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Pathogenic Variability Among the Isolates of *Ascochyta lentis*

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ABSTRACT

The current investigation reported the examination of variation in virulence amongst nine isolates of *Ascochyta lentil* originated from Pakistan. The reactions of ten lentil lines showed considerable differences among isolates in their pathogenicity. The isolates of *A. lentis* could be divided into six distinct pathotypes with variable virulence patterns on the lentil lines; Matodar, Sunrise, ILL 5244, lLL 358, ILL 3537, Digger, Manshra-93, Markaz-2001, Masoor-2002 and Masoor-85. Isolate AL-1 was the most virulent, causing disease on all differentials, while isolates AL-8 and AL-9 were avirulent on all differentials. There were also differences among isolates in their aggressiveness and pathogenicity.

Key Words: Pathogenic; Variability; *Ascochyta lentis*

INTRODUCTION

Ascochyta blight, caused by *Ascochyta lentis* Vassiljevsky, is an important foliar disease of lentil (*Lens culinaris* L.) with worldwide occurrence (Nene *et al.,* 1988) and is a serious threat to lentil production. Yield losses of 70% in Canada (Gossen & Morrall, 1983), 45 - 50% in Pakistan (Malik, 1983) and 40% in United States of America (Kaiser, 1992) have been reported. The use of resistant cultivars is an ideal and the most effective way of controlling *A. lentis.* Some resistant lines have been identified (Singh *et al.,* 1982; Iqbal *et al*., 1990; Sugha *et al*., 1991; Erskine *et al*., 1996; Nasir & Bretag, 1996), but the existence of virulence forms of *A. lentis* (Ahmed *et al*., 1996) may limit their usefulness.

Pathogenic variability within *Ascochyta* spp. causing blight of peas (Jamali *et al*., 2005), chickpeas (Iqbal *et al*., 2004) and faba bean (Rashid *et al*., 1991) is well documented and accomplished. Differences in cultural characters among the isolates of *A. lentis* from 24 different countries have been described (Kaiser *et al*., 1994). Ahmed and Morrall (1995) reported variation in the virulence of isolates of *A. lentis* form Canada. Different mating types of *A. lentis* have also been reported (Ahmed *et al*. 1996a). Discrepancies among lentil lines in their reaction to Ascochyta blight have been observed (Tay, 1989; Andrahennadi, 1994; Ahmed & Morrall, 1996; Nasir & Bretag, 1996). Additional information on pathogenic variability among isolates of *A. lentis* should lead to the development of cultivars with strong and durable resistance. Therefore, the objective of this study was focused to determine the extent of pathogenic variability amongst the isolates of *A. lentis* prevalent in Pakistan.

MATERIALS AND METHODS

Ascochyta lentis was isolated from naturally infected lentil plants in various locations of Pakistan, using an agar plate method (Nasir & Bretag, 1997). Single-spore lines of representative isolates were derived from conidial suspensions and maintained on lentil seed extract agar (LSA). Inoculum was also produced by growing isolates on LSA. Seeds were sown 1 cm deep in pots filled with sterilized clay and sand (1:1) soil mixture. Plants were grown under glasshouse conditions (temperature 20 ± 2^0C) and ten seedlings per pot were maintained. Preliminary experiments were carried out to determine the optimum conditions for disease screening. Plants were sprayed with aqueous spore suspensions of 10^5 conidia mL^{-1} and incubated for 72 h.

Ten plants (2 weeks old) of each of the cultivars; Matodar, Sunrise, ILL 5244, lLL 358, ILL 3537, Digger, Mansehra-93, Markaz-2001, Masoor-2002 and Masoor-85 were sprayed with a freshly prepared spore suspension (10^5 conidia mL^{-1}). In each experiment, as a control, only un-inoculated set of plants was sprayed with sterile distilled water. After inoculation, plants were transferred in to a chamber, where relative humidity and temperature were kept at 95 - 100% and 20 ± 2^0C, respectively. After 48 h, plants were transferred back to the glasshouse. To maintain humidity, all plants were sprayed for 5 min twice a day with a humidifier.

Disease symptoms were scored 10 days after inoculation. All plants were evaluated according to the following disease scale: 1 = no visible lesion, 3 = small flecks on leaves, 5 = many lesions on leaves with or without chlorotic zones, flecks on stems, 7 = extensive lesions on leaves and defoliation, many stem lesions with sporulation, 9 = collapsed leaves and girdled stems, plant death. Lines with the disease rating of 1 - 3 were designated as resistant (R), while the others were categorized as susceptible (S).

RESULTS AND DISCUSSION

The observations of the present study revealed a significant variation among the isolates of *Ascochyta lentis*

for morphological traits as well as the pathogenicity. Substantial differences for linear growth were observed among fungal isolates. Fifteen days after incubation, the colony diameter of 9 isolates ranged from 55.3 – 87.0 mm. Three isolates AL-3, AL-9 and AL-4 with colony diameter of 87.0, 80.0 and 78.8 mm, respectively were found to be fast growing among the tested samples studied. Isolates with linear growth less than 75.0 mm were the slow growing ones. These isolates also displayed some differences in colony colour, pycnidial density, size of pycnidia and pycnidiospores (Table I). The knowledge of variability among the isolates for their pathogenic behavior and other morphological traits is vital for the development of breeding strategy to evolve genotypes with durable disease resistance. Present study revealed variation among the isolates for morphological and cultural traits. Such variability in *Ascochyta rabiei* (Iqbal *et al*., 2004), *A. pisi* (Jamali *et al*., 2005) and *A. lentis* (Kaiser *et al*., 1994; Ahmed & Morrall, 1995) have already been reported. Grewal (1984) reported relatively fast growing and less sporulating isolates as less virulent and slow growing and abundantly sporulating isolates as more virulent. However, the authors of current study were not able to observe this kind of correlation.

Reactions observed on lentil lines 10 days after inoculation ranged from 1 (no visible lesion) to 9 (plant death). All tested isolates produced typical blight symptoms on susceptible plants after 10 days. In addition to leaf lesions, extensive defoliation and stem girdling was observed on most of the susceptible lines. Symptoms differed mainly in the extent of lesions. Disease symptoms were observed first on the most vulnerable lines. To compare the resistance of the different lines, the mean disease scores were computed for each line and expressed as a disease index. On the basis of disease indices, the lentil lines were divided in to two groups i.e. resistant and susceptible (Table II).

To determine the pathogenic variability among the isolates, the lentil lines Matodar, Sunrise, ILL 5244, lLL 358, ILL 3537, Digger, Mansehra-93, Markaz-2001, Masoor-2002 and Masoor-85 were selected as pathogen targets. Isolates exhibited large variation in their pathogenic reaction. Only a few isolates showed the identical reactions for varietal differential of lentil lines. After comparing the reaction of the differentials, the isolates of *A. lentis* could be divided into five pathotypes (Table II). During the experiment, ILL 5244, ILL 358 and ILL 3537 showed a higher level of resistance than any other line.

In a few reports presented on the evaluation of resistance to *A. lentis*, various inoculation techniques and incubation times have been proposed and used (Morrll & Sheppard, 1981; Gossen *et al*. 1986; Kaiser & Hanna, 1986; Cromey *et al*. 1987; Ahmed & Morrall, 1995). These different testing conditions make it difficult to compare the results of various research groups. The development and adoption of a standardized set of differentials and inoculation and incubation procedures for studying the pathogenicity of *A. lentis* would permit the comparison of results among different groups of researchers.

Table I. Cultural variability among the isolates of *Ascochyta lentis* collected from Pakistan

Isolates	Radial growth (mm)	Colony colour	Sporulation	Pycnidial size (um)	Spore size (um)
AL-1	55.3 c	Grey	+	124 x 122 c	10.0 x 5.0 g
AL-2	57.0 c	Light grey	++	113 x 113 g	9.5 x 5.5 f
AL-3	87.0 a	Cream	+	119 x 115 e	11.0 x 5.0 c
AL-4	78.8 a	Black	+++	123 x 113 d	10.0 x 5.4 e
AL-5	60.3 bc	Light brown	+++	114 x 113 f	23.5 x 5.0 a
AL-6	57.8 c	Light grey	+++	101 x 95 i	9.5 x 5.0 h
AL-7	69.0 b	Brown	++	111 x 107 h	11.0 x 5.0 d
AL-8	58.0 c	Grey	++	128 x 121 b	12.0 x 5.0 b
AL-9	80.0 a	Dark brown	+++	132 x 127 a	23.5 x 5.0 a

* Figures having the same letters are non-significant at 5% level of probability.

Table II. Pathogenic variability among the isolates of *Ascochyta lentis* collected from lentil growing areas of Pakistan

	Isolates								
Genotypes	AL-1	AL-2	AL-3	AL-4	AL-5	AL-6	AL-7	AL-8	AL-9
Matodar	S	S	S	R	S	R	R	R	R
Sunrise	S	R	S	R	R	R	R	R	R
ILL.5244	S	R	R	R	R	R	R	R	R
lLL358	R	R	R	R	R	R	R	R	R
ILL.3537	S	R	R	R	R	R	R	R	R
Digger	S	S	S	R	R	S	R	R	R
Mansehra 93	S	S	R	R	R	R	R	R	R
Markaz-2001	S	S	S	R	R	R	R	R	R
Masoor-2002	S	S	S	S	S	S	S	R	R
Masoor-85	S	S	S	S	S	S	S	S	S

R = Resistant, S = Susceptible.

The reactions of the 10 lentil lines to individual isolates of *A. lentis* showed differences in pathogenicity among the isolates. This extremely high variability in the population of *A. lentis* makes it hard to classify pathotypes. More groups could be expected with additional differentials, taking in to account the heterothallic nature of the fungus (Kaiser & Hellier, 1993) and the recent discovery of mating type 1 in Canada (Kemal & Morrall, 1995). Isolate AL-1 can infect all the tested lines suggests the need for additional sources of resistance. Promising levels of resistance have been found in wild species of Lens (Bayaa *et al*., 1994).

Some of these authors designated the pathogenic groups as races, while others 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). Although genetic diversity in isolates was observed and these could broadly be classified into two groups, yet a clear-cut host-pathogen reaction was not observed. This situation did not favour the nomenclature of race rather variation in pathogenicity. This problem might be associated with complex nature of gene-action involved in Ascochyta

resistance (Malik, 1990).

ILL 358 reacted as resistant to all the isolates, which were the confirmation of our data with results of Ahmed and Morrall (1995), and Ahmed *et al*. (1996a). Differences between these results and those of the other authors may be due to the occurrence of different fungal populations in Canada and Australia or to differences in experimental conditions. One dominant gene (Rall) has been identified in ILL 5588 (Tay, 1989; Andrahennadi, 1994) and this line was resistant to all 84 isolates tested by Ahmed *et al*. (1996b). Two dominant genes (Rall, Ra12) have been identified in ILL 5684 (Tay, 1989; Andrahennadi, 1994) and this line was also identified as resistant by Ahmed *et al*. (1996b), but against Australian isolates of *A. lentis* it is susceptible (Nasir & Bretag, 1997). Similarly, Precoz has been identified as a resistant line in Pakistan (Iqbal *et al*., 1990) but is highly susceptible in Canada (Ahmed *et al*., 1996b). These differences in reactions clearly demonstrate the existence of pathogenic variability within populations of *A. lentis* from different countries. Bio-chemical and molecular approaches have been used successfully to provide additional information on the variability of the Ascochyta blight fungus of chickpeas (Weising *et al*., 1991) and the same techniques may be used to compare isolates of *A. lentis*. In addition to biological pathotyping, work on genotyping (DNA fingerprinting) has been recommended in order to compare the genetic variability among the isolates of *A. lentis*.

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