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PATHOGENIC DIVERSITY IN *ASCOCHYTA RABIEI* ISOLATES COLLECTED FROM PAKISTAN

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Abstract

Pathogenecity of 42 isolates derived from single spore cultures representing 15 collecting sites of Pakistan were studied on 7 chickpea varieties. The isolates exhibited variation in morphological and cultural characteristics. Chickpea varieties C-727 and C 44 revealed high degree of susceptibility and suggested to be used as susceptible checks for screening experiments. The factor analysis revealed that first principal component (PC) was more related to blight reaction, second PC contributed more for isolate colony colour. The variability for other morphological traits was distributed among all the three components. The first PC was a weighted average of all the variables. Two clusters were observed using UPGMA that was able to separate *A. rabiei* isolates on the basis of virulence or aggressiveness. The virulent isolates gave same intensity of infection, whereas others were observed with varying degrees of infection. Multivariate analyses were able to distinguish isolates on the basis of virulence rather than origin or morphological/cultural characterization. The susceptible differentials were identified but no variety could be established as resistant that might be due to complex nature of *A. rabiei*. The situation suggests to modify chickpea breeding for blight resistance and to use multiple crosses to build resistance pyramids involving parents with known level of tolerance. Clustering pattern indicated the exchange of breeding material and disease cultures among the researchers or high heterogeneity in the isolates.

Introduction

Chickpea (*Cicer arietinum* L.) is a major legume crop grown under a wide range of agro-ecological conditions of the world and ranks first among food legumes in the Indo-Pakistan Subcontinent and Mediterranean basin (Anon., 1994). Many biotic and abiotic factors are involved in its low production and more than 50 pathogens have been reported so far on chickpea from different parts of the world (Nene *et al.,* 1989). Blight caused by *Ascochyta rabiei* (Pass.) Lab. is one of the most devastating disease of chickpea that causes 20-25% loss annually and may cause total failure to the crop under epidemic conditions. Investigations on this disease were initiated in 1922 in Pakistan (Sattar, 1933), but many aspects are yet undiscovered. Severe epidemics of the disease have been reported from many chickpea growing countries (Cubero, 1984; Anon., 1982; Kaiser, 1972; Malik & Tufail, 1984).

Differences in cultural characteristics and pathogenicity among isolates of this pathogen have been described (Aujla, 1964; Kaiser, 1973; Porta-Puglia, 1992; Vir & Grewal, 1974; Ambardar & Singh, 1996; Jamil, *et al.*, 2000). Reddy & Kabbabeh (1985) reported the existence of 6 races in Syria and Lebanon, while Singh & Reddy (1990) reported the use of 7 differential lines for their identification. On the other hand, Grewal, (1984) observed similar behavior of several cultivars inoculated with different isolates of *A. rabiei* that revealed differences in pathogenicity. The variation in *A. rabiei* is likely to enhance by the presence of teleomorph (*Didymella rabiei* (Kav.) under field conditions (Navas-Cortes *et al.,* 1990; Trapero-Casas & Kaiser, 1992).

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The best method of controlling this disease is through use of resistant cultivars as other control measures are unreliable and uneconomical (Singh *et al.*, 1981). Development of resistant cultivars is a complex phenomenon because of complex nature of the fungus and quick breakdown of varietal resistance (Qureshi & Alam, 1984; Malik, 1990). The present study was thus conducted to understand the genetic diversity in relation with host-pathogen reaction of *A. rabiei* isolates representing chickpea growing areas of Pakistan which is prerequisite for breeding programme aimed at obtaining a durable resistance to *Ascochyta* blight.

Materials and Methods

Isolates of *Ascochyta rabiei*: During chickpea growing season of 1995-96, 42 disease samples (stems, pods and seeds) were collected from farmers' fields and research stations at various districts of North Western Frontier Province (NWFP) and Punjab. Sampling was followed according to the hierarchical sampling strategy (McDonald *et al*., 1989). Diseased samples were surface sterilized in 0.1% mercuric chloride for 2 minutes and plated on chickpea seed meal dextrose agar (CSMDA) containing 40 g chickpea seed meal; 20 g dextrose, 20 g agar and 1 litre water. Each isolate was purified by single spore culture and incubated at 22 ± 2^0C for 2 weeks. Isolates were evaluated for morphological and cultural characteristics and named according to locations from where they were collected.

Single spore cultures of 42 isolates were preserved on CSMDA medium. These isolates were subjected to detailed morphological and cultural characteristics viz., radial growth on medium (mm), colony colour, size of pycnidia (µm) and pycnidiospores (µm). An experiment was conducted to determine the pathogenicity of these isolates on 7 chickpea cultivars viz., C-727, ILC-263, C-44, and CM-72, Piadar, Noor-91 and Punjab-91. Twenty seeds were sown in plastic pots in two-factor complete randomized design with three replications. Seeds surface sterilized with Clorox (0.1% available Chlorine) were sown in pots filled with sterilized sandy loam soil. All the varieties used for experimentation were self pollinated for 2 years to ensure homozygosity. Before inoculation, ten healthy plants were maintained in each pot. Twenty days old plants were inoculated by spraying spore suspension ($5x10^5$ spores per ml) from 15 days old cultures of the isolates and incubated separately under humid chamber for 48 hours in the green house (Singh *et al*., 1981). Relative humidity was maintained in the range of 85-95% for 72 hours. Disease observations were recorded using 1-9 scale as suggested by Singh & Reddy (1993) and designation of pathotypes was followed by Habgood (1970).

The disease data were analyzed for 2 factor design statistics and numerical taxonomic techniques using the procedure of cluster and principal component analyses (Sneath & Sokal, 1973) with the help of computer software "Statistica" and "SPSS" for Windows 98 (Stat Soft, 1995; SPSS 1996). Based on results, similarity index was calculated and then converted to a dissimilarity matrix to construct dendrogram by the UPGMA (Sneath & Sokal, 1973).

Results

Differences in radial growth on medium, colony colour, size of pycnidia and pycnidiospores of isolates were observed. The reaction of chickpea genotypes to 42 isolates

of *A. rabiei* indicated that the cultivars C-727, C 44, ILC 263 and CM 72 were susceptible to all the isolates while remaining three varieties viz., Piada, Noor-91 and Punjab-91 were tolerant and exhibited variation in disease reaction with different isolates. Isolates of *A. rabiei* greatly varied in their pathogenic variation to 7 different genotypes. The analysis of variance showed significant differences ($P < 0.001$) for both the factors. The interaction was also highly significant, this source of variation and that of the isolates represented high proportions of the total sum of squares (Table 1).

Table 1. Two-factor analysis of variance for the reaction of 7 chickpea varieties to 42 isolates of *Ascochyta rabiei* collected from Pakistan.

Source of variation	df	Sum of squares	Mean squares	F-value	Probability	Standard error
Replication	2	1.75	0.87	4.00	0.02	0.02
Varieties	6	264.09	44.01	201.65	0.00	0.04
Isolates	41	1379.97	33.66	154.20	0.00	0.10
Interaction	246	431.51	1.75	8.04	0.00	0.27
Error	586	127.907	0.22			

Coefficient of Variation: 6.22%

The virulence rating of each *A. rabiei* isolate toward all the lines tested exhibited a large but continuous variability. All the cultivars 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 showed that three factors gave eigen values greater than unity, whereas others were <1, hence first three principal components were considered important in contributing variation amongst 42 isolates. First three components contributed 75.9% of the total variability (Table 2). The first PC was more related to blight reaction with different varieties rather than morphological characters of isolates. Pycnidial formation was more related to first PC and second PC contributed more for isolate colony colour, whereas variability for other morphological traits was distributed among all the three components. All the variables except colony colour contributed positively to PC_1: thus this principal component is a weighted average of the characters. Fig. 1 presents the virulence status of *A. rabiei* isolates collected from 15 districts of two provinces (Punjab & North West Frontier Province) that represents the major chickpea growing areas of Pakistan. The isolates collected from six sites (Kaghan, Kohat, Islamabad, Attock, Sialkot & Faisalabad) gave the most virulent reaction consistently.

Two clusters were observed using UPGMA method for constructing dendrogram (Fig. 2). Cluster I consisted of four isolates and these all were less virulent originating from the districts of Jhang, Bakkar (Punjab) and Bannu (North West Frontier Province), whereas cluster II consisted 38 isolates of diverse origin. This cluster could be further separated into two sub clusters i.e., 12 isolates did not infect all the varieties, whereas other 26 isolates infected all the varieties at the same level and could be considered more virulent.

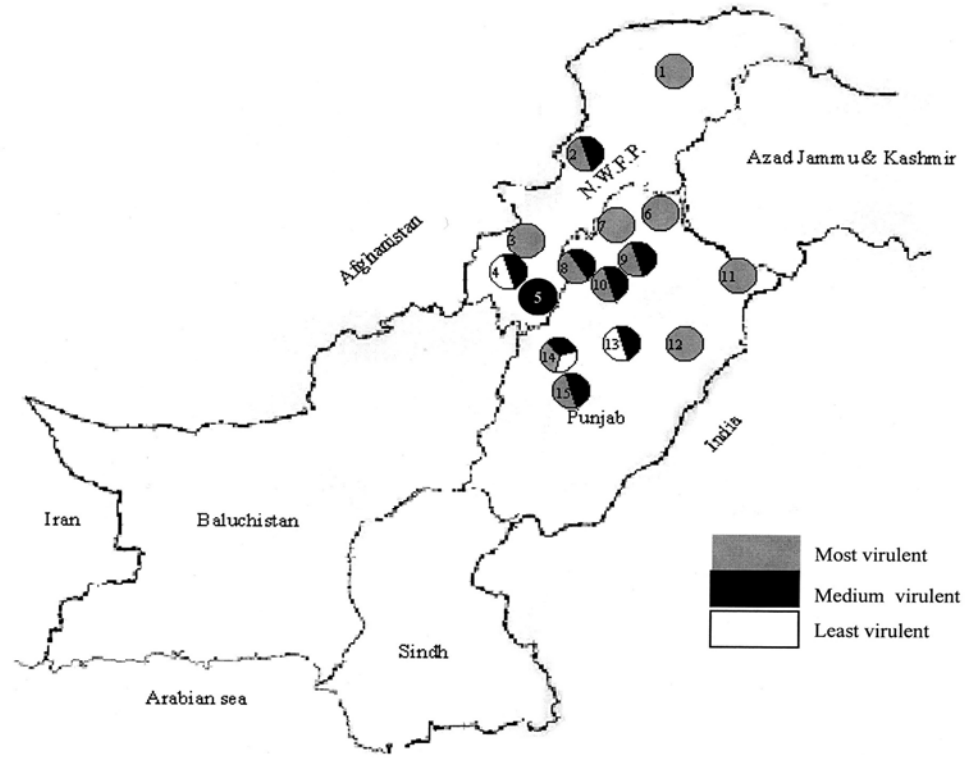

Fig. 1. Collecting sites of *Ascochyta rabiei* in Pakistan. The symbol ◯ represents the collecting site of isolate. The serial numbers represent 1- Kaghan, 2- Kohat, 3- Karak, 4- Bannu, 5- DI Khan, 6- Islamabad, 7- Attock, 8- Mianwali, 9- Chakwal, 10- Khushab, 11- Sialkot, 12- Faisalabad, 13- Jhang, 14- Bhakkar and 15- Layyah.

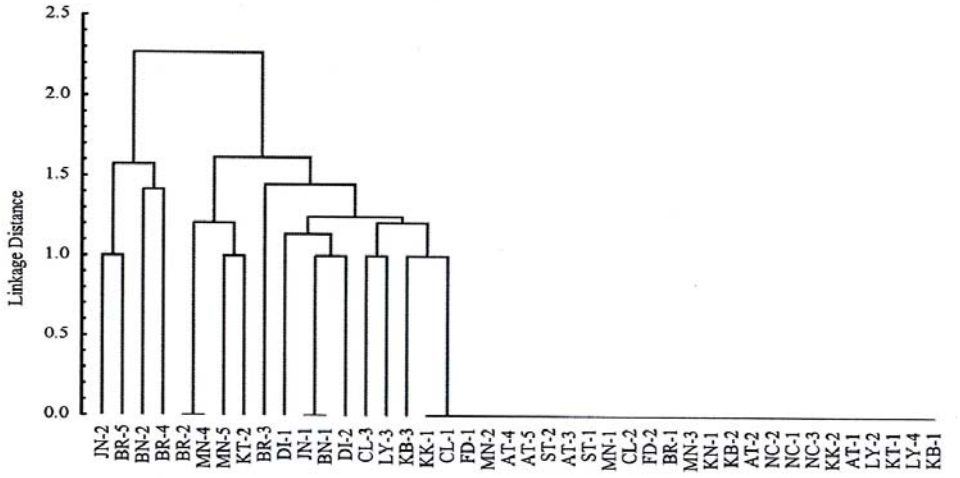

Fig. 2. Cluster diagram of 42 isolates of *Ascochyta rabiei* tested on 7 chickpea varieties.

Table 2. Principal Components (PCs) for cultural characterization and disease reaction of 42 isolates of *Ascochyta rabiei*.

		PC_1	PC_2	PC_3
Eigen value		6.531	1.563	1.015
Proportion of σ^2		54.426	13.025	8.458
Cumulative σ^2		54.426	67.452	75.91
	Range of means	**Eigen vector**		
Colony colour		-0.14	0.86	-0.08
Pycnidial formation		0.88	-0.06	-0.06
Radial growth	2.6-6.7 cm	0.28	-0.77	0.02
Pycnidia	91x85-225x224µm^2	0.50	0.30	0.50
Spore size	8x4-13x5 µm^2	0.37	-0.13	0.80
Disease reaction with variety C-727	4-9†	0.85	0.27	-0.01
Disease reaction with variety ILC-263	5-9	0.78	0.14	0.08
Disease reaction with variety C-44	5-9	0.86	0.09	-0.02
Disease reaction with variety CM-72	3-9	0.87	0.07	-0.19
Disease reaction with variety Piadar	3-9	0.86	-0.14	-0.21
Disease reaction with variety Noor-91	3-9	0.93	-0.05	-0.16
Disease reaction with variety Punjab-91	3-9	0.92	-0.02	-0.05

† Disease rating scale, 1- No infection, 2- Highly resistant (1-5% infection), 3- Resistant (6-10% infection), 4- Moderately resistant (11-15% infection), 5- Tolerant (16-40% infection), 6- Moderately susceptible (41-50% infection), 7- Susceptible (51-75% infection), 8- Highly susceptible (76-100% infection) and 9- Very high susceptible (all plants killed) as defined by Singh & Reddy (1993).

First two factors contributing more than 67% of the variability were plotted for disease reaction and three distinct groups were observed (Fig. 3). The most virulent isolates gave the similar reaction and were observed one point in extreme left of the graph. Least virulent were grouped in the right half of the graph where 3 points were observed because of similar reaction by 2 isolates. The third category i.e., intermediate were ranging from origin to 2 at x-axis, while scattered throughout in the y-axis. It was obvious that only virulent isolates could clearly be identified. Although other two groups were separated but exhibited little breeding value due to non-clear-cut identification. On the whole, multivariate analyses were able to distinguish isolates on the basis of virulence rather than origin or morphological/cultural characterization. Some of the isolates collected from the same area or source behaved differently.

Discussion

Variation in morphological and cultural characters was observed among isolates and similar variability of *A. rabiei* isolates have been reported by Singh & Reddy (1990); Grewal (1984); Gowen (1986); Gowen *et al.,* (1989); Qureshi & Alam (1984) using different isolates and chickpea cultivars. Grewal (1984) reported relatively fast growing and less sporulating isolates as less virulent and slow growing and abundantly sporulating isolates as more virulent. However, no such association was observed in the present studies. Pathogenic variability of *A. rabiei* has been demonstrated by Aujla (1964); Kaiser (1973); Vir & Grewal (1974); Reddy & Kabbabeh (1985); Nene & Reddy (1987); Porta Pulgia *et al.,* (1986 & 1996) and Porta Pulgia (1992), while some of these authors characterize the pathogenic groups as races of different cultivars, other stated the difference in aggressiveness without significant reversal of the ranking order, suggesting variability in aggressiveness rather than in virulence (Gowen, 1986). Available information may not allow the term "race" but to distinguish in pathogenicity of isolates this word could be used (Haware, 1987).

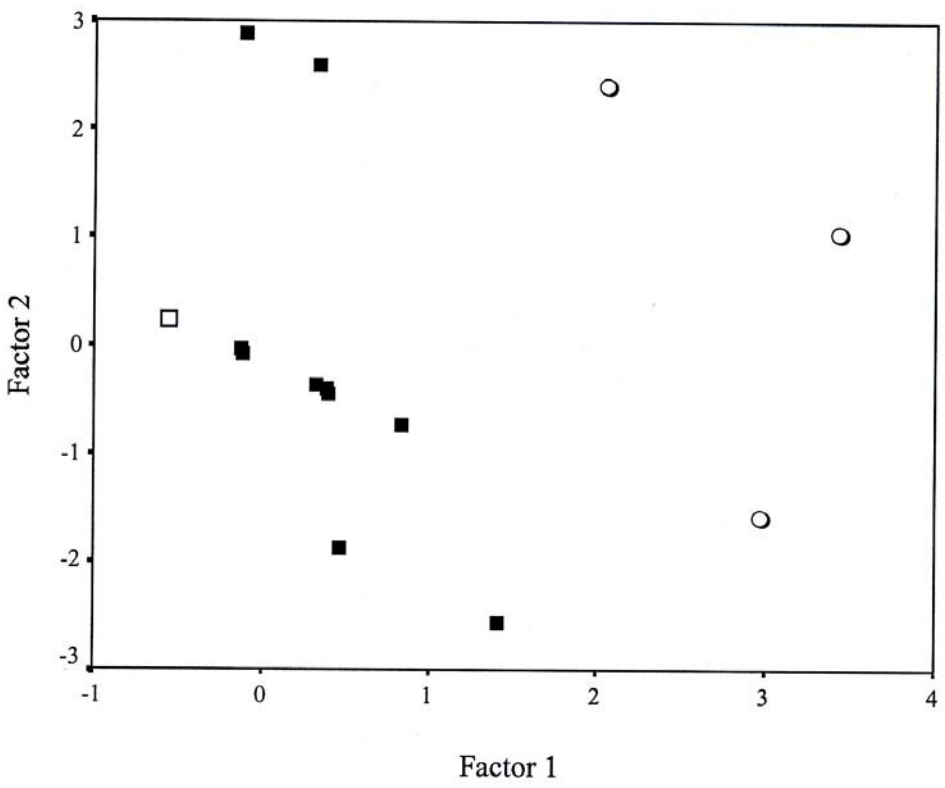

Fig. 3. Scattered diagram for two factors in 42 isolates of *Ascochyta rabiei* collected from chickpea growing areas of Pakistan. μ- least virulent, ν- medium virulent and ο- most virulent.

Although genetic diversity in isolates was observed and they could broadly be classified in three groups but a clear-cut host-pathogen reaction was not observed. This situation did not favour the nomenclature of race rather variation in pathogenicity. Recommendations to standardize race characterization have been made since 1989, but to date standard methodology has still not been agreed upon and no clear differential has been identified that could be acceptable universally. This problem might be associated with complex nature of gene-action involved in *A. rabiei* resistance (Malik, 1990). The need exists to use multiple crosses due to quantitative nature of gene-action involved for disease reaction. This will help to build resistance pyramids that could be obtained by involving parents of diverse origin and known tolerant to disease.

Chickpea cultivars included in the study as shown by multivariate analyses, susceptible genotypes and virulent isolates were identified but, clear-cut standard for resistance was not observed. Inconsistent clustering pattern for various isolates collected from the same origin may be attributed towards frequent exchange of breeding material and disease cultures among the researchers. Several reasons have been reported, such as the increase of chickpea-growing area and the introduction of resistant cultivars that contribute to extending the variability of *Ascochyta* population (Crino *et al.*, 1985; Hussain & Barz, 1997). More variation could be expected, taking into account the

heterothallic nature of the fungus (Trapero-Casas & Kaiser, 1992) and the recent development of new isolates that makes possible the appearance of the teleomoph of the fungus. Variation in isolates originated from same area need to be investigated using biochemical analyses, although isolates collected from the single field could vary for disease infection (Morjane *et al.*, 1994).

The occurrence of isolates belonging to one cluster that are able to infect all the genotypes suggests the need for more suitable sources of resistance. Promising levels of resistance have been reported in wild species of *Cicer* (Singh *et al.*, 1992; Singh & Reddy, 1993) and cultivated chickpea (Iqbal *et al.*, 1989 & 1994; Singh & Reddy, 1989). Even after 90 years of research on chickpea blight, the problem is yet unsolved and further studies on the hostpathogen relationship of *Ascochyta* blight is still needed although effects of environments are well known (Hafiz, 1986). Further study involving biochemical analysis using known material (host and pathogen) should be streamlined for a comprehensive understanding of this complex disease.

A complex pathogenic variability is not surprising since the pathogen has a sexual stage that can generate new recombinants with varying virulence spectrum (Kaiser, 1992). The role of weak pathotypes in generating aggressive pathotypes either through accumulation of virulence and genetic recombination is not yet understood and needs to be explored. The use of field isolates in resistant screening representing populations of the pathogen, rather than individual or mixed races, has been suggested (Mmbaga *et al.*, 1994). The relatedness of the isolates on the basis of host parasite interaction can be determined through multivariate analyses (Shane, 1987). Such results are useful for choosing representative pathotypes that may be used to identify specific resistant groups for utilization in breeding programme. The present study indicated that *A. rabiei* isolates collected from Pakistan were composed of various pathotypes and these cannot be stated as races according to standard definition. A continuous breakdown of resistance in host emphasizes the need for up to date knowledge of physiologic pathotypes prevalent in different regions to develop chickpea cultivars having stable resistance against *Ascochyta* blight. Chickpea variety CM 72 was released as blight tolerant but with the passage of time and by mixing or development of new strains of *A. rabiei*, this variety is no longer tolerant, therefore a need exists to evolve varieties with durable resistance. Similarly, ILC 263 is being used as susceptible check in most of the ICARDA experiments, but two varieties (C 727 and C 44) exhibited higher degree of susceptibility than ILC 263, and these are suggested to use a susceptible check in screening experiments.

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