



Evaluation of urdbean (*Vigna mungo*) genotypes for mungbean yellow mosaic virus resistance through phenotypic reaction and genotypic analysis

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ABSTRACT

Mungbean yellow mosaic virus (MYMV) is a whitefly-transmitted major destructive virus affecting urdbean productivity in India. The objective of this research was to identify urdbean genotypes resistant against MYMV based on the phenotypic reaction and genotypic analysis. A total 48 urdbean genotypes were evaluated for resistance to MYMV by visual scoring of symptoms in the field under natural conditions. Disease severity was assessed using 0-9 rating scale, according to the mean disease score, the urdbean genotypes were categorized into five groups resistant (R, 14 genotypes), moderately resistant (MR, 4 genotypes), moderately susceptible (MS, 10 genotypes), susceptible (S, 18 genotypes) and highly susceptible (HS, 2 genotypes). These results were confirmed through genotyping based on MYMV-resistance tagged molecular markers CEDG180, ISSR811₁₃₅₇ and YMV1 FR. In addition, biochemical analysis was carried out in the genotypes of each category (R-HS). Results showed that MYMV resistance was significantly and positively correlated with the phytic acid and total phenol contents, whereas negative correlation was observed with total sugars in susceptible genotypes. The new identified genotypes (resistant sources) can be utilized in the urdbean breeding programme for improving resistance to MYMV.

Key words: Biochemical analysis, Molecular markers, Mungbean yellow mosaic virus, Urdbean.

INTRODUCTION

Urdbean (*Vigna mungo* L.), also known as black gram, is one of the most ancient and important pulse crops of Asia particularly India, due to its nutritional quality and the suitability to cropping systems. It is the third important pulse crop of India and contributes 70% of world's total urdbean production. However, Yellow Mosaic Disease (YMD) is formidable threat to the flourishing urdbean production in India. Mungbean Yellow Mosaic Virus (MYMV) and Mungbean Yellow Mosaic India Virus (MYMIV) are the main viral pathogens causing YMD in India. MYMIV is most prevalent in northern, central and eastern regions of India Usharani *et al.* (2004), whereas MYMV is predominant in southern and western region (Girish and Usha, 2005). The virus is transmitted by the whitefly and not through sap, seed or soil. Infected plants produce very few flowers and pods, the pods are curled and reduced in size with yield losses ranging from 85–100% (Karthikeyan *et al.*, 2014).

In this context, breeding urdbean cultivars with broad-spectrum and durable resistance is the most cost-effective and eco-friendly approaches for MYMV management in urdbean production. Thus, researchers have put massive efforts in identifying MYMV resistance cultivars in urdbean and many attempts have been made to identify and explore diverse MYMV resistance source in urdbean (Ganapathy *et al.*, 2008; Basamma *et al.*, 2015). After resistance source identification, molecular marker based applications, popularly known as Marker Assisted Selection (MAS) is an effective method to rapidly increase the selection authenticity

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and efficiency (Maiti *et al.*, 2011; Chen *et al.*, 2012). MAS is one of the key methods assisting both traditional breeding practices as well as resistance gene-mediated transgenic breeding approaches. From this approach, already reported molecular markers associated with resistance gene used to verify the resistance in cultivars. Molecular markers linked to the resistance of urdbean Anjum *et al.* (2010) from some resistant sources are valuable information that already available in literatures. Thus, breeders can utilize to exploit

MYMV resistance in a more efficient manner to identify the resistance cultivars. With this background knowledge, the present study was aimed to identify the resistant cultivars by combination of phenotypic reaction and genotypic analysis.

MATERIALS AND METHODS

Plant genetic materials

The experimental materials consisted of 48 urdbean genotypes (Table 1). The seeds were obtained from Department of Pulses, Center for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore and Department of Plant Breeding and Genetics, Agriculture College and Research Institute, Madurai. Among the 48 urdbean genotypes, three lines were derived from mutant line of MDU 1 with different mutagens treated *viz.*, gamma radiation doses 400 Gy and 300 Gy.

Field screening

The seeds of 48 urdbean genotypes were sown in two replications in a randomized complete block design (RCBD) during *Kharif*, 2016. Each genotype was sown in a single row of 3 m length with the spacing of 30 cm x 10 cm. One row of infector line CO-5 (susceptible cultivar) was raised after every test entry. All the recommended cultural practices were followed to maintain the experiment except that insecticide sprays were not given to encourage the white fly population for spread of the disease. When 80% of the plants showed MYMV symptoms, scoring of the test materials was done. The disease was scored on 0-9 arbitrary scale, as

suggested by Mayee *et al.* (1986). The mean disease score was calculated on the basis of disease rating and frequency of diseased plants per total number of plants. The disease scoring was recorded from initial flowering to harvesting by weekly intervals.

Genomic DNA isolation and PCR analysis

The genomic DNA was isolated from the young leaves of urdbean genotypes by the cetyltrimethyl ammonium bromide (CTAB) method as described by Karuppanapandiyan *et al.* (2006). PCR amplification was performed in a 10 µl reaction volume containing 25 ng of DNA template, 10 µM of forward and reverse primer, 1X master mix (Ampliqon, Denmark) and sterile double distilled water. The PCR reaction was carried out in a thermal cycler (Eppendorf) programmed to run the following temperature profile: 94°C for 4 min and 40 cycles at 94°C for 1 min, 45-59°C (depending on marker type) for 1min, 72°C for 1 min followed by 7 min at 72°C. Agarose gel (1.5 % and 3%) electrophoresis was performed to separate the amplified products.

Biochemical analysis

The total free phenol content, from the leaves of 48 urdbean genotypes at 30 DAS, was estimated using the Folin-Ciocalteu reagent. The concentration of total phenol was calculated by referring to the standard curve of catechol and expressed as 'mg/g' of sample. Total sugar content was estimated by anthrone method, as described by Sadasivam and Manickam (1996). Using glucose, total sugars content standard curve was prepared and expressed in mg/g of leaf sample. Using the modified calorimetric (Wade Reagent)

Table 1: Phenotypic reaction of urdbean genotypes against mungbean yellow mosaic virus.

Genotypes	Disease Score	Grade	Genotypes	Disease Score	Grade
VBG 11-053	0.71	R	R 15-008	23.50	S
LBG 808	0.76	R	R 15-001	28.99	S
CO-6	0.76	R	KU-003	24.74	S
VBN-4	0.95	R	LBG-645	25.18	S
VBG-10-019	0.90	R	KU-42	38.89	S
R 15-006	0.86	R	KU-50	28.91	S
R 15-011	0.94	R	ACM-015-14	32.65	S
R 15-009	0.96	R	KU-51	49.53	S
VBG-11-010	0.89	R	LBG 752	8.89	MR
KU-52	0.94	R	ADBG 13-004	7.17	MR
KU-24	0.94	R	RU 15-12	9.09	MR
ACM 14001	0.96	R	VBG 12-062	9.22	MR
ACM -015-30	0.54	R	COBG 653	11.75	MS
ACM-015-29	0.66	R	VBN-6	11.39	MS
LBG 792	28.50	S	IPU 10 -26	11.21	MS
RU 15-1	27.44	S	TU 13	11.5	MS
COBG 10-06	25.19	S	DKU 98	11.28	MS
COBG 11-03	30.99	S	R 15-012	11.67	MS
ADT-3	26.84	S	R 15-004	19.44	MS
RU 15-8	22.67	S	R II-009	20.03	MS
ADT-5	22.00	S	MDU-1	14.53	MS
TADT-26	35.64	S	KU-47	20.44	MS
TNJ-13-029	30.65	S	CO-5	78.75	HS
COBG-11-02	30.67	S	KU-46	50.28	HS

Table 2: PCR amplification of molecular markers linked to MYMV resistance in diverse urdbean genotypes.

Genotypes	Reaction to YMV	CEDG 180		ISSR811 ₁₃₅₇	YMV1-FR
		136 bp	163 bp	1357bp	1357bp
VBG 11-053	Resistant	+	-	+	+
LBG 808	Resistant	+	-	+	+
CO-6	Resistant	-	-	+	+
VBN-4	Resistant	+	-	+	+
VBG-10-019	Resistant	+	-	-	+
R 15-006	Resistant	+	-	+	-
R 15-011	Resistant	-	-	-	-
R 15-009	Resistant	+	-	+	+
VBG-11-010	Resistant	-	-	-	+
KU-52	Resistant	-	-	-	-
KU-24	Resistant	+	-	-	+
ACM 14001	Resistant	+	-	+	-
ACM-015-29	Resistant	+	-	+	+
ACM-015-30	Resistant	+	-	+	-
ACM-015-14	Susceptible	-	+	-	-
ADT-3	Susceptible	-	+	-	-
ADT-5	Susceptible	-	+	-	-
COBG 11-03	Susceptible	-	+	-	-

'+' = Presence of fragment and '-' = Absence of fragment.

Table 3: Mean values of the biochemical traits in 48 urdbean genotypes.

Genotypes	Phytic acid content (mg/g)	Total Phenol (mg/g)	Total Sugars (mg/g)	Genotypes	Phytic acid content (mg/g)	Total Phenol (mg/g)	Total Sugars (mg/g)
LBG 792	7.91	2.46*	26.27	KU-003	6.36	1.61	35.90*
LBG 645	6.50	2.09	29.77	RU 15-1	6.21	1.88	38.36*
COBG 653	9.60*	1.98	33.06*	KU-42	5.31	2.28	40.12*
LBG 752	8.00	2.86*	32.98*	R 15-006	8.68*	3.15*	28.90
COBG 10-06	8.34	2.64*	37.41*	R 15-011	5.98	2.74*	34.59*
COBG 11-03	8.22	2.19	35.99*	KU-46	6.38	1.82	41.92*
IPU 10 -26	9.17*	3.33	31.48*	KU-50	6.20	1.77	38.91*
ADBG 13-004	10.19*	3.20*	26.45	R 15-009	8.98*	2.63*	21.27
TU 13	10.00*	2.87*	32.47*	VBG-11-010	10.77*	2.83*	19.48
DKU 98	13.66*	2.20	28.20	KU-52	5.39	2.37	18.37
VBG 11-053	11.31*	2.72*	16.70	KU-51	6.58	0.97	31.37*
LBG 808	11.77*	2.32	21.72	KU-47	7.88	2.62*	23.71
RU15-12	9.40*	1.52	30.30	KU-24	6.01	2.87*	17.77
VBG 12-062	12.59*	2.19	30.76	ACM-14001	8.24	3.27*	15.22
RU15-8	6.41	1.80	37.52*	ACM-015-14	10.03*	2.07	31.12
TADT-26	7.43	1.97	40.71*	ACM-015-30	9.32*	3.02*	16.70
TNJ-13-029	8.55	2.07	35.88*	ACM-015-29	12.42*	2.42	21.50
R15-012	7.95	2.30	29.76	CO-5	7.23	1.81	31.35*
COBG-11-02	6.80	1.93	35.62*	VBN-6	11.71*	2.58*	29.95
R15-004	5.57	2.09	33.96*	CO-6	10.51*	2.99*	24.72
R15-008	5.62	1.50	35.34*	ADT-3	8.08	2.45*	33.77*
R15-001	7.00	1.93	37.77*	VBN-4	9.01*	2.51*	24.54
VBG-10-019	6.39	2.15	21.82	ADT-5	8.26	1.64	39.77*
R11-009	9.39	2.39	33.32*	MDU-1	8.02	2.47*	35.27*
MEAN	8.36	2.32	30.20				
CD	0.29	0.68	0.76				
SE	0.30	0.07	1.04				

Note: *Significant at P=0.05.

method explained by Gao *et al.* (2007), phytic acid (PA) content was estimated from the seed. PA content was calculated as PA = 3.552 PAP (Phytic acid phosphorous).

RESULTS AND DISCUSSION

MYMV incidence was recorded in field periodically using 0-9 disease rating scale and the results were summarized in Table 1. The disease incidence varied from 0.54 to 50.28%. 48 genotypes were categorized into five groups resistant (R, 14 genotypes), moderately resistant (MR, 4 genotypes), moderately susceptible (MS, 10 genotypes), susceptible (S, 18 genotypes) and highly susceptible (HS, 2 genotypes). None of the test entries appeared to be immune. Fourteen genotypes exhibited resistance reaction; VBG 11-053, LBG 808, CO-6, VBN-4, R 15-009, R 15-006, VBG-10-019, R 15-011, VBG-11-010, KU-52, KU-24, ACM 14001, ACM-015-29 and ACM-015-30 with the disease incidence of 0.54% to 0.96%. The genotype ACM-015-30 had minimum disease incidence (0.54%) and ACM 14001, VBG-11-053 genotypes exhibited maximum disease incidence (0.96%) among the

resistance categories. In moderately resistance category, the percent disease incidence ranged from 7.17% (ADBG 13-004) to 9.22% (VBG 12-062). In the susceptible category, per cent disease incidence ranged from 22% to 49.53%. The maximum susceptibility reaction showed in KU-51 (49.53%) and minimum susceptibility reaction studied in ADT 5 (22%). KU-47 genotype showed maximum per cent disease incidence of 20.44% and IPU 10 -26 had minimum per cent disease incidence of 11.21% among moderately susceptible categories. The highest per cent of disease incidence was observed in KU-46 (50.28%). The genotype appeared to be resistance to highly susceptible. The results are in substantiation with Mohan *et al.* (2014); Gopi *et al.* (2016) and Manivannan *et al.* (2001).

However, sometimes the screening based on natural occurrence in the hot spot areas also does not yield consistent results. A combination with plant breeding approaches will likely to be needed for the improvement of crops Karthikeyan *et al.* (2011). MAS is one of the key methods assisting and improving the traditional breeding

Table 4: Correlation co efficient among the three biochemical traits and MYMV score.

Characters		Phytic Acid	Total Phenol	Total Sugars	MYMV Score
Phytic Acid	GP	1.001.00	0.399*0.319*	-0.378*-0.377*	-0.412 *-0.411*
Total Phenol	GP		1.001.00	-0.613*-0.489*	-0.703*-0.553*
Total Sugars	GP			1.001.00	0.637 *0.209
MYMV Score	GP				1.001.00

Note: *Significant at P=0.05, G-Genotypic correlation co-efficient and P-Phenotypic correlation co-efficient.

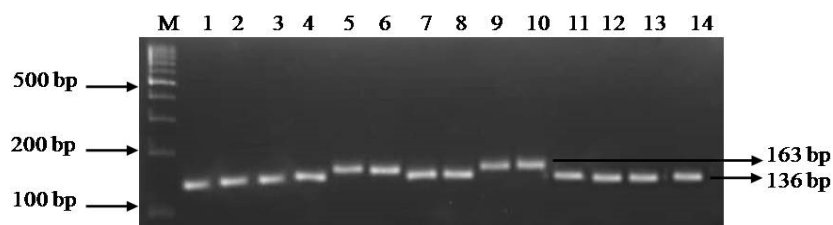


Fig 1: PCR amplification of SSR marker CEDG 180 on urdbean genotypes. Note: VBG 11-053, LBG 808, VBN-4, VBG-10-019, ACM-015-14, ADT-3, R 15-009, R 15-006, ADT-5, COBG 11-03, KU-24, ACM 14001, ACM-015-29 and ACM-015-30.

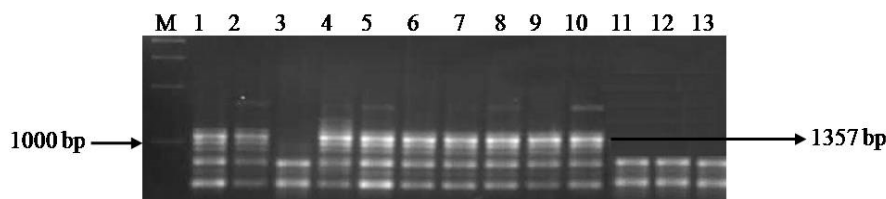


Fig 2: PCR amplification of ISSR marker ISSR811 1357 on urdbean genotypes. Note: VBG 11-053, LBG 808, ACM-015-14, CO-6, VBN-4, R 15-006, R 15-009, ACM 14001, ACM-015-29, ACM-015-30, KU-24, ADT-5 and ACM-015-14.



Fig 3: PCR amplification of SCAR marker YMV1-FR on urdbean genotypes. VBG 11-053, LBG 808 CO-6, VBN-4, VBG-10-019, R-15-009, VBG-11-010, KU-24, ACM-015-29, R 15-006, ACM 14001, ACM-015-30, ACM-015-14 and ADT-3.

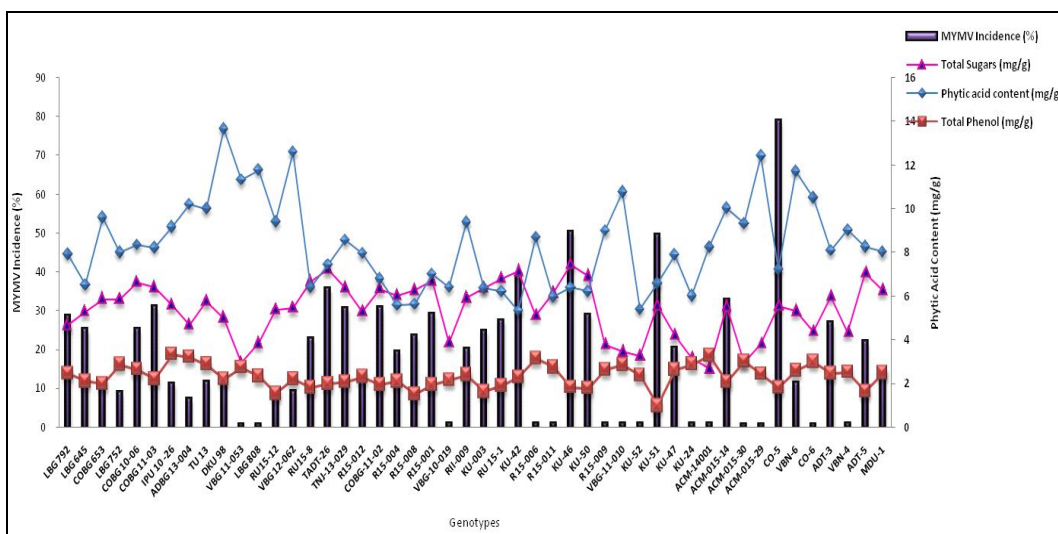


Fig 4: Mean values of the biochemical traits in 48 urdbean genotypes.

practices (Prasanthi *et al.*, 2011). In MAS, already reported molecular markers linked to resistance gene used to confirm the resistance in cultivars. Molecular markers such as CEDG 180, ISSR 811₁₃₅₇ and YMV1 FR (Subramanian and Gopalakrishna, 2006; Gupta *et al.*, 2013) reported to be linked to MYMV resistance in urdbean. These markers are useful to track the MYMV resistance cultivars. Hence, in the present study, three markers were used to confirm the resistance and susceptible lines. Three molecular markers (CEDG180, ISSR811₁₃₅₇ and YMV1FR) were found polymorphic between resistance and susceptible urdbean genotypes studied. SSR marker CEDG 180 produced the 136 bp allele in ten resistance genotypes and in four susceptible genotypes, approximately 163 bp allele linked to susceptibility was amplified (Fig 1). The amplification of resistance linked allele in resistance individual may be attributed to linkage of this marker to MYMV resistance gene. These results were confirmed with earlier studies by Gupta *et al.* (2015) and Vanniarajan *et al.* (2017). ISSR marker ISSR811₁₃₅₇ reported to be linked to MYMV resistance in urdbean (Souframanien and Gopalakrishna, 2006) amplified the 1357 bp marker fragment in nine of the fourteen YMV resistance urdbean genotypes and marker fragment was absent in all four MYMV susceptible urdbean genotypes and five resistance genotypes (Fig 2). Analogousness of upshot reported by Souframanien and Gopalakrishna, (2006) and Gupta *et al.* (2015).

SCAR marker YMV1 FR amplified the nine of the fourteen resistance genotypes (Fig 3) and fragment was absent in susceptible genotypes and in resistance genotypes viz., R15-006, R 15-011, KU-52, ACM 14001, ACM-015-30. In these, R15-006, ACM 14001, ACM-015-30 showed amplification of resistance gene in urdbean germplasm for the marker CEDG 180, ISSR 811₁₃₅₇. The absence of YMV 1FR marker fragment and presence of allele of SSR CEDG180, ISSR 811₁₃₅₇ in germplasm indicated that these germplasm may be carrying different source of MYMV

resistance than the one targeted by YMV 1FR marker and thus two independent genes governing resistance to MYMV are present in urdbean cultivars. This was further confirmed by the position of these two molecular markers on the genetic linkage map developed by Gupta *et al.* (2008). The genotypes VBG 11-053, LBG 808, VBN-4, VBG-11-053, ACM-015-29, ACM-015-30 showed amplification for three molecular markers which is linked to MYMV resistance (Table 2). These resistance genotypes could be used as donor for transfer of MYMV resistance gene by using backcross programme. However, there is a need to test more number of molecular markers to the gene conferring resistance to different races of MYMV.

Moreover, phytic acid, phenols and total sugar are a common phenomenon occurring in plants and play a major role in biotic stresses. In the present study, phytic acid, total phenol and total sugar contents and MYMV disease score in 48 genotypes were correlated. It was noted that the urdbean genotypes exhibited different levels of phytic acid, total phenol and total sugar contents to varying degree of MYMV resistance. The resistance genotypes to MYMV had relatively higher phytic acid, phenol content and low total sugars content in the urdbean genotypes compared to highly susceptible genotypes. These susceptible genotypes exhibited higher total sugars content (Table 3; Fig 4). The present examine indicated that MYMV disease score had significant negative correlation with the phytic acid and total phenol content, whereas positive correlation was noted with total sugars (Table 4). Rapid accumulation of phenols and lower total sugar content in resistance genotypes compared to susceptible genotypes for MYMV disease incidence highlights inducible biochemical pathway of expression of host resistance probably involving synthesis of phenolics, phytic acid content precursor and their further oxidation into toxic quinines and other substrates. The data obtained in the present study well supported this hypothesis that along with high phytic acid, genotypes should possess resistance

genes. Similar kind of result were also obtained by Dhole *et al.* (2015) found that association of seed PA with tolerance to MYMV in mungbean. This research will help to develop a fundamental understanding of the biochemical basis that contributes to plant resistance to insects and also to compare the genotypes for resistance and susceptible ones to MYMV resistance. The results were in conformity with the findings of earlier workers *viz.*, Raghuraman *et al.* (2004); Balakrishnan (2006) in field bean; Murugan *et al.* (2007) in tomato and Taggar *et al.* (2014) in urdbean.

CONCLUSION

In the present study identified the resistance genotypes for MYMV resistance through phenotypic reaction and verified the resistance by molecular markers and biochemical analysis. The new identified genotypes (resistant sources) can be used in the urbean breeding programme for improving resistance to MYMV and they can be used directly as varieties to manage MYMV after evaluation for acceptable agronomic characteristics, adaptation and stability in various regions especially in those regions which are endemic to MYMV.

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