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Research Article

Morphological and biochemical characteristics associated with powdery mildew resistance in M_3 population of blackgram (*Vigna mungo* (L.) Hepper)

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Abstract
Powdery mildew *(Erysiphe polygoni DC.)* is one of the major diseases in blackgram during cold weather season which reduces up to 50 per cent of the yield. The present study deals with the morphological and biochemical characteristics such as trichome density, leaf thickness, stomatal density and total phenol, the enzyme activity of peroxidase and polyphenol oxidase in relation to powdery mildew resistance in blackgram. A total of 59 mutant families selected from the M_2 population were raised as M_3 based on powdery mildew score. Six mutant lines, *viz.,* PM 27, PM 29, PM 48, PM 49, PM 42, PM 34 were identified as moderately resistant mutants against powdery mildew disease incidence based on field and glass house screening of visual scores. The resistant mutants showed higher trichome density, leaf thickness, total phenol content, the enzyme activity of peroxidase and polyphenol oxidase lower total sugar content and stomatal density than susceptible control (CO 6). These results clearly indicated the operation of resistance reaction in the identified mutants. However, the resistant check LBG 17 showed more resistance than the mutants. As the LBG 17 was photosensitive making it unsuitable for cultivation in all the seasons, the resistant mutants identified in this study may be used as resistance sources in place of LBG 17 for powdery mildew resistance studies.

Key words: Blackgram, M_3 population, Powdery mildew, Morphological traits, Biochemical traits.

INTRODUCTION

Blackgram (*Vigna mungo* L. Hepper, 2n =22), is the third most important pulse crop, which belongs to the family Leguminosae and the subfamily Papilionaceae. Blackgram is an abundant source of starch (43.5%), total soluble sugars (4.84%), proteins (22%) and lipids (1.1%) (Suneja *et al.,* 2011). Owing to its high nutritional quality, it has contributed as a major dietary source for the vegetarian population. In India, blackgram is largely cultivated in almost all the states with an overall area and production of about 4.53 million hectares and 2.08 million tonnes, respectively (Indistat, 2020). Yield loss in blackgram is significant due to several viral and fungal disease infestations. Of them, powdery mildew is one of the predominant diseases because of its cosmopolitan distribution. In India, powdery mildew has been catalogued in almost all states and is known to cause a severe yield loss of about 9-50 per cent in blackgram (Reddy *et al.,* 2008; Pandey *et al.,* 2009). Induced mutations have played a vital role in the introduction of new genetic variations of crucial agricultural traits for crop improvement (Tamilzharasi *et al*., 2019). As a natural genetic variation for powdery mildew is very limited in blackgram, mutation breeding could be an ideal method for developing resistant mutants against powdery mildew disease. The variety CO 6 was used for the mutation study. It gives an average yield of 877 kg/ha which matures 60-65 days. It has bold seeds with a 100 seed weight of 5.5gm with good battering quality. It possesses

a determinate plant with synchronized maturity and is recommended for cultivation during the *Rabi* season. It is moderately resistant to yellow mosaic, stem necrosis but susceptible to powdery mildew disease. Hence, this variety has been chosen for mutation work to isolate powdery mildew resistant mutants.

The resistant mechanism of the plant against the pathogen is conferred via the surface and anatomy of the leaves, which includes trichome length, trichome density, stomata morphology, and thickness of the leaf (Soundhiriyan *et al.,* 2018). Trichomes act like a physical barrier that entraps microorganisms such as fungal spores and prevents them from reaching the leaf surface. Additionally, exudates from trichomes consist of secondary metabolites, of which a few metabolites have antimicrobial properties (Lazniewska *et al.*, 2012). Moreover, the higher frequency of trichomes also withstands the penetration of mycelial and different biotrophic fungus infections (Shaik *et al*., 1985). Leaf or epidermal thickness is also correlated with powdery mildew resistance (Commenil *et al.,* 1997). Stomata play a crucial role in disease infestation as the pathogen indirectly penetrates the mesophyll via stomata to form Haustoria (Takamatsu *et al.,* 2008). The biochemical compounds found in plants play an important role in preventing the host plant from getting the disease. Infection with powdery mildew disease causes a significant increase in starch, sugar and sucrose content (Allen, 1942; Pathak *et al*., 2015). Saini *et al.* (1988) reported that total phenol content has a key role to play in the resistance mechanism. The aim of the present study was to identify the resistant mutants in M_3 on the basis of their performance and reliability to powdery mildew disease resistance in terms of morphological and biochemical parameters.

MATERIALS AND METHODS

The study was undertaken at the Department of Pulses at Tamil Nadu Agricultural University in Coimbatore during *Rabi,* 2020. The blackgram variety, CO 6 was used for this study. The seeds were initially irradiated with gamma rays and an EMS combination of 200 Gy + 20mM EMS, 200 Gy + 30 mM EMS, 300 Gy + 20 mM EMS, 300 Gy + 30 mM EMS and 400 Gy + 20 mM EMS and 400 Gy + 30 mM EMS (Tamilzharasi *et al.,* 2019). The M_3 mutants selected from the M_2 generation were utilized in this study. Based on single plant yield and powdery mildew score, M_3 generation seeds were collected from selected 59 M_2 plants and raised as progeny rows with 30×10 cm spacing. Recommended agronomic practices were followed. At 45 DAS (days after sowing), the resistant mutant lines were examined for powdery mildew incidence using 0 to 5 grade scale (AICRP, MULLaRP, 2013).

$$PDI = \frac{\text{Sum of all the rating of infected leaves on plant}}{\text{Number of leaves observed X Maximum disease score}} X\ 100$$

Based on field screening for powdery mildew (visual scoring and the Per cent Disease Index (PDI), six mutant lines PM 27, PM 29, PM 48, PM 49, PM 42 and PM 34 were identified as moderately resistant from 59 M_3 plants. In the present study, CO 6 and LBG 17 were used as susceptible and resistant checks, respectively.

Morphological parameters such as trichome density (5 mm^2), leaf thickness (µm) and stomatal density (mm^2) were assessed for six moderately resistant mutants *viz.,* PM 48, PM 34, PM 42, PM 27, PM 29, PM 49 on a 45-days old plant using a scanning electron microscope. After dehydrating the sample, the Polaron Critical Point Dryer (CPD) from Quorum Technologies dried the intermediate fluids, including liquid carbon dioxide. The samples were coated with electrically conducting material using the EMITECH (SC7620) Super Cool Sputter Coater in a low-vacuum sputtering preheater after the carbon dioxide was fully removed. The specimens were securely attached to a specimen holder, and photographs were taken with an FEI SEM Model "QUANTA 250" at a respective magnification of 20 KV (Dickey and Levy, 1979) and images were recorded in five random fields.

Biochemical parameters, namely total phenol content, total sugars, peroxidase, and polyphenol oxidase were analysed on 45 day old leaves. The Folin-Ciocalteau reagent method was used to determine total phenol content, and the absorbance was measured at 660 nm with a spectrophotometer (Malick and Singh, 1980). The total sugar content calculated was quantified in mg/g of leaf sample by a standard curve made using glucose following the Sadasivam and Manickam's anthrone technique (1996). Peroxidase activity was determined as per the method of Hammerschmidt *et al.* (1982) and reported as a change in absorbance min/g. The method of Mayer *et al.* (1979) was followed to determine the polyphenol oxidase activity which was expressed as the change in absorbance min/g.

Biochemical and morphological data were analysed by completely randomized block design with three replications using the statistical tool package STAR 2.0.1 (IRRI, Philippines). The values were first subjected to an arcsine transformation and then the analysis of variance was calculated. The least Significant Difference (LSD) method was used to compare the treatment means.

RESULTS AND DISCUSSION

Out of the 59 M_3 mutant families studied, six moderately resistant mutants were identified based on visual scoring of a 0 to 5 scale. The results revealed that the per cent disease index (PDI) ranged from 10.25 to 15.5. The six mutants, *viz.,* PM 34, PM 42, PM 48, PM 27, PM 29 and PM 49 recorded PDI of, 11.27, 13.71,10.25, 13.92,12.75, 15, respectively and grouped as Moderately resistant (MR) (**Table 2**). Asif *et al.* (2015), Reddy *et al.* (2017) and Geetha *et al*. (2020) earlier reported similar results.

The six randomly selected moderately resistant mutants were further used for morphological characterization for the traits such as trichome density, leaf thickness and stomatal density to understand their association with powdery mildew resistance and the results are presented in **Table 1**.

Among the resistant mutants, PM 48 recorded a higher trichome density (**Fig. 1a**). Tulasi Korra and Manoj kumar (2020) and Soundhiriyan *et al.* (2018) reported similar results of resistant mutants which had higher trichome density than the susceptible check variety. All the moderately resistant mutants had higher trichome density than the susceptible check variety CO 6 (26.27/5 mm^2) (**Fig.1b**).The resistant check variety LBG 17 (76.36 mg/g) had significantly higher trichome density (**Fig. 1c**), followed by selected moderately resistant mutants PM 48 (62.34/5 mm^2), PM 49 (50.40/5 mm^2), PM 34 (49.82/5 mm^2), PM 27 (46.20 /5 mm^2), PM 42 (43.42/5 mm^2) and PM 29 (38.92/5 mm^2).

Leaf thickness was found to be 84.50, 81.10, 79.30, 75.32, 72.46 and 71.27µm in the resistant mutants PM 48, PM 34, PM 42, PM 49, PM 27 and PM,29 respectively (**Table 1**). PM 48 showed higher leaf thickness among the resistant mutants (**Fig. 2a**). Soundhiriyan *et al*. (2018) and Tulasi Korra and Manoj kumar (2020) reported that leaf thickness was more in resistant genotypes than the susceptible ones. The leaf thickness observed in moderately resistant mutants were higher than the susceptible check variety CO 6 which was recorded (57.74µm) (**Fig. 2b**). The resistant check variety LBG 17 had a significantly higher leaf thickness of 101.80 µm (**Fig. 2c**) than the moderately resistant mutants.

Stomatal density was inversely related to powdery mildew resistance. The stomatal density was lower in selected moderately resistant mutant PM 49, which was 105.32 mm^2 (**Fig. 3a**) followed by PM 27 (107.36 mm^2), PM 42 (109.10 mm^2), PM 34 (111.27 mm^2), PM 29 (114.26 mm^2), PM 48 (123.92 mm^2) and it was significantly higher in susceptible check CO 6 which was 170.63 mm^2 (**Fig. 3b**). Lower stomatal density was observed in resistant check (**Fig. 3c**) and mutants than in the susceptible check variety. Similar results were observed by TulasiKorra and Manoj kumar (2020) and Lima *et al.* (2010) who reported that the highly resistant genotypes showed significantly lower stomatal frequency than resistant genotypes while the highest stomatal frequency was found in the highly susceptible genotype.

Characterization of biochemical parameters in blackgram involves analysis of total phenols, total sugar, Peroxidase activity and Polyphenol Oxidase activity. Results of the biochemical analysis are presented in **Table 2.**

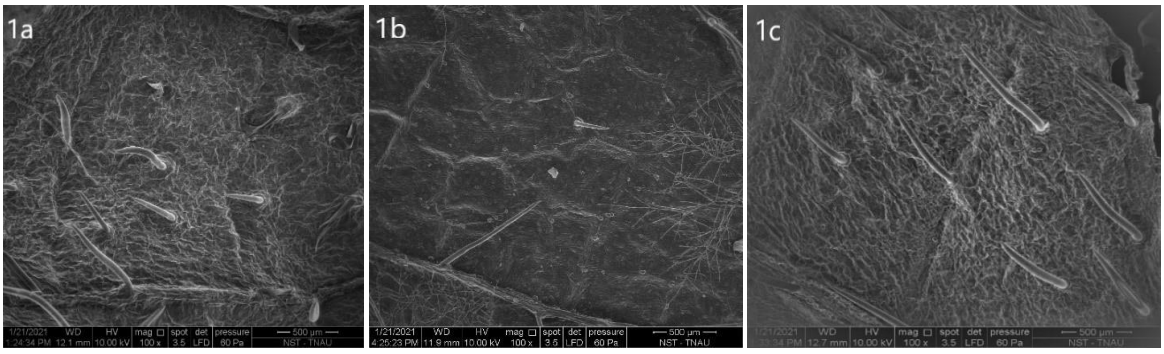
The phenolic content of moderately resistant mutants along with resistant and susceptible check varieties revealed a significant difference between them. The phenol content of moderately resistant mutants were PM 48 (5.97 mg/g), PM 42 (5.75 mg/g), PM 34 (5.87 mg/g), PM 27(5.25 mg/g), PM 49 (5.37 mg/g,) and PM 29(4.74 mg/g) showed higher value than the susceptible check variety CO 6 (3.25 mg/g). LBG 17 (6.25 mg/g) recorded higher phenol content than susceptible check variety. Significantly lower phenol content was observed in susceptible check variety CO 6. Soundhiriyan *et al.* (2018) and Kalia and Sharma (1988) stated that the resistant genotypes recorded higher phenolics in response to the disease when compared to susceptible genotypes.

The sugar content of moderately resistant mutants ranged from 23.2 (PM 48) to 30.0 mg/g (PM 29), while the resistant genotype LBG 17 recorded 24.8 mg/g of sugar content. The susceptible check variety CO 6 showed a higher level of sugars (32.1 mg/g) than resistant mutants and resistant check varieties. The study revealed that powdery mildew infected plants recorded higher levels of

Table1. Morphological variation in the M_3 mutant population of blackgram

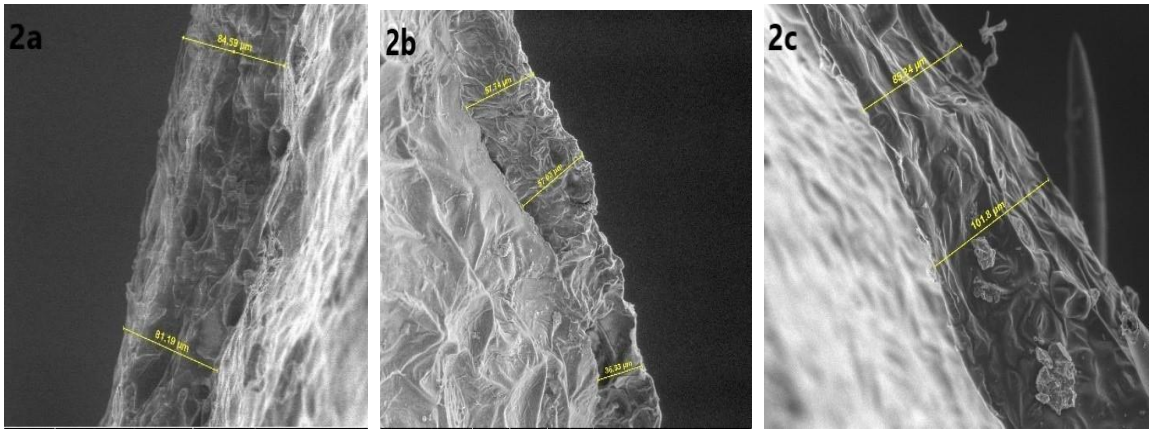
S. No	Name of the entries	Trichome density (5mm^2)	Leaf thickness (µm)	Stomatal density (mm^2)
1	PM 48	62.34^{b}	84.50^{b}	123.92^{b}
2	PM 34	49.82^{c}	81.10^{b}	111.27^{c}
3	PM 42	43.42^{d}	79.30^{c}	109.10^{d}
4	PM 49	50.40^{c}	75.32^{cd}	105.32^{e}
5	PM 27	46.20^{d}	72.46^{e}	107.36^{e}
6	PM 29	38.92^{e}	71.27^{e}	114.26^{c}
7	CO 6	26.27^{f}	57.34^{f}	170.63^{a}
8	LBG 17	73.36^{a}	101.80^{a}	106.30^{e}
	SEd	1.45	2.29	1.77
	CD (P<0.05)	3.12	3.51	3.80
	CV %	3.62	2.57	1.83

By LSD, the column means followed by a similar letter are not statistically different at the 5% level.



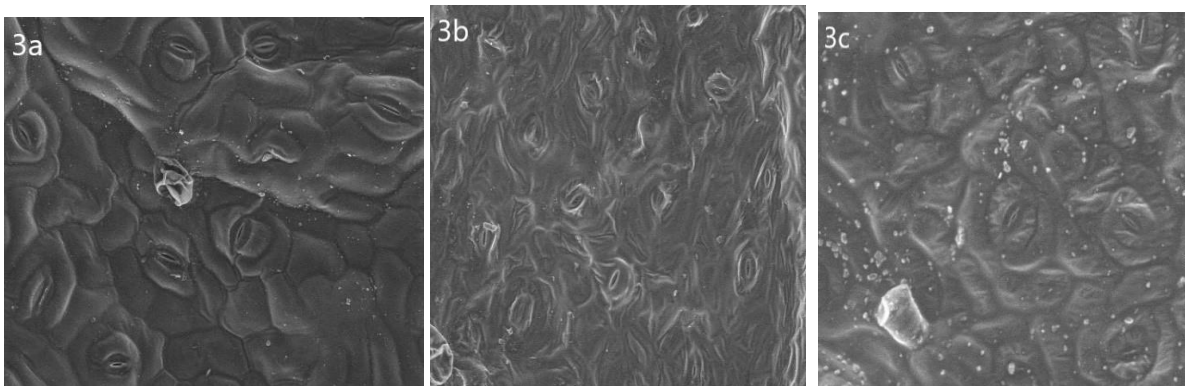
PM 48 CO 6 LBG 17

Fig. 1. Variation in trichome density in the mutants and check varieties of blackgram as revealed by scanning electron microscopy



PM 48 CO 6 LBG 17

Fig. 2. Scanning electron microphotographs showing variation in leaf thickness in the mutants and check varieties of blackgram



PM 49 CO 6 LBG 17

Fig. 3. Variation in Stomatal density in the mutants and check varieties of blackgram as revealed by scanning electron microscopy

Table 2. PDI and Biochemical parameters in M_3 mutant population

S. No	Name of the entries	Reaction	PDI	Total phenol	Total sugars	Peroxidase (POX)	Polyphenol Oxidase (PPO)
1	PM 48	MR	10.25^{d} (18.67)	5.97 ^{ab} (14.14)	23.20^{d} (28.79)	0.74 ^{ab} (4.93)	0.58^{c} (4.37)
2	PM 34	MR	11.27^{de} (19.61)	5.87 ^{b} (14.02)	24.00 ^{d} (29.33)	0.58 ^{c} (4.37)	0.66 ^{b} (4.66)
3	PM 42	MR	13.71^{c} (21.73)	5.75 ^{b} (13.87)	25.20 ^{d} (30.13)	0.66 ^{cd} (4.66)	0.52 ^{cd} (4.14)
4	PM 49	MR	12.75 ^{d} (20.92)	5.37 ^{c} (13.39)	28.00^{bc} (31.94)	0.48 ^{d} (3.97)	0.43^{e} (3.76)
5	PM 27	MR	13.92 ^{c} (21.90)	5.25 ^{c} (13.24)	28.80 ^{b} (32.45)	0.50 ^{d} (4.05)	0.48 ^{e} (3.97)
6	PM 29	MR	15.15 ^{b} (22.90)	4.74 ^{d} (12.57)	30.00 ^{b} (33.21)	0.42 ^{e} (3.72)	0.41 ^{e} (3.67)
7	CO 6	S	65.54 ^{a} (53.72)	3.25 ^{e} (10.38)	32.10 ^{a} (34.51)	0.31 ^{f} (3.19)	0.38^{f} (3.53)
8	LBG 17	R	0.00 ^{f} (0.00)	6.25 ^{a} (14.47)	24.80^{d} (29.86)	0.87^{a} (5.26)	0.76^{a} (5.00)
	SEd		0.58	0.12	0.59	0.01	0.01
	CD(P<0.05)		1.09	0.26	1.28	0.02	0.03
	CV%		0.58	2.87	2.71	2.43	3.70

Values followed by different superscripts in the same column are significantly different according to LSD test. Values in parenthesis are arc sine transformed values

sugar as compared to uninfected plants. These findings were in confirmation with Ramasami and Shanmugan (1977), Azmat and Khan (2014) and Dakshayani *et al.* (2005).

Variation was observed for peroxides activity in the moderately resistant mutants *viz.,*PM 48 (0.74 change in absorbance/min/g), PM 42 (0.66 change in absorbance/min/g), PM 34 (0.58 change in absorbance/min/g, PM 49 (0.48 change in absorbance/min/g), PM 27 (0.50 change in absorbance/min/g), PM 29 (0.42 change in absorbance/min/g) and also in the susceptible check variety CO 6 (0.31 change in absorbance/min/g). Higher significant peroxidase activity was observed in resistant check variety LBG 17 (0.87 change in absorbance/min/g) as compared to mutants and susceptible check variety. Similar findings were reported by Tirupathiswamy *et al.* (2017) and Arora *et al.* (1985)

The activity of polyphenol oxidase showed a significant difference between the resistant mutants and susceptible check variety CO 6. The highest polyphenol oxidase activity was observed in resistant check LBG 17 (0.76 change in absorbance/min/g) compared to the resistant mutants, viz., PM 34 (0.66 change in absorbance/min/g), PM 48 (0.58 change in absorbance/min/g), PM 42 (0.52 change in absorbance/min/g), PM 49 (0.43 change in absorbance/min/g), PM 27 (0.48 change in absorbance/min/g) and PM 29 (0.41 change in absorbance/min/g). Polyphenol Oxidase activity was significantly lower in susceptible check variety CO 6 (0.38 change in absorbance/min/g) when compared to the resistant mutants and check. Similar results were found by Bairwa *et al.* (2014) and Tirupathiswamy *et al.* (2017).

It is concluded that moderately resistant mutants and the resistant check variety LBG 17 showed higher phenol content, peroxidase activity, polyphenol oxidase activity and low sugar content than the susceptible check variety CO 6. It is clearly indicated the existence of resistance reaction in the selected mutants. However, the resistant mutants identified will be further confirmed during M_4 generation for powdery mildew resistance. In addition, the moderately resistant mutants and resistant check, LBG 17 exhibited higher trichome density, leaf thickness and lower stomatal density compared to susceptible check variety CO 6. Since the resistant check variety LBG 17 is known for photosensitivity nature, it may not be suitable for cultivation during *rabi* season. Since the identified mutants are insensitive and could be used as donors in the place of LBG 17 for powdery mildew resistance.

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