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PATHOGENIC DIVERSITY IN *ASCOCHYTA RABIEI* (PASS.) LIB., OF CHICKPEA

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Abstract

Ten isolates of *Ascochyta rabiei* derived from single spore cultures were studied for their morphological characters and pathogenic variability. These isolates exhibited variation in morphological and cultural characteristics. Variation in rating of each *A. rabiei* isolates towards all the test cultivars exhibited in a continuous manner. Susceptible cultivars showed symptoms involving lesions on the leaves and stem and even in severe cases resulted in plant mortality. Reaction of 19 chickpea genotypes to all the isolates of *A. rabiei* indicated that Venhar was resistant to most of the isolates and tolerant to AR 1 and AR 9 isolates, whereas as it was susceptible to the isolates AR 2. Cultivars AUG 424, C 44 and NIFA 95 showed susceptible response to all the isolates. The remaining cultivars acted as differentials and showed considerable variation in disease reaction. The grouping of isolates is expected to have indication for virulence response to various cultivars that is yet to be investigated.

Introduction

Chickpea (*Cicer aretinum* L.) is one of the major pulse crop grown in rainfed areas of Pakistan. It is a rich source of vegetable protein for human nutrition (Hulse, 1991). Among the various factors contributing to its low production, biological constraints particularly diseases are the most important. Of several diseases affecting this crop, blight caused by *Ascochyta rabiei* (Pass) Lab., teleomorph *Didymella rabiei* Kovachevski is the most threatening. It is known to occur in almost all countries where chickpea is grown (Nene, 1982). A number of epiphytotics of *Ascochyta* blight have been reported in Pakistan (Aslam, 1984). Chickpea breeders in Pakistan have concentrated their efforts to develop blight resistant cultivars that gave rise to promising grmplasm (Iqbal *et al*., 1993). A number of research studies have been undertaken on the multilateral aspects of the disease in the various parts of the world in order to understand and manage the disease. Different parameters have been explored including severity, pathogenicity, life cycle, disease cycle, epidemiology, breeding for resistance as well as cultural and chemical control of chickpea blight (Kaiser, 1992).

Differences in cultural characteristics and pathogenicity among isolates of this pathogen have been described by various workers (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.*, 1995; Trapero-Casas & Kaiser, 1992).

Table 1. Morphological characteristics of various isolates of *Ascochyta rabiei.*

Isolate	Locality170 x 175 f	Colony colour	Pycnidial size	Spre size
AR-1	National Agricultural Research Center, (NARC),Islamabad	Light brown	164 x 167 g	10.0 x 5.1 c
AR-2	Barani Agriculture Research Institute, (BARI), Chakwal	Grey	152 x 150 i	9.5 x 5.5 f
AR-3	Nuclear Institute for Food & Agriculture, (NIFA), Peshawar	Black	142 x 132 j	10.0 x 5.0 b
AR-4	Ayub Agriculture Research Institute, (AARI), Faisalabad	Light brown	225 x 224 a	13.0 x 5.0 a
AR-5	Gram Research Station, (GRS), Kalor kot	Dark brown	170 x 175 f	10.0 x 5.5 c
AR-6	Farmer's field, Chakwal	Grey	194 x 196 c	10.0 x 6.0 b
AR-7	Groundnut Research Station (GRS), Attock	Dark brown	184 x 186 d	10.0 x 5.1 f
AR-8	Barani Agriculture Research Station, (BARS), Kohat	Dark brown	155 x 153 h	10.0 x 5.0 f
AR-9	Agriculture Research Institute, (ARI), D.I. Khan	Light brown	184 x 186 d	10.0 x 5.4 d
AR-10	Farmer's field, Mainwali	Grey	183 x 177 e	13.5 x 5.0 b

*Figures having the same letters are non-significant at 0.05% level of probability.

Although blight can be controlled by the use of disease free seeds, destruction of plant disease debris, seed dressing and foliar fungicides but under certain conditions these approaches are not feasible (Bashir & Ilyas, 1983; Malik *et al*., 1991; Rauf *et al*., 1996*).* Therefore importance of resistant cultivars is an established fact recognized by the researchers. The breakdown of genetic resistance may be attributed to the genetic variability in the pathogen (Qureshi & Alam, 1984). The present study was conducted to understand genetic diversity in relation with host-pathogen reaction of *A. rabiei* isolates representing chickpea growing areas of Pakistan. This information is a prerequisite for initiating a breeding programme aimed at obtaining a durable resistance against *Ascochyta* blight in chickpea.

Materials and Methods

During chickpea growing season of 2003-04, 10 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 done according to the hierarchical sampling strategy as described by McDonald *et al*., 1989. Diseased samples were surface sterilized in 0.1% Mercuric chloride for 2 minutes and plated in chickpea seed meal dextrose agar (CSMDA) containing 40 g chickpea seed meal; 20 g dextrose, 20 g agar and 1 liter water. Each isolate was purified by single spore culture and incubated at 22±2ºC for 2 weeks. Isolates were grouped according to the sites of collection (Table 1).

Single spore cultures of 10 isolates were preserved on CSMDA medium. These isolates were subjected to detailed morphological and cultural characterization viz., radial growth on medium (mm), colony colour, size of pycnidia (µm) and pycnidiospores (µm). An experiment was conducted in order to determine the pathogenicity of these isolates to 19 cultivars; Parbat, Dashat, C 727, ILC 263, Noor 91, 88194, 90395, C 44, Bittle, Punjab 91, Paidar 91, C 235, Wanhar, Balksar, CM 88, CM 98, NIFA 88, NIFA 95, AUG 424.

Table 2. Reaction of nineteen cultivars/lines to the 10 isolates of *Ascochyta rabiei*.

Cultivars/ lines	AR1	AR2	AR3	AR4	AR5	AR6	AR7	AR8	AR9	AR10
C- 727	S	S	R	R	R	S	S	S	S	S
ILC-263	S	S	S	R	R	S	R	S	S	S
Noor-91	S	S	R	R	R	S	R	S	S	S
88194	S	S	R	R	R	S	S	S	S	S
90395	S	S	R	R	R	R	R	S	S	S
C-44	S	S	S	S	S	S	S	S	S	S
Bittle	S	S	S	S	R	S	S	S	R	R
Punjab-91	S	S	S	R	R	S	S	S	R	R
Paidar-91	S	S	S	R	R	R	R	S	S	R
C-235	S	S	R	S	R	R	R	S	S	R
Vanhar	R	S	R	R	R	R	R	S	R	S
Balksar	S	S	R	R	R	R	R	S	R	R
CM-88	S	S	R	R	R	S	R	S	S	S
CM98	S	S	R	R	S	S	S	S	S	S
NIFA-88	S	S	S	R	S	S	S	S	S	S
NIFA-95	S	S	S	S	S	S	S	S	S	S
AUG-424	S	S	S	S	S	S	S	S	S	S
Parbat	S	S	S	R	R	R	R	S	S	R
Dashat	R	S	R	R	R	R	R	S	R	R

*R= Resistant, S= Susceptible

Ten seeds of each cultivar were sown in plastic pots arranged in two-factor complete randomized design with three replications. Prior to sowing, seeds were surface sterilized with Clorox (0.1% available Chlorine) and pots were filled with sterilized sandy loam soil. Homozygosity in all the cultivars used for experimentation was ensured. Before inoculation, five 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.,* 1982). Relative humidity was maintained in the range of 85-95% for 72 hours. Disease observations were recorded using 1-9 rating scale as suggested by Singh & Reddy (1993) and designation of pathotypes was followed by Habgood (1970).

Results and Discussion

A significant difference was recorded on the basis of cultural and morphological characters of 10 isolates of *A. rabiei*. In case of morphological traits of the isolates, a great variation was observed. Fourtemm days after incubation, the colony diameter of 10 isolates ranged from 9.7–75.4 cm. The maximum mean mycelial growth shown by isolates AR 6, AR 9 and AR 10 is statistically non significant with 73.5, 71.9 and 64.2 mm respectively after 21 days followed by AR 5 and AR 1, 44.3 and 43.8 mm respectively, while isolate AR 7 showed minimum growth 11.2 mm (Fig. 1). These isolates also showed some differences in colony colour, size of pycnidia and pycnidiospores (Table 1). The isolates also showed some differences in colony colour that varied from light brown to black. The colony colour of one isolate was black where as there are three isolates in each of grey, light and dark brown category. The data on size of pycnidia and pycnidisopres also varied significantly. Maximum size of pycnidia was obtained from isolates of AR 4 (225x224µm) and AR 7 (204x205µm) respectively.

The isolate AR 10 has larger spore size (13.5x5.0µm) as compared to others. Cultural characteristics could not be correlated with pathogenic variability and diseased development. The molecular techniques may be helpful for further study to confirm the association and correlation in this respect. Molecular markers, such as isozymes, RAPD and RFLP, are based on the identification of protein or DNA polymorphisms and have most of the properties of an ideal marker (Mayer *et al*., 1997).

Variation in rating of each *A. rabiei* isolate towards all the cultivars tested exhibited in a continuous manner. Susceptible cultivars showed symptoms involving lesions on the leaves and stem and even in severe cases resulted in plant mortality. Reaction of 19 chickpea genotypes to the 10 isolates of *A. rabiei* indicated that cultivar Venhar showed resistant reaction to most of the isolates, and susceptible response to AR-1 and AR-9 isolates while they exhibited variable susceptible reaction to the isolate AR-2. Cultivars AUG-424, C 44 and NIFA-95 showed susceptible response to all the isolates. The remaining cultivars acted as differentials and showed considerable variation in disease reaction.

Two clusters were observed for complete linkage using Euclidean distances for cultural and morphological characteristics of 10 isolates of *A. rabiei* (Fig. 3). When the clusters were subdivided at 50% dissimilarity, the isolates, AR 5, AR 4 and AR 3 were not grouped with any other indicating some particular characteristics that might be related to level of virulence Two isolates in each case were grouped into fourth and fifth subcluster, whereas all the other three isolates (AR 8, AR 2 and AR 1) were grouped together in sixth subcluster. The reaction of 19 chickpea genotypes to 10 isolates of *A. rabiei* used in the present study revealed that AUG 424, C 44 and NIFA 95 were susceptible to all the isolates and the remaining 16 cultivars acted as differentials and showed considerable variation in disease development with different isolates. It was observed that all the genotypes were susceptible when inoculated with isolate AR 2 and AR 8, and both of these isolates were grouped together. There was also a significant pathogenic variability between isolates with respect to their capability of disease development on 19 chickpea genotypes (Fig. 2).

The virulence rating of each *A. rabiei* isolate toward all the test lines exhibited a large but continuous variability. All the cultivars showed symptoms involving both leaves and stems. On leaves, circular spots appeared that were followed by drying of a part or the whole lamina. Extensive lesions ranging from flecks to large lesion (>5mm) were recorded on the stem. The most virulent isolates were AR-1, AR-2, AR-6, AR-7, AR-9 and AR-10 whereas AR-8 was less virulent. The least virulent were AR-3, AR-4 and AR-5.

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 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. This 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. The varieties released as blight resistant did not remain resistant with the passage of time and by mixing or development of new strains of *A. rabiei*, that is therefore a dire need to evolve varieties with durable resistance.

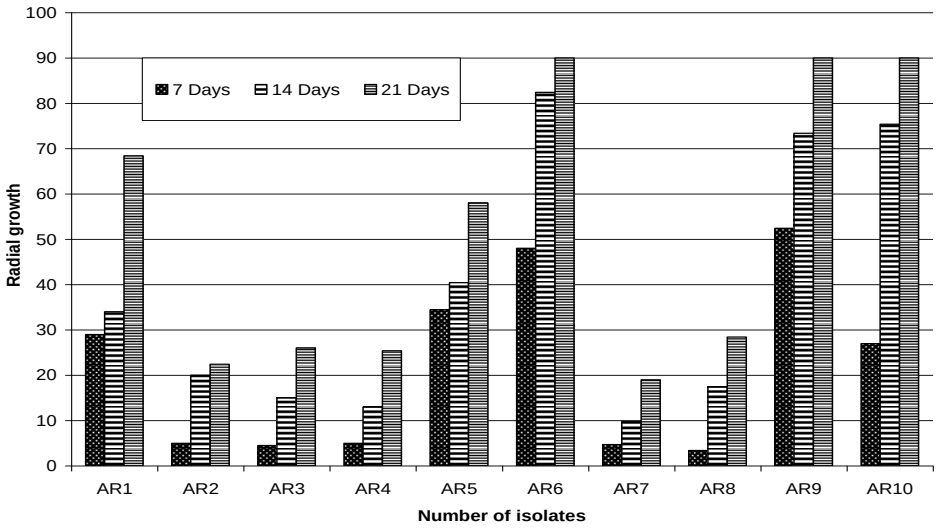

Fig. 1. Comparison of radial growth (mm) of different isolates of *Ascochyta rabiei* on chickpea seed meal agar medium.

Fig. 2. Variation of growth pattern of the isolates of *Ascochyta rabiei* on chickpea seed-meal agar medium.

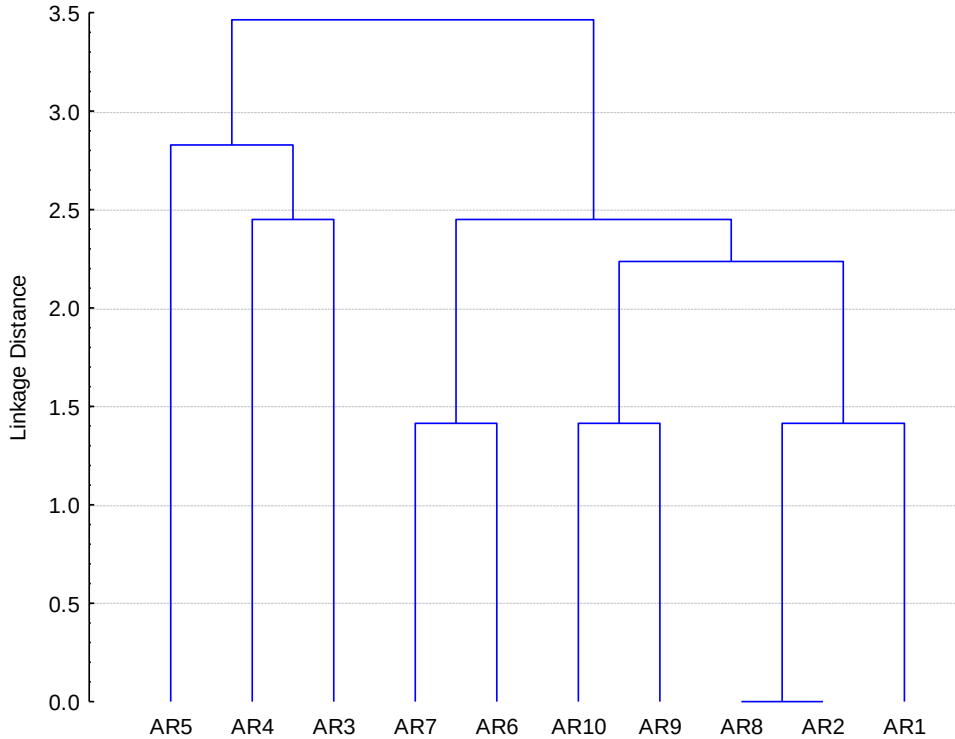

Fig. 3. Cluster diagram of ten isolates of *Ascochyta rabiei* based on cultural and morphological characters.

References

Ambardar, V.K. and S.K. Singh. 1996. Identification and elucidation of *Ascochyta rabiei* isolates of chickpea in Jammu. *Indian Journal of Mycology and Plant Pathology*, 26: 4-8.

Aslam, M. 1984. A review of research on chickpea blight fungus in Pakistan.In: *Chickpea*, (Eds.): M.C. Sexena and K.B Singh, ICARDA, Aleppo, Syria, pp. 255.

Aujla, S.S. 1964. Study on eleven isolates of *Ascochyta rabiei* (Pass.) Lab., the causal agent of gram blight in the Punjab. *Indian Phytopathology*, 17: 83-87.

Bashir, M. and M.B. Ilyas. 1983. Chemical control of gram blight. *Pak. J. Agri. Sci.*, 20:152-158.

Grewal, J.S. 1984. Evidence of physiological races in *Ascochyta rabiei* of chickpea. In: *Ascochyta blight and winter sowing of chickpea* (Eds.): M.C. Saxena, M.C. and K.B. Singh. ICARDA/ Martinus Nijhoff Publishers, 255-259.

Habgood, R.M. 1970. Designation of races of plant pathogens. *Nature*, 227: 1269-1290.

Hulse, J.H. 1991. Nature, composition and utilization of grain legmes. p. 11-27. In: *Uses of tropical legumes*: Proceedings of a Consultants meeting, 27-30 March 1989, ICRISAT Patanch eru. AP 502 324, India.

Iqbal, M.J., K. Iftikhar and M.B. Ilyas. 1993. Evalution of chickpea germplasm for resistance against blight disease. *J. Agri. Res.*, 31: 449-453.

Jamil, F.F., N. Sarwar, M. Sarwar, J.A. Khan, J. Geistlinger and G. Jkahl. 2000. Genetic and pathogenic diversity within *Ascochyta rabiei* (Pass.) Lab. populations in Pakistan causing blight of chickpea (*Cicer arietinum* L.). *Physiological and Molecular Plant Pathology*, 57: 243-254.

Kaiser, W.J. 1973. Factors affecting growth, sporulation, pathogenicity and survival of *Ascochyta rabiei*. *Mycologia*, 65: 444-457.

Kaiser, W.J. 1992. Epidemiology of *Ascochyta rabiei.* In: *Disease Resistance Breeding in Chickpea*. K.B. Singh & M.C Saxena. Aleppo Syria: ICRDA, 117-143.

Malik, M.R., S.M. Iqbal and B.A. Malik. 1991. Economic losses of *Ascochyta* blight in chickpea. *Sarhad J. Agri.*, 8: 765-768.

Mayer, M.S., A. Tullu, C.J. Kumar, W.J. Kaiser, J.M. Craft and F. J. Muehlbauer. 1997. Development of DNA markers for *Fusarium* wilt resistance in chickpea. *Crop Sci*., 37: 1625-1629.

McDonald, B.A., J.M. McDonald, S.B. Goodwin and R.W. Allard. 1989. DNA restriction fragment length polymorphism among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from single wheat field. *Phytopathology*, 80: 1368-1373.

Mmbaga, M.T., S. Kabbabeh and K.B. Singh. 1994. Pathogenic variability of *Ascochyta rabiei* and *Ascochyta* blight resistance in chickpea (*Cicer arietinum* L.). *Proceedings of the Ninth Congress of the Mediterranean Phytopathological Union.* September 19-25, Turkey.

Navas-Cortes, J.A., A. Trapero-Casas and M. Jimenez-Diaz. 1995. Survival of *Didymella rabiei* in chickpea straw debris in Spain. *Plant Pathology*, 44: 332-339.

Nene, Y.L. 1982. A review of ascochyta blight of chickpea. *Tropical Pest Management*, 28: 61-70.

Porta Pulgia, A. 1992. Variability in *Ascochyta rabiei*. pp. 135-143. In: *Disease Resistance Breeding in Chickpea*. (Eds.): K.B. Singh and M.C. Saxena. ICARDA.

Qureshi, S.H. and S.S. Alam. 1984. Pathogenic behavior of *Ascochyta rabiei* isolates on different cultivars of chickpea in Pakistan. *International chickpea Newsletter*, 10: 29-31.

Rauf, C.A., M.R. Malik, S.M. Iqbal, S. Rahat and S.hussain. 1996. Fungicides; an economic tool to enhance productivity and net returns in chickpea crop. *Sarhad J. Agri.*, 12: 445-448.

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

Shane, W.W. 1987. Use of principle component analysis and cluster analysis in crop loss assessment. pp 139-149 in *Crop Loss Assessment and Pest Management.* (Ed.): P.S. Teng. American Phytopathological Society, St Paul, Minnesota, USA

Singh, G., K. Singh and S. Kapoor. 1982. Screening for sources of resistance to *Ascochyta* blight of chickpea. *International Chickpea Newsletter*, 6: 15-17.

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: 127-12.

Singh, K.B. and M.V. Reddy. 1993. Resistance to six races of *Ascochyta rabiei* in the world germplasm collection of chickpea. *Crop Science*, 33: 186-189.

Trapero-Casas, A. and W.J. Kaiser. 1992 Development of *Didymella rabiei*, the telomorph of *Ascochyta rabiei* on chickpea straw. *Phytopathology*, 82: 1261-1266.

Vir, S. and J.S. Grewal. 1974. Physiologic specialization in *Ascochyta rabiei* the causal organism of gram blight. *Indian Phytopathol*, 27: 355-360.

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