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Reaction of Chickpea Genotypes to the Isolates of *Ascochyta rabiei* (Pass) Lab.

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Abstract: Two isolates of *Ascochyta rabiei* (Pass) Lab. derived from single spore cultures representing the most and least aggressive nature were studied separately and in combination for pathogenic aggressiveness on sixteen chickpea varieties. A great deal of variation was observed in the pathogenic reaction of isolates for inducing disease development. The cultural traits, radial growth and pycnidial size, were also significantly different for the two isolates. Similarly, a significant difference between chickpea genotypes was observed for their response to isolates regarding disease development. Five varieties, C-727, C-44, Noor-91, Punjab-91 and ILC-263 revealed high degree of susceptibility and are suggested to be used as susceptible checks for screening experiments. Two other genotypes, Dasht and Balkasar showed high degree of tolerance to both the isolates when applied separately or as 1:1 mixture. The aggressiveness of mixture of two isolates was reduced to the level of least aggressive isolate instead of having synergic effect for blight development.

Key words: Chickpea, varieties, *Ascochyta rabiei*, isolates, pathogenic, aggressiveness

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 in the Indian Subcontinent and Mediterranean basin (Anonymous, 1994). A number of biotic and abiotic factors are responsible for its low production. More than 50 pathogens have been reported from different parts of the world that attack chickpea (Nene *et al.*, 1989). Blight caused by *Ascochyta rabiei* (Pass) Lab. is considered to be the most devastating disease of chickpea not only in Pakistan but in different parts of the world. Yield losses caused by Ascochyta blight are inevitable with susceptible cultivars and may go up to 100% on a worldwide basis (Haware, 1998). Under epidemic conditions (high humidity, windy and rainy weather) 100% yield losses can occur within three weeks. A number of blight epidemics have been encountered in Pakistan (Malik and Tufail, 1984) and from many chickpea growing countries (Cubero, 1984; Kaiser, 1972).

Differences in cultural characteristics and pathogenicity among isolates have been described by Aujla (1964), Kaiser (1973), Porta-Puglia (1992), Vir and Grewal (1974) and Jamil ***et al.*** (2000). Reddy and Kabbabeh (1985) reported the existence of six races in Syria and Lebanon. Singh and Reddy (1989) reported the use of seven differential lines for their identification. On the other hand, Grewal (1984) observed 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. The variation in *A. **rabiei*** is likely to enhance by the presence of teleomorph ***Didymella rabiei*** (Kavach.) under field conditions (Navas-Cortes ***et al.***, 1990; Trapero-Casas and Kaiser, 1992).

It is believed that the best method of controlling Ascochyta blight is the development of resistant cultivar as the other control methods are unreliable and uneconomical (Singh ***et al.***, 1981). Although development of cultivars with durable resistance is a difficult task due to complex nature of the fungus that may cause rapid breakdown of varietal resistance (Kaiser, 1973; Qureshi and Alam, 1984; Malik, 1990) but use of resistance is the most applicable and practicable method to control the disease. Before initiating a breeding programme aimed at the development of genotypes with reliable and stable resistance it is imperative to understand the pathogen with respect to variation between its different isolates for host pathogen interaction. This study was therefore, conducted to understand variability behaviour in isolates of ***A.rabiei*** for the development of disease on chickpea cultivars.

Materials and Methods

The isolates of *A. **rabiei*** used in this study were obtained from blight infected chickpea plants collected during growing season of 1995-96. Both the isolates were categorized on the basis of their morphological and cultural characteristics. Their aggressiveness was determined using a set of 16 chickpea cultivars. Single spore cultures of the isolates were preserved on CSMA medium. Two isolates representing the most aggressive (AT-2) and the least aggressive (BK-5) groups, were used to determine the pathogenicity when applied separately and as 1:1 mixture on sixteen chickpea cultivars; Dasht, Parbat, C-727, C-44, C-235, CM-72, NIFA-88, NIFA-95, Bittle-98, Noor-91, Punjab-91, Piadar-91, Bulkasar, Wanhar, ILC-263 and DC-1.

Ten seeds of each genotype were sown in plastic pots in complete randomized design. Prior to sowing, seeds were surface sterilized with clorox (0,1% available Chlorine) and pots were filled with sterilized sandy loam soil. After germination, five healthy plants were maintained in each pot. Twenty days old plants were inoculated by spraying spore suspension with concentration of 5×10^5 spores per ml, that had been prepared from 15 days old cultures of the isolates. The inoculated seedlings were incubated under humid chamber for 72 h (Singh ***et al.***, 1982). The relative humidity in the chamber was maintained in the range of 85-95% following which plants were sprayed with water twice a day till the recording of disease observation. Fourteen days after inoculation the disease observations were recorded on 1-9 scale as suggested by Singh ***et al.*** (1981).

Results and Discussion

The reaction of chickpea genotypes when tested against individual isolates as well as in combination of both (1:1 ratio) was recorded on 1-9 scale (Single ***et al.***, 1981). There were significant differences between the isolates and between the genotypes for disease development (Table 1). The disease score on genotypes inoculated with least aggressive isolate ranged from 2.2 to 7 whereas in the case of aggressive isolate and mixture of two it ranged from 5.4 to 9 and 2.4 to 7, respectively. The isolate means of disease scores on 16 genotypes were 7.95, 5.66 and 4.90, respectively for aggressive, least aggressive and mixture of both (Table 1). The isolate means of disease score for least aggressive and most aggressive isolates were significantly different from each other. However, the mean disease score of the mixture of two isolates (4.90) was not highly significant from that of least aggressive isolate. The genotypic mean for disease of Balkasar and Dasht were 3.20 and 3.33, respectively. The maximum mean disease with score 7.33 was exhibited by C-235, which was followed by C 44 and C 727 with respective disease scores of 6.87 and 6.80. The intensity of disease on each genotype was different under three different treatments. The disease score of Dasht increased from 2.2 (in the case of mixture of two) to 5.4 (in the case of aggressive isolate). Similarly for Balkasar it increased from 2.2 to 5.

The cultural traits showed significant differences between the two isolates for radial growth and pycnidial size (Table 2). The spore size in both the isolates was however same.

All the cultivars subjected to disease infection (single or combined) showed symptoms involving both leaves and stems. On the leaves, circular spots appeased 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. Isolates of *A. **rabiei*** greatly varied in their pathogenic reaction in 16 genotypes. Analysis of variance showed significant differences ($P<0.001$) between genotypes as well as between treatments. The aggressiveness rating of each *A. **rabiei*** isolate toward all the lines tested exhibited a large but continuous variability. The results showed that there was remarkable variation in pathogenicity between two isolates for disease development. This was obvious from the genotypic means of disease scores for individual isolates. The disease development on individual genotypes (irrespective of their resistance level) under each isolate also showed variation between the two. A consistent trend of increased disease rating under aggressive isolate as compared to that of least aggressive isolate was observed in all the genotypes. A similar grouping of *A. **rabiei*** isolates on the basis of aggressiveness have been reported by Singh (1985; 1987; 1990), Vir and Grewal (1974), Grewal (1984), Qureshi and Alam (1984) using different isolates and chickpea cultivars. Similarly pathogenic variability of *A. **rabiei*** has been demonstrated by Aujla (1964), Kaiser (1973), Grewal (1984), Vir and Grewal (1974), Reddy and Kabbabeh (1985), Nene and Reddy (1987), Porta Pulgia ***et al.*** (1986) and Porta Pulgia (1992). Some of these authors designated the pathogenic groups as races of pathogen while others stated that

only the difference in aggressiveness does not qualify the condition required to designate them as different races. They argued that variability was in aggressiveness rather than in virulence (Gowen, 1986 and Haware, 1987).

The overall reaction of the sixteen chickpea genotypes to the isolates of *A. rabiei* showed variability for the degree of aggressiveness. Several reasons have been reported for such variation. For example the increase of chickpea-growing area and the introduction of resistant cultivars may have contributed to extending the variability of *Ascochyta* population (Crino ***et al.***, 1985 and Porta-Puglia ***et al.***, 1996). More variation could be expected, taking into account the heterothallic nature of the fungus (Trapero-Casa and Kaiser, 1992) and the recent development of new isolates that make possible the appearance of the teleomoph of the fungus. Variation in isolates originated from same area need to be investigated as isolates collected from the single field could vary for disease infection (Morjane ***et al.***, 1994). Biomolecular approaches could also provide useful information on the variability of the fungus (Nene and Reddy, 1987). The further study involving biochemical analysis using known material (host and pathogen) should be streamlined for a comprehensive understanding of this complex disease.

The present results and previous studies provide evidence that isolates of *A. rabiei* differ in both aggressiveness and in their specific virulence patterns. The occurrence of 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). When the most aggressive and the least aggressive isolates were applied as 1:1 mixture, the aggressiveness of this mixture was similar to that observed in least aggressiveness isolate. This indicated the dominance of the less aggressive isolate over the most aggressive isolate. The chickpea cultivars Parbat, C-235, CM-72, NIFA-88 and NIFA-95, which were susceptible to the aggressive isolate appeared to be resistant/ tolerant to the mixed population of isolates as observed for least aggressive isolate. Similarly, the resistance behavior of other cultivars became similar to that observed for least aggressive isolate when subject to the mixture of isolates. In other words, the aggressive isolate lost its aggressiveness when applied in combination with the least aggressive isolate. This may be due to weak isolate having occupied the site of infection (Ali ***et al.***, 1993) that did not allow the aggressive isolate to cause severe infection, or due to the rapid multiplication/growth of the less aggressive isolate as observed on host plant, suppressing the growth of more aggressive isolate and reducing the aggressiveness of mixture to the level of less aggressive isolate. Since disease is caused through the production of toxins (Alam ***et al.***, 1989; Hohl ***et al.***, 1991 and Kaur, 1995) the capability of aggressive isolate to produce this substance may have reduced in mixture. Since capability of isolates varies for the production of phytotoxins (Kaur, 1995) that cause disease in the host plant it appears that less aggressive isolate retarded the multiplication rate and toxin production capability of aggressive isolate through the production of some other chemical that may have retarding effect on multiplication and on the production

Table 1: Effect of isolates of *Ascochyta rabiei* representing the most and the least aggressive nature when applied separately and in combination

Cultivars	Most aggressive isolate	Least aggressive isolate	Mixed isolate	Means
Dasht	5.4	2.2	2.4	3.33
Parbat	7.0	3.2	3.6	4.60
C 727	9.0	4.4	7.0	6.80
C 44	9.0	5.0	6.6	6.87
C 235	9.0	7.0	6.0	7.33
CM 72	9.0	5.0	3.4	5.80
NIFA 88	7.0	2.6	3.8	4.47
NIFA 95	9.0	4.2	5.0	6.07
Bittle 98	7.0	5.4	5.8	6.07
Noor 91	9.0	3.4	5.4	5.93
Punjab 91	9.0	4.0	7.0	6.67
Piadar 91	9.0	3.4	5.8	6.07
Bulkasar	5.0	2.2	2.4	3.20
Wanhar	6.0	3.0	3.2	4.07
ILC 263	9.0	4.6	7.0	6.87
DC 1	5.6	4.0	4.0	4.53
LSD (P<0.05%)	0.9096	0.7431	0.7601	
EMS	0.517	0.345	0.361	
G. means	7.95	5.66	4.90	

Duncan's Multiple Range Test (DMRT) was performed at P<0.05.

Table 2: Comparison of virulent and avirulent isolates of *Ascochyta rabiei*

		Most aggressive isolate	Least aggressive isolate
Radial growth	$\bar{x}$±SD	4.20+1.00	3.00+1.00
	t-value	$1.4x10^{7}$ **	
	Probability	0.000	
Pycnidual size	$\bar{x}$±SD	41826.7±165.10	1807.0±175.00
	t-value	170.97 *	
	Probability	0.000	
Spore size	$\bar{x}$±SD	45.0±12.0	45.0±12.0
	t-value	0.00 NS	
	Probability	1.00	

* Significant, ** highly significant and NS non-significant

of toxins from the less aggressive isolate itself. This was further supported when radial growth of isolates was compared.

It would be appropriated to conduct more studies on different mixtures of aggressive and less aggressive isolates to confirm these results. If it is confirmed that the least aggressive isolate reduces the disease developing capability of more aggressive isolate (as observed in the present study), the introduction of less aggressive isolate in the areas of more aggressive isolates would reduce the risk of disease development in that area and chickpea lines with moderate resistance level would be appropriate for that area. This will give an advantage of introducing genotypes with relatively high yield potential as blight resistance and yield potential are negatively

correlated. Pizano (1997) also proposed the introduction of less aggressive isolate Fusarium wilt of carnation in the areas where more aggressive isolates exist to reduce the severity wilt disease. The role of weak pathotypes in suppressing the aggressiveness of virulent pathotypes either through inactivation of virulence or genetic recombination is not yet understood and needs to be explored. In this study it was observed that the pattern of resistance in chickpea genotypes under all the treatments (aggressive, least aggressive and mixture of isolates) was similar. The most resistant and the most susceptible genotypes were same under the three treatments. The only difference was that of disease severity. However, previous studies indicated that resistant behavior of a host plant to a specific isolate might not be similar for all the isolates. These differences in the results may be either due to only two isolates used or due to newly developed tolerant genotypes used in this study. Further investigation is needed to confirm these findings.

Previously, 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). However, broad resistance that is effective against entire population is not always available and must be developed through breeding (Singh ***et al.***, 1992). The relatedness of the isolates on the basis of host parasite interaction can be determined through multivariate analysis (Shane, 1987). Such results could be useful for choosing representative pathotypes that may be used to identify specific resistant groups for utilization in breeding programme.

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