Effect of Temperature and pH on Morphological and Cultural Variation of *Ascochyta rabiei*, the Blight Pathogen of Chickpea in India

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Abstract

Ascochyta rabiei, causal agent of blight in chickpea is a highly variable pathogen because of the existence of two mating types. Study was conducted on the temp and pH dependent cultural and morphological variation among seven *A. rabiei* isolates from the north western plain zone (NWPZ) and north hill zone (NHZ) of India. The optimum temp and pH for the growth of majority of the isolates studied was 20-25 C and 6.0-7.0, respectively. The isolates differed in their colony colour, mycelial intensity, pycnidia and pycnidiospore production and these variations were characteristic of each isolate with respect to temp and pH.

Key words: *Ascochyta rabiei*, cultural characters, pH and temperature

Citation: Basandrai KA, Pande S, Kishore GK and Sharma M. 2007. Effect of temperature and pH on morphological and cultural variation of *Ascochyta rabiei*, the blight pathogen of chickpea in India. 254-258.

Chickpea (*Cicer arietinum* L.) is one of the important post-rainy season legume crops in the sub- tropical environments of western Africa and northern Asia (WANA) region, and Indo Gangetic plains (IGP) of India and Pakistan. The winter rains coupled with low temp during the crop season prolongs the vegetative and reproductive periods of the crop. These conditions are also favourable for the development of foliar diseases, of which *Ascochyta* blight is of primary concern. The disease caused by fungus *Ascochyta rabiei* (perfect stage *Mycosphaerella rabiei* (Syn. *Didymella rabiei*) Kovachevski) Arx is endemic in most of the WANA and IGP countries (Pande et al 2005). *A. rabiei* is considered as a highly variable fungus as the sexual reproduction involves two mating types, *MAT-1* and *MAT-2* reported from majority of the chickpea growing areas globally (Armstrong et al 2001; Kaiser and Kusmenoglu 1997). The sexual recombination leads to rapid evolution of the fungus that cause frequent breakdown of host plant resistance (Malhotra et al 2003; Pande et al 2005). Frequent occurrence of *Ascochyta* blight prompted us to characterize different pathotypes or races among the *A. rabiei* isolates. Morphological and cultural variation of *A. rabiei* isolates from the NWPZ and NHZ with respect to growth, temp and pH has been reported.

Materials and Methods

Ascochyta rabiei was isolated from infected chickpea plants in seven locations of NWPZ viz, Ambala [(Ar (AMB)]/ Ganganagar [(Ar (GGR)], Dhaulakuan [(Ar (DLK)], Gurdaspur [(Ar (GDP)], Hisar [(Ar (HIS)], Ludhiana [(Ar (LUD)] and Pantnagar [(Ar (PNT)]; one location in north hill zone (NHZ) Palampur [(Ar (PMR)]. Single spore isolates of individual culture was used to study the morphological and cultural variation. From actively growing culture of individual isolates of *A. rabiei,* a 5-mm disc was inoculated at the centre of a 90-mm petri plate with chickpea dextrose agar media (chickpea meal extract - 40g, dextrose - 20g and agar - 15g /l, pH 5.5). Inoculated plates were incubated separately and kept at 5, 10, 15, 20, 25, 30 and 35 C to determine the effect of temp on fungal growth. To determine the effect of pH, the cultures were inoculated on chickpea dextrose agar with pH adjusted to 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 using 1N HCl or NaOH, and incubated at 20 C with 12 h photoperiod. Each treatment consisted of three replications arranged in a completely randomized block design.

Inoculated plates were regularly observed for colour of the colony, pycnidia and mycelium, mycelial intensity (thickness; measured as '+', to '+++++'). The radial growth was measured in mm in two different directions perpendicular to each other, up to 14 days after inoculation (DAI). For quantification of pycnidia, a 1-cm diam disc was cut at 1-cm distance from the center of 14 –d- old cultures and observed under Gallen kamp colony meter magnifying glass. The same disc was macerated in 10 ml of sterile distilled water and the spore suspension so obtained was observed under a

photomicroscope using a haemocytometer and pycnidiospores/ml of water were counted. The experiments were conducted in three replications and repeated twice. Data from different experiments were subjected to ANOVA using Genstat 5 statistical package.

Results and Discussion

Variation in colony, mycelial and pycnidial colour. The seven *A. rabiei* isolates tested varied in their colony colour and mycelial intensity with respect to incubation temp (Table 1). The pycnidial colour remained as black or brown and mycelial colour as off- white or light cream in all the treatments. In general, colonies of all the *A. rabiei* isolates were gray with varying colour intensities depending on pH. Isolates Ar (LUD) and Ar (PNT) had deep gray colonies with dark brown centers at all pH levels. Pycnidia of all the isolates varied from gray to brown in colour depending on the pH (Table 2).

The colour of the mycelium remained off-white or light cream in all the isolates at all the incubation temp, while it varied from off-white to gray at different pH levels. Isolates Ar (AMB) and Ar (PMR) developed ash coloured abundant mycelium at different pH levels.

Variation in radial growth. The optimum growth temp was 20 C for all the isolates and the growth at temp above and below the optimum, varied with respect to the isolate (Table 3). The radial growth of all the isolates was significantly ($P = 0.05$) less at 15 C than at 20 C. At 25 C, the radial growth of four isolates Ar (HIS), Ar (DLK), Ar (PMR) and Ar (LUD) was comparable ($P = 0.05$) to that of at 20 C. All the isolates failed to grow at 35 C. Isolate Ar (HIS) showed statistically more ($P = 0.05$) radial growth even at 10 and 30 C. Radial growth of all the isolates did not differ significantly ($P = 0.05$) within a pH range of 5.5 to 7.5, but was significantly less at pH 8.0 compared to neutral pH.

Pycnidia and pycnidiospore production. In contrast to the temp requirement for radial growth, all the *A. rabiei* isolates, except for Ar (DLK) had the highest pycnidial production at 25 C, which drastically dropped by >10-folds at 30 C (Table 3). At 5 and 35 C, no pycnidia were formed in any of the *A. rabiei* isolates tested. Pycnidiospore formation was almost similar in Ar (GDP) and Ar (DLK) at 20 and 25 C, while in the other five isolates the number of pycnidiospores formed at 20 C were more than 50% compared to that at 25 C. Marginal or no pycnidiospore formation was observed in all the isolates at 5 and 35 C.

Table 1. Morphological and cultural characters of *Ascochyta rabiei* isolates at different temperatures

Temp. (C)	Colony colour	Mycelium intensity
Ar (AMB)		
10	Gray with green hue	++++
15	Brown with greenish hue	+++++
20	Gray brownish with green hue	+++
25		+++
30	Light margin, jet black centre	+++
Ar (DLK)		
10	Light with greenish hue	+++++
15	Gray	+++
20	Gray with greenish brown hue	+++
25	Brownish with greenish hue	+++
30	Pink light margins, slate gray centre	++++
Ar (GDP)		
10	Gray with green hue	++++
15	Gray with green hue	++++
20	Gray with greenish hue, centre black brown	+++
25	Blackish brown with greenish hue,	+++
30	Brown with blackish centre, next line	+++
Ar (HIS)		
10	Light	++
15	Reddish , dark brown centre.	++
20	Light margin, with steel gray centre	++
25	Jet black with concentric rings	+++
30	Pinkish with irregular margins	++++
Ar (LUD)		
10	Gray with greenish hue	++
15	Light	++++
20	Light with greenish hue	+++++
25	Light gray	++++
30	-	
Ar (PNT)		
10	Light with greenish hue	+++
15	Light with greenish hue	++++
20	Black pycnidia gray with light green hue.	++
25	Gray	+++
30	Centre dark brown to light gray	++
Ar (PMR)		
10	Gray coloured	+++
15	Gray coloured light red	++++
20	Light reddish	+++
25	Light with greenish hue	++++
30	Dull pink	++++

- Data not available; Ar (AMB) = Ambala, Ar (DLK) = Dhaulakuan, Ar (GDP) = Gurdaspur, Ar (HIS) = Hisar, Ar (LUD) = Ludhiana, Ar (PNT) = Pantnagar, Ar (PMR) = Palampur

Table 2. Cultural and morphological characters of seven isolates of *Ascochyta rabiei* at different pH levels

pH	Colony color	Pycnidial color	Mycelial intensity
Ar (HIS)			
5.5	Gray, dark brown centre with green hue	Gray to brown	++
6.0	Gray	Brown	+++
6.5	Gray with slate black centre	Gray to brown	+++
7.0	Dark gray with dark brown centre	Steel gray	+++
7.5	Gray	Brown	+++
8.0	Gray with brown black centre	Brown	++++
Ar (LUD)			
5.5	Dark gray with dark brown centre	-	+++
6.0	Light gray	Light brown to brown	++++
6.5	Light gray	Gray to brown	++++
7.0	Light gray		+++
7.5	Gray	Steel gray	+++
8.0	Dark almond	Grayish	+++
Ar (DLK)			
5.5	Dark gray	Gray to brown	++
6.0	Dark gray with blackish brown centre with green hue	Gray to brown	++
6.5	Dark gray, with blackish brown centre with brownish hue	Brown to gray	++
7.0	Dark gray, with blackish brown centre	Slate gray	++
7.5	Light gray with brown centre	Slate black	++++
8.0	Dark gray with greenish hue	Slate gray to brown	++++
Ar (PNT)			
5.5	Black	Black	+++
6.0	Dark	Slate gray to brown	++++
6.5	With blackish brown centre	Slate gray to brown	+++
7.0	Gray with dark brown centre	Slate gray to brown	+++
7.5	Light , dark brown with greenish hue	Brown to black	+++
8.0	Light gray	Black	++++
Ar (PMR)			
5.5	Dark	Gray to brown	+++
6.0	Dark grayish	Gray to brown	++++
6.5	Dark gray	Gray to brown	+++
7.0	Steel gray, gray	Gray to brown	++++
7.5	Gray	Gray	++++
8.0	Light	Light slate to brown	++++
Ar (GDP)			
5.5	gray with jet black centre	Gray	+++++
6.0	Jet black in centre, brown with green hue in margin	Gray	+++++
6.5	Steel gray	Gray	+++++
7.0	Black in centre and light gray on periphery	Gray	+++++
7.5	Blackish brown	Gray	++++++
8.0	Grayish brown	Gray	+++++
Ar (GGR)			
5.5	-	-	
6.0	Light gray, brown centre	brown	+++
6.5	Gray with greenish hue, with brownish centre	Gray	++++
7.0	Gray, centre blackish brown with green hue	Gray brown	++++
7.5	Gray with brown centre, green hue	Gray to brown	+++
8.0	Gray with greenish hue	Black	+++

- = Data not available; Ar (HIS) = Hisar, Ar (LUD) = Ludhiana, Ar (DLK) = Dhaulakuan, Ar (PNT) = Pantnagar, Ar (PMR) = Palampur, Ar (GDP) = Gurdaspur, Ar (GGR) = Ganganagar

Pycnidiospore formation (101.1 x 10^3 pycnidiospores/ml) was high in numbers in the isolate Ar (HIS) grown at 10 C. Among all the treatments, a max. pycnidia were observed in Ar (PNT) grown at 25 C, while a max. of pycnidiospores were observed in Ar (HIS) incubated at 20 C. Isolate Ar (HIS) produced the statistically more number of pycnidiospores/ ml even at 10 and 30 C also. This was found to be the highly virulent isolate (Basandrai et al 2005).

Pycnidia and pycnidiospore formation in the seven *A. rabiei* isolates varied with respect to pH, and the production was max. within a pH range of 6.0-7.0, except in isolates Ar (HIS) and Ar (PNT), that produced a max. number of pycnidia at pH 5.5. These isolates produced max. pycnidiospores at pH 5.5 (Table 4).

Table 3. Variation in growth and production of pycnia and pycniospores of *Ascochyta rabiei* isolates at different temperatures

Isolate	5 C	10 C	15 C	20 C	25 C	30 C	35 C
Radial growth (mm)							
Ar (GDP)	3	24	42.3	58.3	48.7	33	0
Ar (AMB)	3	31	38	56.2	39.7	22	0
Ar (HIS)	3	35.7	48.3	63	58.7	37	0
Ar (DLK)	7	26	42.3	62	60	26	0
Ar (PNT)	11	22.3	38.3	56.7	55.7	22	0
Ar (PMR)	0	28	43.7	65	52.3	25	0
Ar (LUD)	16	27.3	39.7	55.3	51	24	0
CD ($P = 0.05$)				8.27			
Pycnidia/cm^2							
Ar (GDP)	-	0	13.7	82.3	51.3	2.3	-
Ar (AMB)	-	12.7	20.3	46	51.3	0	-
Ar (HIS)	-	15.3	65.7	77.7	90.7	8.7	-
Ar (DLK)	-	11.7	49	90.7	52.7	3	-
Ar (PNT)	-	18.7	25.7	57.7	130	6.3	-
Ar (PMR)	-	7.7	18	42.3	53.7	6	-
Ar (LUD)	-	19	47.3	49.7	73	0.3	-
CD (P = 0.05)				13.8			
Pycnidiospores (x 10^3)/cm^2							
Ar (GDP)	-	0	4.5	57.2	58.3	0.2	-
Ar (AMB)	-	2.5	6.8	54.7	34.5	0	-
Ar (HIS)	-	101	119	301	156	0	-
Ar (DLK)	-	0.3	3.7	71.4	68.1	0	-
Ar (PNT)	-	0.5	46.2	84.3	44.5	0.8	-
Ar (PMR)	-	0.8	14.3	183	108	1.5	-
Ar (LUD)	-	25.7	32	97.1	47.2	0	-
CD ($P = 0.05$)				38			

- = No pycnidial and pycnidiospores formation in any of the isolates at 5 C and 35 C

Table 4. Effect of pH on pycnidial production in different isolates of *Ascochyta rabiei*

Isolate	5.5	6	6.5	7	7.5	8
Pycnidia/cm^2						
Ar (GDP)	34.7	26	34.7	23.3	49.3	31
Ar (GGR)	94	93	96.7	106	65.3	38.7
Ar (HIS)	100	95.3	76	97.3	88.7	57.7
Ar (DLK)	48.3	57.3	84.3	33.3	34.7	46
Ar (PNT)	106	68.3	87.7	74	83.7	65.3
Ar (PMR)	39	65.3	68.7	44.7	66.3	26
Ar (LUD)	73	83.3	66	70.7	66	41.3
CD ($P = 0.05$)			14.9			
Pycnidiospores (x 10^3/cm^2)						
Ar (GDP)	23	16.7	67.2	10	16.7	15.3
Ar (GGR)	69.8	32.7	49.5	76.5	31.5	58.7
Ar (HIS)	128	163	90.5	143	130	7.5
Ar (DLK)	36.3	56.2	97.8	28.3	56.2	19.7
Ar (PNT)	209	129	102	147	200	139
Ar (PMR)	69	143	129	92.8	135	27.1
Ar (LUD)	153	148	108	183	195	78.8
CD ($P = 0.05$)			22.5			

This study clearly indicated the effect of temp and pH on the cultural and morphological variability of *A. rabiei*. The optimum temp and pH required for the growth of majority of the isolates was in agreement with the earlier reports (Kaiser 1973; Ram and Mahinder 1993). As observed in the present studies, the pH level has been reported to influence the fungal growth, pycnidial formation and sporulation of different phytopathogenic fungi (Maheshwari et al 2000). In general, the fungi grow well between pH 4.0 - 8.8, but pH of 6.4 -7.6 has been found to be the best (Bedi and Aujla 1970). The fungus developed faster at pH levels between 5.5 - 7.5, and slowed at pH 8.0. Temp and pH are the two crucial components that influence the in vitro growth and various biological activities of microorganisms e.g. fungicide sensitivity (Shrestha et al 2005). These two parameters also influence the in vivo pathogenicity of *A. rabiei* (Chauhan and Sinha 1973).

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Accepted: Aug 18, 2007