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Morphological and phenotypic variability in blackgram genotypes with varying reaction to *Mungbean* yellow *Mosaic* virus infection

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Abstract
Sixteen blackgram [*Vigna mungo* (L.) Hepper] genotypes were screened for *Mungbean* yellow mosaic disease under field conditions and identified nine genotypes as resistant, as moderately resistant, four genotypes as susceptible and two as highly susceptible based on modified MULLaRP scale (0-9). No genotypes were found immune or highly resistant against yellow mosaic disease. Morphological characters namely leaf thickness, epicuticular wax content, trichome density and stomatal frequency were evaluated among the genotypes having different degree of resistance. Leaves of *MYMV* resistant genotypes were thick when compared to highly susceptible genotypes. Similarly, the amount of epicuticular wax and trichome density in resistant genotypes were comparatively high to that of highly susceptible genotypes. However, stomatal frequency was high in highly susceptible genotypes when compared to resistant genotypes. Significant negative correlation was observed among morphological characters with disease severity except the stomatal frequency which was positively correlated. Low disease severity were found in the genotypes with purple petiole colouration or purple splash and having resistant or moderately resistant reaction to *MYMV* infection while genotypes with green colour petiole were found to be either susceptible or highly susceptible.

Keywords: Blackgram, *Mungbean* yellow mosaic virus, morphological characters

Introduction
Blackgram [*Vigna mungo* (L.) Hepper] is an excellent source of easily digestible protein with low flatulence. It supplies 26% protein, 57% carbohydrate, 1.2% fat and is a good source of phosphoric acid, calcium, thiamine (B1), riboflavin (B2) and niacin (B3) (Singh and Awasthi, 2004) [32]. Yellow mosaic disease is caused by *Mungbean yellow mosaic virus (MYMV)*, *Mungbean yellow mosaic India virus (MYMIV)*, *Horsegram yellow mosaic virus (HgYMV*) and *Dolichos yellow mosaic virus (DoYMV)*. In south India the usually disease is being caused by *MYMV*, a whitefly (*Bemisia tabaci*)-transmitted gemini virus and is one of the serious viral disease of blackgram that occur. It is a serious constraint in blackgram cultivation and could result up to 100% yield losses due to yellowing of leaves (Biswas *et al*., 2009) [9]. As the disease could not be managed satisfactorily by insecticides or any chemical applications, other alternatives of controlling the disease should be designed. Therefore, the present study was conducted to study the morphological basis of resistance to *MYMV* infection in blackgram genotypes.

Material and Methods
The experiment was conducted during *kharif* 2014-15 at the Regional Agricultural Research Station (RARS), Lam, Guntur using 16 black gram genotypes namely KPU-1, KPU-9, KPU-6, KPU-29, KPU-21, KPU-22, KPU 12-133, KPU 12-1731, OBG-32, LBG-752, DKU-87, DKU-102, UG-281, PU 12-11, Co5 and LBG-623 (susceptible check) obtained from RARS, Lam. A Randomised Block Design with two replications in a micro plot of 5 x 4 m with spacing of 30 x 10 cm was followed and percent disease incidence was recorded weekly using the formula

$$\text{Per cent MYMV incidence} = \frac{\text{Number of plants infected in a micro plot}}{\text{Total number of plants in a micro plot}} \text{x } 100$$

By using 0-9 modified scale of All India Coordinated Research Project on MULLaRP (Alice and Nadarajan, 2007) [2], *MYMV* severity was recorded weekly and per cent disease index (PDI) was computed using the formula given by Wheeler (1969) [37].

$$PDI = \frac{\text{Sum of all the numerical ratings}}{\text{Number of observations} \times \text{Maximum disease rating}} \times 100$$

The genotypes were assigned different disease reactions based on the categorization given by Gantait and Kantidas (2009) [17] (Table-1).

Table 1: Categorization of blackgram genotypes based on MYMV disease severity

PDI	Rating	Reaction
0.1-5	1.0 to 2.0	Resistant (R)
5.1-15	2.1 to 4	Moderately resistant (MR)
15.1-30	4.1 to 5	Moderately susceptible (MS)
30.1-75	5.1 to 7	Susceptible (S)
75.1-100	7.1 to 9	Highly susceptible (HS)

Leaf Thickness (µm) was measured using ocular micrometer as described by Tagger and Gill (2012) [35]. The stomatal frequency was determined following the method of Varadarajan and Wilson (1973) [36]. Leaf epicuticular wax content was determined as per the procedure given by Fernandes *et al.* (1964) [15]. Trichome Density (5 mm dia leaf disc) was carried out according to the procedure described by Tagger and Gill (2012) [35].

Results and Discussion

Mungbean Yellow mosaic disease varied from 3.73 (DKU-87) to 96.15% (LBG-623) in 16 genotypes tested (Table-2) and based on 0-9 scale, genotypes *i.e.*, DKU-87, KPU 12-133, DKU-102, UG-281, KPU-21, KPU-6, KPU-29, KPU 12-1731 and PU 12-11 were categorized as resistant and the disease rating varied from 0.85 to 1.50. Genotype LBG-752 was categorized as moderately resistant with 2.75 disease rating, genotypes KPU-1, KPU-22, KPU-9 and OBG-32 were categorized as susceptible (5.65 to 6.65 rating) and genotypes Co5 and LBG-623 as highly susceptible (7.60 to 7.85 rating).

Leaf Thickness (µm)

Significant differences in leaf thickness were observed among black gram genotypes and varied between 160.41 to 97.09 µm. In *MYMV* resistant genotypes, it ranged from 126.31 (KPU 12-1731) to 160.41 µm (DKU-87) and in moderately resistant genotype (LBG-752) it was 120.01 µm thick. Leaves of *MYMV* susceptible genotypes were thin and thickness ranged between 108.40 (OBG-32) and 114.17 µm (KPU-9) and in highly susceptible genotypes thickness was 97.09 (LBG-623) and 101.51 µm (Co5) (Table 2). Significant negative correlation (-0.902) existed between leaf thickness and disease severity (Table 3). Kunkalikar *et al*. (2007) [21] reported histological changes in papaya leaf due to *Papaya Ring spot Virus* infection. Pilic *et al*. (2013) [26] reported differences in pepper leaf thickness infected with *Cucumber Mosaic Virus* (*CMV*).

Reduction in the size of a virus infected leaf may be due to the hydrolytic enzymes secreted from the virus that cause disintegration of cell walls forming large intercellular spaces (Singh, 1971) [30]. Hypotrophy and destruction of spongy cells, loss of columnar nature and distortion of palisade cells was reported to occur due to metabolic changes induced by virus (Kunkalikar *et al*., 2007) [21].

Stomatal Frequency (number of stomata/mm^2)

Stomatal frequency of 16 blackgram genotypes differed significantly and it ranged from 71.71 to 155.64 mm^2. Among *MYMV* resistant genotypes stomatal frequency was low in DKU-87 (71.71) and high in PU 12-11 (102.10) per mm^2. In moderately resistant genotype (LBG-752) it was 113.75 mm^2, in susceptible genotypes it varied from 142.82 (KPU-9) to 148.53 mm^2 (KPU-1) and in highly susceptible genotypes stomatal number was 150.15 (Co5) and 155.64 (LBG-623) mm^2 (Table 2). A significant positive correlation (0.961) existed between stomatal frequency and disease severity (Table 3).

The results were in agreement with the findings of Gagandeep *et al*. (2013) [16] who reported significantly higher number of stomata in *CMV* susceptible watermelon genotypes than resistant one and reported significant positive correlation between stomatal size, density, index and PDI. Ishak and El-Deeb (2004) [19] reported decrease in stomatal number on viral infection compared to healthy plant. Lindsey and Gudauskas (1974) [22] measured diffusive resistance of leaves and indicated that stomatal apertures were reduced in leaves of *Maize Dwarf Mosaic Virus* infected plants. However, in the present study, variation in the number of stomata among the blackgram genotypes could be due to the variation in the genetic makeup.

Epicuticular Wax Content (mg dm^{-2})

Epicuticular wax content in blackgram genotypes varied significantly and it ranged from 0.20 (LBG-623) to 0.58 mg dm^{-2} (DKU-87 and DKU-102). It was recorded high in *MYMV* resistant genotypes which ranged between 0.45 (KPU 12-1731) and 0.58 mg dm^{-2} (DKU-87 and DKU-102). In moderately resistant genotype (LBG-752) 0.44 mg dm^{-2} of epicuticular wax was recorded. In susceptible genotypes it ranged between 0.27 (OBG-32) to 0.33 mg dm^{-2} (KPU-9) and in highly susceptible genotypes it was 0.20 in LBG-623 and 0.24 mg dm^{-2} in Co5. Significant negative correlation (-0.958) existed between epicuticular wax and disease severity (Table 2 and 3).

These results were in agreement with the findings of Chand and Verma (1980) [11], who reported thick cuticle in *MYMV* resistant blackgram and *mungbean* varieties than the susceptible ones. *Cotton Leaf Curl Virus* (*CLCuV*) resistant cotton cultivars were reported to have high epicuticular wax than susceptible cultivars (Ashraf *et al*., 1999; Zafar *et al*., 2010) [4, 39].

Waxes are triterpenoids which impart slippery character (Bass and Fidgor, 1978) [6], contaminate insects pad surface and create hindrance for their contact with plants (Eigenbrode, 2004) [13]. Leaf waxes and their effect on insect interactions were reported with reference to *Brassica* sp. and flee beetle, *Phyllotreta* sp. (Stoner, 1990; Bodnaryk, 1992; Eigenbrode *et al*., 2000) [34, 10, 14] and pea and aphid, *Aphiduservi* (Rutledge *et al*., 2003) [27]. Susceptibility of certain genotypes to *MYMV* infection could be due to insufficient amount of wax that allowed insect to contact the host thus resulting in the transmission of virus.

Trichome Density (5 mm dia leaf disc)

Significant variation in the trichome density was recorded among different blackgram genotypes which ranged from 6.13 (LBG-623) to 16.88 (DKU-87). In *MYMV* resistant genotypes, trichome density ranged between 12.25 (KPU-6) and 16.88 (DKU-87) and in moderately resistant genotype, LBG-752 it was 9.25. In susceptible genotypes, trichome density ranged between 7.13 (OBG-32) and 8.50 (KPU-1) and in highly susceptible genotype (LBG-623), the trichome number was 6.13 and in Co5 it was 6.75 (Table 2). Negative

correlation (-0.899) existed between trichome density and disease severity (Table 3).

Results were in agreement with the findings of Arora *et al*. (2011) [3] who reported significantly high trichome frequency in *Tomato Leaf Curl Virus* resistant tomato genotypes than susceptible one. Chand and Varma (1980) [11] reported whitefly resistant blackgram varieties with more leaf hairs per cm^2 than susceptible varieties and pubescent genotypes with less whitefly population than non-pubescent genotypes. Negative correlation between leaf trichomes and whitefly was reported in brinjal (Soundararajan and Baskaran, 2001; Ayyasamy and Baskaran, 2005; Singh *et al*., 2002) [33, 5, 31] and blackgram (Taggar and Gill, 2012) [35]. Leaf trichome density has defensive character that prevents the infestation of whitefly by deterring or limiting their establishment (Sanchez-Pena *et al*., 2006) [28] and thus making the movement, feeding and oviposition difficult (Noris and Kogan, 1980) [25].

Population of *B. tabaci* was high in cotton genotype having low trichome density and long hair length (Naveed *et al*., 2011) [23]. However, positive correlation between whitefly population and trichome density was reported in cotton (Butler *et al*., 1986; Ozgur and Sckeroglu, 1986; Navon *et al*., 1991; Ashraf *et al*., 1999) [24, 4] which are contradictory with the present study.

Table 2: Morphological variability in blackgram genotypes with varying reaction to MYMV infection during kharif 2014-15

S. No.	Genotypes	Disease Incidence (%)	PDI	Disease reaction	Leaf thickness (µm)	Stomatal frequency (mm^2)	Epicuticular wax content (mg/dm^2)	Trichomes density (5 mm dia leaf disc)
1	DKU-87	3.73* (11.13)	5.00 *(12.89)	R	160.41	71.71	0.58	16.88** (4.15)
2	DKU-102	4.19 (11.80)	6.66 (14.90)	R	154.33	80.56	0.58	15.63 (4.01)
3	KPU-21	5.79 (13.59)	7.78 (15.81)	R	146.67	85.56	0.56	12.50 (3.59)
4	UG-218	7.01 (15.32)	8.89 (17.04)	R	134.62	78.45	0.55	14.88 (3.92)
5	KPU-6	5.97 (14.11)	9.44 (17.88)	R	144.68	97.30	0.56	12.25 (3.56)
6	KPU-29	5.57 (13.65)	10.00 (18.32)	R	131.39	75.40	0.49	12.63 (3.61)
7	KPU 12-1731	6.97 (15.30)	10.00 (18.40)	R	126.31	99.25	0.45	12.75 (3.62)
8	KPU 12-133	6.78 (15.06)	11.10 (19.43)	R	149.65	86.64	0.55	16.13 (4.08)
9	PU 12-11	6.63 (14.91)	11.67 (19.92)	R	141.83	102.10	0.46	12.50 (3.60)
10	LBG-752	14.47 (22.34)	21.11 (27.22)	MR	120.01	113.75	0.44	9.25 (3.12)
11	OBG-32	67.38 (55.15)	58.48 (49.87)	S	108.40	147.54	0.27	7.13 (2.75)
12	KPU-1	64.37 (53.36)	60.33 (50.94)	S	106.01	148.53	0.31	8.50 (2.98)
13	KPU-22	56.68 (48.84)	62.15 (52.08)	S	112.16	144.96	0.32	8.25 (2.96)
14	KPU-9	49.21 (44.53)	62.94 (52.49)	S	114.17	142.82	0.33	8.13 (2.93)
15	Co5	93.53 (76.90)	75.55 (60.55)	HS	101.51	150.15	0.24	6.75 (2.69)
16	LBG-623	96.15 (79.08)	79.98 (63.53)	HS	97.09	155.64	0.20	6.13 (2.57)
SEm±		1.58	1.78	-	8.38	8.18	0.03	0.17
CD (P ≤ 0.05%)		4.76	5.38	-	25.13	24.53	0.08	0.51
CV%		10.01	11.16	-	13.09	14.70	12.57	10.11

*Figures in parentheses are arcsine transformed values

**Values in parentheses are square root transformed values

Table 3: Correlation coefficients between morphological characters and mungbean yellow mosaic disease severity

Morphological characters	MYMV severity
Leaf thickness	-0.902
Stomatal frequency	0.961
Epicuticular wax	-0.958
Trichome density	-0.899

Pigmentation of Petiole

Petiole colouration was light green in the genotypes KPU-9, KPU-1, KPU-22, OBG-32, Co5 and LBG-623 while it was greenish with purple splashes or streaks in five genotypes *viz*., KPU-6, KPU-29, LBG-752, UG-218 and PU 12-11 and in the remain genotypes *i.e*., DKU-87, DKU-102, KPU-21, KPU 12-133 and KPU 12-1731 petiole was dominated with purple pigmentation. The genotypes with purple petiole pigmentation and purple splash were found with low *MYMV* disease severity, whitefly population and showed resistance to moderately resistant reaction and the genotypes with green colour petiole were found to be either susceptible or highly susceptible to *MYMV* infection (Table 4).

Davies (2004) [12] emphasized the production of anthocyanins or tannins, quinones and phytomelanins and their involvement in plant defence. Konczak and Zhang (2004) [20] and Wrolstad (2004) [38] reported that certain anthocyanins have demonstrable antiviral, antibacterial and fungicidal activities. Purple pigmentation observed in certain genotypes in present study substantiates earlier reports as they recorded low *MYMV* incidence than green pigmentation.

Similarly it was reported that colour plays an important role in host-plant selection by whiteflies from a far of distance of the host. Whiteflies were reported to be more attracted to yellow-green colour when compared to red, orange/red, dark green and purple (Husain and Trehan, 1940) [18] and female whiteflies were reported to show high colour preferences (Ahmad and Harwood, 1973; Berlinger, 1980; Berlinger, 1986; Sharaf, 1982) [1, 7, 8, 29]. Hence, genotypes having light green coloured petioles were preferred more than dark coloured petioles by whiteflies in the present situation rendering then to more *MYMV* infection.

Seed Colour Characters

Seed colour was dark black in KPU-1 and KPU 12-133, dull black in the genotypes *viz*., KPU-6, KPU-9, KPU-21, KPU-22, OBG-32, UG-18, PU 12-11 and Co5, dull black with mosaic pattern in KPU 12-1731, light brown in DKU-87, DKU-102 and KPU-29, shiny black in LBG-752 and shiny greenish marble tinge in LBG-623 (Table 4). The genotypes that were found resistant to *MYMV* infection had light brown seeds.

Table 4: Variation in phenotypic characters in mungbean yellow mosaic disease resistant and susceptible blackgram genotypes during kharif 2014-15

S. No.	Genotypes	Petiole colouration	Seed colour
1	DKU-87	Purple	Light brown
2	DKU-102	Purple	Light brown
3	KPU-21	Purple	Dull black
4	UG-218	Greenish with purple splashes	Dull black
5	KPU-6	Greenish with purple splashes	Dull black
6	KPU-29	Greenish with purple splashes	Light brown
7	KPU 12-1731	Purple	Dull black with mosaic pattern
8	KPU 12-133	Purple	Dark black
9	PU 12-11	Greenish with purple splashes	Dull black
10	LBG-752	Greenish with purple splashes	Shiny black
11	OBG-32	Light green	Dull black
12	KPU-1	Light green	Dark black
13	KPU-22	Light Green	Dull black
14	KPU-9	Light green	Dull black
15	Co5	Light green	Dull black
16	LBG-623	Light green	Shiny with greenish marble tinge

Conclusions

Leaves of *MYMV* resistant genotypes were thicker (126.31 to 160.41 µm) when compared to highly susceptible genotypes (97.09 µm - 101.51 µm). Similarly, the amount of epicuticular wax varied significantly among genotypes and was found high in resistant genotypes (0.45 to 0.58 mg dm^{-2}) when compared to highly susceptible genotypes (0.20 to 0.24 mg dm^{-2}). Trichome density ranged between 12.25 (KPU-6) and 16.88 (DKU-87) in resistant genotypes and in highly susceptible genotypes it varied from 6.13 (LBG-623) to 6.75 (Co5). However, higher stomatal frequency was recorded in highly susceptible genotypes that varied between 150.15 (Co5) and 155.64 (LBG-623) per mm^2 compared to resistant genotypes which ranged from 71.71 (DKU-87) to 102.10 per mm^2 (PU 12-11). Significant negative correlation was observed among morphological characters with disease severity except the stomatal frequency which was positively correlated. The genotypes with purple petiole pigmentation and purple splash were found with low *MYMV* disease severity and showed moderately resistant reaction and the genotypes with green colour petiole were found to be either susceptible or highly susceptible to *MYMV* infection.

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