saturated aqueous solution of 1,4-dichlorobenzene for 2.5 hours at 15°C), and the use of certain cultivars of reference, will undoubtedly allow comparable results to be obtained not only by various authors but also using different techniques, as has occurred in other species (Venora et al. 1995c; Pignone et al.1995).

Almost all the cytologic techniques currently available in plant cytology have been used in the genus *Cicer*. The results do not often allow description of the relationships between different species on the basis of specific techniques that emphasize chromosomal structure. C-banding and fluorochromes have shown the existence of different heterochromatic classes, but there is not enough variability among the examined species. Such studies have confirmed the results obtained both by genetic analysis (Ladizinsky and Adler, 1976) and or karyologics (Ocampo et al. 1992) as regards the phyletic relations among the different species.

The techniques of *in-situ* hybridization (F.I.S.H.) have demonstrated the possibility of cytologic localization of rDNA probes. The availability in the imminent future of many probes of DNA and genes cloned for specific traits, also for the leguminous plant (Kahl et al. 1994), will allow us to outline relationships of homoeology between chromosomes and/or the single arms of the different species (Galasso et al. 1995).

Although great karyotypic differences have not been found, all the authors are in agreement in underlining structural differences (centromere position) among the varieties and accessions studied. It is worth noting that, except for the two papers by Mukherjee and Sharma (1986) and Venora et al. (1995), which specifically studied intraspecific variability with cytologic methods, all the other works only incidentally refer to it. A systematic study is required, which will provide precise information with quantitative estimates of the different classes of heterochromatin, together with the application of molecular cytology techniques and genetic analysis of interesting genotypes. This will allow a more definite estimate of the variability within each of the nine annual species of the *Cicer* genus, and will enable the choice of particular accessions also in possession of specific cytologic characteristics.

As illustrated in this brief review, cytogenetics in general, and that concerning *Cicer* in particular, is currently flourishing, thanks also to the availability of computerized technologies and new analytical methods. This makes us hope that in the imminent future it will be possible to achieve 'macroscopic' results, that is, the insertion of useful genes from wild species, by means of better 'microscopic' knowledge.

References

Abbo, S., T.E. Miller, S.M. Reader, R.P. Dunford and I.P. King. 1994. Detection of ribosomal DNA sites in lentil and chickpea by fluorescent in situ hybridization. *Genome* 37: 713–716.

Ahmad, F. 1988. Interspecific hybridisation and genetic relationships among the annual *Cicer* L. species. PhD thesis.

University of Saskatchewan, Canada.

Ahmad, F. and T. Hymowitz, 1993. The fine structure of chickpea (*Cicer arietinum* L.) chromosomes as revealed by pachytene analysis. *Theoretical and Applied Genetics* 86: 637–641.

Ahmad, F., A.E. Slinkard and G.J. Scoles. 1987. The cytogenetic relationship between *Cicer judaicum* Boiss. and *Cicer chorassanicum* (Bge.) M.Pop. *Genome* 29: 883–886.

Ahmad, F., A.E. Slinkard and G.J. Scoles. 1988. Investigations into the barrier(s) to interspecific hybridization between *Cicer arietinum* L. and eight other annual *Cicer* species. *Plant Breeding* 100: 193–198.

Bahl, P.N. 1987. Cytology of chickpea. Pages 83–97 *in* The Chickpea (M.C. Saxena and K.B. Singh ed.). CAB International, Wallingford, UK.

Bassiri, A., F. Ahmad and A.E. Slinkard. 1987. Pollen grain germination and pollen tube growth following in vitro and in vivo self and interspecific pollination in annual Cicer species. *Euphytica* 36: 667–675.

Galasso, I. and D. Pignone. 1992. Characterization of chickpea chromosomes by banding techniques. *Genetic Resources and Crop Evolution* 39: 115–119.

Galasso, I., M. Frediani, M. Maggiani, R. Cremonini and D. Pignone. 1995. Chromatin characterization by banding techniques, in situ hybridization and nuclear DNA content in *Cicer* L. (Leguminosae). *Genome.* (In press.)

Giorgi, B. and C. Ceoloni. 1988. Citogenetica e miglioramento genetico. Pages 333–376 *in* Miglioramento genetico Vegetale (G.T. Scarascia-Mugnozza, ed.). Pàtron, Bologna, Italy.

Greilhuber, J. and F. Speta. 1976. C-Banded karyotypes in the *Scilla hohenackeri* Group, *S. persica* and *Puschkinia* (Liliaceae). *Plant Systematics and Evolution* 126: 149–188.

Kabir, G. and R.M. Singh. 1989. Interphase nuclear structure and heterochromatin in *Cicer* species. *Cytologia* 54: 27–32.

Kahl, G., D. Kaemmer, K. Weising, S. Kost F. Weigand and M.C. Saxena. 1994. The potential of gene technology and genome analysis for cool-season food legume crops: theory and practice. *Euphytica* 73: 177–189.

Ladizinsky, G. and A. Adler. 1976. Genetic relationships among the annual species of *Cicer* L. *Theoretical and Applied Genetics* 48: 197–203.

Malhotra, R.S., R.P.S. Pundir and A.E. Slinkard. 1987. Genetic Resources of Chickpea. Pages 67–81 *in* The Chickpea (M.C. Saxena and K.B. Singh, ed.). CAB International, Wallingford, UK.

Muehlbauer, F.J., W.J. Kaiser and C.J. Simon. 1994. Potential for wild species in cool season food legume breeding. *Euphytica* 73: 109–114.

Mukherjee, S. and A.K. Sharma. 1987. Structure and behavior of chromosomes in the strains of *Cicer arietinum* L. *Cytologia* 52: 707–713.

Ocampo, B., G. Venora, A. Errico, K.B. Singh and F. Saccardo. 1992. Karyotype analysis of the *Cicer* genus. *Journal of Genetics and Breeding* 46: 229–240.

Ohri, D. and M. Pal. 1991. the origin of chickpea (*Cicer arietinum* L.): karyotype and nuclear DNA amount. *Heredity 66*: 367–372.

Pignone, D., I. Galasso and G. Venora. 1995. Karyomorphological and heterochromatin similarities among four *Vigna* species. *Genetic Resources and Crop Evolution* 42: 57–60.

Sharma, P.C. and P.K. Gupta, 1986. Cytogenetics of legume genera *Cicer* L. and *Lens* L. Pages 321–340 *in* Genetics and Crop Improvement (P.K. Gupta and J.R. Bahal, ed.). Rastagi and Company, Meeret, India.

Singh, K.B. and M.V. Reddy. 1993. Sources of resistance to *Ascochyta* blight in wild *Cicer* species. *Netherlands Journal of Plant Pathology 99:* 163–167.

Singh, K.B., M. Di Vito, N. Greco and M.C. Saxena. 1989. Reaction of wild *Cicer* species lines to *Heterodera ciceri*. *Nematologia Mediterannea* 17: 113–114.

Singh, K.B., R.S. Malhotra and M.C. Saxena. 1990. Sources of tolerance to cold in *Cicer* species. *Crop Science* 30: 1136–1138.

Singh, K.B. and B. Ocampo. 1993. Interspecific hybridization in annual *Cicer* species. *Journal of Genetics and Breeding* 47: 199–204.

Stebbins, G.L. 1971. Chromosomal Evolution in Higher Plants. Edward Arnold, London.

Tayyar, R.I., A.J. Lukaszewski and J.G. Waines. 1994. Chromosome banding patterns in the annual species of *Cicer*. *Genome* 37: 656–663.

van der Maesen, L.J.G and R.P.S Pundir. 1984. Availability and use of wild *Cicer* germplasm. *Plant Genetic Resources Newsletter* 57: 19–24.

Venora, G., C. Conicella, A. Errico and F. Saccardo. 1991. Karyotyping in Plants by an Image Analysis system. *Journal of Genetics and Breeding* 45: 233–240.

Venora, G., I. Galasso and D. Pignone. 1995a. Quantitative heterochromatin determination by means of image analysis. *Journal of Genetics and Breeding* 49: 187–190.

Venora, G., B. Ocampo, K.B. Singh and F. Saccardo. 1995b. Karyotype of kabuli-type chickpea (*Cicer arietinum* L.) by image analysis system. *Caryologia* 48: 147–155.

Venora, G., I. Galasso and D. Pignone. 1995c. Retrospects and Perspectives of cytogenetical studies in Vigna. *Biologisches Zentralblatt* 114: 231–241.

Introgression of Progressive Genes from Wild *Cicer* Species: Interspecific Hybridization

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Summary

Over the last decade, conventional breeding techniques have generated chickpea cultivars with appreciable agronomic features; nevertheless, there has not been a breakthrough in high-yielding varieties. Yields continue to be erratic and production unstable. These are the main reasons why this crop is unattractive to farmers.

Wild genetic resources have proved to be dramatically important for the genetic improvement of crops. Attempts to cross the annual wild *Cicer* species with kabuli-type chickpea cultivars at ICARDA have shown that two species, *C. echinospermum* and *C. reticulatum*, are readily crossable with the cultigen. Their F_1s have exhibited vigor over the cultivated parent for a number of agronomic traits and the F_2s have shown transgressive segregants for seed yield and related traits. Superior progenies were carried on following the pedigree method of selection. Replicated trials in F_7 have shown that both *C. echinospermum* and *C. reticulatum* when in hybrid combination with *C. arietinum* ILC 482, were able to produce lines with superior agronomic features compared to the cultigen parent. Seed yield was improved by 39%, biological yield by 49%, 100-seed weight by 39%, plant height by 28% and seed protein content by 11%. The superior lines had the value of being phenologically and phenotypically kabuli-like. Thus, this material has a practical utility for the improvement of chickpea kabuli-type either used directly for cultivar release or as prebreeding.

Introduction

The genus *Cicer* belongs to the family Leguminoseae, subfamily Papilionaceae and tribe Cicereae Alef. (Kupicha 1981). It comprises 43 species: nine annuals, 33 perennials and 1 unspecified (*C. laetum* Rass & Sharip.) (van der Maesen, 1987). They are grouped into four sections on the basis of morphological traits and life cycle: *Cicer* (or *Monocicer*), *Chamaecicer*, *Polycicer* and *Acanthocicer*. The annual *Cicer* species comprise nine taxa: *C. arietinum* L. (the cultivated taxon), *C. bijugum* K.H.Rech., *C. cuneatum* Hochst. ex Rich, *C. echinospermum* P.H.Davis, *C. judaicum* Boiss., *C. pinnatifidum* Jaub. & Sp., *C. reticulatum* Ladiz. and *C. yamashitae* Kitamura (grouped into the section *Cicer*), and *C. chorassanicum* (Bge.) M.Pop. (included in the *Chamaecicer*).

Chickpea is the third most important pulse in the world and first in the Mediterranean basin. It is grown on 10.3 million hectares with a production of 7.0 million tonnes (FAO 1995), this is 15.1% of the world area devoted to pulses, and 12.1% of the total pulse production. The crop is mainly grown in regions with unfavorable pedo-climatic conditions, poor agricultural systems and unpredictable occurrences of biotic and abiotic stresses, which severely affect yield potentials. World average yields (about 0.7 t/ha) are far from being improved and stabilized, because of the continuous shift of chickpea to marginal areas caused by increases in more strategic and lucrative crops.

Conventional breeding over the last decade has produced cultivars with significant agronomic improvements, nevertheless, the crop continues to be economically unattractive to farmers, because of low revenues associated with erratic yields, although chickpea unit prices are higher than those of cereals.

Wild genetic resources have been dramatically effective in improving a number of crops (Harlan 1984; Prescott-Allen 1988). ICARDA, which has a world mandate to serve as a center for the collection, evaluation and maintenance of kabuli-type chickpea and the wild *Cicer* species, maintains a collection of 268 accessions of the eight annual wild *Cicer* species. These species have been shown to possess desirable resistance against major biotic and abiotic stresses responsible for

severe yield losses and production instability (Singh et al. 1981, 1989 a, b, 1990; Singh and Reddy 1993; Singh and Weigand 1994). A few accessions are multiple sources of resistance. Furthermore, resistance against cyst nematode and seed beetle are only available within the wild taxa (ICARDA 1993).

Annual wild Cicero species have been used in distant hybridizations with the goal of improving agronomic traits in chickpea (Singh et al. 1984; Jaiswal and Singh 1989; Pundir et al. 1992; Sheila et al. 1992), but as yet there have been no reports of acceptable lines that are ready for direct use in breeding programs.

During the last decade, chickpea breeding activities in ICARDA have devoted increasing attention to the exploitation of wild *Cicer* germplasm. Several interspecific hybridizations carried out at Tel Hadya (ICARDA headquarters, Northwest Syria) have shown significant F_1 hybrid vigor over best parent for morpho-agronomic traits (Singh and Ocampo 1993; Ocampo 1995). Positive transgressive segregants were observed in F_2 (Singh and Ocampo 1993; Ocampo 1995).

These promising results led us to ask whether alleles of wild origin could be selected in transgressive combinations with those of domesticated origin, for the agronomic improvement of kabuli chickpea.

Materials and methods

This investigation was carried out in the field at Tel Hadya (36^0 35' E, 36^0 51' N and 284 m asl), ICARDA's main research station in Northwest Syria, during 1987–95. The experimental material is shown in Table 1. The annual wild *Cicer* spp. were chosen at random. The nine accessions were crossed in a diallel fashion.

The material was protected from ascochyta blight by periodic spraying of chlorothalonil and the experimental area was hand and chemically weeded. The crossing technique consisted of simultaneous emasculations and pollinations made from morning up to the first hours of the noon. The putative F_1s were grown in the field flanked by their two parents during 1988/89 to ascertain their hybrid authenticity. Selection was carried out within successful matings, which involved the cultigen, i.e., *C. arietinum* x *C. echinospermum*, *C. arietinum x C. reticulatum*, and their reciprocals. Selection (pedigree method) started in F_2 and sought the largest possible combination of the following desirable characters: high seed yield; erect or semi-erect growth habit; tall plants; absence of pod shattering; heavy, beige-colored seeds; owl or round seed shape; and uniform maturity. Based on these criteria, selection pressure in F_2 was 18% in *C. arietinum x C. echinospermum*; 8% in *C. echinospermum* x *C. arietinum*, and 6% in the two cross-combinations involving *C. reticulatum*. A preliminary yield trial was carried out in F_6 during 1993/94, where ninety-five F_6 lines and parents (*C. arietinum* [ILC 482], *C. echinospermum* [ILWC 35/s-1] and *C. reticulatum* [ILWC 21-15]) were evaluated following an incomplete block design with two replications. Twenty-two F_6 progenies superior for seed yield, seed size and plant height and 100-seed weight were selected and evaluated, together with parents, during the 1994/95 season following a partially balanced triple lattice design. Plot size was 2.45 m^2. Spacings between and within rows were 45 cm and 8 cm, respectively. Evaluations done during 1994/95 were taken on 14 characters, namely:

- days to 50% flowering (DFL), number of days from sowing to the stage when about 50% of plants had begun to flower
- days to maturity (DMA), number of days from sowing to the stage when about 90% of plants had 100% mature pods
- plant height (HGT), average height in cm of three randomly selected plants at the end of flowering from ground level to the highest point in the plant
- biological yield (BYD), weight (kg/ha) of all parts of plants of the plot at maturity, after discarding plants included in the heads of 25 cm of the plots to avoid border effect
- seed yield (SYD), weight (kg/ha) of the seeds of plants of the plot at maturity, after discarding plants included in the heads of 25 cm of the plots to avoid border effect
- growth habit (GRH), angle of primary branches at mid-pod filling stage visually scored on the basis of performance of the majority of plants in plots as erect = 0–15° from vertical, semi-erect = 16–25°, semi-spreading = 26–60° and spreading = 61–80°
- uniformity to maturity (UMAT), unvarying maturity visually scored as u = uniform maturity and nu = no uniform maturity
- pod dehiscence (PDD), at maturity visually

Table 1. Passport information of the experimental material used in the interspecific hybridizations carried out during 1987/88 at ICARDA's Tel Hadya station in Northwest Syria.

ILC/ILWC[†]	Species	Origin	Donor[‡]	Collection No.
482	*C. arietinum* L. pure line	TUR	PIC	26780-68
34	*C. bijugum* K.H.Rech. population	TUR	WRPIS	NO 200
147	*C. chorassanicum* (Bge) M.Pop. population	AFG	ALAD/ICRISAT	JM 2230 (sel.)
187	*C. cuneatum* Hochst. ex Rich pure line	ETH	-	-
179	*C. echinospermum* P.H.Davis pure line	TUR	-	NO 204 (sel.)
94	*C. judaicum* Boiss. population	PAL	-	NO 182 (sel.)
29	*C. pinnatifidum* Jaub. & Sp. population	TUR	ICRISAT	NO 189
124	*C. reticulatum* Ladiz. pure line	TUR	ICRISAT	JM 2106a (sel.)
214	*C. yamashitae* Kitamura pure line	AFG	ALAD/ICRISAT	JM 2021

[†] = ILC = International Legume Chickpea accession number; ILWC = International Legume Wild *Cicer* accession number.

[‡] = ALAD = Arid Land Agricultural Development, Beirut, Lebanon; ICRISAT = International Crop Research Institute for Semi-Arid Tropics, Patancheru, A.P., India; PIC = PIC, Menemen, Izmir, Turkey; WRPIS = Western Region Plant Introduction Station, USDA, Pullman, Washington, USA.

- scored as nd = no dehiscence and d = dehiscence
- seed color (SCOL), scored as beige, orange, copper and brown
- seed shape (SSH), scored as angular, owl and round
- seed protein content (PRO), following Kjeldahl procedure and expressed in percent germinability (GRM), following Anonymous (1985)

Results

A total of 5280 flowers were pollinated in 68 cross combinations (Singh and Ocampo 1993). The successful cross-combinations were: *C. arietinum* x *C. echinospermum*, *C. arietinum* x *C. reticulatum*, *C. echinospermum* x *C. reticulatum*, their reciprocals and *C. bijugum* x *C. judaicum* and *C. pinnatifidum* x *C. judaicum*. All F_1s were fully fertile except those involving *C. judaicum* because of structural flower aberrations.

Estimates of F_1 seed yield hybrid vigor over best parent among hybrids derived by crossing *C. echinospermum* and *C. reticulatum* to *C. arietinum*, ranged from 28 to 153% (Singh and Ocampo 1993).

Transgressive positive segregation for agronomic traits was plentiful in F_2 generation. For instance, seed yield (g/plant) ranged from <1 to 55 in *C. arietinum* x *C. echinospermum*, <1 to 30 in the reciprocal, from <1 to 30 in *C. arietinum* x *C. reticulatum* and <1 to 65 in the reciprocal (Singh and Ocampo 1993). Exemplified F_2 transgressive segregants for few morpho-agronomic traits are shown in Table 2, referring to the two cross-combinations involving *C. arietinum* and *C. echinospermum*. Transgressive positive segregants for seed yield and related traits were also present across successive generations.

Differences between reciprocals were significant only in matings involving *C. echinospermum*, where the best performances were achieved by *C. arietinum* x *C. echinospermum*. Already in F_5, genotypes *C. echinospermum* x *C. arietinum* were not present, because they did not conform to selection criteria.

The agronomic mean estimates of the 22 F_7 progenies grown during 1994/95 are shown in Table 2. Nine progenies showed higher seed yield compared to the cultigen, nevertheless, only progeny 55 (*C. arietinum* x *C. reticulatum*) significantly out-yielded ILC 482 (by a margin of 39%). This progeny was semi-erect, uniform in maturity, free from pod shuttering, and had kabuli-like seeds. Moreover, it did not differ significantly from ILC 482 for days to maturity, plant height, harvest index, 100-seed weight and protein content.

Fifteen progenies showed heavier seeds compared to those of the cultigen ILC 482, but eight showed significant weight increases ranging from 15 to 39%. They showed kabuli-type seed, were uniform in maturity and free from pod shuttering.

Most progenies were numerically taller than the cultigen.

Although most progenies had high seed protein content compared to ILC 482, only one (67, a *C. reticulatum* x *C. arietinum* genotype) showed a significant increase by a margin of 8%. *C. reticulatum* showed the highest level of seed protein content.

Seed germination was satisfactory for almost all the progenies. The relatively low percentage of germination within the F_7 progenies was caused by bacterial and fungal contamination. The low germination percentage showed by the wild parents was due to the hard and thick seed coat, because scarified seeds showed germination higher than 90%. Seeds of the recombinants with owl and angular seed shape had to be scarified during the first generations because of their thick and hard seed coat. Round seeds never demanded scarification. Furthermore, many of these later progenies displayed the detachment of the seed coat with the consequent splitting of the two cotyledons. Seeds of the wild parent always required scarification.

Discussion

The production of agronomically superior F_7 progenies, coupled with the absence of undesirable agronomic and phenotypic attributes from the wild parents, has shown the great potential of interspecific hybridization in chickpea for the genetic improvement of the crop. This is despite the overall poor agronomic features of the wild parents (Robertson et al. 1995) and partial sterility, which affected the crosses involving *C. echinospermum* (Singh and Ocampo 1993).

Undesirable wild gene components of *C. echinospermum* and *C. reticulatum* were less of a problem than expected. Therefore,

Table 2. Mean estimations, ranges and skewness† of morpho-agronomic traits of F_2 populations involving *Cicer arietinum* and *C. echinospermum*.

Characters	Cross-combination and parents	Mean	Range	Skewness
Days to maturity	AE‡	186	164–193	-0.8**
	EA	183	163–198	-0.2
	A	183	181–183	
	E	176	166–181	
Plant height (cm)	AE	23	3–37	0.0
	EA	21	8–35	0.3
	A	20	16–30	
	E	10	5–17	
100-seed weight (g)	AE	21	8–48	0.8**
	EA	18	6–31	0.0
	A	26	17–31	
	E	14	11–16	
Biological yield (g/plant)	AE	40	2–162	1.5**
	EA	38	1–195	2.3**
	A	20	9–32	
	E	18	6–31	
Seed yield (g/plant)	AE	10	0–50	1.6**
	EA	9	0–30	1.0**
	A	9	9–32	
	E	7	6–31	
Harvest index (%)	AE	24	2–82	0.6**
	EA	26	2–80	0.3
	A	47	40–54	
	E	32	9–39	

† = skewness only for F_2 populations

‡ = A= *C. arietinum* (ILC 482), R= *C. reticulatum* (ILWC 21–15), E= *C. echinospermum* (ILWC 35/s-1), AE= *C. arietinum* (ILC 482) ' *C. echinospermum* (ILWC 35/s-1) and AR= *C. arietinum* (ILC 482) ' *C. reticulatum* (ILWC 21–15)

** = value significantly different from 0 at $P= 0.01$

backcrosses not being imperative, segregating populations can conserve their high genetic variability while advancing through generations by means of straight pedigree selection. Moreover, the chances of loosing minor effects of the desired polygenic units from the wild forms were reduced.

Few attempts to improve chickpea via interspecific hybridizations carried out in India (Singh et al. 1984; Jaiswal et al. 1986; Jaiswal and Singh 1989) have produced genetic variation for a few important agronomic traits, nevertheless, the superior transgressive segregants have been plagued by undesirable traits which are peculiarities of the wild forms such as: pod shattering; hard seed coat; prostrate growth habit; and, unacceptable seed color.

Cicer echinospermum and *C. reticulatum*, both contributed alleles for the improvement of kabuli.

Significant differences between the two cross-combinations involving *C. echinospermum* suggest the strong maternal effects of this species when in combination with the cultivated genotypes (Ocampo 1995).

The presence of transgressive segregants for seed yield and most important agronomic traits would suggest allelic complementarity between *C. echinospermum*, *C. reticulatum* and *C. arietinum*, epistatic effects and/or modifying factors. This would foster hopes for the production of better recombinants when using elite germplasm as cultigen parents.

Poor consistency between mean estimates of selected material across seasons and generations (correlation data not shown) rendered selections frustrating (Jaiswal et al. 1986). This was particularly true for seed yield, which is a polygenic character with low heritability, and less for characters, such as plant height and seed weight, which usually show a higher degree of heritability. Environmental interferences, the heterozygous condition of genes ruling important physiological activities, and nonadditive gene effects could have made a great contribution to the poor correlations (Jaiswal et al. 1986; Jaiswal and Singh 1989; Ocampo 1995). Selections could be started on genetically more homogeneous material (F_5 or F_6), after advancing in bulk the segregant populations and carrying out slight negative selections. Nevertheless, positive selections for relatively high heritable traits, like 100-seed (Ocampo 1995), could start in the early generations.

Introgression into the cultigen of annual wild *Cicer* species which were not crossed with it is highly desirable, because these taxa are a unique source of good levels of resistance to biotic and abiotic stresses, which the crop lacks (Singh et al. 1981; 1989 a,b; Singh et al. 1990; Singh and Reddy 1993; Singh and Weigand 1994). Unfortunately, the lack of cross-compatibility between *C. reticulatum* and *C. echinospermum*, and the remaining annual wild *Cicer* species seems to preclude their introgression into the cultigen even by using bridge-crossing.

Assuming that the high rates of cross success obtained in this work (Singh and Ocampo 1993) were chiefly due to the harmonious combination of the genotypes crossed and to the environment, more matings have to be attempted taking into account interactions with the environment. Diverse crossing techniques like the use of hormones, *of mentor pollen*, etc. (Stalker, 1980) could aid the gene flow in crosses involving the annual *Cicer* species. Rescue of ovules and their *in vitro* culture could prove a valid solution for the cross-success, whenever genetic incompatibilities between taxa don't preclude the total gene flow.

The unpredictable ability of both *C. echinospermum* and *C. reticulatum* to improve agronomic traits in chickpea, indeed call for profound evaluation of chickpea wild genetic resources. Moreover, wider surveys of different genotypic combinations have to be attempted as well as optimization of breeding methodologies for a more effective exploitation of *C. echinospermum* and *C. reticulatum*.

References

Anonymous. 1985. Pages 300–528 *in* International rules for seed testing. Seed Science Technology.

FAO. 1995. *Quarterly Bulletin of Statistics* 8(1/2): 51, 54.

Harlan, J.R. 1984. Evaluation of wild relatives of crop plants. Pages 212–222 *in* Crop Genetic Resources: Conservation & Evaluation (J.H.W. Holden and J.T. Williams, ed.). G. Allen & Unwin, London, UK.

ICARDA. 1993. Kabuli Chickpea Improvement *in* Food Legume Improvement Program, Annual Report for 1989. ICARDA, Aleppo, Syria.

Jaiswal, H.K., B.D. Singh, A.K. Singh and R.M. Singh. 1986. Introgression of genes for yield and yield traits from *C. reticulatum* to *C. arietinum*. *International Chickpea Newsletter* 14: 5–8.

Jaiswal, H.K. and B.D. Singh. 1989. Analysis of gene effects for yield traits in crosses between *C. arietinum* L. and *C. reticulatum* Ladz. *Indian Journal of Genetics* 49(1): 9–17.

Kupicha, F.K. 1981. Cicereae *Alefed.* Page 382 *in* Advances in legume systematics, Part 1 (R.M. Polhill and P.H. Ravens, ed.). Royal Botanic Gardens, Kew, UK.

Ocampo, B. 1995. Estudio de las especies anuales del género *Cicer* L.: aplicación a la mejora genética de *Cicer arietinum* L. Unpublished PhD thesis, University of Córdoba, Córdoba, Spain.

Prescott-Allen, R. 1988. Genes from the wild. Earthscan Publication, London, UK.

Pundir, R.P.S., M.H. Mengesha and G.V. Reddy. 1992. Interspecific hybridization in *Cicer*. *International Chickpea Newsletter* 26: 6–8.

Robertson, L.D., K.B. Singh and B. Ocampo. 1995. A catalog of annual *Cicer* species. ICARDA, Aleppo, Syria.

Sheila, V.K., J.P. Moss, C.L.L. Gowda and van H.A. Rheenen. 1992. Interspecific hybridization between *C. arietinum* and wild *Cicer* species. *International Chickpea Newsletter* 27: 11–13.

Singh, B.D., H.K. Jaiswal, R.M. Singh and A.K. Singh. 1984. Isolation of early-flowering recombinants from the interspecific cross between *C. arietinum* and *C. reticulatum*. *International Chickpea Newsletter* 11: 14–16.

Singh, K.B., G.C. Hawtin, Y.L. Nene and M.V. Reddy. 1981. Resistance in chickpea to *Ascochyta rabiei*. *Plant Disease* 65: 586–587.

Singh, K.B., S. Weigand, M.P. Haware, M. Di Vito, R.S. Malhotra, O. Tahhan, M.C. Saxena and L. Holly. 1989a. Evaluation of wild species to biotic and abiotic stresses in chickpea. Abstract no. 14–13. XII Eucarpia Congress, European Association for Research on Plant Breeding, 27 Feb to 4 Mar 1989, Gottingen, Germany. European Association for Research on Plant Breeding, Wageningen, the Netherlands.

Singh, K.B., M. Di Vito, N. Greco and M.C. Saxena. 1989b. Reaction of wild *Cicer* spp. lines to *Heterodera ciceri*. *Nematologia Mediterranea* 17: 113–114.

Singh, K.B., R.S. Malhotra and M.C. Saxena. 1990. Sources of tolerance to cold in *Cicer* species. *Crop Science* 30: 1136–1138.

Singh, K.B. and M.V. Reddy. 1993. Sources of resistance to ascochyta blight in wild *Cicer* species. *Netherlands Journal of Plant Pathology* 99: 163–167.

Singh, K.B. and B. Ocampo. 1993. Interspecific hybridization in annual *Cicer* species. *Journal of Genetics and Breeding* 47: 199–204.

Singh, K.B. and S. Weigand. 1994. Identification and use of resistant sources in *Cicer* species to *Liriomyza cicerina*. *Genetic Resources and Crop Evolution* 41: 75–79.

Stalker, H.T. 1980. Utilization of wild species for crop improvement. *Advances in Agronomy* 33: 111–147.

Van der Maesen, L.F.G. 1987. Origin, History and taxonomy of Chickpea. Pages 11–34 *in* The chickpea (M.C. Saxena and K.B. Singh, ed.). CAB International, Wallingford, UK.

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