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Identification of partial resistant genotypes of Indian mustard against *Alternaria brassicicola* under late sown condition in Bihar

Matlooba Naseem, Chanda Kushwaha*, Abhijeet Ghatak, JN Srivastava, Chandan Kishore[1] and Saurabh Bharti

Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur 813210, India
[1]Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur 813210, India
**Corresponding author: chanda.kushwaha@gmail.com*
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Abstract

The present investigation was undertaken to identify the partial resistant sources to Alternaria blight (*Alternaria brassicicola)* in Indian mustard under late sown environmental conditions. Fifteen genotypes of Indian mustard were evaluated in split plot design under artificial inoculation at four different dates of sowing during *Rabi* season, 2019-20 (12th Nov, 19th Nov, 26th Nov and 3rd Dec). The data obtained from study show the significant effect of date of sowing on percent disease index and area under disease progress curve (AUDPC) under late sown condition due to early onset of diseases at early growth stages coupled with warmer environmental condition. Among different genotypes of Indian mustard, BRRM-101, BRRM-102, BRRM-103, BRRM-104, BRRM-106 line shows less percent disease index as well as area under the disease progress curve.

Keywords: *Alternaria blight, AUDPC, mustard genotypes, partial resistant, PDI*

Introduction

Brassica juncea (L.) Czern & Coss., also known by the name of Indian mustard, belongs to the brassicaceae (cruciferae) family, is the most predominant crop out of rapeseed-mustard in India and accounts for more than 90 % of the area. Oilseeds are the second most agricultural economy in India next to cereals growing at a pace of 4.1 % per annum in the last three decades. Oilseed brassica shares 24.4 % area and 26.8 % production of total oilseeds in the country (Choudhary *et al.,* 2021). Its oil content varies from 38 to 46 % (Meena *et al.,* 2016). The oilseed brassica usually contains 38-57 % of erucic acid, 4.7-13 % linolenic acid and 27 % of oleic and linoleic acid, which are of high nutritive value required for human health (Singh *et al.,* 2011). The area, production and productivity of rapeseed-mustard during 2018-19 in the world were 36.59 million ha, 72.37 million tonne, and 1980 kg/ha, respectively (Anonymous, 2020). Globally, India accounts 6.23 million ha area with production 9.34 million tonne and productivity 1499 kg/ha. In Bihar, rapeseed-mustard is cultivated in 0.08 million ha leading to production of 0.11 million tonne with a productivity of 1305 kg/ha (Agricultural Statistics at a Glance, GOI, 2018-19). In the world, India stands 3rd in terms of area and production after Canada and China and 5th in terms of productivity after Germany, France, Canada and China. In the last few years, India emerged as a largest importer of edible oilseed which ranked 7th in the world and meets 57 % of the domestic edible oil requirements through imports. The import has increased mainly due to increasing consumption of edible oils with the rising population and changing lifestyle of the people and production constraints like cultivation under rainfed situation, biotic and abiotic stresses, and resources limitation (Jat *et al.,* 2019).

Among the biotic stresses, Alternaria blight disease has been reported from all the of the world and is one among the important diseases of Indian mustard causing up to 47 % yield losses (Kolte, 1985). In mid-eastern India, yield losses were reported from 20 to 36 % in different *Brassica* spp. (Kumar *et al.,* 2014). Reports of occurrence of *A. brassicae* at relatively low temperatures whereas higher temperature regimes promote growth of *A. brassicicola* is well dominated (Humpherson-Jones and Phelps, 1989). Additionally, *A. brassicicola* can become more aggressive under warmer condition and can causes potential losses (Sinha *et al.,* 2021). Among various disease management tactics, the use of genetic resistance in rapeseed-mustard is considered to be the best way to reduce the incidence of Alternaria blight. Screening of genotype is a prerequisite for identification of tolerant sources to Alternaria blight in rapeseed-mustard under late sown environmental conditions.

Materials and Methods

Planting material

A set of fifteen different genotypes of mustard planting material were procured from Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, were sown on different date during *Rabi* season 2019-20. The field trial was conducted at Bihar Agricultural College Research Farm, BAU, Sabour for disease assessment under artificially inoculated conditions. They were sown on four different dates *viz.*, 12th November, 2019, 19th November, 2019, 26th November, 2019 and 03rd December, 2019 in a split plot design with two replication and 2 rows per genotype. The crop spacing was maintained 30×10 cm following standard package of practices. Each row length was 5 meters. Susceptible check 'Varuna' was raised after each genotype. Observation for initial appearance of disease symptoms and disease index at 30 to 90 days after sowing were recording, using 0 to 5 rating scale given by Wheeler (1969). Randomly five plants from each genotype were selected and tagged for recording the appearance of disease symptoms and per cent disease index at 7-day interval. Based on disease severity taken at different time interval, AUDPC was calculated.

Disease assessment

Percent disease Index was calculated by the formula given by McKinney (1923): Per cent disease Index (PDI) = (Sum of all numeric ratings)/ (Total no. of leaves × Maximum grade) × 100

PDI = [{n (0) + n (1) + n (2) + n (3) + n (4) + n (5)}/ { t (n) x 5}] × 100

Where, n (0), n (1), n (2), n (3), n (4), n (5) = Number of leaves showing severity score of 0, 1, 2, 3, 4 and 5.

t (n) = total number of leaves scored.

The area under the disease progress curve (AUDPC) value was calculated according to the formula used by Wilcoxson *et al.* (1975), who quantified the

$$\mathbf{AUDPC} = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \mathbf{x}(t_{i+1} - t_i)$$

Where, y_i = the Disease severity at i^{th} observation
t_i = time (in days) at the i^{th} observation
n = total number of observations.

Isolation, purification and maintenance of the pathogen

The pathogen isolated from infected leaves of mustard showing characteristic symptoms of Alternaria blight from BAU, Sabour farm. The bits of diseased portion of infected plant parts along with healthy portion were cut into bits of 3-5 mm, surface sterilized with sodium hypochlorite solution (1%) for 30 seconds, washed thrice with sterilized distilled water and dried the samples using sterilized blotter papers to remove excess moisture thereafter three-four bits were placed in each petri plates of size 90 mm containing potato dextrose agar (PDA) medium. The inoculated plates were incubated in BOD incubator at 25 ± 2 ºC for 7 days. Slide culturing was done to determine the microscopic features for morphological characterization. For this slide of the cultures were prepared in lacto phenol and cotton blue and examined under EVOS® fluorescent microscope. Purification of isolated pathogen was carried out using single spore isolation method according to the method described by Dhingra and Sinclair (1985). After that sub-culturing was done using hyphal tip method and pure cultures were maintained in slants at 4 ºC for further work.

Preparation of spore suspension for artificial inoculation

The crop sown in field was artificially inoculated by foliar spray with spore suspension. Which was prepared from freshly developed conidial growth using sterile distilled water and then strained through muslin cloth. The spore concentration was adjusted to 1×10^5 conidia mL^{-1} distilled water using haemocytometer and then sprayed using an atomizer at the rate of 5 mL/plant at 45 DAS (Vishunavat and Kolte, 2008).

Statistical analysis

The results were analysis using statistical package IBM SPSS Statistics for Windows, version 23.0 for Analysis of variance (ANOVA) for the test of significance of mean at 5% level (P =0.05).

Results and Discussion

It was observed that the crop sown on D1 (12th November, 2019) recorded appearance of Alternaria blight disease among all the genotypes at later growth stages (78.9 DAS) and crop sown on D2 (19th November, 2019) and D3 (26th November, 2019) recorded appearance of disease at 77.13 and 68.6 DAS respectively. However, crop sown on D4 (03rd December, 2019) recorded appearance of Alternaria blight disease among all the genotypes at an early growth stage (50.3 DAS) (Table 1).

Table 1: First appearance of Alternaria blight disease in different genotypes of mustard under different date of sowing

Genotype	First appearance of Alternaria blight (DAS)			
	D1(12th Nov, 2019)	D2(19th Nov, 2019)	D3(26th Nov, 2019)	D4(03rd Dec, 2019)
BRRM-101	83.5 ± 0.5*	77.5 ± 0.5	72.5 ± 0.5	49.5 ± 0.5
BRRM-102	82.5 ± 0.5	86.0 ± 0.0	71.0 ± 0.0	53.0 ± 0.0
BRRM-103	79.0 ± 0.0	74 .0 ± 0.0	70.5 ± 0.5	54.0 ± 0.0
BRRM-104	72.5 ± 0.5	79.5 ± 0.5	67.0 ± 0.0	49.5 ± 0.5
BRRM-105	72.5 ± 0.5	80.0 ± 0.0	66.5 ± 0.5	52.0 ± 0.0
BRRM-106	79.5 ± 0.5	77.5 ± 0.5	68.0 ± 0.0	51.0 ± 0.0
BRRM-107	80.5 ± 0.5	75.5 ± 0.5	69.0 ± 0.0	47.0 ± 0.0
BRRM-108	79.5 ± 0.5	76.5 ± 0.5	65.5 ± 0.5	50.0 ± 0.0
BRRM-109	85.5 ± 0.5	77.5 ± 0.5	66.0 ± 0.0	50 .0 ± 0.0
Varuna	70.0 ± 0.0	64.5 ± 0.5	65.0 ± 0.0	47.5 ± 0.5
Pusa Mustard-25	84.0 ± 0.0	82.5 ± 0.5	69.5 ± 0.5	50.0 ± 0.0
Rajendra Anukul	80.5 ± 0.5	78.5 ± 0.5	68.0 ± 0.0	52.5 ± 0.5
Pusa Bold	72.0 ± 0.0	82.5 ± 0.5	68.5 ± 0.5	50.0 ± 0.0
Rajendra Suflam	82.5 ± 0.5	72.5 ± 0.5	68.5 ± 0.5	50.0 ± 0.0
Kranti	80.0 ± 0.0	72.5 ± 0.5	74.0 ± 0.0	49.0 ± 0.0
Mean	78.9	77.1	68.6	50.3
CD (p = 0.05) Date of sowing			2.1	
CD (p = 0.05) Genotypes			4.0	
CD (p = 0.05) Date of sowing × Genotypes			NS	

* ± Standard deviation

The effect of sowing date on the incidence of Alternaria blight severity presented in Table 2 &3 which show significant effects of date of sowing on Alternaria blight incidence. The mean value of Percent Disease Index (Table 2) was found lowest from the genotypes of mustard sown on D1 (12th November, 2019) i.e., 17.9 while it

Table 2: Effect of date of sowing on percent disease index and area under the disease progress curve in different genotypes of mustard infected with *A. brassicicola*

Genotype	Per cent disease index (85-90 DAS)				Area under the disease progress curve			
	D1	D2	D3	D4	D1	D2	D3	D4
BRRM-101	12.8	23.9	26.1	53.2	64.2	155.2	105.9	196.6
BRRM-102	16.7	15.6	28.3	60.0	99.2	92.3	123.4	213.5
BRRM-103	14.4	25.6	27.8	57.2	75.8	119.6	110.8	207.4
BRRM-104	13.3	22.8	32.2	61.1	90.6	111.4	131.3	207.4
BRRM-105	16.7	23.3	33.3	58.9	117.8	94.9	157.5	230.8
BRRM-106	15.0	30.0	31.7	55.1	110.8	161.4	140.2	203.4
BRRM-107	19.4	28.3	28.4	70.0	114.7	146.4	110.4	232.5
BRRM-108	15.6	27.8	37.8	68.9	94.5	169.8	204.8	243.8
BRRM-109	15.0	25.6	31.7	65.0	75.0	74.4	137.7	249.5
Varuna	26.1	37.7	35.6	84.4	128.3	198.3	118.2	306.0
Pusa Mustard-25	19.4	22.2	27.6	75.6	60.3	120.5	114.3	269.7
Rajendra Anukul	18.9	24.4	28.3	74.4	79.7	110.8	121.1	276.7
Pusa Bold	23.3	22.8	28.9	74.4	110.0	87.5	157.1	269.7
Rajendra Suflam	20.6	36.7	30.0	70.0	75.0	173.6	161.4	261.1
Kranti	21.1	35.0	23.9	75.9	56.4	148.3	102.7	263.7
CD (p = 0.05) Date of sowing			1.9		8.1			
CD (p = 0.05) Genotypes			3.6		15.7			
CD (p = 0.05) Date of sowing × Genotypes			9.7		31.4			

increased significantly with the delays in sowing. Genotypes of mustard sown on D4 (3rd December, 2019) recorded maximum disease severity i.e., 66.9. Among the genotypes of mustard sown on different dates BRRM-101 recorded minimum diseases severity (12.6, 53.2) while Varuna recorded highest disease severity (26.1, 84.4) on both first and fourth date of sowing.

Area under disease progress curve was calculated from disease severity data collected at two different assessments for mustard genotypes under different dates of sowing. The Area under the disease progress curve (AUDPC) value ranged from 64.2 to 128.3 on first date of sowing (12th November, 2019) as compared to 92.3-173.6 and 105.9-204.8 on second (19th November, 2019) and on third date of sowing (26th November, 2019) respectively. It was clearly observed from Table 2, that the maximum value of AUDPC (196.6 to 306.0) was observed on fourth date of sowing (03rd December, 2019). Among the genotypes maximum AUDPC value was observed in Varuna (306.0) and minimum was in genotypes BRRM-101 (196.6) under late sown condition. From the given data, mustard genotype sown under late sown condition, the performance of BRRM-102, BRRM-103, BRRM-104, BRRM-106 lines show less disease severity similar to the line BRRM-101 in comparison to another genotype. Analysis of variance for PDI and AUDPC (Table 3) indicated significant difference among genotypes, date of sowing and its interaction.

Results showed that plant growth stages significantly influence the onset of Alternaria blight on mustard and disease severity increases with the age of plant and highest was recorded at 85 DAS. Similar results were reported by Gupta *et al.* (2003), Khatun *et al.* (2011). Meena *et al.* (2020) and Kumar *et al.* (2019) also reported that first appearance of Alternaria blight disease on mustard leaves occurred between 65-98 days after sowing.

The present study on effect of date of sowing and genotype on disease severity of Alternaria blight revealed significant interactions between the factors. Generally, early infection was observed when the crop was sown later than 2nd week of November and this might be attributed largely due to concurrence of favourable weather factors with susceptible growth stage of mustard. When the crop is sown on the 2nd week of November, siliqua formation starts from the end of December and is completed within middle of January which can escape heavy infection of Alternaria blight at a time when weather conditions are not favourable for Alternaria blight development due to severe winters. On the other hand, crops sown later than 2nd week of November receives

Table 3: Analysis of variance for the effect of date of sowing and genotypes on PDI and AUDPC in mustard infected by *Alternaria brassicicola*

Source of error	df	Mean sum of squares	
		PDI	AUDPC
Replication	1	16.6	482.8
Date of sowing	3	14043.7*	129192.2*
Genotype	14	147.9*	2559.9*
Date of sowing × Genotype	42	49.1*	1725.2*
Error	59	13.1	245.6

*Significant difference at 5 % probability ($p \leq 0.05$)

Table 4: Meteorological data from October 2019 to April 2020

Month	Rainfall (mm)	Temperature (°C)		RH (%)	
		Max	Min	7.00 AM	2.00 PM
October 2019	23.4	30.8	21.7	89.4	78.5
November 2019	0.0	28.9	16.2	87.6	76.1
December 2019	2.5	21.2	9.3	91.4	75.9
January 2020	6.0	21.9	8.8	93.0	71.0
February 2020	38.4	24.1	9.7	88.4	66.7
March 2020	48.0	30.5	16.8	86.6	59.1
April 2020	71.4	33.1	20.2	88.7	65.0

heavy infection of Alternaria blight at the time of siliqua formation due to onset of Alternaria blight at susceptible growth stages of crop development.

Generally, the mustard crop matures in about 103-110 days and the most susceptible stages of Alternaria blight is flowering and rossetting stages i.e., 40-75 days after sowing (Meena *et al.*, 2004). In the present study it was observed that 1st date of sowing show less disease severity followed by 2nd and 3rd date of sowing however it was found maximum on 4th date of sowing. Less disease severity was observed because the susceptible stages of crop escape the disease development due to timely sowing of crops in comparison to delay date of sowing which provide favourable weather condition at the susceptible stage of crop. This finding is also supported by reports of Meena *et al.* (2004), Meena *et al.* (2010), Kaur *et al.* (2006), Khatun *et al.* (2011) and Sohi *et al.* (2020) who also found that mustard sown in end of October or 2nd week of November received heavy infection with delay in sowing date therefore, yielded higher levels of the disease. Additionally, warmer condition at susceptible crop stages at later date of sowing may lead to higher disease as a result of enhanced virulence in the pathogen (Sinha *et al.*, 2021).

The disease severity as well as, area under disease the progress curve (AUDPC) was observed maximum at 4th date of sowing. Crop sown during December experienced favourable conditions at the susceptible crop growth stages for development of Alternaria blight. Thereby resulting in higher level of disease development. These results were also in accordance with the Mahapatra and Das (2015) and Meena et al. (2011) who also revealed enhanced disease development with delayed sowing.

Conclusion

Recently, changes in preferences have increased the demand of oilseed in the domestic market leading to increase in imports in edible oils. In order to meet the required demand productivity is also required to be improved. Mitigation strategies involving disease management tactics hold a key position in this regard. Exploiting genetic resistance/tolerance to Alternaria blight still holds a significance in respect to changing climate over years toward warmer environments. A shift towards warmer climate may lead to dominance of *A. brassicicola* on mustard. Under such situation genotype BRRM-101, BRRM-102, BRRM-103, BRRM-104, BRRM-106 may provide a significant gain while reducing the disease level and can be used in crop improvement programme.

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