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Resistance to Six Races of *Ascochyta rabiei* in the World Germplasm Collection of Chickpea

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ABSTRACT

Ascochyta blight [caused by *Phoma rabiei* (Pass.) Khune & J.N. Kapoor; syn. *Ascochyta rabiei* (Pass.) Lab.] is the most important disease of chickpea (*Cicer arietinum* L.) in many countries. Progress in breeding blight-resistant cultivars has been hampered by the absence of dependable sources of resistance. An effort was made to screen the world germplasm collection against races of *P. rabiei* with the objective of identifying resistant sources. A total of 19 343 germplasm accessions of chickpea (12 749 desi and 6594 kabuli types) were evaluated for resistance to six races of *P. rabiei* at Tel Hadya, Syria, between 1979 and 1991. Germplasm accessions were sown in the field during the winter season and inoculated by scattering ascochyta blight-diseased chickpea debris and spraying a spore suspension of a mixture of six races of *P. rabiei* prevalent in Syria and Lebanon. In greenhouse evaluations, germplasm accessions were grown in pots and inoculated by spraying the spore suspension of a composite of the six races; inoculated plants were incubated in plastic moist chambers for 1 wk. Blight severity was scored on a scale of 1 to 9, where 1 to 4 = resistant, 5 = tolerant, and 6 to 9 = susceptible. Only three desi accessions (ICC 4475, ICC 6328, and ICC 12004) and two kabuli accessions (ILC 200 and ILC 6482) were resistant in repeated field and greenhouse evaluations. Another six desi and three kabuli accessions were resistant in repeated field tests but tolerant in greenhouse evaluations. These accessions will be used as sources of resistance in the ascochyta blight-resistance breeding programs.

Ascochyta blight is the most important foliar disease of chickpea in many countries. The disease develops in epiphytotic proportions when the relative humidity is >60% and temperatures between 10 and 20 °C (Reddy and Singh, 1990b). Despite efforts to control this disease since it was reported early in the 20th century (Butler, 1911), it still causes serious losses in Southwest Asia and the Mediterranean region (Nene and Reddy, 1987). In the past 60 yr, considerable efforts have been made to control the disease using host-plant resistance (Singh and Reddy, 1991), but with only limited success. All blight-resistant cultivars developed in the Indian subcontinent became susceptible, probably due to the appearance of new races (Singh and Reddy, 1991). A few resistant lines reported from Iran (Kaiser, 1972) and Bulgaria (Solel and Kostrinski, 1964, Ganeva and Matsov, 1977) were found susceptible in ICARDA screening.

In recent years, a few sources of resistance to blight have been reported from ICARDA and successfully used in its breeding program (Singh et al., 1981, 1984, Reddy and Singh, 1984, Singh and Reddy, 1991). In view of both the importance of ascochyta blight-resistant cultivars in stabilizing chickpea production and the frequent breakdowns of resistant sources identified in the past, a large-scale evaluation of the world germplasm collection maintained in gene banks at the ICRISAT Center in India and ICARDA in Syria was undertaken in search of new and better sources of resistance for use in breeding. Evaluations in the past were (i) conducted in the field or greenhouse and (ii) used blight-infected chickpea debris or one race of the pathogen as inoculum. In this paper, we report the results of evaluation of ≈20 000 accessions, both desi (angular, small, dark-colored seeds) and kabuli (ram-head-shaped, large, beige-colored seeds) types for resistance to six races of *P. rabiei* at Tel Hadya, Syria.

MATERIALS AND METHODS

Seed of 6594 kabuli ILC accessions was from the Legume Program and Genetic Resources Unit of ICARDA in Tel Hadya, Syria. Seed of 12 749 desi ICC accessions were obtained from the Genetic Resources Unit, ICRISAT, India. Evaluation for

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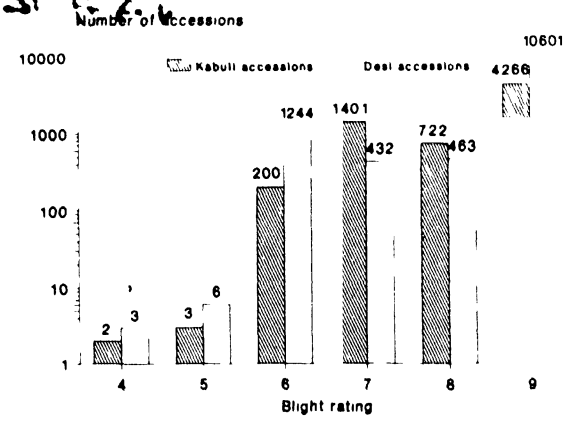

Fig. 1. Field and greenhouse evaluations of kabuli and desi chickpea germplasm accessions for resistance to ascochyta blight at ICARDA, Syria, 1979 to 1991.

blight resistance was conducted in the field between 1979 and 1991 The number of accessions evaluated each year ranged from 366 to 4306

Blight severity in the accessions was scored using a 9-class scale (Reddy and Singh, 1984, Reddy et al , 1984), where 1 = immune reaction no visible lesions on stems and leaves, 2 = highly resistant no lesions on stems, but lesions on leaves, with few pycnidia, 3 = resistant 5% stems, leaves, and pods infected and stems broken, stem lesion ≤5 mm long, with few pycnidia, 4 = moderately resistant 15% stems, leaves, and pods infected and stems broken, stem lesion >5 mm long, with few pycnidia, 5 = tolerant 40% stems, leaves, and pods infected and stems broken, stem lesion >5mm long, with more pycnidia, 6 = moderately susceptible 50% stems, leaves, and pods infected and stems broken, stem lesion >5 mm, with more pycnidia, 7 = susceptible 75% stems, leaves, and pods infected and stems broken, stem lesion >5 mm long, with more pycnidia, 8 = highly susceptible 100% stems, leaves, and pods infected and stems broken, stem lesion >5 mm long, with more pycnidia, and 9 = very highly susceptible all plants killed Scoring was done twice, the first score was taken when all plants in the susceptible check had died, and the second at harvest, mainly for pod infection

Except for 1991, accessions rated 1 to 5 in the first year of evaluation were reevaluated in subsequent years, along with new accessions All of the 721 lines with a rating of 1 to 5 before 1990 were evaluated under greenhouse conditions dur ing 1990 These 721 lines were reevaluated in the field by inoculating with diseased debris and spore suspensions, which were prepared by mixing six races in 1991

In field evaluations, generally 40 seeds were sown per acces sion in a single 4-m row in the first year Inter- and intrarow spacings were 45 and 10 cm, respectively A row of a known blight-susceptible line, ILC 263 or ILC 1929, susceptible to all the six races was sown as an indicator row for the disease at an interval of 4 to 10 test rows In subsequent years, the selected accessions were sown in two replications in a random ized complete-block design and the susceptible controls were planted after every two test rows In addition, a strip of I I C 1929 or ILC 263, 3 m wide, was sown around the evaluation plot Germplasm accessions were sown between 10 November and 10 December This period is the beginning of the winter rainy season, a more favorable period for blight development in the Mediterranean region than in the spring when chickpeas are normally sown

In February, plants were inoculated with *P rabiei* by scattering blight-diseased chickpea debris collected from the pre vious season (Singh et al , 1981) For the 1979 inoculations, diseased debris collected from naturally infested fields at the ICARDA farm was used In subsequent seasons, the debris used was collected from the disease nursery and stored in a field shelter Accessions were also inoculated with a spore suspension of the prevailing races of the pathogen in Syria and Lebanon The fungus was multiplied on chickpea–dextrose broth (Reddy and Singh, 1984) During 1979, 1980, and 1981, dis eased debris and a spore suspension of a single race (the pre vailing isolate of *P rabiei* at the ICARDA farm, later designated as Race 3) were used for inoculation (Reddy and Kabbabeh, 1985) Between 1982 and 1988, diseased debris and a spore suspension of a mixture of Races 1, 2, 3, and 4 was used During 1989, 1990, and 1991, diseased debris and a spore suspension mixture of Races 1, 2, 3, 4, 5, and 6 were used The races uere mixed in equal proportion to obtain a spore concentration of 100 000 spores ml^{-1} of water, which was sprayed with knapsack sprayers Between inoculation and the time required for full disease development (a score of 9 for the susceptible check ILC 263 or II C 1929), the nursery was sprinkler irrigated (1979–1988) or mist irrigated (1989–1991) for 3 h d^{-1} during dry periods to increase relative humidity to >60% Irrigation was repeated at the early podding stage of the crop for 1 wk to promote pod infection Spore suspension inoculations were continued until the susceptible control line was killed Depending on the weather, the number of spore suspension inoculations varied from three to seven (a greater number of inoculation sprays being required during a dry spring)

In the greenhouse, accessions were sown in sterilized soil in 20-cm plastic pots during 1990 in two replications In each pot, 10 seeds were sown per accession After every 10 pots of test accessions, a pot of the susceptible cultivar ILC 263 was grown Plants were inoculated when they were 15 d old with a spore suspension of the mixture of the six races and covered with air tight plastic cages (170 cm long, 135 cm wide, 60 cm high) for 7 d to increase the relative humidity to >85% After removal of cages, plants were irrigated twice a day to raise the humidity Accessions were scored for blight severity 30 d after inoculation when the susceptible checks were killed Sur viving plants were reinoculated at the podding stage, covered with plastic cages for 7 d, and scored for pod infection at harvest The greenhouse temperature was maintained at 20 ± 2 °C

RESULTS AND DISCUSSION

Of the 13 yr in which the germplasm was evaluated in the field (1979 to 1991), screening was affected by waterlogging during 1985, an unknown race of *P rabiei* during 1987, and severe cold during 1990. Results from these years were not considered. Evaluation for blight resistance was very effective in the remaining 10 years. The known blight susceptible lines ILC 263 and ILC 1929 were uniformly killed indicating high disease pressure. The distribution of accessions was skewed towards susceptibility. Only 1138 accessions were scored 5 or less in the first year of field evaluation. In reevaluations, many of these accessions had higher disease scores and were discarded.

Only nine desi (0.07%) and five kabuli (0.08%) accessions were resistant or tolerant in repeated evaluations against a composite of six races (Fig. 1). Only three desi (ICC-4475, ICC-6328, and ICC-12004) and two kabuli accessions (ILC-200 and ILC-6482) were resistant in both field and greenhouse evaluations (Table 1).

A large number of germplasm accessions that showed resistance in the early years of evaluation (1979–1988) were susceptible in later years (1989–1991). Also, most of the lines reported resistant earlier to a mixture of four

Table 1 Mean and range of blight score of chickpea desi and kabuli accessions resistant or tolerant to a mixture of six races of *Phoma rabiei* (the causal agent of ascochyta blight) from Syria, ICARDA, 1979 to 1991

Accession no	Field evaluation, 1979 to 1991		Greenhouse evaluation, 1990
	Mean blight score† (range)	Years no	Mean blight score†
	Desi		
ICC 3606	3 0 (2–4)	2	5 0
ICC 4286	4 0 (4–5)	2	5 0
ICC 4475	3 5 (3–4)	4	4 0
ICC 4828	4 0 (3–5)	2	5 0
ICC 6328	3 5 (3–4)	2	4 0
ICC 8540	4 0 (4–4)	2	5 0
ICC 8566	4 0 (4–4)	2	5 0
ICC 9584	4 0 (4–4)	2	5 0
ICC 12004	3 0 (3–3)	3	4 0
	Kabuli		
ILC 187	3 0 (2–4)	9	5 0
ILC 200	3 0 (2–4)	9	3 5
ILC 3856	3 0 (3–4)	6	5 0
ILC 5913	3 0 (2–4)	2	5 0
ILC 6482	4 0 (4–4)	2	2 0

† 1–4 = resistant, 5 = tolerant, 6–9 = susceptible

races (Reddy and Singh, 1984; Singh et al., 1984) were susceptible when later tested against a composite of six races. The nine desi and five kabuli germplasm accessions resistant or tolerant in both field and greenhouse evaluations to a mixture of six races should be very useful as sources of resistance in the ascochyta blight–resistance breeding programs. Iranian and Bulgarian lines reported resistant earlier were susceptible in this screening. Some of the kabuli accessions such as ILC 187 and ILC 200 also showed resistance in the field over nine seasons indicating stability of their resistance (Table 1). Two kabuli accessions, ILC 200 and ILC 3856, which were resistant in the present study were also reported resistant earlier (Singh et al., 1984, Reddy and Singh, 1985), whereas other lines earlier reported as resistant were susceptible. This may be entirely due to screening against a composite mixture of six races.

The higher susceptibility of the lines in the greenhouse test than in the field could be due to more favorable conditions, especially temperature, for disease development. In the field, relative humidity is favorable during most of the crop season (November to June), but because of low temperatures (mean weekly temperatures < 10 °C) blight development in the early stages of crop growth up to March is retarded (Reddy and Singh, 1990b). From May onward, day temperatures are higher (> 25°C) and not favorable for blight infection. Relative humidity >60% and temperatures between 10 and 20 °C are favorable for blight development (Reddy and Singh, 1990b; Weltzien and Kaack, 1984). Therefore, field screening of germplasm accessions should be used as a preliminary test to discard the bulk of susceptible lines.

Many lines that show resistance in the vegetative stage are susceptible in the podding stage (Reddy and Singh, 1984) and it is necessary to have lines resistant in both vegetative and podding stages, as the conditions for blight development are often favorable at podding time also. Earlier reports on resistance to blight were based mainly on evaluations at the vegetative stage (Reddy and Singh, 1984). Generally, desi types have shown higher resistance in the vegetative stage than kabuli types, but higher susceptibility at the podding stage. The lines identified in the present study have resistance at both the vegetative and podding stages and will be more useful in breeding programs

Some of the lines identified as resistant in the field in this study showed < 10% yield loss under severe disease pressure (Reddy and Singh, 1990a), while the susceptible lines had no yield. The fact that these lines do not have much yield loss with infection makes them useful in blight-resistance breeding programs

Phoma rabiei, the causal organism of the blight, is known to be a highly variable fungus (Nene and Reddy, 1987). Breakdown of resistance to the disease is a frequent phenomenon (Singh and Reddy, 1991). Earlier sources of resistance to the disease were identified mainly using a single isolate of the fungus The lines that are identified in this study using diseased debris and a mixture of six races may prove to be more durable. The diseased debris used for inoculation came from a screening nursery where several genotypes with different levels of susceptibility to blight have been grown; therefore, it comprises a mixed population of the blight pathogen As differential susceptible checks for different races were not used in the evaluation, the relative resistance of the accessions to individual races was not ascertained.

Five of the nine resistant or tolerant desi germplasm accessions identified in this study originated in India or Iran; the origin of four lines is unknown These nine accessions exhibit significant variation in maturity (52–91 d to 50% flowering) and plant height (31–66 cm) Mean canopy height of the resistant accessions was 48 cm, as compared with a mean of 38 cm for all of the accessions evaluated. The resistant or tolerant accessions were mainly of erect or semierect type, with a large variation in seed size (9.7–35 0 g 100-seed weight) The mean time to 50% flowering of the resistant and tolerant accessions was 75 d, as compared with 36 d for all the accessions evaluated under blight-free conditions at ICRISAT Center, India (Pundir et al , 1988). These results suggest positive association between blight resistance, maturity, plant height, and erect growth habit in desi chickpea resistant lines. Seed color also varied greatly from yellow to black. The observation made by Kaiser (1972) that blight resistance is associated with black seed color was not substantiated

Three of five kabuli resistant or tolerant accessions originated from the USSR and one from Morocco. The origin of the other is not known. They were all late types with days to 50% flowering ranging from 142 to 145. They were mainly tall (50–78 cm height), with semierect growth habit. Their seed size was comparatively smaller (21.1–35.5 g 100-seed weight), and they are pea shaped. Mean time to 50% flowering, plant canopy height, and 100-seed weight of the resistant and tolerant accessions recorded at ICARDA in Syria were 143 d, 62 cm, and 25 g, as compared with 137 d, 54 cm, and 30 g, respectively, for all accessions evaluated (Singh et al., 1991). While there seems to be some positive relationship between maturity, plant height, and blight resistance in kabuli germplasm, seed size is negatively associated; however, a large number of accessories with late matu-

rity and tall plant stature were susceptible to ascochyta blight.

Screening of chickpea blight resistance on such a large scale had not been done before. The work was undertaken because of frequent breakdowns of resistant sources owing to inadequacy of screening procedure. It is believed that resistant sources identified in this study will prove more useful in the ascochyta blight–resistance breeding programs.

Small quantities of seed of these five resistant lines are available from the Legume Program of ICARDA, Syria.

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