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Mungbean Yellow Mosaic Infection and Biochemical Variability in Blackgram Genotypes

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ABSTRACT

Mungbean yellow mosaic disease varied from 3.73 (DKU-87) to 96.15% (LBG-623) in 16 blackgram [Vigna mungo (L.) Hepper] genotypes tested. Of the 16 genotypes nine genotypes were categorised as resistant, one as moderately resistant, four genotypes as susceptible and two as highly susceptible genotypes. Significant variation was observed among the genotypes tested with respect to the amount of total phenols, total proteins, total chlorophyll, peroxidases and phenylalanine ammonia lyase activity. High amount of biochemical substances were recorded in resistant genotypes than other genotypes.

Key words : Blackgram, Chlorophyll, *Mungbean Yellow Mosaic Virus*, Peroxidases, Phenols, Phenylalanine Ammonia Lyase and Proteins.

In India, blackgram is cultivated in 2.29 M ha with 1.90 M t production and 500 kg ha^{-1} productivity (Department of Agriculture and Cooperation, Government of India, 2012-13) and in Andhra Pradesh, it is grown in an area of 2.65 lakh ha with a production of 2.05 lakh tonnes and 774 kg ha^{-1} productivity (Department of Agriculture, Government of Andhra Pradesh, 2013-14). Blackgram 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).

Mungbean Yellow Mosaic Virus (*MYMV*), a whitefly (*Bemisia tabaci*) transmitted gemini virus, causes yellow mosaic disease and is one of the most serious viral diseases of blackgram that occurs in South Asia. It is a serious constraint in blackgram cultivation and could result up to 100% yield losses due to yellowing of leaves (plate 1,2) (Biswas *et al*., 2009). 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 biochemical 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 blackgram 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 microplot of 5 m x 4 m with spacing of 30 cm x 10 cm was followed and per cent disease incidence was calculated using the formula

$$\text{Per cent } MYMV \text{ incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

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

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

The genotypes were assigned different disease response / reactions based on the categorization given by Gantait and Kantidas (2009) (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 4.0	Moderately resistant (MR)
15.1-30	4.1 to 5.0	Moderately susceptible (MS)
30.1-75	5.1 to 7.0	Susceptible (S)
75.1-100	7.1 to 9.0	Highly susceptible (HS)

Total phenols were determined following the method described by the Association of Analytical Chemists colorimetric method (AOAC), (1965). Protein estimation was carried out according to the procedure described by the Lowry *et al*. (1951), total chlorophyll was calculated by using the formula,

Total chlorophyll (mg g^{-1} tissue) = [20.2 (D645) + 8.02 (D663)] x V/1000 x W

Where, D is optical density at respective wave length (nm), V is final volume of chlorophyll extract in 80% acetone and W is fresh weight of the tissue extracted (Bruinosa, 1963). Peroxidase activity was determined according to Hammerschmidt *et al.* (1982) and phenylalanine ammonia lyase activity was determined as described by Dickerson *et al*. (1984). The amount of transcinnamic acid synthesized was calculated using the extinction coefficient of 9630 $M^{-1}cm^{-1}$.

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) (Table 2).

Estimation of Total Phenols

Total phenols differed significantly among blackgram genotypes tested and varied from 0.36 (LBG-623) to 0.90 mg/100 mg (DKU-87). They were recorded high in *MYMV* resistant genotypes (0.79 to 0.90 mg/100 mg) and low in highly susceptible genotypes (0.36 to 0.40 mg/100 mg) (Table 2). Rathi *et al*. (1986) reported high amount of total phenols in *Pigeonpea Sterility Mosaic Virus* resistant pigeonpea varieties while Prabu and Warade (2009) had reported increased phenols in *Okra Yellow Vein Mosaic Virus* resistant, wild and inter-specific hybrids of okra. High amount of total phenols in the resistant genotypes were accompanied due to increased activities of polyphenol oxidases and peroxidases, resulting in increased oxidation of phenolic substances to form more toxic quinones and others oxidative products that might aid to combat the pathogen in the resistant host (Jabeen *et al*., 2009).

Estimation of Total Proteins

Total proteins varied significantly among the genotypes tested and ranged from 1.40 (LBG-623) to 1.99 (DKU-87). The amount of total proteins were high in *MYMV* resistant genotypes and ranged between 1.83 (PU 12-11) and 1.99 mg/100 mg (DKU-87) compared to susceptible genotypes (1.53 in OBG-32 and KPU-1 - 1.63 mg/100 mg in KPU-29) and in highly susceptible genotypes, amount of proteins had reduced to 1.40 mg/100 mg (LBG-623) and 1.48 mg/100 mg (Co5) (Table 2). The present results were in agreement with the reports of Chand and Varma (1980) where *MYMV* resistant *urdbean* varieties were reported with high total protein content than that in highly susceptible and susceptible varieties. The occurrence of high amount of total proteins could be due to the increased synthesis of proteins for the activation of enzymes that are essential for defence activities (Vidyasekaran, 2001).

Estimation of Total Chlorophyll Content

Significant variation in total chlorophyll was recorded among the blackgram genotypes and it varied from 0.036 (LBG-623) to 0.134 mg/100 mg (DKU-87). The chlorophyll content was high in resistant genotypes (0.113 to 0.134 mg/100 mg) and low in highly susceptible genotypes LBG-623 (0.036

Table 2. Biochemical variability in blackgram genotypes with varying reaction to *MYMV* infection during *kharif* 2014-15.

S. No.	Genotypes	Disease Incidence (%)	PDI	Disease reaction	Total phenols (mg/100 mg)	Total proteins (mg/100 mg)	Total chlorophyll (mg/100 mg)	Peroxidase (Δ Abs/min/ g)	Phenylalanine ammonia lyase activity activity (η moles of transcinnamic acid/min/g)
1	DKU-87	3.73	5.65	R	0.90	1.99	0.134	0.36	147.98
2	DKU-102	4.19	1.13	R	0.89	1.95	0.131	0.34	127.21
3	KPU-21	5.79	1.30	R	0.86	1.90	0.114	0.32	127.21
4	UG-218	7.01	5.90	R	0.85	1.90	0.124	0.34	137.59
5	KPU-6	5.97	1.05	R	0.83	1.89	0.113	0.30	142.78
6	KPU-29	5.57	5.70	R	0.88	1.94	0.121	0.34	124.61
7	KPU 12-1731	6.97	0.90	R	0.80	1.87	0.119	0.31	140.19
8	KPU 12-133	6.78	1.30	R	0.82	1.88	0.127	0.31	132.40
9	PU 12-11	6.63	6.65	R	0.79	1.83	0.116	0.31	134.99
10	LBG-752	14.47	2.75	MR	0.65	1.74	0.094	0.25	132.40
11	OBG-32	67.38	0.85	S	0.43	1.53	0.051	0.18	101.25
12	KPU-1	64.37	0.95	S	0.44	1.52	0.053	0.18	90.86
13	KPU-22	56.68	1.00	S	0.46	1.56	0.054	0.19	98.65
14	KPU-9	49.21	1.50	S	0.51	1.63	0.060	0.20	106.44
15	Co5	93.53	7.60	HS	0.40	1.48	0.043	0.14	85.67
16	LBG-623	96.15	7.85	HS	0.36	1.40	0.036	0.14	83.07
	SEm±	1.58	0.19	-	0.04	0.03	0.006	0.01	8.06
	CD (Pd ≤ 0.05)	4.76	0.58	-	0.12	0.08	0.018	0.18	24.16
	CV%	10.01	11.91	-	11.93	12.89	12.69	0.30	13.48

R-Resistant; S-susceptible; HS-Highly susceptible

mg/100 mg) and Co5 (0.043 mg/100 mg) (Table 2). Similar variations were reported by Mali *et al*. (2000) in *mothbean* and Ajmal *et al*. (2011) in cotton. Virus induced chlorosis in plants is generally attributed to inhibition of chloroplast development and stimulation of the enzyme, chlorophyllase which attack chlorophyll (Esau, 1956). It was reported that the virus infected pigeon pea leaves had much higher chlorophyllase activity than the healthy areas (Ramakrishnan *et al*., 1968).

Peroxidase Activity (Δ Abs/min/g)

Peroxidase activity differed significantly in blackgram genotypes and its activity varied from 0.14 (LBG-623) to 0.36 Δ Abs/min/g (DKU-87). In *MYMV* resistant genotypes, its activity ranged from 0.31 (PU 12-11, KPU-12-133 and KPU-12-1731) to 0.36 Δ Abs/min/g (DKU-87) and in highly susceptible LBG-623 and Co5 genotypes peroxidase activity was 0.14 Δ Abs/min/g (Table 2). Ashfaq *et al*. (2010) reported similar results in blackgram and Seyyed *et al.* (2012) reported in corn. Peroxidases play an important role in plant defence mechanism due to their involvement in the removal of hydrogen peroxide from cells, involvement in cell wall lignifications and in the formation of barrier substances at the site of pathogen penetration (Almagro *et al*., 2009). It was reported that they play a major role in the oxidation of phenolic compounds, increased antimicrobial activity and also trigger programmed cell death close to the site of infection that result in arresting pathogen development (Shimizu *et al*., 2006).

Phenylalanine Ammonia Lyase Activity (PAL) (η moles of transcinnamic acid/min/g)

Phenylalanine ammonia lyase activity differed significantly among blackgram genotypes and its activity ranged from 83.07 to 147.98 η moles of transcinnamic acid/min/g. Phenylalanine ammonia lyase activity was high in *MYMV* resistant genotype (124.61 to 147.98η moles of transcinnamic acid/min/g) compared to highly susceptible genotypes (83.07 to 85.67 η moles of transcinnamic acid/min/g) (Table 2). High PAL activity in resistant genotypes over susceptible genotypes and increase in PAL activity has frequently been mentioned as a defence reaction of plants in response to pathogen attack (Logemann

Plate 1. Healthy blackgram plant

Plate 2. *MYMV* infected blackgram plant

et al., 2000). An increase in PAL activity results in the accumulation of phenolic compounds that act as substrates for oxidative enzymes like polyphenol oxidases and peroxidases. PAL catalyzes first reaction of phenylproponoid pathway where, sequence of reactions are involved in the conversion of phenylalanine to t-cinannamic acid (Slatnar *et al.*, 2010), which is an immediate precursor for the biosynthesis of salicylic acid, a signal molecule in systemic acquired resistance (Klessig and Malamy, 1994). It was reported that phenylalanine is required for the growth and development of pathogen (Liu and Rahe, 1997) and decrease in its levels due to PAL accumulation may restrict the pathogen.

From the present study it can be concluded that high total phenols, total proteins and total chlorophyll content were present in *MYMV* resistant genotypes than highly susceptible genotypes. High peroxidase and phenylalanine ammonia lyase activity was recorded in *MYMV* resistant genotypes when compared to highly susceptible genotypes.

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