Variability in Virulence to Chickpea of an Italian Population of *Ascochyta rabiei*

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ABSTRACT

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

The reaction of 13 chickpea (*Cicer arietinum* L.) genotypes tested separately with 41 Italian isolates of *Ascochyta rabiei* showed variability in the degree of virulence of the fungus. Three groups of isolates were identified by cluster analysis. The high percentage of isolates infecting all genotypes tested showed the need for more durable sources of resistance than those available in Italian chickpea cultivars.

Additional keywords: *Cicer,* physiological specialization

Under Mediterranean growing conditions, *Ascochyta rabiei* (Pass.) Labrousse causes severe blight epidemics on chickpea (*Cicer arietinum* L.), particularly when sowing is done during the winter (7). Differences in cultural characters and pathogenicity among isolates of this pathogen have been described (1,10,11, 13,24). Reddy and Kabbabeh (15) reported the existence of six races in Syria and Lebanon, and Singh and Reddy (17) proposed the use of seven differential lines for their identification. On the other hand, Gowen et al. (6) showed a constant ranking of several cultivars inoculated with different isolates of *A. rabiei* and concluded that the differences in pathogenicity were attributable to variation in virulence of the isolates. Also, 102 Pakistani *Ascochyta* isolates fell into eight pathogenic groups differing in their virulence (8). The amount of variability of *A. rabiei* is likely enhanced by the presence of the teleomorph (*Didymella rabiei* (Kovachevski) v. Arx, syn.: *Mycosphaerella rabiei* (Pass.) Kovachevski) under field conditions (12,22).

The use of resistant cultivars represents the most effective way of controlling Ascochyta blight. Some resistant chickpea lines have been developed, but the possible existence of different pathotypes of *A. rabiei* limits their utilization. The reactions of some chickpea lines in different Italian locations (2) suggested the need for a survey of the variability of the fungus in our country, where the diversity of environmental conditions is considered representative of most Mediterranean climatic zones (23). Therefore, the objective of this study was to evaluate the pathogenic variability of an Italian population of *A. rabiei*, in order to contribute to the elucidation of the host–pathogen relationship. This knowledge is a prerequisite for breeding programs aimed at obtaining a durable resistance to Ascochyta blight.

MATERIALS AND METHODS

Forty-one isolates of *A. rabiei* were obtained from samples collected in different locations in Italy and maintained on potato-dextrose agar (PDA) slants at 10°C following single-spore isolation. Subcultures for plant inoculations were grown on PDA in plastic petri dishes at 22 ± 1°C exposed to alternating 12 h of near-UV light (peak at 360 nm) and 12 h of darkness until good sporulation was obtained.

A set of 13 lines (11 from the germ plasm collection of the International Center for Agricultural Research in the Dry Areas [ICARDA] and two Italian land races) distributed in three replicates of 10 plants each was used to test each isolate. Inside a conditioned greenhouse (22 ± 3°C), the plants were grown in plastic trays filled with a 5-cm layer of a sterile 3:1 soil–sand mixture. The seeds were surface-disinfested with sodium hypochlorite (active Cl 2%) for 15 min and then rinsed with tap water and planted into the soil at 5-cm intervals in the row with 6 cm between the rows.

Preliminary experiments were carried out to develop a suitable method of inoculation and incubation (4; *unpublished data*). Inside the above-mentioned greenhouse, plastic cabinets (250 cm long, 95 cm wide, and 63 cm high) with electric heating elements and refrigerating pipes running through the bottom under a 10-cm layer of moist perlite, proved suitable to maintain carefully controlled temperature and relative humidity (RH). The repeatability of the testing method had been demonstrated on a set of chickpea lines inoculated separately with five isolates of *A. rabiei* and incubated in identical cabinets located in two diverse greenhouses (*unpublished data*).

Inoculations were performed by spraying 15-day-old seedlings with a spore suspension of the fungus prepared by gently rubbing a glass rod on the surface of a mature colony soaked with sterile water. The suspension was filtered through a double layer of cheesecloth; the spore density was measured by a Bürker hemacytometer and diluted to the concentration of 1.8×10^5 spores ml^{-1}. The isolates were tested within a few months after being collected in the field. After inoculation, the plants were incubated inside the plastic cabinets, where the RH was maintained above 90% and the temperature at 21 ± 1°C for 5 days by keeping the cabinets closed. Then the cabinet tops were opened gradually and the perlite layers were kept constantly wet.

Disease development was recorded 15 days after inoculation on each individual plant according to the following evaluation scale, modified from a 0 to 4 scale established by Vir and Grewal (24): 0 = no visible lesions; 1 = a few small (up to 5 mm^2) lesions on stem and/or foliage; 2 = superficial stem lesions exceeding 5 mm^2 and absence of stem girdling; 3 = deep and extensive stem lesions, stem girdling that

Table 1. Two-factor analysis of variance for the reaction of 13 chickpea genotypes to 41 isolates of *Ascochyta rabiei*[a]

Source of variation	df	Sum of squares	Mean square	*F* value	Probability
Chickpea genotypes (A)	12	520.619	43.385	27.064	0.0001
Ascochyta isolates (B)	40	971.994	24.3	15.159	0.0001
A × B	480	769.356	1.603	5.821	0.0001
Error	1.066	293.520	0.275		

[a] Coefficient of variation: 15.97%.

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Accepted for publication 23 May 1995.

Publication no. D-1995-1130-06R

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; 5 = plant killed.

The averages of individual records were classified as follows: 0–2.5 = resistant; >2.5 = susceptible. We considered the 2.5 average value as a suitable discriminant between susceptible and resistant reactions because it results from scores in which the stem-girdling symptom, which is of great impact on yield losses, is represented.

The experiment was designed according to a two-factor completely randomized design in which the level of the isolate factors was split in replicates and chickpea lines. The data were statistically analyzed by analysis of variance (ANOVA) as a mixed model in which the isolates were considered the fixed factor and the chickpea lines the random factor; the most appropriate error to test the hypothesis of the differences of the *Ascochyta* isolate means is the interaction A × B, if significant. The factor analysis of the matrix isolate × chickpea genotypes (41 × 13) relative to disease index was carried out to have the orthogonal transformation by VARIMAX, in function of correlation groups of chickpea genotypes (i.e., by the genotypes that have the same behavior with respect to the isolates). Euclidian distance between isolates was computed using the first three factors that described more than 70% of the total variability. To represent the relationship between isolates, a cluster analysis was performed using the distance matrix. For the statistical procedures, SPSS 6.0 software package was used.

RESULTS

The analysis of variance showed highly significant differences among both chickpea genotype and *Ascochyta* isolate effects. The interaction genotype × isolate was also highly significant; this source of variation and that of the isolates represented high proportions of the total sum of squares (Table 1). The virulence rating of each *A. rabiei* isolate toward all the lines tested showed a large but continuous variability. All the chickpea genotypes showed symptoms involving both leaves and stems. On the leaves, circular spots appeared, soon followed by drying of a part or the whole lamina. On the stems, more or less extensive lesions were observed, ranging from flecks to larger lesions (>5 mm^2), which in the case of severe attacks evolved into complete and deep girdling.

The factor analysis shows that three factors describe 57.9, 7.9, and 7.1% of the total variability, respectively (Table 2). In the rotated factor matrix, the chickpea lines that account for the major variability are: ILC 1929 and ILC 200 for factor 1, ILC 482 and ILC 484 for factor 2, and ILC 191 and ILC 3279 for factor 3 (Table 3).

The results of cluster analysis on the isolates are shown in Figure 1. At a distance of around 13, three main clusters can be distinguished. The three clusters include 13, 11, and 17 isolates, respectively. Taking into account the geographical origin of the isolates, they seem to be randomly distributed among the groups.

Table 2. Eigenvalue and proportion of variance relative to the three first factors

Factor	Eigenvalue of 3 factors	Variation (%)	Cumulative (%)
1	7.528	57.9	57.9
2	1.022	7.9	65.8
3	0.925	7.1	72.9

Table 3. Correlation matrix between original variables and factors after VARIMAX rotation

Genotype	Factor 1	Factor 2	Factor 3
Calia	0.559	0.295	0.605
ILC 191	0.262	0.083	0.868
ILC 1929	0.850	0.228	0.266
ILC 200	0.798	0.078	0.348
ILC 202	0.283	0.622	0.508
ILC 3279	0.322	0.340	0.803
ICC 5127	0.365	0.417	0.358
ILC 482	0.097	0.900	0.193
ILC 484	0.569	0.672	0.055
ICC 3996	0.543	0.457	0.309
ILC 72	0.233	0.606	0.643
NEC 138/2	0.458	0.490	0.193
Principe	0.641	0.428	0.308

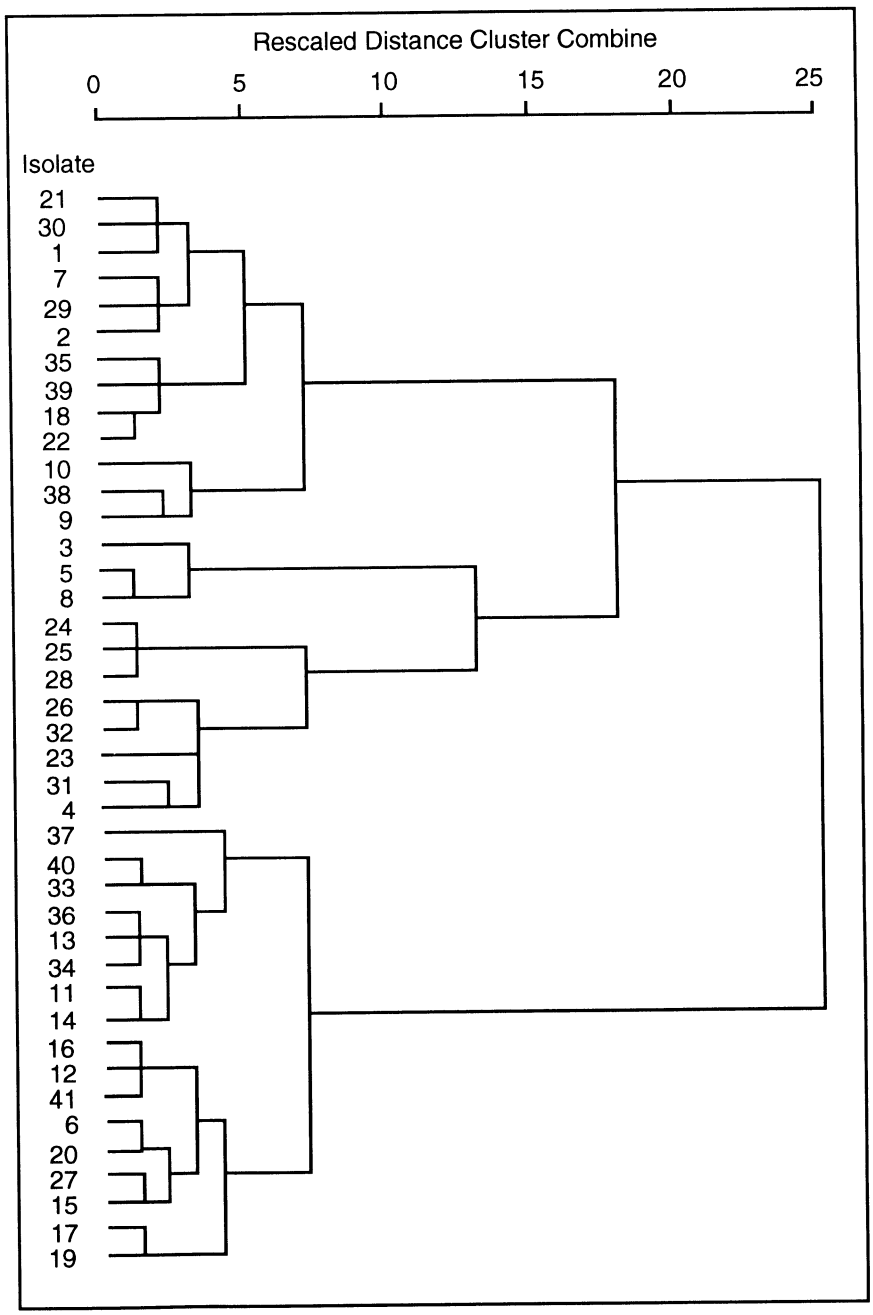

Fig. 1. Dendrogram drawn from cluster analysis (Ward minimum variance method) on the virulence of 41 isolates of *Ascochyta rabiei* tested on a set of 13 chickpea genotypes.

DISCUSSION

The overall reaction of the 13 chickpea genotypes to each isolate of *A. rabiei* showed variability for the degree of virulence. The lines ILC 1929 or ILC 200, ILC 482 or ILC 484, and ILC 191 or ILC 3279, as shown by the component analysis, could be considered differentials for separating the isolates of *A. rabiei* into three groups corresponding to different degrees of virulence.

Several reasons, such as the increase of chickpea-growing area in Italy and the introduction of resistant cultivars Sultano and Califfo (3), may contribute to extending the variability of Italian *Ascochyta* populations. More variation could be expected, taking into account the heterothallic nature of the fungus (22) and the recent discovery of *Ascochyta* mating type 1 among Italian isolates (one out of 14 isolates tested; W. J. Kaiser, *personal communication*), which makes possible the appearance of the teleomorph of the fungus, not yet observed in the field in Italy.

The occurrence of isolates belonging to cluster 1 that are able to infect all the genotypes tested suggests the need for more suitable sources of resistance. Promising levels of resistance were found in wild species of *Cicer* (14,19), although the incompatible interspecific barriers make it difficult to transfer this resistance to Ascochyta blight into *C. arietinum*. The high percentage of highly virulent isolates, also observed by Reddy and Kabbabeh (15), is in apparent conflict with field results concerning ILC 3279. Up to now, this line has shown a good level of resistance under field conditions in different years and locations in Italy (3).

The grouping of our isolates in three clusters agrees to some extent with the results of Reddy and Kabbabeh (15). Also in our experiments, the genotype ILC 1929 was the most susceptible to the isolates belonging to all the groups; and this line, together with the line ILC 3279 (only susceptible to the most virulent isolates), was among the differentials proposed by these authors. Comparing our data with the results of Singh and Reddy (17), only the susceptible line ILC 1929 of the four used in both sets of experiments reacted in the same way. In our experiments, we tested the plants at the juvenile stage, while the latter authors inoculated the plants both at the vegetative and at the reproductive stages (18). Also, the differences with the data of other authors could be explained either by the different fungal populations in the Middle East and in Italy or by the diverse experimental conditions used. As far as the experimental conditions are concerned, previous reports (4,9,21) showed the effects of plant age, inoculum concentration, temperature, and humidity on the host reaction. The development and adoption of a standardized set of differentials as well as inoculation and incubation procedures for studying the virulence of *A. rabiei* would permit the comparison of results among international workers dealing with this host–pathogen interaction. Biomolecular approaches could also provide useful information on the variability of the fungus (25). Further studies on the host–pathogen relationship and on the effect of environment on Ascochyta blight of chickpea are still needed. According to the more recent literature, resistance to *A. rabiei* is controlled by a complex system involving more than one gene and is influenced by interallelic interactions (5,16,20).

ACKNOWLEDGMENTS

This work was carried out within the collaborative project "Development of chickpea germplasm with combined resistance to Ascochyta blight and Fusarium wilt using wild and cultivated species" between Italian Institutions and ICARDA (1988–1994). We thank W. J. Kaiser for the critical reading of the manuscript, S. Benedettelli and A. Sonnino for help in the statistical analyses, and F. Saccardo and A. Quacquarelli for their useful suggestions during the research.

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