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Chickpea breeding for resistance to ascochyta blight

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SUMMARY - Blight disease caused by *Ascochyta rabiei* (Pass.) Lab. represents one of the limiting factors for chickpea production under Mediterranean growing conditions, particularly when the sowing date is advanced to winter period. The utilization of resistant cultivars is the most effective way for the control of the pathogen but the knowledge of ascochyta races is a very important prerequisite in chickpea breeding programs. In order to obtain blight resistant chickpea lines, since 1982, breeding strategies have been developed in Italy either evaluating international and indigenous collections of germplasm coming from cultivated and wild species or crossing agronomically interesting lines with the resistant ones. Mutagenesis methodologies are utilized as useful tool to induce new genetic resistances not available in nature. Screenig methods for the selection of a large number of lines were developed firstly under greenhouse conditions, by artificial inoculation, then in the field where the presence of inoculum at low densities is assured. This methodology is based on the dipping of some control seeds in a conidial suspension of *A. rabiei* and their subsequent intercropping with the lines to be screened.

RESUME - "Amélioration du pois chiche pour la résistance à l'anthracnose". L'anthracnose causée par Ascochyta rabiei *(Pass.) Lab. est l'un des facteurs limitants de la production du pois chiche dans les conditions méditerranéennes, particulièrement lorsque la date de semis est avancée vers l'hiver. L'utilisation de cultivars résistants est le moyen le plus sûr pour contrôler ce pathogène; mais la connaissance des races d'anthracnose est un pré-requis très important pour tout programme de sélection. Afin d'obtenir des lignées de pois chiche résistantes à l'anthracnose, on a développé en Italie depuis 1982 différentes stratégies d'amélioration: évaluation de ressources génétiques locales ou internationales composées d'espèces cultivées ou sauvages, ou croisement des lignées agronomiquement intéressantes avec les lignées résistantes. La mutagénèse a été utilisée pour induire de nouvelles résistances génétiques non disponibles dans la nature. Des méthodes permettant de cribler un grand nombre de lignées ont été développées en serre avec inoculation artificielle, puis au champ en présence d'inoculum à faible concentration. Cette dernière méthode est basée sur le trempage des graines d'un témoin dans une suspension de conidies* d'A. rabiei, *mises ensuite en culture alternée avec des lignées à tester.*

Introduction

Blight caused by *Ascochyta rabiei* (Pass.) Lab. is the most important disease of chickpea crop in many parts of the world and mainly in Mediterranean area. It becomes very destructive particularly with early sowing in winter, when high relative humidity favours severe epidemics. Disease control by the use of fungicides in such situations is inadequate. Hence, use of resistant cultivars is the only reliable and effective way available up to date (Singh *et al.*, 1984). Several sources of resistance were identified in the last 50 years (Table 1) but sometimes the appearance of new races os *A. rabiei* has broken their resistance. For instance, resistant cvs. F8 and C 12-34 became susceptible. They were substituted by the cv. C 235 obtained in 1960. Other resistant cultivars have been released in many countries and recently ICARDA reported resistant lines among several germplasm accessions of different origins. Some of these sources have been utilized in hybridization programs with adapted and high yielding cultivars and some lines have been released for commercial production.

Very few studies on the genetic control of resistance to ascochyta blight are reported in the literature, representing a limitation for chickpea breeding programs. Work done so far shows that the resistance is controlled by a single dominant or recessive gene (Table 2). Three dif-

Table 1. Chickpea resistant cultivars released in different countries.

Cultivar	Country	Reference
F8	India and Pakistan	Luthra *et al.*, 1938
C 12-34	India and Pakistan	Ahmad *et al.*, 1949
C 235	India and Pakistan	Bedi and Athwal, 1962
BULGARIA	–	Solel and Konstrinski, 1964
C727	Pakistan	Asiz, 1972
P 1528-1-1, I-13	India	Grewal and Vir, 1974
AYELET, OFRA	Israel	Retig and Lehrer, 1977
Sovkhozuyi 14, Kubanskii 199, Resusi 216, VIR32	Bulgaria	Ganeva and Matson, 1977
GALBEN	Bulgaria	Singh, 1978
GHAB-1	Syria	ICARDA, 1982
CM72, C44, AUG-480	Pakistan	Haq *et al.*, 1983
YIALOUSA	Cyprus	Hadjichristodoulou, 1984
FARDON	Spain	Singh and Saxena, 1987
GHAB-2	Syria	Singh, personal communication
AJAY, ATUL	India	Kharkwal *et al.*, 1988

Table 2. Inheritance of resistance to ascochyta blight in chickpea.

Cultivars	Controlling Gene	Reference
F8, F10	Single dominant	Hafiz & Ashraf, 1953
I-13	Single dominant	Vir. *et al.*, 1975
Code no. 72-92	Single dominant	Eser, 1976
ILC 72, ILC 183, ILC 200, ICC 4935	Single dominant	Singh & Reddy, 1983
ILC 191	Single recessive	Singh & Reddy, 1983
ILC 200, ILC 201	Single dominant	Açikgöz, 1984
72012, ILC 195, NEC 138-1	Single recessive	Açikgöz, 1984
P 1252-1, EC 26446, PG 82-1	Single dominant	Tewari & Pandey, 1986
BRG-8	Single recessive	Tewari & Pandey, 1986

ferent independently segregating genes were recently reported by allelic tests (Tewari and Pandey, 1986).

Some reports on the mechanism of resistance published so far, indicate that a large number of stomata per unit area of leaf, stem hairyness as well as a small number of tertiary branches are correlated to ascochyta resistance (Ahmad, 1952; Hafiz, 1986). The higher secretion of malic acid as a cause of resistance in cultivar F8 than in susceptible material (Hafiz, 1952) was not confirmed by subsequent studies. Biochemical comparisons between resistant and susceptible cultivars showed a higher peroxidase and catalase activity and more L-cystines and phenolic content after inoculation of the resistant ones (Vir and Grewal, 1974 a,b; 1975 b,c). A high level of phytoalexin medicarpin and maackiain in resistant plants has been recently reported by Weigand *et al.* (1986). Studies on the possible correlation of resistance to phytoalexin production are also currently in progress at Stazione Sperimentale di Granicoltura per la Sicilia in collaboration with ENEA and Istituto Sperimentale per la Patologia Vegetale of Rome.

Screening methods

Although considerable progress has been made in chickpea breeding in recent years, a proper standardization of both field an greenhouse screening techniques has not occurred to internationally compare the results. The first attemps of screening revealed their inefficiency but recently new greenhouse (Reddy and Nene, 1979) and field (Reddy *et al.*, 1980) procedures for screening germplasm have been proposed.

Also in Italy, greenhouse and field screening techniques were developed in order to test large number of lines. In greenhouse the tests are done in thermostatically (t=22+4°C) controlled plastic chambers where 15-day old seedlings are sprayed with a spore suspension (1.8 10^5 ml^{-1}) of the fungus. The relative humidity is maintained at about 90% keeping the chambers closed for 5 days. After 12-15 days, disease incidence is recorded according to a 0-5 rating scale; the average of individual records are classified as follows: 0-2.5 = resistant; 2.5-5 = susceptible. This technique was also utilized for the characterization of *Ascochyta rabiei* races.

Preliminary experiments established that 15 day old plants can give useful results to differentiate the susceptibility or resistance of the tested lines at an inoculum concentration between 2 10^4 ml^{-1} and 2 10^5 ml^{-1} (Crinò, unpublished data; Del Serrone *et al.*, 1987).

A screening technique field conditions and in winter sowing, was also developed to confirm greenhouse results (Crinò *et al.*, 1987). Some seeds of the susceptible line are dipped for one night in a conidial suspension of *A. rabiei* and, after drying in an oven with forced ventilation, they are intercropped between 2 single rows of the material to be tested. The lines are scored following 1-9 scale proposed by Singh *et al.* (1981). Resistant lines are reevaluated in the subsequent seasons for further assessment.

Strategies of breeding

Breeding for resistance to ascochyta blight started almost 50 years ago with the selection of pure lines from the existing germplasm accessions and hybridization programs. Intensive work has been recently done by the ICRISAT-ICARDA Cooperative Project for the development of blight resistant lines with a large variability for plant type, length of the biological cycle, cold tolerance and seed size. These lines are supplied to different countries for their utilization in the national chickpea improvement programs.

Evaluation of some wild species of *Cicer* for resistance to Ascochyta blight has been reported (Sandhu, 1980; Van der Maesen, 1984) but only few results are available indicating their use in the interspecific hybridization (Mercy and Kakar, 1975; Pundir and Van der Maesen, 1983; Bassiri *et al.*, 1987). Most crosses proved unsuccessful except for the combination *C. arietinum* x *C. reticulatum.*

Some Indian and Pakistani chickpea breeders advocated the use of mutation breeding to confer a specific improvement in a variety without altering its other characters. In particular, mutants resistant to *A. rabiei* have been induced by gamma rays and chemical mutagen (EMS) at NIAB in Pakistan (Haq *et al.*, 1983) and India (Kharkwal *et al.*, 1988).

Considering the breakdown of resistance, a breeding program for horizantal resistance to blight, supported by FAO, was started in 1975 in Morocco (Pieters an Tahiri, 1986). Significant correlations between some suggested screening tests and the field score were observed indicating a good estimate of resistance in field. A similar program for horizontal resistance was started at ICARDA as well in 1979-80 (Singh *et al.*, 1984).

No progress was made for the development of chickpea multilines due to little information so far available on physiological races of *A. rabiei* (Singh *et al.*, 1984).

Current research in Italy

In the central and southern regions of Italy, ascochyta blight attacks represent one of the major limiting factors for winter sown chickpea crop causing very severe losses of yield. In order to obtain resistant lines, since 1982, breeding strategies were developed (Fig. 1) either by evaluating ICARDA and other germplasm as well as local landraces or by crossing agronomically interesting lines with the resistant ones. Mutation breeding is utilized for the induction of new resistance (against race 6 of *A. rabiei*) not existing in the available 'gene pool' (Fig. 1).

A wide collection of indigenous and foreign germplasm (569 lines) (Table 3) was evaluated for resistance to *A. rabiei* either in greenhouse or under field conditions in Central and South Italy.

Table 3. Number of germplasm lines evaluated at ENEA since 1981-82 to 1987-88.

Origin	Analysed genotypes (No.)	Origin	Analysed genotypes (No.)
ICARDA	235	Algeria	4
Italy	86	Egypt	4
India	59	USA	4
USSR	35	Jordan	3
Turkey	31	Irak	3
Iran	19	Afghanistan	3
Spain	10	Irsael	3
Morocco	9	Yugoslavia	3
France	7	Mexico	2
Bulgaria	6	Ethiopia	1
Tunisia	6		
Unknown	5	Total	569

After 5 years of selection in different localities, 2 ICARDA lines (ILC 3279 and ILC 72) showed a good reaction of resistance even under high disease incidence in open field. Because of their additional agronomical traits for winter sowing, they have been released in 1987 as "Sultano" and "Califfo" (Calcagno *et al.*, 1988) and they are now under agronomical trials for adding to variety list.

Among 280 lines tested for ascochyta resistance in field during 1987-1988, 174 lines proved resistant (Table 4) including also 31% of F_5, F_6, F_7 and F_8 material selected for genetic and agronomical traits as well as for ascochyta resistance from F_4 ICARDA nurseries received every year at ENEA. Selected resistant material is also being tested by artifical inoculation with each race of *A. rabiei,* in greenhouse, to assess the type of resistance.

With the aim to combine favourable agronomical characters with the resistance, a hybridization program was started in 1984. F_1 generation has been multiplied in greenhouse and then two different procedures have been followed on F_2 and F_3 progenies: selection for resistance and agronomical characters in field and only selection for resistance in greenhouse. In the next generations, the lines obtained by both procedures will be evaluated for agronomical and resistance characters and then, in F_6, F_7 and F_8, multilocation trials will be carried out (Fig.1).

For some crosses, a characterization of genes controlling the resistance to ascochyta blight has been tried in the varieties "Sultano" and "Califfo". Further studies are necessary to confirm the present data and to obtain

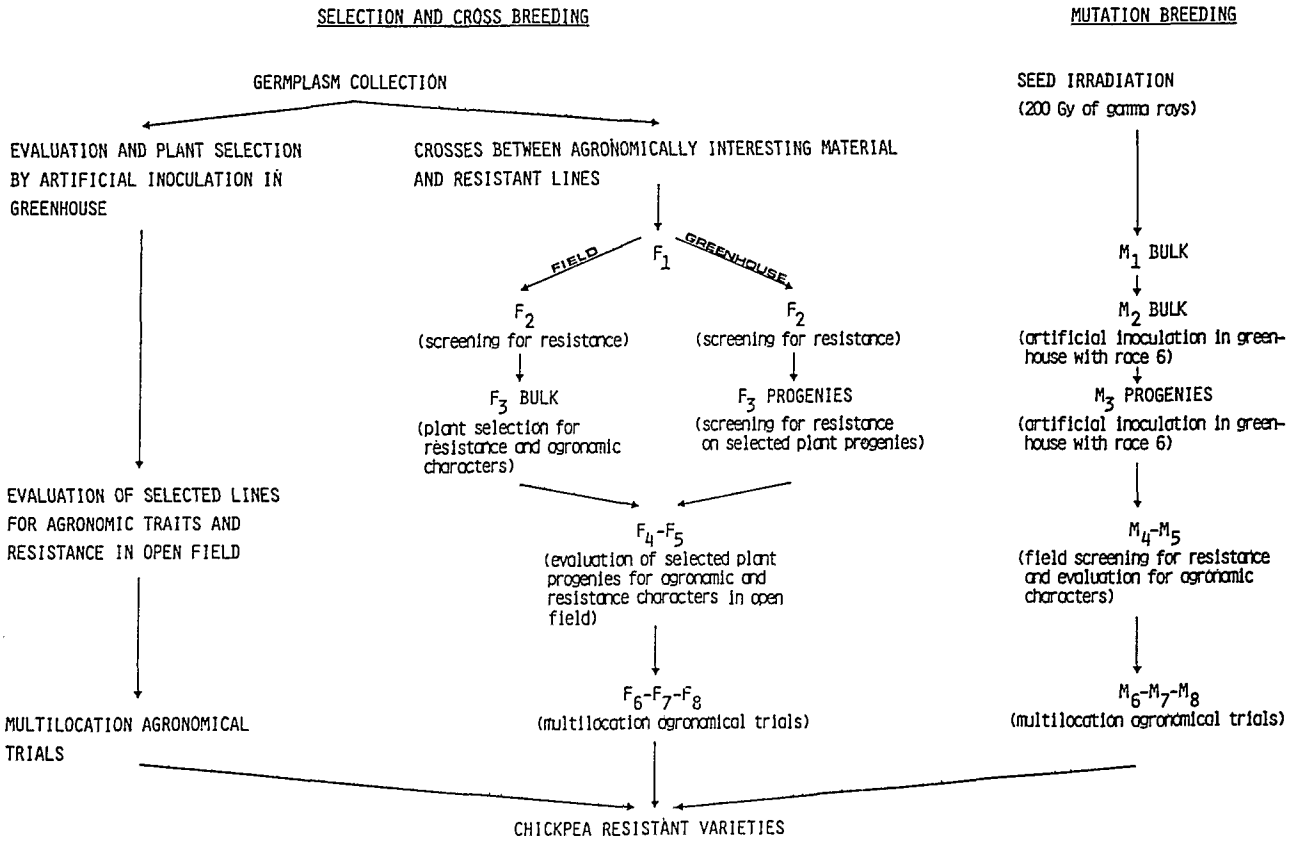

Fig. 1. Strategies of chickpea breeding for resistance to *A. rabiei* in Italy.

Table 4. Chickpea lines selected in field for resistance to ascochyta blight during 1987-88.

Material	Analysed lines (No.)	Resistant lines (No.)
F_5	10	2
F_6	38	31
F_7	14	12
F_8	10	9
ICARDA nurseries	122	112
ICRISAT nurseries	6	–
Italian ecotypes	67	7
French ecotypes	2	1
American collection	11	–
Total	280	174

Table 5. Response to ascochyta infection of some accessions of chickpea wild species coming from the regional plant introduction station of Washington State University (USA).

Wild species	Origin	Accessions	Aschochyta score		Reaction to *A. rabiei*
			Greenhouse (0-5)	Field (1-9)	
C. reticulatum	Turkey	489777	3.31	5	S
C. pinnatifidum	Turkey	458556	2.44	1	R
C. echinospermum	Turkey	489776	–	1	R
C. cuneatum	Ethiopia	458554	–	2	R
C. bijugum	Turkey	458550	2.00	3	R
	Turkey	458551	–	3	R
C. judaicum	Lebanon	458558	0.86	1	R
	Israel	458559	0.42	1	R
CALIA	Italy		4.37	8	S

S = susceptible; R = resistant

more information on the inheritance of resistance in other lines.

Eight accessions of chickpea wild species coming from the Regional Plant Introduction Station of Washington State University (USA) were analysed for resistance to the 2nd race of *A. rabiei* in greenhouse and under field conditions of infection (Table 5). It could be useful to screen them against the most aggressive race of *A. rabiei* and then to try to transfer the character of resistance into the cultivated species. New techniques (embryo rescue in particular) should be developed to overcome the incompatibility barriers. This point and the induction of resistance to race 6 are objectives of a collaborative project started in 1988 between ICARDA and different Italian Research Institutions.

A mutation breeding program has also been started in 1986-87. 20 kg. of seeds of Italian variety "Calia" were treated with gamma rays at the dose of 200 Gy. After a multiplication of M_1 material in the field, M_2 plants were artificially inoculated with the race 6 of *A. rabiei* in greenhouse. The experiment is still in progress but out of 5,000 plants, tested so far 32 were without symptoms. Other studies under field conditions in the past proved unsuccessful (Crinò *et al.*, 1987).

Conclusions

Many lines resistant to ascochyta blight have been identified and are available now in some chickpea germplasm collections. Their use may be limited by the existance of different races of the pathogen and, in this respect, breeding efforts should be concentrated on the lines maintaining the resistance at several locations. Therefore, intensive work is necessary on sources of stable resistance as well as for the study of the inheritance and mechanism of resistance to blight. The identification of lines resistant to each physiological race, as reported by Porta-Puglia in this meeting could suggest the possible presence of different genes in the existing cultivars which could be incorporated into a single genotype.

The occurrence of new races for which resistance does not appear to be existing in the available cultivars necessitates search for new sources of resistance including mutation breeding. Interspecific hybridization could also be usefully utilized but more emphasis is necessary for the development of *in vitro* techniques for this purpose. A greater collaboration between different Institutions working on chickpea breeding for ascochyta blight resistance is necessary.

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