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IDENTIFICATION OF PATHOTYPES IN *ASCOCHYTA RABIEI* (PASS.) LAB., THE CAUSE OF CHICKPEA BLIGHT IN PAKISTAN

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Abstract

One hundred and two isolates of *Ascochyta rabiei* differing in their cultural characters were selected for pathotype analysis from a large collection of isolates obtained from chickpea growing areas of Pakistan during 1984 - 92. On the basis of pathogenicity on 11 chickpea differentials, the isolates were classified into 8 pathogenic groups. Out of 102 isolates tested, only 6 were highly virulent, 8 least virulent whereas others were in between the two extremes.

Introduction

Chickpea (*Cicer arietinum* L.), an important pulse crop is cultivated over 1 m ha in Pakistan with an average yield of 550 kg/ha (Anon., 1991). It is a major source of protein (20 - 26%) for the poor sections of the population in the country. Chickpea blight caused by *Ascochyta rabiei* (Pass.) Lab., first reported from North Western province of United India in 1911 (Butler, 1918) has been appearing in epidemic proportions after every 5 to 8 years in Pakistan where the epidemic of 1979-80 alone produced a loss of US$ 90 millions (Malik & Tufail, 1981). Continued cool and wet weather particularly at the time of flowering and fruiting may result in total loss of crop (Kaiser, 1973; Hafiz, 1986). Although some fungicides have been reported to control the disease but their use at commercial level is uneconomical and not possible under epiphytotic conditions (Reddy & Singh, 1983).

Breeding for disease resistance/tolerance may control the disease, but host resistance does not persist for a long time. Chickpea blight resistant varieties viz., F8, C12/34, C612, C727, C235, AUG480 (Kauser, 1965; Nene & Reddy, 1987) lost their resistance with the passage of time, either due to genetic breakdown or due to change in the pathogen in the form of new strains or races (Anon., 1963; Reddy & Singh, 1983). It necessitates a continuous breeding programme for evolving resistant varieties after careful screening against the virulent isolates of the pathogen. Cultural variations in growth, morphology and sporulation of *A. rabiei* (Luthra *et al.*, 1939; Aujla, 1964; Kaiser, 1973) as well as pathogenic variability on different chickpea cultivars has been reported with isolates from India (Aujla, 1964; Grewal, 1984), Iran, Pakistan and Turkey (Kaiser, 1973, Hussain & Malik, 1991).

For the identification of resistant/tolerant chickpea genotypes against the prevalent pathotypes/strains or races of *A. rabiei,* information about the pathotypes/strains or races in a particular region is necessary in the breeding programme. Experiments were

Table 1. Pathogenic groups of *Ascochyta rabiei* Isolates.

Pathogenic groups		Disease rating range	Name of the isolate and location
1	(2)*	6.1-6.5	A-21(Talagang, Fatehjang); A-23(Chakwal Farm).
2	(4)	5.6-6.0	A-4, A-8, A-10 (NARC); Italy-1.
3	(16)	5.1-5.5	56, A-12, A-19, AB48, (NARC); B-18 (Dali Shahar near Kundian); Tarquina (Italy); B-7 (Chak 46, Bhakar); 54-A (Kot Feteh Khan, Fateh Jang); 46 (Attock Bridge); 45-B (Attock farm); Italy -5; Italy-3; N-18 (Dadhyal, Distt. Chakwal); N-25 (Sang Kalan, Distt. Chakwal); Ab38 (Bhown Farm, Chakwal); AB39, (Govt. Barani Farm, Chakwal).
4	(25)	4.6-5.0	A-7, A-11, N-34, AB47, (NARC); 34-F (Kaloor Kot Seed Farm); B-23 (Roda, Khushab); 36 (Mapel Sharif, Bhakar); B-3 (Chak Mohan Wala); B-6 (Mankera); 53-A (Dhok Pelian, Feteh Jang); 73 (Chak Loya, Athara Ha zari); KB-89 (Kaghan); N-8 (Moona, Distt, Chakwal); N-2, AB61 (Kundian); N-3, AB-45, (Distt. Attock); N- 4 (Choai Bungla Basali Road, Attock); N-27 (Chak Bazair Distt. Chakwal); N-13 (Kundian Farm); AB-4, AB-5, AB-6 (Chakwal).
5	(37)	4.1-4.5	B-17 (Piplan Seed Farm); B-19 (Nawan Jandan- wala, Bhakar); A-9, AB-22, AB-46, AB-49, (NARC); Italy-4; B-2 (Chak Shakarwala); Italy-2, N-33 (Adhi Kot Distt. Khushab); N-20, N-24 (Govt. Farm Kaloor Kot); N-6, AB43, AB44 (Attock); N-10 (Roda, Distt. Khushab); N-32, AB-42 (AB-42 (Bhown Farm Distt. Chakwal); N-5 (AECF Kundian); N-9, N-16 (Kot Fateh Khan Distt. Attock); N-29 (Nurpur Thal); P-9, P-10 (Peshawar); AB-1 (Kalar Kahar). AB-3 (Chakwal), AB-7, AB-8, AB-9, AB-10, (Attock); AB-11, (NIFA); AB-16, AB-20, AB-21 (Rawalpindi), KB-91-1 (Kaghan), AB-50, AB-55 (Karak).
6	(10)	3.6-4.6	D-Institute (NIAB); N-7 (Bhown Farm, Chakwal); N-12, (NARC); P-16 (Peshawar); AB-19 (Mianwali); KB-91-2 (Kaghan); AB-51, AB-56, (Karak); AB-57 (Banu); AB-64 (Layyah).
7	(7)	3.1-3.5	N-17, N-23 (Govt. Kaloor Kot Farm); N-14, (Fatehjang); N-21 (Kalar Kahar); AB-17, AB-23 (NARC); AB-60 (KNC, Kundian).
8	(1)	2.6-3.0	N-11 Warisabad, Groat Road, Distt. Khushab).

*Figures in Parenthesis are the number of isolates under each pathogenic group.

therefore, carried out to determine the existence of pathotypes in *A. rabiei* prevalent in different chickpea growing areas of Pakistan. Information about the variability of pathotypes will facilitate the selection of suitable chickpea cultivars resistant/tolerant to specific pathotypes to be grown in a specific region.

Materials and Methods

Collection, isolation and purification of fungal cultures: Chickpea grown in different geographic regions of the country was surveyed from 1984 to 1992 and blight infected plant material collected. The fungus was isolated as single spore pure culture. Based on variability in colony colour, growth and sporulation, 102 isolates were selected for further studies. *A. rabiei* cultures were grown on chickpea seed meal agar at 20°C in 250 ml flasks (Kaiser, 1973; Bedi & Aujla, 1970; Chauhan & Sinha, 1973). Sterilized water was poured in 10 day old cultures, mixed with a sterilized spatula and the spore suspension filtered through a double layer of muslin cloth was adjusted to 10^6 cfu/ml. One drop of Tween-20 as wetting agent was added per 100 ml of spore suspension to prevent run-off of inoculum from plant surface. Each isolate was tested for its pathogenicity on a set of 11 chickpea genotypes.

Pathogenicity test: Seeds of 11 chickpea genotypes viz., Aug-424, Pb-1, 6153, C727, C235, Aug480, C44, CM2, CM72, CM88 and ILC191 were treated with 0.2% benlate and grown in 12" diam., earthen pots filled with sterilized soil @ 5 seeds/pot. The pots were kept in the field during the crop growing season. At flowering stage when the temperature was around 18 - 22°C, the healthy plants were inoculated with spore suspension (10^6cfu/ml) of each isolate using an automizer until run off (keeping 3 pots/cultivar/isolate). One set of uninoculated differential host cultivars was kept as control. The inoculated plants were kept under 95 - 100% RH for 48 h after inoculation. The high humidity was maintained by keeping the pots under iron cages covered with wet coarse cloth and by frequent sprinkling of water both inside the cages and on the coarse cloth.

Disease scoring: Infection of leaf, branch, pod, number of stem girdlings and broken stems including size of lesions were recorded at 15 - 20 days after inoculation. Disease reaction of each isolate on chickpea genotypes was recorded on 1 - 9 scale developed for *A. rabiei* (Reddy *et al.*, 1984).

Results and Discussion

Although cultural differences among various isolates were observed but these could not be correlated with the virulent forms of the isolates, as also reported by Jan & Wiese (1991). Based on average disease reactions on chickpea genotypes, the isolates were divided into 8 pathogenic groups (Table 1).

Differential disease reaction of different chickpea cultivars to various isolates of *A. rabiei* confirmed the existence of pathogenic variability. The isolates falling under group 1 proved to be more virulent than others (Table 2). Isolates of group 2 were slightly less virulent than those of the preceding group, with a tolerant response of 3 genotypes and resistant response of 2 others, but more virulent than the succeeding

Table 2. Disease response of 8 pathogenic groups comprising 102 isolates of *Ascochyta rabiei* on individual chickpea differentials.

Pathogenic groups		Aug-424	Pb-1	6153	C-727	C-235	Aug-480	C-44	CM-2	CM-72	CM-88	ILC-191
1	(2)*	S	S	S	S	S	S	S	S	S	S	R
		(9)**	(8)	(7)	(5.75)	(6.25)	(6.5)	(6.5)	(6)	(5.75)	(5.75)	(3.5)
2	(4)	S	S	S	S	S	S	T	T	T	R	R
		(8.5)	(7.75)	(6.63)	(5.63)	(5.75)	(6)	(5.38)	(4.75)	(4.75)	(4)	(4)
3	(16)	S	S	T	T	T	T	T	T	R	R	R
		(8.54)	(7.46)	(5.46)	(4.85)	(4.96)	(5.46)	(5.31)	(4.23)	(3.96)	(4)	(3.43)
4	(25)	S	S	T	R	T	T	T	R	R	R	R
		(8)	(7)	(5)	(3.8)	(4.4)	(5)	(4.7)	(3.7)	(3.7)	(3.7)	(3.7)
5	(37)	S	S	R	R	R	T	R	R	R	R	R
		(7.83)	(6.27)	(3.92)	(3.78)	(3.78)	(4.53)	(4.08)	(3.17)	(3.47)	(3.42)	(3.22)
6	(10)	S	T	R	R	R	R	R	R	R	R	R
		(7.75)	(4.88)	(3.38)	(3.25)	(3.25)	(3.88)	(3.63)	(3)	(3)	(3.25)	(2.63)
7	(7)	T	T	R	R	R	R	R	R	R	R	R
		(4.13)	(4.13)	(3.63)	(3.5)	(2.63)	(3.88)	(3.5)	(3.25)	(3.25)	(3.13)	(2.88)
8	(1)	S	R	R	R	R	R	R	R	R	R	R
		(6)	(4)	(2)	(2.5)	(3)	(4)	(3)	(1)	(3)	(2.5)	(1)

*Number of isolates in each pathogenic group.

**Mean of disease ratings of isolates within a pathogenic group against individual differential.

R (Resistance) = 1.0 - 4.0, T (Tolerant) = 4.1 - 5.5, S (Susceptible) = 5.6 - 9.0

groups. Similar trend was observed in all the remaining groups. Isolate N-11 of Group 8 appeared to be least virulent since it failed to induce disease even on the susceptible chickpea differentials. Very little differences between the isolates of group 7 and 8 were observed, as on most of the genotypes the disease reactions were similar (Tables 1&2).

A large number of isolates falling in group 3, 4 and 5 (5th being the largest comprising of 37 isolates) showed moderate reaction whereas the number of highly virulent isolates (Group 1) and least virulent isolates (group 8) was very small. Most virulent isolates (group I) were collected from north western parts of the country presumably due to cool wet weather conducive for the development of the disease. It could also be due to intensive cultivation of more diverse genotypes (Gowen *et al.*, 1989). There seems to be no correlation between pathogenicity level and geographic distribution or ecological conditions of the isolates, as is evident by the grouping of isolates belonging to widely separated sites in the same pathogenic group (Table 1). Such dislocation may result from concurrent but separate development of the virulence forms, dispersal of infected seed or transport of other infested material (Jan & Wiese, 1991). Similar observations on the existence of pathogenic variation in *A. rabiei* has been reported (Aujla, 1964; Bedi & Aujla, 1969; Kaiser, 1973; Vir & Grewal, 1974; Grewal, 1984; Reddy & Kabbabeh, 1985; Porta-Puglia *et al.*, 1985, Porta-puglia, 1989; Nene & Reddy, 1987; Gowen *et al.*, 1989; Jan & Wiese, 1991; Hussain & Malik, 1991). There is need to develop a uniform testing system and naming the pathogenic variants since different terms like race (Aujla, 1964), physiologic races (Bedi & Aujla, 1969), strains (Kaiser, 1973) and pathogenic variants as groups of isolates (Vir & Grewal, 1974) have been used. An assortment of 50 isolates collected in Syria and Labanon were grouped into 6 races according to their aggressiveness against a set of host cultivars (Reddy & Kabbabeh, 1985; Weising *et al.*, 1991). Similarly Porta-Puglia *et al.*, (1985) confirmed the existence of pathogenic variability in *A. rabiei* in Italy and they called the variants as pathogenic groups.

Lack of uniform nomenclature and testing procedures resulted in adoption of different methods by various investigators to study the pathogenic variability of the fungus. Host differentials, inoculum concentration, inoculation techniques, environmental conditions were variables, even the rating scales used for disease assessment varied (Nene, 1984; Reddy *et al.*,1984). Therefore, Haware (1987) considered that the differences in host reaction were not clear enough to classify the isolates into race.

In the present study the *A. rabiei* isolates showed difference in their virulence but the different pathogenic groups could not be designated as races because uniform techniques and host differentials for race identification in *A. rabiei* are not yet available as also suggested by Jan & Weise (1991). There is need for the adoption of an internationally recognized standard technique, uniform set of host differentials for studying pathogenic variability and uniform rating scales for disease assessment. The application of molecular marker techniques for genotyping of isolates from each pathogenic group has been initiated which will supplement our present work on biological pathotyping and will enable us to clearly identify the physiologic races if exist in *A. rabiei* isolates prevalent in Pakistan.

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