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Short Communication

Differential response of Indian mustard (*Brassica juncea*) genotypes to white rust disease-causing fungi *Albugo candida* over a two-year interval under controlled epiphytotic conditions

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Abstract

White rust in oilseed *Brassicas* is caused by obligate oomycete pathogen *Albugo candida* and is considered an important disease in Indian mustard (*Brassica juncea*) reducing seed yield significantly. In the present study, twenty-five genotypes of *B. juncea* were screened at the cotyledonary stage against Wellington isolate of *A. candida* (*Ac-Wltn*) for two years (2016–18) at ICAR–Indian Agricultural Research Institute (IARI), New Delhi and one year (2021–22) at IARI–Regional Station, Wellington under controlled conditions. Ten genotypes (Donskaja, Heera, MSTWR-17-13, MSTWR-17-15, PDZ-1, PDZ-3, PDZ-4, PDZ-5, PM-24, and Pusa Karishma) of *B. juncea* were found highly resistant at ICAR–IARI, New Delhi during 2016–18. However, all the genotypes including resistant ones were found to be susceptible with different levels of susceptibility under controlled conditions at IARI–Regional Station, Wellington during 2021–22. It might be due to the prevalence of more than one isolate in the Nilgiris hills or the breakdown of resistance in the resistant genotypes against *Ac-Wltn*.

Keywords: *Albugo candida, differential response, Indian mustard, white rust*

Introduction

India ranks second in consuming and first in importing vegetable oil in the world (Chand *et al.,* 2022). Unprecedented population growth, urbanization, changing demographic patterns, increased per capita income, changing dietary habits with more affinity towards processed packed foods, etc. are increasing the demand for vegetable oil. Among oilseeds, the *Brassica* genus contains six interrelated species that have global commercial significance, three of which are diploids *viz., B. campestris* (2n=2x=20; AA), *B. nigra* (2n=2x=16; BB) and *B. oleracea* (2n=2x=18; CC) and three are amphidiploid *viz., B. juncea* (2n=4x=36; AABB), *B. carinata* (2n=4x=34; BBCC), and *B. napus* (2n=4x=38; AACC).

Among different oil-yielding *Brassica* species, Indian mustard [*Brassica juncea* (L.) Czern. & Coss.] is the most important and widely cultivated species in India occupying about 90% of the area (8.1 million ha) with a production of 11.7 million tonnes and a productivity of 1458 kg/ha during 2021–22 (Anonymous, 2023). The productivity of Indian mustard is highly affected by biotic (diseases, insects, parasitic weeds, etc.) and abiotic factors (heat, drought, frost, salinity, etc.). Among biotic factors, white rust caused by an obligate oomycete pathogen [*Albugo candida* (Pers. Ex. Lev.) Kuntze] is considered a highly destructive disease. Both vegetative and reproductive phases of the plants are affected by this fungal pathogen due to localized and systemic infection, respectively (Saharan and Verma, 1992; Barbetti *et al.,* 2016). At least 13 races of *A. candida* have been identified based on their specificity to different cruciferous species of which race-2 predominantly infects *B. juncea* (Verma *et al.,* 1999). However, this species-specific race pathogenicity is not absolute. Most races can also infect related *Brassica* species, especially those sharing their genome with the hosts from which they were originally collected (Saharan and Verma, 1992).

In the past, many isolates of *A. candida* have been reported and used for screening the *B. juncea* genotypes in India (Singh *et al.,* 2021). The possibility of the prevalence of more than one isolate at a location cannot be denied. Therefore, in the present study, we studied disease reactions on the same set of *B. juncea* genotypes against *A. candida* isolate (*Ac-Wltn*) at two locations under controlled conditions during 2016–18 and 2021–22.

Materials and Methods

A set of twenty-five genotypes of *B. juncea* including indigenous and exotic lines were used, and their detailed pedigrees were presented in Table 1. The Nilgiris isolate of *A. candida* from Wellington (*Ac-Wltn*) was collected from fresh and new leaves of diseased plants that had very severe white rust blisters. The white rust-infected leaves were spread on the bloating paper sheets for proper aeration and to remove the excess moisture. The inoculum of white rust zoosporangia was prepared from fresh sporangium by scrapping the pustules with a sterile scalpel and then transferring them into glass-based petriplates having sterile distilled water. The petriplates were kept at 4°C for 3–4 hrs for zoospore release, and the concentration of zoospores was adjusted to 7–8 × 10^4 zoospores per microliter of distilled water using a hemocytometer (Sachan *et al.,* 2004; Chand *et al.,* 2022).

Table 1: Description of twenty-five genotypes of Indian mustard used for screening against the Wellington isolates of *A. candida* collected from the Nilgiris hills

S.N.	Genotypes	Parentage	Genotypic class
1.	BEC-144	Exotic collection from Poland	Exotic collection
2.	Bio-YSR	A somaclonal variant of *B. juncea*	Registered germplasm
3.	Donskaja	Exotic collection from the Russian Federation	Exotic collection
4.	Durgamani	Local collection from Rajasthan	Released cultivar
5.	EC-399299	Exotic collection from China	Exotic collection
6.	Heera	Derived from East European germplasm line	Registered germplasm
7.	JM-1	Pusa Bold / L 6	Released cultivar
8.	JM-2	Mutant of RL 9	Released cultivar
9.	JM-3	Varuna/YRT-3	Released cultivar
10.	MSTWR 17-13	Pusa Vijay/Bio YSR// Pusa Vijay	Advanced elite line
11.	MSTWR 17-15	BEC-286/Bio YSR	Advanced elite line
12.	NPJ-181	BCEF-1-00-18-1-6/NPJ-119//NPC-9	Advanced elite line
13.	PDZ-1	LES-27/NUDHYJ-3	Advanced elite line
14.	PDZ-3	Pusa Karishma/EC597325	Advanced elite line
15.	PDZ-4	Pusa Mustard -21/ EC597325	Advanced elite line
16.	PDZ-5	Pusa Mustard -21/ EC597325	Advanced elite line
17.	PM-24	Pusa Bold/LEB-15//LES-29	Released cultivar
18.	Pusa Karishma	Pusa Barani / ZEM 1	Released cultivar
19.	Pusa Vijay	Synthetic B. juncea / VSL 5	Released cultivar
20.	RL-1359	RLM 514 / Varuna	Released cultivar
21.	Rohini	Selection from the natural population of Varuna	Released cultivar
22.	Rust 17-304	Pusa Vijay/Bio YSR//Pusa Vijay	Advanced elite line
23.	Rust 17-305	BEC-286/Bio YSR	Advanced elite line
24.	Rust 17-306	PM-30/Bio YSR// Bio YSR	Advanced elite line
25.	Varuna	Selection from Varanasi local	Released cultivar

In artificial epiphytotic conditions, 2–3 untreated seeds of 25 genotypes were sown in plastic trays (34×22×9 cm) each having 40 wells of 4 cm diameter. 8–10 days old seedlings at two cotyledon stages were used and 5 µl zoospore suspension was drop inoculated manually on the adaxial surface of each lobe of cotyledon employing a micropipette. The inoculated trays were kept in a humid chamber covered tightly by a thin polythene sheet (700-gauge thickness) for maintenance of relative humidity. For the onset of the disease, the dark condition was maintained inside the plastic chamber for the first 24 hrs after inoculation by covering the polythene sheet with a light-blocking thick cloth made sheet. Low temperature (15–20°C) and high humidity (>65%) are prerequisites for a congenial white rust disease development in Indian mustard entries (Mehta, 2021). Therefore, low temperature and high humidity inside the polythene chamber were maintained by supplying water (up to 2–3 cm height) at regular intervals once in 2–3 days and a hand atomizer was used to wet the surrounding area of the chamber during the entire period of disease development.

The experiments on disease screening were conducted under artificially controlled epiphytotic conditions at ICAR–Indian Agricultural Research Institute (IARI), New Delhi during 2016–18, and IARI–Regional Station, Wellington during 2021–22 for *Ac-Wltn* isolate. The disease scoring was done using a 0–9 scale at the cotyledonary stage modified from Fox and Williams (1984). The stepwise stages of phenotypic screening starting from sample collection to disease appearance have been illustrated briefly in Fig. 1. After scoring, the percent disease index (PDI) was calculated using the following formula:

$$\text{PDI} = \frac{\Sigma\text{ (numerical ratings)}}{\text{(No. of samples scored} \times \text{Maximum score)}} \times 100$$

The mean values of selected and tagged plants at each replication for each genotype were used for analyzing the statistical and final interpretation. Genotypic mean values were also compared using critical differences at a 5% level of significance.

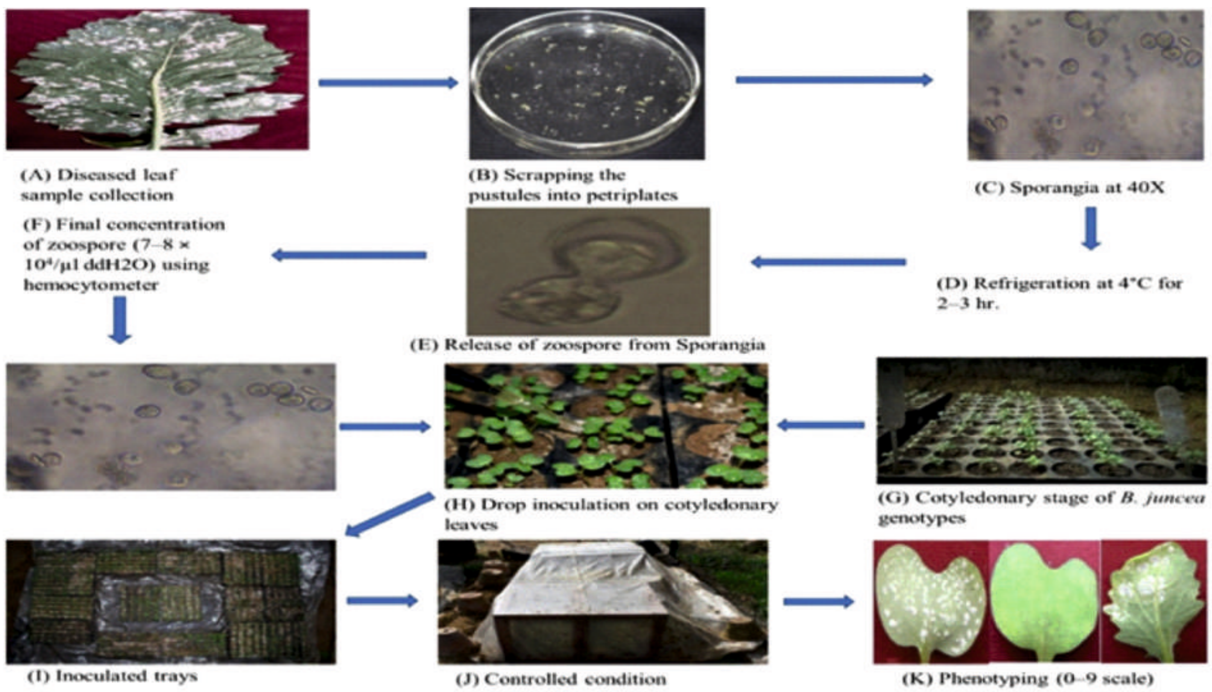

Fig. 1: Schematic illustration of different stages from white rust diseased leaf collection to phenotyping after disease development

Results and Discussion

Screening under artificially controlled conditions at ICAR–IARI, New Delhi (2016–17 & 2017–18)

A set of twenty-five genotypes was screened against the Wellington isolate (*Ac-Wltn*) of *A. candida* at the cotyledonary stage under artificial epiphytotic conditions during *Rabi* 2016–17 and 2017–18, and PDI was calculated to categorize the genotypes into different disease response groups (Table 2). In 2016–17, the PDI ranged from 0% (Donskaja, Heera, MSTWR-17-13, MSTWR-17-15, PDZ-1, PDZ-3, PDZ-4, PDZ-5, PM-24, and Pusa Karishma) to 62.98% (Varuna). Based on PDI values, ten genotypes were categorized as highly resistant (Donskaja, Heera, MSTWR-17-13, MSTWR-17-15, PDZ-1, PDZ-3, PDZ-4, PDZ-5, PM-24, and Pusa Karishma), ten as susceptible (BEC-144, Bio YSR, Durgamani, EC-399299, JM-1, JM-2, RL-1359, Rohini, Rust-17-305, and Rust-17-306), one as moderately susceptible (NPJ-181), and four as highly susceptible (Rust-17-304, JM-3, Pusa Vijay, and Varuna). Likewise, in 2017–18, the PDI ranged from 0% (Donskaja, Heera, MSTWR-17-13, MSTWR-17-15, PDZ-1, PDZ-3, PDZ-4, PDZ-5, PM-24, and Pusa Karishma) to 62.59% (Varuna). Thirteen genotypes (BEC-144, Bio YSR, Durgamani, EC-399299, JM-1, JM-2, JM-3, NPJ-181, RL-1359, Rohini, Rust-17-304, Rust-17-305, and Rust-17-306) were reported as susceptible, and two (Varuna and Pusa Vijay) were highly susceptible; however, highly resistant genotypes were the same as reported in the year 2016–17. Based on combined mean PDI values, the genotypes were classified into three major classes such as highly resistant (Donskaja, Heera, MSTWR-17-13, MSTWR-17-15, PDZ-1, PDZ-3, PDZ-4, PDZ-5, PM-24, and Pusa Karishma), susceptible (BEC-144, Bio YSR, Durgamani, EC-399299, JM-1, JM-2, NPJ-181, RL-1359, Rohini, Rust-17-304, Rust-17-305, and Rust-17-306) and highly susceptible (JM-3, Pusa Vijay and Varuna). During this time, the isolate was maintained in the living host (Varuna) followed by storing at –20°C and screening the same set of genotypes against the same isolate for two years. Likewise, Singh *et al* (2021) screened 30 *B. juncea* genotypes at the cotyledonary stage to *Ac-Wltn* isolate under the artificial conditions at ICAR–IARI, New Delhi and 11 genotypes including Pusa Karishma, PM-24, PDZ-1, PDZ-3, PDZ-4, and Donskaja were reported as highly resistant (PDI=0).

Table 2: Screening of Indian mustard genotypes against the Wellington isolate (*Ac-Wltn)* of *A. candida* under artificially controlled epiphytotic at IARI, New Delhi, and Wellington conditions

S.N.	Genotypes	Cotyledonary stage				
		PDI at New Delhi				PDI at Wellington
		2016 -17	2017 -18	Mean	2020 -21	2022
1.	BEC-144	36.03 (S)	39.70 (S)	37.86 (S)		59.51 (HS)
2.	BIO-YSR	40.46 (S)	31.14 (S)	35.80 (S)	37.34 (S)	56.14 (HS)
3.	Donskaja	0.00 (HR)	0.00 (HR)	0.00 (HR)	7.20 (MR)	46.90 (S)
4.	Durgamani	28.26 (S)	40.87 (S)	34.57 (S)		53.87 (HS)
5.	EC-399299	42.11 (S)	32.23 (S)	37.17 (S)		35.90 (S)
6.	Heera	0.00 (HR)	0.00 (HR)	0.00 (HR)		14.46 (MS)
7.	JM-1	48.81 (S)	40.77 (S)	44.79 (S)		48.52 (S)
8.	JM-2	39.92 (S)	44.58 (S)	42.25 (S)		44.64 (S)
9.	JM-3	56.75 (HS)	47.91 (S)	52.33 (HS)		47.39 (S)
10.	MSTWR 17-13	0.00 (HR)	0.00 (HR)	0.00 (HR)		44.13 (S)
11.	MSTWR 17-15	0.00 (HR)	0.00 (HR)	0.00 (HR)	8.37 (MR)	39.50 (S)
12.	NPJ-181	24.42 (MS)	34.93(S)	29.67 (S)		48.98 (S)
13.	PDZ-1	0.00 (HR)	0.00 (HR)	0.00 (HR)		49.72 (S)
14.	PDZ-3	0.00 (HR)	0.00 (HR)	0.00 (HR)		30.66 (S)
15.	PDZ-4	0.00 (HR)	0.00 (HR)	0.00 (HR)		42.39 (S)
16.	PDZ-5	0.00 (HR)	0.00 (HR)	0.00 (HR)		31.43 (S)
17.	PM-24	0.00 (HR)	0.00 (HR)	0.00 (HR)	7.85 (MR)	64.63 (HS)
18.	Pusa Karishma	0.00 (HR)	0.00 (HR)	0.00 (HR)		13.03 (MS)
19.	Pusa Vijay	57.86 (HS)	55.84 (HS)	56.85 (HS)	46.49 (S)	50.20 (HS)
20.	RL-1359	42.98 (S)	30.33 (S)	36.66 (S)		58.49 (HS)
21.	Rohini	39.99 (S)	48.81 (S)	44.40 (S)		62.00 (HS)
22.	Rust 17-304	57.57 (HS)	42.25 (S)	49.91 (S)		38.20 (S)
23.	Rust 17-305	29.74 (S)	38.58 (S)	34.16 (S)		30.33 (S)
24.	Rust 17-306	41.05 (S)	45.14 (S)	43.10 (S)		37.44 (S)
25.	Varuna	62.98 (HS)	62.59 (HS)	62.78 (HS)		58.72 (HS)
	CD (p=0.05)	5.65	5.94		6.06	13.03
	CV	13.23	14.20		13.86	17.89

Letters in parentheses represent disease reaction: HR-highly resistant; R-resistant; MR-moderately resistant; MS-moderately susceptible; S-susceptible; HS-highly susceptible

Screening under artificially controlled conditions at ICAR–IARI, New Delhi (2020–21)

The five genotypes (BIO YSR, Donskaja, MSTWR-17-15, PM-24, and Pusa Vijay) were screened against the *Ac-Wltn* isolate at the cotyledonary stage under artificial epiphytotic conditions in *Rabi* 2020–21, and PDI was calculated to categorize the genotypes into two disease response groups (Table 2). The PDI ranged from 7.20% (Donskaja) to 46.49% (Pusa Vijay). Based on PDI values, five genotypes were categorized as moderately resistant (Donskaja, MSTWR-17-15, and PM-24) and susceptible (Pusa Vijay and BIO YSR). While screening fresh inoculum was collected from Wellington as previously collected inoculum could not be maintained. Resistant genotypes had expressed different levels of disease incidence under controlled conditions at ICAR–IARI, New Delhi.

Screening under artificially controlled conditions at ICAR–IARI, RS, Wellington (*Kharif* 2022)

The same set of genotypes was again screened at the cotyledonary stage under controlled epiphytotic conditions at IARI, Regional Station, Wellington during 2021–22 (Table 2) in two different experiments conducted at 10-day intervals. The PDI ranged from 13.03% (Pusa Karishma) to 64.63% (PM-24). Based on PDI, the genotypes were mainly grouped into three categories viz., moderately susceptible (Heera and Pusa Karishma), susceptible (JM-1, JM-2, JM-3, MSTWR-17-13, MSTWR-17-15, Donskaja, EC-399299, NPJ-181, PDZ-1, PDZ-3, PDZ-4, PDZ-5, Rust-17-304, Rust-17-305, Rust-17-306) and highly susceptible (BEC-144, Bio YSR, Durgamani, PM-24, RL-1359, Rohini, Varuna, and Pusa Vijay).

There might be a few probable reasons for the differential response of genotypes to the *Ac-Wltn* isolate such as (i) the prevalence of more than one isolate in The

Nilgiris hills that has more pathogenicity than the previous isolate or (ii) the breakdown of resistance in the resistant genotypes against *Ac-Wltn*. Breakdown of resistance might be due to– (1) the narrow genetic base of *B. juncea* genotypes as only a few east-European genotypes are being used frequently in white rust resistance breeding programs, (2) sexual recombination in *A. candida* as the different host *Brassica* spp. (*B. juncea*, *B. oleracea*, etc.) are continuously available at the vicinity of Wellington station due to year-round cultivation or as stray plants (3) mutation in *A. candida* towards virulence.

It has also been reported that there was a shortage/moderate level of annual rainfall at Wellington during 2016–19 but a good amount of rain was received after *Kharif* 2019 which might be the main reason for the evolution of new isolate(s) of *A. candida* (unpublished). Therefore, it might be possible that there is more than one isolate in the Nilgiris hills as they differ in pustule shape and size. In the past, Kiyosawa (1982) reported the breakdown of resistance in different crop–pathogen associations. He stated that the longevity of resistance to rice blast disease in rice varieties, in Japan, is below 3 years and less than 6 years for different rusts (stem, leaf, and stripe) in wheat varieties in the USA. There might be different reasons for the breaking down of resistance in resistant cultivars and the following factors may be the major cause for the breakdown of resistance: (1) mutation of pathogen leading to virulence, (2) sexual and asexual recombination, (3) acreage of resistant cultivars, (4) genetic homogeneity of the cultivated varieties, and (5) lowering of field resistance.

Conclusion

Many isolates of *A. candida* have been reported in different rapeseed-mustard-growing regions across the country. The probability of the prevalence of more than one isolate in a single location cannot be denied, especially in the Nilgiris hills. Therefore, there is a dire need to generate comprehensive information on the racial spectrum of *A. candida* existing in the mustard-growing regions. It might be possible that increasing the area under a mustard-based cropping system can accelerate the pathogenic evolution to overcome the host resistance due to high selection pressure. Therefore, new differential hosts from *B. juncea* must be used for the identification of new pathotypes and, then a large accession must be screened against *A. candida* isolates for the identification of new resistance genes.

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