

Studies on the Pathogenic Variability in *Ascochyta pisi*

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ABSTRACT

Cultural and morphological characteristics of 10 isolates of *Ascochyta pisi* (Lib.) collected during winter 1997-98 from farmers' fields located in Murree, Lahore, Faisalabad, Hazro, Sialkot, Khanewal, Sheikhpura, Rawalpindi and Islamabad were studied. It was observed that isolates of districts Sheikhpura and Lahore were fast growing and the maximum mean mycelial growth was 73.4 and 71.8 mm, respectively, after three weeks of inculcation. Abundant pycnidia and pycnidiospores were obtained from the isolates of Murree and Islamabad, respectively. Reaction of eight pea genotypes to ten isolates of *A. pisi* exhibited a considerable degree of variability in disease reaction. Based on disease reaction six pathotypes of *A. pisi* were identified viz, Race-1, Race-2, Race-3, Race-4, Race-5 and Race-6. All the genotypes showed susceptible response to the Race-1 the most virulent one, whereas the least virulent was Race-6.

Key Words: Pea; *Ascochyta pisi*; Blight; Pathogenic variability; Isolates; Resistance

INTRODUCTION

Pea crop is grown in Pakistan on an area of 142.9 thousands hectares with a production of 78.3 thousand tonnes (Anonymous, 2003). Its national average yield 551 kg/ha is considered to be the lowest in the world. Besides other causes of low yield, diseases play a major role. The first description of the organism associated with pea blight was made in Europe by Liberto in 1830 who designated it as *Ascochyta pisi* Lib. Further descriptions were made by Berkeley (1841). *Ascochyta* blight is a serious pea crop disease, which caused severe losses in the past. Bottcher (1954) reported the yield losses in Germany up to 25 to 75%. In Canada during 1974, yield losses were 50 to 75% due to the disease (Wallen, 1974).

Up to now five physiologic races of *A. pisi* have been reported from the United States, the USSR and Canada. In Canada, the four races identified were generally localized in separate pea growing districts (Wallen, 1957). These races of *A. pisi* apparently vary geographically. Genetic study revealed that the resistance in a pea cultivar "Rondo" is practically dominant and is controlled by one recessive major gene. It gives plants a good level of resistance against the most prevalent strains of *A. pisi*. The resistance found in cultivar "Rondo" is effective against nearly all physiological strains of *A. pisi* and showed high heritability. Some strains can infect "Rondo" but new resistant lines can be found. Thus, the pathotypes can be distinguished by different hosts (Cousin *et al.*, 1985). Vladimirseva *et al.* (1989) worked on the isolation and identification of races of *A. pisi* in Altai region. Out of 31 races of *A. pisi* found on peas, 14 were receded in the Altai region of the USSR during 1989-87. Among these, seven were found every year and races 7, 8 and 29 were predominant.

Pea crop suffered generally in the northern area of Pakistan by *A. pisi*, which has been first reported by Sattar in 1933. The disease is gaining importance day by day and can appear in an epidemic form at any time in Pakistan. So far, no significant work has been done on blight of pea. Therefore, this study was planned to identify the pathogenic variability of *A. pisi*, which will ultimately help in developing resistant varieties of pea against blight disease.

MATERIALS AND METHODS

Pea samples infected by blight were collected from Murree, Lahore, Faisalabad, Hazro, Sialkot, Khanewal, Gujranwala, Sheikhpura, Rawalpindi and Islamabad districts during 1997-98 in winter sown crops from farmer's fields and research stations. Pieces of diseased stems, leaves, pods, and seeds were surface sterilized with 0.1% mercuric chloride for one minute and washed thrice in distilled water, were plated on pea seed meal agar (PSMA) medium (40 g pea seed meal, 20 g dextrose, 20 g agar and 1 liter water). Single spore isolation was maintained on slants of PSMA medium. Cultural and morphological characters of ten isolates were studied on PSMA plates incubated at 20±2°C. Ten pycnidia and pycnidiospores were measured for each isolate for size of pycnidia and pycnidiospores with the help of stage and an ocular micrometer (Dollar & Gurcan, 1992). Colony diameter was measured at weekly intervals for three weeks. Data were analyzed by using complete randomized design.

For studying physiologic specialization, eight pea cultivars namely Meteor, Green feast, Samarina, Climaz, Nevona, Carawella, M-105 and 122A5 were used. Five seeds of each cultivar were sown in triplicate in plastic pots (6 x 12 x 6 cm). Inoculum of *A. pisi* isolates was multiplied

on PSMA medium. Twenty-five days old plants of each cultivar were inoculated separately by spraying spore suspension (5×10^4 spores/ml) prepared from 15 days old culture of each isolate in sterilized water. Lady hand sprayers were used for inoculation of each isolate and the plants inoculated with individual isolate were kept in separate humidity chamber for 48 h. Observations on disease incidence were recorded 15 days of inoculation according to 1-9 disease rating scale designated by Grewal (1984). Finally, the isolates were categorized into different groups by considering the disease reaction 1-3 as resistant and above 5, susceptible.

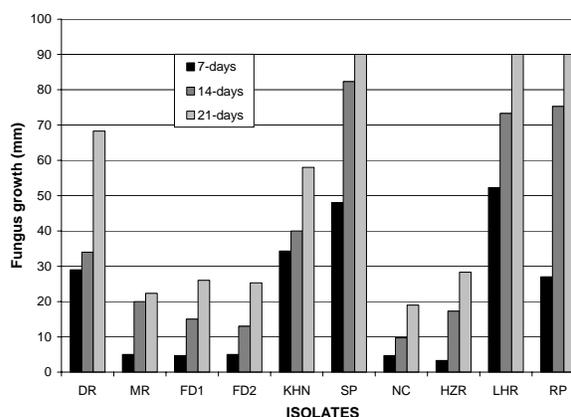
RESULTS AND DISCUSSION

The maximum mean mycelial growth of isolates SP and LHR is statistically non-significant with 73.4 and 71.9 mm, respectively after 21 days followed by RP, 64.1 mm, KHN, 44.2 mm and GR, 43.8 mm. Rest of the isolates viz; HZR, MR, FD-1 FD-2 and NC showed the least and statistically at par mycelial growth of 16.3, 15.8, 15.2, 14.4 and 11.1 mm, respectively (Fig. 1). Interaction between number of days and mean isolate growth of 90 mm after 21 days and the growth of LHR, SP and RP isolates after 21 days was statistically at par with mycelial growth of isolate SP 82.3 mm after 14 days and growth of isolate SP was statistically at par with isolate RP after an interval of 14 days. Isolate HZR, MR, FD-1, FD-2 and NC showed the least and almost at par mycelial growths after 7, 14 and 21 days, respectively.

Data on cultural and morphological characteristics of the aforementioned 10 isolates of *A. pisi* in respect of pycnidial formation, size of pycnidia and pycnidiospores revealed that abundant formation of pycnidia were obtained on isolate MR (Table I). Intermediate pycnidial formation was observed in isolates RP, NC, HZR and LHR while the least pycnidial formation was observed in the isolates SP, KHN, FD-1, FD-2 and GR.

The maximum size of pycnidia and pycnidiospores was obtained from isolate RP (233 x 253 μm) and FD-1 (5 x 11.8 μm), respectively. Size of pycnidia of isolates SP, MR and NC i.e. 238 x 240, 236 x 240 and 229 x 245 μm , respectively and size of pycnidiospores of these three isolates i.e. 4.5 x 10.9, 5 x 11.7 and 4.5 x 11.9 μm , respectively was statistically non significant. There was statistically significant difference between sizes of pycnidia of isolates FD-2, KHN, HZR and LHR with sizes of 232 x 230, 221 x 235, 217 x 226 and 207 x 217 μm respectively, however, sizes of pycnidia of isolate FD-1 and GR (210 x 220 & 205 x 230 μm) which were showing statistically significant differences in size of pycnidiospores, of all the rest eight isolates were statistically non significant. Our results are similar to the findings of Susuri (1984) about size of pycnidia and pycnidiospores which indicated that the differences in size of pycnidia and pycnidiospores of

Fig. 1. Comparison of radial growth of different isolates of *Ascochyta pisi* on pea seed meal agar medium



different isolates of *A. pisi* exist in nature. The reaction of eight pea genotypes to ten isolates of *A. pisi* indicated that cultivars Meteor, Carawella and 122A5 showed susceptible reacting to all the isolates tested while remaining six cultivars acted as differential and showed a considerable degree of variation in disease reaction with different isolates (Table II). Isolates of *A. pisi* greatly varied in their pathogenic variation on eight different genotypes. The variation supports the evidence that the races exist in *Ascochyta* spp. (Sangwan, 1989).

Based on disease reaction production by ten isolates on eight different cultivars of pea, six groups were identified viz, Race-1 (KHN), Race-2 (SP & FD-1) Race-3 (HZR & MR), Race-4 (NC & RP). Race-5 (GR & LHR) and Race-6 (FD-1). All the genotypes showed susceptible response to the Race-1, thus this race was the most virulent, whereas the least virulent was Race-6, because most of the genotypes exhibited resistant response toward this race.

The findings of the study are coinciding with that of Wallen (1957) who has reported races of *A. pisi* generally localized in separate pea growing districts, which vary geographically. The present study also supported the findings of previous workers (Cousin *et al.*, 1985). Vladimirseva *et al.* (1989) based on the pathogenic behavior of the isolates of *A. pisi*, identified 31 races of the fungus. Our results emphasize the need for upto date knowledge of physiologic races prevailing in different regions to develop pea varieties having stable resistance towards blight disease.

The present study indicated that there is a significant variability in *A. pisi* and also existed a sufficient genetic variation and resistance to *Ascochyta* blight in the pea cultivars that can be exploited for disease control. Keeping in view the findings of present study, the resistant sources should be incorporated in prevailing pea varieties through sound breeding programme so that resistant varieties of pea against *Ascochyta* blight could be obtained.

Table I. Characterization of *Ascochyta pisi* isolates on the basis of morphological characteristics (size of pycnidia and pycnidiospores)

Isolate	Locality of collection	Pycnidial formation	Size of pycnidia (μm)	Size of pycnidio- spores (μm)
DR	Gujranwala	++	205 x 230 f	4.6 x 11.7 ab
MR	Murree	++++	236 x 240 b	5 x 11.7 ab
FD-1	Faisalabad	++	210 x 220 f	4.7 x 11.9 ab
FD-2	Faisalabad	++	232 x 230 c	5 x 11.8 a
KHN	Khanewal	++	221 x 235 d	4 x 11.5 ab
SP	Sheikhupura	++	238 x 240 b	4.5 x 10.9 ab
NC	Islamabad	++++	229 x 245 b	4.5 x 11.9 ab
HZR	Hazro	+++	217 x 226 e	5.5 x 10 ab
LHR	Lahore	++	207 x 217 g	4.8 x 10 b
RP	Rawalpindi	+++	236 x 240 b	5 x 11.7 ab

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

** ++ (Fair), +++ (Good), ++++ (Abundant)

Table II. Reaction of ten isolates of *Ascochyta pisi* on eight pea cultivars

Cultivars	Isolates									
	GR	MR	FD-1	FD-2	KHN	SP	NC	HZR	LHR	RP
Meteor	S	S	S	S	S	S	S	S	S	S
Green feast	S	S	S	R	S	S	S	S	S	S
Samarina	R	S	R	R	S	R	S	S	R	S
Climax	R	S	R	R	S	R	R	S	R	R
Nevona	S	R	R	R	S	R	R	R	S	R
Carvella	S	S	S	S	S	S	S	S	S	S
M-105	R	S	R	R	S	R	S	S	R	S
I22A5	S	S	S	S	S	S	S	S	S	S

* R = Resistant, S = Susceptible

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