Original Research Article

"Enhancing Mungbean Resistance to Yellow Mosaic Virus: Insights from Biochemical Studies."

ABSTRACT

Mungbean is one among the important and major pulse crop for supplementation of protein in subtropical zones of the world. Apart from providing protein in the diet, it also has the appreciable quality of helping the symbiotic root rhizobia by contributing to fix atmospheric nitrogen enriches the fertility of soil. Among the biotic factors, Mungbean yellow mosaic virus (MYMV) is the major bottleneck for pulse producers. The current study is aimed at estimation of biochemical enzymes like peroxidase, polyphenol oxidase, catalase, phenols and sugars contributing for resistance in mungbean genotypes. Fourteen Mungban genotypes including one susceptible check MYB-11 and KKM-3 (UASB released variety) were screened during *kharif* 2022 and summer 2023.To determine MYMV induced biochemical changes in mungbean, the infected leaf samples were collected from resistant, moderately resistant, moderately susceptible and susceptible genotypes. The varied level of different defense inducing enzymes from all the 14 mungbean genotypes was estimated. The biochemical constituents such as total sugar, total phenol content and enzyme activity of Peroxidase (PO), Polyphenol oxidase(PPO) and catalase activity were estimated. The present study indicated that the defence inducing enzymes, i.e, peroxidase, polyphenol oxidase, catalase, phenols and sugars, showed comparatively higher level of resistance in genotypes *viz*., MYB- 6, 7, 8 and 9 when compared to the susceptible check (MYB-11).

Key words: Enzymes, genotypes, Mungbean, MYMV, sugars, phenols, resistance, susceptible

1. INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek] is one of the important pulse crops in India. It is an important source of dietary protein across South Asia where the diet is mostly cereal based. It is a short duration crop in humid and sub-humid regions (Jayappa *et al*. 2017). 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). MYMV is a major threat

to legume crops for successful cultivation. In pulses, the yield loss from viral diseases accounts for up to 80%, while in mungbean, the MYMV alone causes up to 80 to 100% loss (Bhanu *et al*. 2017).

The major bottleneck in the mungbean production is its massive gap in the productivity though it is very popular crop throughout the world with the introduction of improved varieties, breeding for several resistant cultivars, and better management for the production techniques, mungbean can take up its stand in the world to continue to be an excellent choice for farmers. Breeding for resistant varieties is considered to be the most important and environmental friendly approach to come back from several drawbacks which are presently witnessing in the mungbean. Most of the efficient cultivars are prone to susceptibility over a period of time due to several factors. Thereby screening of resistant genotypes based on biochemical and molecular approaches are gaining importance.

As the disease could not be managed satisfactorily by insecticides or any chemical application, other alternatives of controlling the disease should be designed. Therefore, the present study was conducted for the confirmation of biochemical basis of resistance to MYMV infection in mungbean genotypes.

2. MATERIAL AND METHODS

2.1 Evaluation of MYMV under field conditions

The field experiments were conducted at the experimental plot, Main Research Station, Hebbal, Bengaluruto identify the potential resistance genotypes against MYMV. Fourteen Mungban genotypes including one susceptible check MYB-11 and KKM-3 (UASB released variety) were screened during *kharif* 2022 and summer 2023. These 13 genotypes were obtained from Asian Vegetable Research and Development Center (AVRDC) Taiwan formerly which is known as World Vegetable Centre. The Mungbean genotypes were sown with spacing of 30 × 10 cm, with a row length of 2.5 m in RCBD design with two replications. The recommended package of practices was followed for raising the crop. Plants were allowed for natural infection of MYMV. The observations were recorded on symptom expression, disease incidence, severity and yield during the experiment. Mungbean yellow mosaic disease severity was recorded at 30, 45 and 55 DAS from all the plants from each row. Severity of disease was scored according to phenotypic disease severity

scale developed from AVRDC. The mungbean genotypes were categorized based on modified phenotypic disease severity scale of AVRDC. The per cent disease index (PDI) was computed by using the following formula (Wheeler, 1969)

$$\text{Per cent disease index (\%)} = \frac{\text{Sum of numerical observation} \times 100}{\text{Maximum disease scale} \times \text{No. of observations}}$$

2.2 Biochemical variation studies

To determine MYMV induced biochemical changes in mungbean, the infected leaf samples were collected from resistant, moderately resistant, moderately susceptible and susceptible genotypes. The varied level of different defense inducing enzymes from all the 14 mungbean genotypes was estimated. The biochemical estimations were carried out from 15 to 20 days old freshly collected leaf samples from the mungbean sown at the experimental field Main Research Station, Hebbal, Bengaluru. The biochemical constituents such as total sugar, total phenol content and enzyme activity of Peroxidase (PO), Polyphenol oxidase(PPO) and catalase activity were estimated. The total sugar was estimated by Hedge and Hofreiter (1962), Dubois *et al*. (1956) and total phenol by Sadasivam and Manickam (1996), peroxidase enzyme activity was estimated by spectrophotometer method as described by Hartee(1955), polyphenol oxidase and catalase enzyme activity by Mayer *et al*. (1965)

3. RESULTS AND DISCUSSION

The genotypes were categorized into different groups by using modified AVRDC scale. The MYB - 6, 7, 8, 9 and 12 belongs to resistant group with per cent disease index in the range of 10.01- 25 in all the seasons. The other genotypes with MYMV infection in different seasons were categorized into different groups as indicated in the Table .

Table 1: Grouping of mungbean genotypes evaluated against MYMV during different seasons (AVRDC Scale)

Score	Reaction	% Disease index	*Kharif* 2022	Summer 2023
1	Highly resistant (HR)	0.01-10	Nil	Nil
2	Resistant (R)	10.01 -25	MYB 6, 7, 8 ,	MYB 6, 7, 8 , 9

			9 & 12	& 12
3	Moderately Resistant (MR)	25.01 - 40	MYB 1,5,10, 13 & KKM -3	MYB 1, 3, 5 & KKM -3
4	Moderately Susceptible (MS)	40.01 – 60	MYB 2, 3 & 4	MYB 2, 4, 10 & 13
5	Susceptible (S)	60.01 – 80	MYB - 11	MYB -11
6	Highly Susceptible (HS)	>80	Nil	Nil

Gupta and Mishra (2014) screened mungbean and urdbean lines of around 54 and 43, respectively against MYMV in field conditions. Among them, 11 genotypes of mungbean and 16 of urdbean were found resistant to MYMV. Mohan *et al*. (2014) evaluated the resistance source against MYMV in two different locations. They screened for 120 mungbean genotypes and found that the genotypes TM-11-07, EC 398897, TM11-34, IPM-02-03, PDM-139, IPM-02-14, Pusa-0672, MH-521, Pusa-0871, and CO-7 showed resistance in both the locations and these genotypes would be utilized as donors to bring MYMV resistant lines.

3.1 Variation of different enzymes activity in mungbean genotypes due to MYMV infection.

Table 2: Estimation of peroxidase activity (PO) (ΔAbs $min^{-1}g^{-1}$) in mungbean genotypes

Line	Peroxidase activity (PO) (ΔAbs min^{-1} g^{-1})	Group acc to R software
MYB - 1	0.60459	FG
MYB - 2	0.25433	G
MYB - 3	0.22412	G
MYB - 4	0.66222	FG
MYB - 5	2.38711	DE
MYB - 6	3.34316	B
MYB - 7	4.22167	A
MYB - 8	3.13427	BC
MYB - 9	2.07086	DE

MYB - 10	1.75290	E
MYB - 11	0.41309	FF
MYB - 12	0.96904	F
MYB - 13	2.49897	CD
KKM - 3	0.96640	F
S.Em±	0.11735	
C.D. (P = 0.01)	0.7400642	
CV	31.26241	

The activity of peroxidase was tested in all the 14 mungbean genotypes where we can find the significant difference of peroxidase activity with respect to its resistance levels. The highest activity is found in the MYB-7 i.e. 4.22167 ΔAbs min^{-1} g^{-1} ,and the least activity is seen in the MYB-3 with 0.22412 ΔAbs min^{-1} g^{-1} , and o.41309 ΔAbs min-1 g -1 was noticed in the susceptible check MYB-11 which is less compared to the resistant lines MYB-6, 7, 8, 9, and 12. (Table 2). Increased peroxidase activity in resistant genotypes than susceptible genotypes because cell wall of plants appears to be a major site for defense-related peroxidase polymerization reactions such as lignification, suberization and cross-linking of structural cell wall proteins. The increased PO activity observed in the present study may be triggered by cellular damage caused by virus replication (Rathi *et al*. 1986). Present experimental results were in accordance with the reports of Singh *et al*. (2003) they found that values of peroxidase activity in muskmelon plants infected with downy mildew pathogen. It showed greater activity of the peroxidase in resistant plants than the susceptible plants.

Table 3: Estimation of Polyphenol oxidase (PPO) (ΔAbs min^{-1} g^{-1}) in mungbean genotypes

Line	**Polyphenol oxidase (PPO) (ΔAbs min^{-1} g^{-1})**	**Group acc to R software**
MYB - 1	1.350	BCD
MYB - 2	0.950	DEF

MYB - 3	0.780	DEFG
MYB - 4	0.920	CDEFG
MYB - 5	0.570	EFG
MYB - 6	1.655	ABC
MYB - 7	2.120	A
MYB - 8	1.440	BCD
MYB - 9	1.110	CDE
MYB - 10	0.765	DEFG
MYB - 11	0.195	G
MYB - 12	2.020	AB
MYB - 13	0.370	FG
KKM - 3	0.202	G
S.Em±	0.11735	
C.D. (P = 0.01)	0.7400642	
CV	31.26241	

The highest level of PPO was found in the MYB-7 followed by MYB-12, 6, 8 and the least activity was observed as 0.195 c in MYB-11 which is a susceptible check (Table 3). PPO activity has been reported to increase following infection by yellow mosaic disease in mungbean and urdbean (Rao *et al*. 1988) and it may be due to activation of latent host enzyme, solubilization of host PPO which is normally particulate or even due to de novo synthesis (Pitts,1975).Similar results were also reported by Anuradha *et al*. (1999) where they studied the biochemical modifications in banana due to Banana Bunchy Top Virus and observed that the level of Polyphenol oxidase enzyme activity was more in resistant plants compared to the susceptible plants

Table 4: Estimation of Catalase activity (ΔAbs min^{-1} g $^{-1}$) in mungbean genotypes

Line	**Catalase activity (ΔAbs min-^{1} g $^{-1}$)**	**Group acc to R software**

MYB - 1	3.663500	CD
MYB - 2	2.335000	E
MYB - 3	3.771250	CD
MYB - 4	3.242400	DE
MYB - 5	4.556500	BC
MYB - 6	5.504000	AB
MYB - 7	6.012475	A
MYB - 8	6.422350	A
MYB - 9	6.101100	A
MYB - 10	5.280450	AB
MYB - 11	2.956600	AE
MYB - 12	5.524050	AB
MYB - 13	5.607950	AB
KKM - 3	3.810350	CD
S.Em±	0.32199296	
C.D. (P = 0.01)	1.217406	
CV	12.26187	

The catalase is the very important enzyme which will protect the cell from oxidative damage from Reactive oxygen species (ROS). These acts as scavengers. ROS highest activity of catalase is found in the lines like MYB-7, 8, and 9 which is almost nearly equal and lowest was found in the MYB-11 and MYB-2 (Table 4).

Table 5: Estimation of Phenols (ΔAbs min^{-1} g^{-1}) in mungbean genotypes

Line	**Phenols (ΔAbs min^{-1} g^{-1})**	**Group acc to R software**
MYB - 1	87.36460	K
MYB - 2	123.73735	H
MYB - 3	136.27755	G
MYB - 4	81.30100	L
MYB - 5	116.10890	I
MYB - 6	302.43063	A

MYB - 7	286.78740	B
MYB - 8	272.52480	C
MYB - 9	250.63445	D
MYB - 10	198.78490	E
MYB - 11	87.19785	K
MYB - 12	194.02470	F
MYB - 13	105.25590	J
KKM - 3	77.72440	M
S.Em±	1.879004	
C.D. (P = 0.01)	2.940004	
CV	0.8271537	

Phenols are the defense inducing molecules where the highest amount of phenolic compounds will be present in the resistant lines when compared to the susceptible ones. The highest value of phenol was found to be 302.43063 ΔAbs $min^{-1}g^{-1}$ in MYB-6 followed by 286.78740 ΔAbs min^{-1} g^{-1}, in MYB-7. The lowest amount of phenols was observed in MYB-4 and then followed by MYB-11 which is a susceptible check (Table 5). Resistant plants were synthesis more of phenolic when it has interactions with the virus infection. Phenolic content enhances the mechanical activity of the host cell walls by the production of lignin and suberin which are the main components in the formation of physical barriers that block the spread of virus. Patel *et al*. (2013) investigated the biochemical changes in mungbean induced by MYMV and reported that phenol was found in decreasing trend in the susceptible leaves as compared to resistant genotypes.

Table 6: Estimation of Total sugars (ΔAbs min^{-1} g^{-1}) in mungbean genotypes

Line	**Total sugars (ΔAbs min^{-1} g^{-1})**	**Group acc to R software**
MYB - 1	0.8965	E
MYB - 2	0.9523	DE
MYB - 3	0.5065	F
MYB - 4	0.5342	F
MYB - 5	0.5698	F

MYB - 6	3.3082	A
MYB - 7	2.5478	BB
MYB - 8	2.5471	BC
MYB - 9	1.2285	CCD
MYB - 10	0.9635	D
MYB - 11	0.2863	GH
MYB - 12	2.2587	C
MYB - 13	0.6574	F
KKM - 3	0.3592	G
S.Em±	0.5526	
C.D. (P = 0.01)	0.95234	
CV	32.5417	

As there is more infection there will be reduced amount of sugars in contrast to healthy ones. The highest amount of sugars was present in the MYB-6 of around 3.3082 ΔAbs min^{-1} g^{-1} followed by MYB-7, 8, and 9, which are categorised under resistant lines. The lowest amount of sugars was present in the susceptible check MYB-11 of about 0.2863 ΔAbs min^{-1} g^{-1} (Table 6). These results were supported by the results given by Thind *et al*. (1996) as the reduction in amount of starch, reducing sugars, non-reducing sugars, and total sugars in plants infected with yellow mosaic virus as compared to the healthy ones. Sinha and Srivastava (2010) reported that the three mungbean genotypes *viz*., HUM-2, ML-192 and Pusa Baisakhi affected by the Mungbean Yellow Mosaic Virus infection and virus infection had significant impact on total sugar content in infected leaves.

4. CONCLUSION

Fourteen Mungban genotypes including one susceptible check MYB-11 and KKM-3 (UASB released variety) were screened during *kharif* 2022 and summer 2023. To determine MYMV induced biochemical changes in mungbean, the infected leaf samples were collected from resistant, moderately resistant, moderately susceptible and susceptible genotypes. The varied level of different defense inducing enzymes from all the 14 mungbean genotypes was estimated. The biochemical constituents such as total sugar, total phenol content and enzyme activity of Peroxidase (PO), Polyphenol oxidase(PPO) and catalase activity were estimated. The present study indicated that the defence inducing enzymes, i.e, peroxidase,

polyphenol oxidase, catalase, phenols and sugars, showed comparatively higher level of resistance in genotypes *viz*., MYB- 6, 7, 8 and 9 when compared to the susceptible check (MYB-11).

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