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Pathogenicity Diversity in *Albugo candida* Isolates causing White Rust Disease in Rapeseed-Mustard

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A B S T R A C T

Albugo candida causing white rust of rapeseed-mustard is a highly variable fungus and cause great economic losses. In this study 44 *A. candida* isolates belongs to 17 different states of India has been used for the pathogenicity diversity. Symptomatology of the pathogen i.e. pustule pattern, pustule size and pustule colour has taken as criteria for the pathogenicity diversity. All the 44 *A. candida* isolates were inoculated on susceptible *Brassica juncea* cultivar Varuna for the study. In the study of white rust symptoms of 44 *A. candida* on Varuna cultivar five different types of pustule pattern has been observed viz. viz. Separate circular; Coalesced circular, Scattered and pin head; Restricted near veins and veinlets and Restricted near veins and veinlets separate or coalesced circular type pustules. Pustules size among 44 *A. candida* isolates was varied from 0.5-2.5mm while pustule colour of all the isolates was creamy white. The study very less pathogenicity diversity in pustule size and colour has been observed among 44 *A. candida* isolates, while in pustule pattern some diversity was observed.

Introduction

Oilseed brassica is one of the most important oilseed crop of tropical and subtropical countries. Rapeseed-mustard affected by different biotic stress, among them white rust caused by *A. candida* is a major disease, causing heavy economic losses. It is estimated that the floral and leaves infection can cause losses from 20-60 per cent (Kolte, 2002; Khunti *et al.*, 2003; Sachan *et al.*, 2004; Kumar and Kalha, 2005) and up to 36.88 per cent (Bal and Kumar, 2014) in mustard. White rust symptoms are characterised as local and systemic. In case of local infection, symptoms of the disease appear primarily on the lower surface of leaves and manifested by the appearance of minute or small, white or

creamy yellow raised scattered and circular pustules (1 to 2 mm in dia.) which later coalesce to form patches. Systemic infection symptoms include distortion, hypertrophy, hyperplasia and inflorescence sterility. This infection phase has been known as the stag head.

The flowers affected show malformation, the petals become green sepals and the stamens can be transformed into a leaf-like structure or a carpel.

The petals and stamens persist in the flower, as in normal flowers, rather than falling early. Sometimes the stamens become thick club-shaped sterile bodies. Usually the ovules and pollen grains are atrophied and result in incomplete sterility (Kolte, 1985). Yield loss is greater in case of systemic than local infection (Harper and Pittman, 1974; Petrie, 1973; Verma and Petrie, 1980). At maturity stage, stag heads are with thick-walled brown oospores that have been appeared to survive in dry form for a more than 20 years (Verma and Petrie, 1975).

A. candida isolates from different geographical regions and different host may be different in their incubation period, latent period and production of sporangia and zoospores, pustule size, shape and texture and aggressiveness (Lakra and Saharan, 1988; Gupta and Saharan, 2002; Patni *et al.,* 2005 and Mishra *et al.,* 2009). This indicated that there may be the possibilities of variability within *A. candida* isolates. The high variability of *A. candida* makes it a highly virulent pathogen, which is difficult to manage. The study of variability is needed to find out the status of variability, there occurrence and evolution pattern so that it will be helpful in the development of resistant varieties against the disease. Therefore, in this study pathogenicity variability of 44 *A. candida* isolates has been done.

Materials and Methods

Forty four *A. candida* isolates collected from Oilseed Pathology Laboratory, Department of Plant Pathology were belonging to 17 states of India and also from 10 different *Brassica* spp. The description of *A. candida* isolates used in the studies is given in the table below (Table 1).

Isolation and purification of white rust inoculum

The isolation and purification of all *A. candida* isolates were done on susceptible *B. juncea* cv. Varuna grown under glasshouse. The seedlings were raised in plastic pots containing sterilized soil. The fresh sporangial suspension was prepared with single pustule in a glass vial contained 1-2ml sterile distilled water. The suspension was mixed properly and kept it at 10°C for overnight for the release of zoospores. Although, freshly prepared sporangial suspension has also used for the inoculation without any incubation period. The sporangial concentration in the suspension was maintained about 2.5×10^5 zoosporangia per ml using haemocytometer. The seedlings at cotyledonary stage (7-8DAS) were inoculated with 10µl spore suspension through inoculating micropipette on each and every lobes of cotyledon of the seedlings. After inoculation plants were transfer to plant propagator box and kept the box in a dark room for 72hrs, with a relative humidity of more than 90 per cent and of 18 ± 2^0C temperature. After three days plants were taken out and kept in glasshouse where, proper temperature (18 ± 2^0C) and humidity (>80%) were maintained during the entire plant growth and disease appearance period. When disease appeared (10-15DAI), infected leave samples were collected before yellowing of the leaves and preserved at low temperature (4°C- 10°C) by

keeping it in butter paper bags for further studies. The pustule pattern of all 44 *A. candida* isolates were observed and recorded. Likewise, pustule dia. of 10 randomly selected leaves of an isolate were taken in mm. Analysis of mean pustule size of all 44 *A. candida* isolates has been done through Duncan's Multiple Range Test (DMRT) using SPSS 16.0 version software to find out whether there is significant difference between the mean pustule size. Pustule colour in symptomatic leaves of each isolate was observed and recorded.

Results and Discussion

The pathogenicity diversity of 44 *A. candida* isolates has been done on the *B. juncea* cv. Varuna. The colour of pustules of all the isolates was creamy white in colour. The pustule pattern of all the isolates has been observed visually and categorized in 05 different types of pustule pattern viz. Separate circular; Coalesced circular, Scattered and pin head; Restricted near veins and veinlets and Restricted near veins and veinlets separate or coalesced circular type pustules.

Nine showed separate circular type, 16 of coalesced circular type, 12 of scattered pin head type, 04 of restricted near vein and veinlet type and 03 of restricted near vein and veinlet separate or coalesced circular type of pustule (Table 2).

The variation in pustule pattern of 33 *A. candida* isolate indicated different level of pathogenicity diversity. The isolates that formed different pustule pattern could be of different pathotypes. The similar type of observations has been reported by Pandey *et al.*, (2013) where pinhead raised pustules were observed in isolates of Pantnagar, Delhi, Bharatpur, Hisar, Kangra, and Jammu, while pinhead scattered pustules in isolates of Ludhiana. Based on pustules pattern 33 *A. candida* isolates were grouped in 05 pathotypes.

In Duncan's multiple range test (DMRT) of mean pustule size of *A. candida* isolates varied from 0.5-2.5mm in dia. The lowest mean pustule size i.e. 0.5mm dia. was observed in Ac-MMP (*B. juncea* cv. Maya, Morena, MP) while, highest mean pustule size i.e. 2.5mm dia. was observed in Ac-BjV (*B. juncea* cv. Varuna, Pantnagar, Uttarakhand), Ac-Knp (Kanpur, UP) etc. The mean pustule size was 1.59mm dia. In DMRT of mean pustule size of 44 *A. candida* isolates, less variability has been observed (Table 3).

The similar type of observations has been reported by Pandey *et al.*, (2013) the small size pustules (0.5-1.5 mm) was observed in New Delhi and Hisar isolates of *A. candida*, while large size pustules (1-3 mm) in Pantnagar and Ludhiana isolates of *A. candida*.

In this study pathogenicity diversity of *A. candida* isolates has been observed based on symptomatological observations. The significant diversity has been observed in pustule pattern of different *A. candida* isolates which could be utilized for the further study. While, in case of pustule colour and pustule size of *A. candida* isolates, there was no significant difference observed.

Table.1

S.No.	Isolate Name	Host	Place	State	Latitude & Longitude
1.	Ac-BjV	*B. juncea* cv. Varuna	Pantnagar	Uttarakhand	29° 02' 60" N 79° 30' 59" E
2.	Ac-Brai	Banarasi rai			
3.	Ac-Brys	*B. rapa* yellow sarson			
4.	Ac-BjBio	*B. juncea* cv.Bio-YSR			
5.	Ac-Br	*B. rugose*			
6.	Ac-Bni	*B. nigra*			
7.	Ac-Bna	*B. napus*			
8.	Ac-BjK	*B. juncea* cv.Kranti			
9.	Ac-Orai	Ornamental rai			
10.	Ac-Kw	Kattili weed			
11.	Ac-Knp	*B. juncea*	Kanpur	Uttar Pradesh	26°26'59.6"N 80°19'54.8"E
12.	Ac-Met	*B. juncea*	Meerut		28° 59' 4" N 77° 42' 21" E
13.	Ac-Fzb	*B. juncea*	Faizabad		26° 46' 12" N 82° 9' 0.00" E
14.	Ac-Alw	*B. juncea*	Alwar	Rajasthan	27° 33' 39" N 76° 37' 30" E
15.	Ac-Bhrtp	*B. juncea*	Bharatpur		27° 13' 1" N 77° 29' 22" E
16.	Ac-Kot	*B. juncea*	Kotputli		27° 42' 13"N 76° 12' 4" E
17.	Ac-Sgn	*B. juncea*	Sri Ganganagar		29° 54' 13" N 73° 52' 37" E
18.	Ac-Knc	*B. juncea*	Kannahalli	Karnataka	12° 52' 15"N 76° 47' 18"E
19.	Ac-Bnglr	*B. juncea*	Bangalore		12° 58' 20" N 77° 34' 50" E
20.	Ac-Ldh	*B. juncea*	Ludhiana	Punjab	30° 54' 3" N 75° 51' 26" E
21.	Ac-Abr	*B. juncea*	Abohar		30° 08' 40" N 74° 11' 43" E
22.	Ac-VND	*B. juncea* cv. Varuna	IARI	New Delhi	28° 37' 55" N 77° 8' 19" E
23.	Ac-PbND	*B. juncea* cv. PusaBahar	IARI		
24.	Ac-BcND	*B. carinata* cv. BCS-1	IARI		
25.	Ac-UD	*B. juncea*	UDSC		28° 38' 41" N 77° 13' 0" E
26.	Ac-Hsr	*B. juncea*	Hisar	Haryana	29° 9' 6.69" N 75° 43' 16" E
27.	Ac-Krnl	*B. juncea*	Karnal		29° 41' 8.2" N 76° 59' 25" E
28.	Ac-Srsa	*B. juncea*	Sirsa		29° 32' 11" N 75° 1' 31" E
29.	Ac-BR	*B. juncea*	Pusa,	Bihar	25°51′39′′N

			Samastipur		85°46’56’’E-
30.	Ac-Kng	*B. juncea*	Kangra	Himachal Pradesh	32° 5' 59" N 76° 16' 8" E
31.	Ac-Smr	*B. juncea*	Sirmaur		30° 38' 24"N 77° 26' 24"E
32.	Ac-Psb	*B. juncea*	Ponta Sahib		30° 26'16"N 77°37' 26"E
33.	Ac-PbMP	*B. juncea* cv. Pusa bold	Morena	Madhya Pradesh	26° 30' 0.0" N 78° 0' 0.00" E
34.	Ac-VdMP	*B. juncea*cv. Vardan			
35.	Ac-MMP	*B. juncea*cv. Maya			
36.	Ac-VMP	*B. juncea*cv. Varuna			
37.	Ac-Jam	*B. juncea*	Chatha	Jammu & Kashmir	32° 43' 58" N 74° 51' 51" E
38.	Ac-Meg	*B. juncea*	Umiam	Meghalaya	25° 34' 0.1" N 91° 52' 59" E
39.	Ac-WB	*B. juncea*	Kolkata	West Bengal	22° 34' 21" N 88° 21' 50" E
40.	Ac-AWB	Amaranthus	Kolkata		
41.	Ac-Asm	*B. juncea*	Jorhat	Assam	26° 45' 28" N 94° 12' 35" E
42.	Ac-MZ	*B. juncea*	Aizawl	Mizorum	23° 43' 37" N 92° 43' 3.4" E
43.	Ac-Mnp	*B. juncea*	Imphal	Manipur	24° 48' 50" N 93° 57' 1.0" E
44.	Ac-SK	*B. juncea*	Marchak	Sikkim	27° 19' 48" N 88° 37' 12" E

Table.2 Grouping of *A. candida* isolates based on pustule pattern

Group	Pustules Pattern	***A. candida*isolate**
1.	Separate and circular type pustules	Ac-Brai, Ac-Brys, Ac-Bna, Ac-Orai, Ac-Met,Ac-Alw,Ac-Bhrtp, Ac-Ldh, Ac-VND
2.	Coalesced circular type pustules	Ac-BjV, Ac-BjK, Ac-Br,Ac-Kw, Ac-Knp, Ac-BcND, Ac-Hsr, Ac-Krnl, Ac-BR, Ac-Kng, Ac-PbMP, Ac-VMP, Ac-MMP, Ac-Jam, Ac-Meg, Ac-Mnp
3.	Scattered, pin head type pustules	Ac-BjBio, Ac-Bni, Ac-Knc, Ac-Bnglr, Ac-PbND, Ac-Smr, Ac-WB,Ac-AWB, Ac-Sgn, Ac-Asm, Ac-MZ, Ac-SK
4.	Restricted near veins and veinlets type Pustules	Ac-Fzb, Ac-Psb, Ac-VMP, Ac-UD
5.	Restricted near veins and veinlets separate or coalesced circular type Pustules	Ac-Abr, Ac-Srsa, Ac-Kot

Table.3 Duncan's multiple range tests for mean pustule diameter

Sr. No.	*A. candida* isolate	Average pustule size (mm)
1	Ac-BjV	2.5^{c}
2	Ac-Brai	1.0^{ab}
3	Ac-Brys	1.5^{abc}
4	Ac-BjBio	1.5^{abc}
5	Ac-Br	1.5^{abc}
6	Ac-Bni	2.0^{bc}
7	Ac-Bna	2.0^{bc}
8	Ac-BjK	1.5^{abc}
9	Ac-Orai	1.0^{ab}
10	Ac-Kw	1.5^{abc}
11	Ac-Knp	2.5^{c}
12	Ac-Met	2.5^{c}
13	Ac-Fzb	1.0^{ab}
14	Ac-Alw	2.0^{bc}
15	Ac-Bhrtp	2.0^{bc}
16	Ac-Kot	1.0^{ab}
17	Ac-Sgn	0.8^{a}
18	Ac-Knc	2.5^{c}
19	Ac-Bnglr	2.5^{c}
20	Ac-Ldh	2.0^{bc}
21	Ac-Abr	1.0^{ab}
22	Ac-VND	1.0^{ab}
23	Ac-PbND	1.0^{ab}
24	Ac-BcND	1.0^{ab}
25	Ac-UD	2.5^{c}
26	Ac-Hsr	2.5^{c}
27	Ac-Krnl	1.0^{ab}
28	Ac-Srsa	0.5^{a}
29	Ac-BR	2.5^{c}
30	Ac-Kng	1.5^{abc}
31	Ac-Smr	2.5^{c}
32	Ac-Psb	1.0^{ab}
33	Ac-PbMP	2.0^{bc}
34	Ac-VdMP	1.0^{ab}
35	Ac-MMP	0.5^{a}
36	Ac-VMP	2.0^{bc}
37	Ac-Jam	2.5^{c}
38	Ac-Meg	1.0^{ab}
39	Ac-WB	2.0^{bc}
40	Ac-AmWB	2.0^{bc}
41	Ac-Asm	0.8^{a}
42	Ac-MZ	0.8^{a}
43	Ac-Mnp	1.0^{ab}
44	Ac-SK	1.5^{abc}
Mean		1.59
SEM		0.07

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