RESEARCH ARTICLE Legume Research- An International Journal, Volume 44 Issue 2: 152-157 (February 2021)

Deployment of yellow mosaic disease resistance in mungbean [*Vigna radiata* (L.) Wilczek] through interspecific hybridization

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10.18805/LR-4082

ABSTRACT

Mungbean yellow mosaic virus [Urdbean: New Delhi: 2011] Accession no. JQ398669.1, was found to be prevalent in both summer and *kharif* season causing Yellow Mosaic Disease (YMD) of mungbean. Since similar strain was observed in both the seasons, the short duration interspecific derivative lines of mungbean suitable for summer season were evaluated for YMD resistance in *kharif* season- a highly favourable environment for YMD epidemics during 2014-2016. Out of fourteen lines showing stable resistant reaction, few lines promising for other economically important traits were selected and again evaluated by whitefly mediated artificial inoculation technique for confirmation of resistance during 2017. The selected promising interspecific derivative lines showed no YMD symptoms except SML 1829. SML 1827 derived from interspecific cross between mungbean and rice bean showed highest yield among all interspecific derivatives. Further, rice bean specific segments were also identified in SML 1827 with SSR markers.

Key words: Begomoviruses, Host resistance, Hybridization, Interspecific, Mungbean, Yellow mosaic disease.

INTRODUCTION

Crop diversification is a relevant option to rejuvenate green revolution in North India. For diversification, pulses are one of the best candidates. India is producing 23.13 million tons of pulses from an area of 29.46 million hectares (Anonymous, 2018). Mungbean [*Vigna radiata* (L.) Wilczek], also known as green gram, is one of the important pulse crops grown in India. Recently, development of short duration varieties of mungbean with synchronous maturity, reduced photosensitivity and high yield has offered a great scope for their introduction as a catch crop in rice-wheat or rice-potato cropping system in Punjab. Mungbean is a rich source of protein (24%) and iron (6 mg/100gm of raw seed in improved varieties), improves soil health and also offers the potential for increased income for small-scale farmers (Chadha 2010). However, the area under mungbean is significantly less *i.e*. 4.32 m ha with 2.17 m tones production in India (Anonymous 2018). One of the major bottle neck in mungbean production is yellow mosaic disease (YMD) resulting in 10-100 per cent losses in yield (Nene 1972). In North India, high incidence of YMD occurs in *kharif* season; however, few instances of severe disease incidence on summer mungbean have also been observed in last several seasons. Management of yellow mosaic disease through deployment of host resistance remained unsuccessful due to highly variable nature of pathogen and lack of reliable screening techniques. Evaluation of large number of mungbean germplasm at hot spot locations has resulted in identification of few mungbean lines *viz;* ML 818, ML 1349, IPM 02-14 and ML 1628 with stable resistance (Parihar *et al.,* 2017; Nair *et al.,* 2017). However, higher level of resistance has been observed in urdbean [*Vigna mungo* (L.) Hepper] and rice bean [*Vigna umbellata* (Thunb) Ohwi & Ohashi]. Earlier studies on interspecific hybridization in *Vigna* species have suggested the possibility of successful hybridization of *V. radiata* with *V. mungo* and *V. umbellata* with 0.7-11.5 per cent pod set and 22-34.21 per cent hybrid germination (Subramianian 1980; Shanmugam *et al*. 1983; Sehrawat and Yadav 2014; Lekhi *et al.,* 2017). A large set of interspecific derivative lines have been developed from interspecific crosses between mungbean × urdbean and mungbean × rice bean at PAU, Ludhiana with aim of introgression of YMD resistance and other economically important traits into mungbean. Since spring/summer season represents the largest pulse cultivation segment in Punjab, breeding efforts were focused more on developing short duration interspecific lines of mungbean. Further, these short duration interspecific derivatives needed to be screened extensively against YMD, keeping in view the high incidence of disease in summer season for last several years in few pockets of state. On pathogen side also, characterization of begomovirus species causing YMD in both seasons was a pre-requisite for devising screening strategies for summer/ spring mungbean germplasm. In present investigation, virus species associated with YMD was characterized from both

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How to cite this article: Ludhar, N.K., Sirari, A., Bains, T.S., Sharma, A., Patel, S.A.H. Sharma, P., Rani, U. and Cheema, H.K. (2021). Deployment of yellow mosaic disease resistance in mungbean [*Vigna radiata* (L.) Wilczek] through interspecific hybridization. Legume Research. 44(2): 152-157. DOI: 10.18805/LR-4082.

Submitted: 17-09-2018 **Accepted:** 29-03-2019 **Online:** 14-08-2019

kharif and summer season, interspecific derivative lines were extensively evaluated for YMD resistance and promising lines were evaluated for yield trials in target season.

MATERIALS AND METHODS

Characterization of begomovirus species associated with YMD

YMD infected mungbean leaf samples from *kharif* and summer season were collected during 2013-14. Total DNA was extracted from YMD infected leaf samples and amplified with Mungbean yellow mosaic India virus (MYMIV) and Mungbean yellow mosaic virus (MYMV) species specific primers (Naimuddin *et al.* 2011). PCR reaction mixture was prepared with 40 ng of total genomic DNA along with 1.5 mM magnesium chloride, 1.0X of PCR buffer, 0.15 of dNTP mix, 10 p mol of each forward and reverse primers and 1 U of Taq DNA polymerase as final concentration. Visualization of viral components was done on 1% agarose gel and all the samples were eluted, purified by gel purification kit (Favor Prep GEL/PCR Purification mini kit) and sequenced (Eurofins Genomics India Pvt. Ltd.). Retrieved sequences were further analyzed using BLASTn programme.

Screening of interspecific derivatives lines of mungbean for YMD resistance

Short duration interspecific derivative lines along with three resistant checks *viz;* ML 818, ML 2037 and ML 2056 were evaluated for YMD resistance by modified double infector row technique in *kharif* season during 2014-16. The observations on whitefly incidence were made twice in the season at 40 and 55 days after sowing. Whitefly population was recorded using Split cage for counting adult whiteflies (Kooner and Cheema 2007). The promising lines were further evaluated by whitefly mediated artificial inoculation technique for confirmation of resistance during 2017. Pedigree of promising interspecific derivative lines of mungbean is given in Table 1.

Modified double infector row technique

The test entries along with checks were sown in *kharif* season for three consecutive years (2014-2016). Two rows (three meter length) of each test entry were sown between two infector rows of susceptible check SML 1082. Each entry was sown with three replications. All recommended package and practices were followed for raising experimental material. YMD score was recorded on 1-9 scale (Singh and Bhan 1998) at weekly intervals after first appearance of disease on susceptible check till 12th week (Table 2). Data was also recorded on per cent disease incidence (number of infected plants) and disease severity (number of infected leaves per plant). The formulas used for calculation of per cent disease incident and disease severity were as follows:

$$\text{Per cent disease incidence (PDI)} = \left\{ \frac{\text{Number of disease plants}}{\text{Number of total plants examined}} \right\} \times 100$$

$$\text{Disease severity} = \left\{ \frac{\text{Total number of leaves affected}}{\text{Total number of leaves per plant}} \right\} \times 100$$

Based on the disease severity, symptom severity grades, designated with numerical values of 0-4 and a scale of response value (0-1) corresponding to such grades was

Table 1: Pedigree of promising interspecific derivative lines of mungbean and checks.

Name of the cross	Line	Pedigree
	SML 1808	ML 1349×Mash 1-1
	SML 1828	SML 668×Mash 1-1
	SML 1829	SML 668×Mash 1-1
	SML 1837	ML 1349×Mash 1-1
Mungbean × Urdbean	SML 1839	ML 1349×Mash 1-1
	SML 1840	ML 1349×Mash 1-1
	SML 1841	ML 1349×Mash 1-1
	SML 1843	SML 740×Mash 1008
	SML 1822	ML 267×Mash 114
	SML 1809	ML 1265×RBL 35
	SML 1810	ML1265×RBL 35
	SML 1811	SML 668×RBL 35
Mungbean × Rice bean	SML 1824	(ML 881×RBL 35)×ML 881
	SML 1825	(ML 881×RBL 35)×ML 881
	SML 1826	(ML 881×RBL 35)×ML 881
	SML 1827	(ML 881×RBL 35)×ML 881
Mungbean × Wild Moong	SML 1815	ML 818×*Vigna radiata var. sublobata*
	SML 1844	ML 5×ML 881
	ML 818	5145/87×ML 267
Mungbean × Mungbean	ML 2037	ML 818×VC-6372-45-8-1
	ML 2056	ML 1165×ML 1191
	SML 1082	SML 357×SML 470

Table 2: Rating scale for YMD (Singh and Bhan 1998).

Grade	Description	Reaction
1	No visible symptoms or minute yellow specks covering 1.1-5 per cent leaf area	Resistant (R)
3	Mottling of leaves covering 5.1-15 per cent leaf area	Moderately resistant (MR)
5	Yellow mottling and discoloration of 15.1-30 per cent leaf area	Moderately susceptible (MS)
7	Pronounced yellow mottling and discoloration of leaves and pods, reduction in leaf size and stunting of plants covering 30.1-75 per cent of foliage	Susceptible (S)
9	Severe yellow discoloration of leaves covering 75.1-100 per cent of foliage, stunting of plants and reduction in pod size	Highly susceptible (HS)

Table 3: Scale for classifying reaction of YMD (Singh and Singh 2000).

Symptoms	Severity grade	Response value	Coefficient of infection (CI)	Disease reaction
Symptoms absent	0	0	0-4	Highly resistant
Very mild symptoms upto 25% leaves	1	0.25	5-9	Resistant
Appearance of symptoms in 26-50% leaves	2	0.5	10-19	Moderately resistant
Appearance of symptoms in 51-75% leaves	3	0.75	20-39	Moderately susceptible
Severe disease infection in symptoms (>75% leaves)	4	1	40-69	Susceptible
			70-100	Highly susceptible

given to each entry. Coefficient of infection was calculated by multiplying the percent disease incidence to the response value. Disease reaction categories were assigned to each test entry on the basis of coefficient of infection as per scale given by Singh and Singh (2000), described in Table 3. The data was analysed using CPCS software.

Whitefly mediated inoculations under controlled conditions

Pure culture of whitefly (*Bemisia tabaci*) was maintained on cotton plants in pots and kept in cages made up of muslin cloth and black chart to provide dark condition for whitefly to establish on cotton plants. YMD infected plants from field were transplanted in pots and kept in insect proof cages. Non-viruliferous whiteflies from cotton were allowed to feed on YMD infected plant for 24 hours. After 24 hours acquisition access period, these viruliferous whiteflies were released on healthy mungbean plants of susceptible variety SML 1082 for 48 hours kept in insect proof cages separately. The inoculated plants expressed YMD symptoms after 10 days and were further used as inoculum for screening. The viruliferous whiteflies were released on test entries for 48 hours of inoculation access period (IAP) at true leaf stage of seedlings. The YMD inoculum maintained on SML 1082 was also kept among test entries to enhance disease development. After IAP, whiteflies were removed and test entries were transferred to insect proof glasshouse. YMD score was recorded on 1-9 scale (Singh and Bhan 1998) after 2 days of inoculations and at every 2 days interval till 24 days.

Confirmation of interspecific derivative lines with SSR marker

Genomic DNA was extracted from interspecific derivative line SML 1827 along with parents *i.e.* ML 881 and RBL 35. A set of 121 *Vigna* specific SSR markers were used to amplify the genomic DNA of parents. Out of 121, three markers *viz*; VR 0293, CedG-048 and MBM-050 were polymorphic between parents. These three markers were used to amplify the genomic DNA of SML 1827. The sequence of these SSR markers is given in Table 4. PCR reaction mixture was prepared with 40 ng of total genomic DNA along with 1.5 mM magnesium chloride, 1.0X of PCR buffer, 0.15 of dNTP mix, 5 p mol of each forward and reverse primers and 1 U of Taq DNA polymerase as final concentration. PCR products were visualized under 6 per cent PAGE (Poly Acrylamide Gel Electrophoresis).

RESULTS AND DISCUSSION

Begomovirus species associated with YMD

DNA A component of begomovirus amplified as 1000 bp size amplicon and showed 94-99% sequence homology with *Mungbean yellow mosaic virus* [Urdbean: New Delhi: 2011] Accession no JQ398669.1 in all summer as well as *kharif* samples. Recently, in an extensive study, MYMV- Urdbean strain was found prevalent in North India (Nair *et al.* 2017) whereas, both MYMV and MYMIV species were detected at New Delhi (Bag *et al.* 2014). Since similar strain was observed in both the seasons, the summer mungbean

Table 4: List of polymorphic primers used for PCR amplification of SML 1827, ML 881 and RBL 35.

Name of Primer	Sequence
VR-0293F	GTGGCTCACAAGGTAGTGCTAA
VR-0293R	GAGAGAAACAACCAACCAAAGG
CedG-048F	TCTCTTCCTCTATGGCTTGG
CedG-048R	GCTCCTCCTTTTGCTGCATC
MBM-050F	TAACCTCTTTTTCATGGCGG
MBM-050R	CACGAAGACCCACCAAACTT

Table 5: Reaction of interspecific lines to YMD in modified double infector row screening method.

Entries	YMD score (1-9)	PDI	Disease severity	Response value	Coefficient of infection	Disease reaction	Number of whiteflies per cage
SML-1808	1.50	27.6	12.25	.25	6.90	R	4.88
SML-1809	1.00	24.12	10.20	.25	6.03	R	4.55
SML-1810	1.83	22.00	17.50	.25	5.50	R	4.66
SML-1811	2.33	26.00	16.25	.25	6.50	R	4.33
SML-1815	2.17	31.20	20.00	.25	7.80	R	6.00
SML-1822	2.67	24.12	13.50	.25	6.03	R	3.66
SML-1824	2.17	36.00	10.50	.25	9.00	R	6.33
SML-1825	2.00	18.00	11.25	.25	9.00	R	6.16
SML-1826	2.00	23.16	15.00	.25	5.79	R	5.33
SML-1827	2.17	34.00	10.00	.25	8.50	R	4.50
SML-1828	3.00	24.9	25.50	.50	12.45	MR	3.00
SML-1829	3.33	19.14	28.25	.50	9.57	MR	4.33
SML-1837	3.00	21.12	26.25	.50	12.06	MR	4.66
SML-1839	2.17	28.48	22.50	.25	7.12	R	4.66
SML-1840	2.00	26.00	14.00	.25	6.50	R	5.33
SML-1841	1.33	34.4	12.50	.25	8.60	R	4.66
SML-1843	3.00	24.12	10.50	.25	6.03	R	4.33
SML-1844	3.00	20.92	27.00	.50	10.46	MR	4.66
SML 1082	9.00	100.00	100.00	1.0	100.00	HS	-
CD (En/Tr/In)	**0.31/0.78/1.36**	**0.87/2.18/3.78**	**NS/1.48/2.56**				

lines were evaluated in *kharif* seasons, which was highly favourable for YMD epidemics.

Interspecific derivative lines of mungbean with stable YMD resistance

In preliminary evaluation by modified double infector row technique for three years (2014-2016), YMD symptoms were observed on fourth week after sowing and disease continued to progress up to 8th week of sowing. The mean YMD score, PDI and disease severity of three years is given in Table 5. The YMD score, per cent disease incidence and disease severity in all lines differed significantly. However, the influence of seasonal variation was non-significant on disease severity. Least per cent disease incidence (PDI) was observed in case of SML 1825 whereas least disease severity was observed in case of SML1827. On the basis of PDI and disease severity, fourteen lines namely; SML: 1808, 1809, 1810, 1811, 1815, 1822, 1824, 1825, 1826, 1827, 1829, 1829, 1841 and 1843 showed resistant reaction with coefficient of infection less than 9 whereas four lines *viz*; SML 1828, 1829, 1837 and 1844 were classified as moderately resistant with coefficient of infection ranging from 10-19. The interspecific lines, showing resistant reaction were derived from either mungbean × urdbean or mungbean × rice bean crosses, whereas highly susceptible line was derived from mungbean × mungbean cross. YMD score of these lines were also recorded for comparison with parental data, which was available in form of YMD score only (Table 6). It was observed that the interspecific derivative lines derived from R × R cross between mungbean and ricebean, showed transgressive segregation for YMD resistance. Similarly, in all R × R crosses between mungbean and urdbean, the derivative lines showed YMD score lower than parents, indicating transgressive segregants. Further, in case of all interspecific R × S crosses, the resistance in derivative lines clearly showed introgression from ricebean and urdbean. Infector row technique has also been used in earlier studies to explore durable resistance in mungbean at hot spot locations over the years. In one study, multi environment testing of mungbean genotypes at 28 hot spot locations was resulted in identification of three genotypes *viz*; ML 818, ML 1349 and IPM 2-14 with stable resistant reaction across the test environments. GGE biplot analysis revealed that out of six, Pantnagar, Vamban and Ludhiana were the best test environment for screening YMD resistance (Parihar *et al.* 2017). YMD resistant interspecific lines with good yield performance were selected and subjected to whitefly mediated artificial inoculations for confirmation of

Table 6: YMD score of parents of interspecific lines used in present study.

Parental line	YMD score*	Parental line	YMD score*
ML 1349	2.5	RBL 35	1.0
SML 668	6.5	ML 881	5.5
Mash 1-1	2.0	ML 5	3.0
Mash 1008	3.0	ML 1268	4.5
Mash 114	2.0	ML 1165	3.0
ML 267	7.0	ML 818	2.0
ML 1265	3.0	VC- 6372-45-8-1	7.0
SML 357	9.0	SML 470	7.0

Table 7: Reaction of interspecific derivative lines of mungbean to YMD under artificial inoculation.

Genotypes	YMD score (1-9)											
	Days after inoculations											
	2	4	6	8	10	12	14	16	18	20	22	24
SML 1808	-	-	-	-	1	1	1	1	1	1	1	1
SML 1810	-	-	-	-	1	1	1	1	1	1	1	1
SML 1811	-	-	-	-	1	1	1	1	1	1	1	1
SML 1822	-	-	-	-	1	1	1	1	1	1	1	1
SML 1827	-	-	-	-	1	1	1	1	1	1	1	1
SML 1829	-	-	-	-	1	1	1	1	1	1	2	2
ML 2037	-	-	-	-	1	1	1	1	1	1	1	1
ML 2056	-	-	-	-	1	1	1	1	1	1	1	1
ML 818	-	-	-	-	1	1	1	1	1	1	1	1
SML 1082	-	-	-	-	2	2	3	3	5	5	5	7

Table 8: Yield and YMD reaction of promising interspecific derivative lines.

Interspecific derivative mungbean lines	YMD score (1-9) yield		(Kg/ha)
	Double infector row method	Whitefly mediated artificial inoculation	
SML 1808	1.50	1	1329
SML 1810	1.83	1	1375
SML 1811	3.33	1	1347
SML 1822	2.67	2	1340
SML 1827	2.67	1	1467
SML 1829	3.33	2	1412

resistance. The disease symptoms were appeared after the 10 days of inoculations and the symptoms were appeared first on susceptible check (SML 1082). All the interspecific derivatives lines along with three resistant checks showed no symptoms except SML 1829 (Table 7). The interspecific lines showed similar disease reaction as in case of infector row technique; however, level of disease severity differed in both the cases. The low level of disease severity, observed in case of artificial screening technique due to less inoculum pressure as compared to field conditions. It suggests that double infector technique is more reliable and efficient technique for screening large number of germplasm at hotspot location. Whitefly mediated inoculation technique can be used for confirmation of resistance in elite lines. The yield performance of promising line is given in Table 8. SML 1827 was found to be most promising with 1427 kg/ha yield followed by SML 1829.

Identification of donor segments in interspecific derivative line SML 1827 with SSR markers

Since SML 1827 was most promising and released as first interspecific mungbean variety for cultivation in Punjab, it was critical to identify donor segments in SML 1827 introgressed from ricebean. Out of 121 markers, three markers, *viz*; VR-0293, CedG-048 and MBM-050 were found to be polymorphic between ML 881 and RBL 35. The rice bean parent (RBL 35) specific segment of ~200 bp and ~250 bp

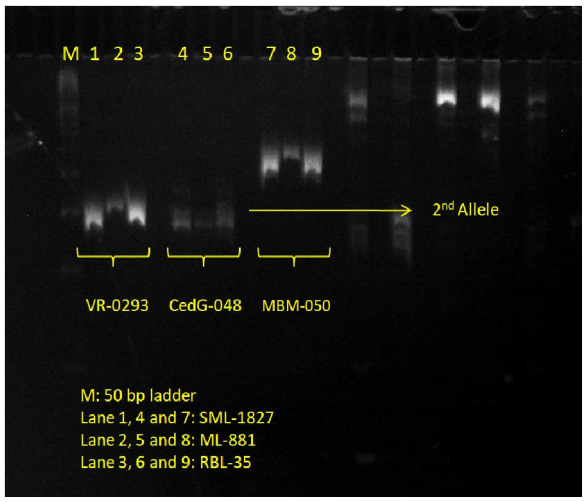

Fig 1: Rice bean (RBL 35) specific segments in SML 1827.

were amplified in promising interspecific line SML 1827 with markers VR- 0293 and MBM – 050 respectively (Fig 1).

CONCLUSION

Despite of several efforts of extensive resistance breeding, stable resistance against YMD could not be achieved due to highly variable nature of the virus. However, in rice bean and few germplasm lines of urdbean, high level of resistance has been observed. There is an urgent need for transferring this resistance into high yielding mungbean lines. Modern short duration varieties of mungbean offer a great scope for diversification as summer/spring crop between rice and wheat/potato. The landmark summer mungbean variety SML 668 became popular in Punjab and adjoining states as catch cum cash crop. Summer mungbean crop encounters low incidence of YMD in North India due to high temperature and low humidity. However, last several seasons have witnessed severe YMD epidemics in few pockets of Punjab. Upsurgence of whitefly during last few years further aggravated the situation and revisiting the population structure of begomovirus species associated with YMD

became urgent. It has implications for breeding YMD resistant short duration lines suitable for summer season-the largest pulse cultivation segment in Punjab. In present study, it was found that *Mungbean yellow mosaic virus* [Urdbean: New Delhi: 2011] Accession no. JQ398669.1 was prevalent in both summer and *kharif* season of mungbean. Therefore, strategy was devised to screen all summer germplasm during *kharif* season which was favourable for YMD epidemic caused by similar viral strain.

A large number of lines derived from interspecific crosses between mungbean x urdbean or mungbean x rice bean, with high level of YMD resistance have been developed and evaluated at PAU for three consecutive years (2014-16). Out of these, few lines promising for YMD resistance and other economically important traits were selected and evaluated extensively for confirmation of resistance during 2017. Fourteen lines were identified with resistant reaction under field conditions. The selected promising interspecific derivative lines showed no symptoms under artificial inoculation conditions except SML 1829. The Present study identifies a set of elite interspecific derivative lines with stable YMD resistance and high yield and can be used immediately in varietal development. One of these lines namely, SML 1827 outperformed in multi-location trials and now released for cultivation in Punjab.

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