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Interspecific Hybridization Between *Vigna radiata* and *Vigna mungo* Towards the Broadening of Genetic Base for MYMV Disease Resistance and Generating Variability

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ABSTRACT

Background: The main cause for low yield in greengram is its susceptibility to Mungbean Yellow Mosaic Virus (MYMV) which is the most prevalent and destructive viral pathogen cause 85% yield lose. Inter specific hybridization between *Vigna radiata* and *Vigna mungo* could be an alternate approach to develop MYMV resistant genotypes in greengram which leads to additional source of creating variability for desirable attributes including yield, nutritional quality, biotic and abiotic stresses.

Methods: The present investigation was carried out at National Pulses Research Centre (NPRC), Tamil Nadu Agricultural University, Vamban during 2016-2017. Interspecific hybridization has been effected between *Vigna radiata* var. VBN (Gg)2, VBN (Gg) 3 (as females) and *Vigna mungo* Var. Mash 114 (as male) during *Summer* 2016. The interspecific F_1 hybrids, F_2 and F_3 populations were evaluated during *Kharif* 2016, *Rabi* 2016-17 and *Summer* 2017 respectively. The F_2 and F_3 populations were evaluated for days to 1st flowering, days to maturity, plant height (cm), number of branches/plant, number of clusters/plant, number of pods/clusters, pod length (cm), number of seeds/pod, number of pods/plant, single plant yield (g). The MYMV resistance has been confirmed under infector row method using CO 5 as susceptible check variety. Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability (h^2) in broad sense, genetic advance as per cent of mean, Skewness and Kurtosis were estimated for yield and yield components.

Result: Four true F_1 plants were recovered in *Vigna radiata* var. VBN (Gg)2 × *Vigna mungo* Var. Mash 114 cross combination. The fertile F_1 had the shallow lobbed leaf of *Vigna radiata* var. VBN (Gg)2 and black colour seed of *Vigna mungo* var. Mash 114 with pollen fertility of 42.0 per cent and crossability of 12.50%. Most interestingly all the four interspecific F_1 plants were free from MYMV disease. In F_2 generation, only one healthy plant was survived which had a pod, stem and branching behaviour of blackgram and greengram characters of lobbed leaf and green seed colour. In F_3 generation, number of branches per plant, number of clusters per plant, number of pods per plant, pod length and seed yield per plant had high GCV, high PCV, high heritability coupled with high genetic advance as per cent of mean. Present study suggests that MYMV resistant cultivars of greengram can be explored through interspecific hybridization with *Vigna mungo* var. Mash 114 as a source of resistance and the hidden transgressive segregants can be recovered in F_3 generation.

Key words: F_3 generation, Greengram × Blackgram, Inter specific hybridization, MYMV resistance, Release of hidden variability.

INTRODUCTION

Greengram [*Vigna radiata* (L.) Wilczek], also known as mungbean, green bean, mash bean, golden gram and green soy which is an important source of dietary protein across Asia. It is widely cultivated in tropical and sub tropical regions as mono crop as well as in the intercropping systems. Almost 90% of the world's greengram production comes from Asia, and India is the world's largest producer. In India greengram is cultivated in an area of 4.32 million ha with an annual production of 2.17 million tones and productivity of 502 kg/ha. In Tamil Nadu, greengram is being cultivated in an area of 1.68 lakh ha with a production of 0.51 lakh tonnes and productivity of 300 kg/ha (Anonymous, 2018). The main cause for low yield is its susceptibility to pests and diseases of which Mungbean Yellow Mosaic Virus (MYMV) is the most prevalent and destructive viral pathogen produces typical yellow mosaic symptoms. The symptoms appear in the form of small irregular yellow specs and spots along the veins, which enlarge until leaves were completely yellowed. Diseased plants were stunted with fewer flowers and pods that bear smaller, occasionally shrivelled seeds in severe cases and other plant parts also become completely yellow. Depending on the severity of the MYMV disease infection, the yield penalty may reach up to 85% (Haq *et al*., 2010).

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MYMV disease is being transmitted by white fly *Bemisia tabaci* and its control is often based on limiting the vector population with insecticides, which are ineffective under severe whitefly infestations (Malathi *et al*., 2008). The use of resistant varieties is the most desirable strategy to manage the disease in an economical and environmentally friendly way.

Interspecific hybridization is one of the methods of creation of genetic variability and widening of genetic base of a crop species. Continuous breeding efforts for improvement of greengram [*Vigna radiata* (L.) Wilczek] and blackgram [*V.mungo* (L.) Hepper] had exhausted the available variability and only limited improvement is possible. Both of these species have some desirable characters like, greengram has early maturity, erect growth habit and long pods with more number of seeds/pod and blackgram possess non shattering pods with synchronous maturity, more clusters/plant, pods with large seeds and comparatively more durable resistance to yellow mosaic virus, which can be transferred in them *via* wide hybridization (Singh, 1990). Mahalingam *et al*., (2019) reported that, interspecific hybridization between *Vigna radiata* and *Vigna mungo* could be an alternate approach to develop MYMV resistant genotypes in greengram. In view of these considerations the present investigation was under taken with the objective of developing high yielding and MYMV resistant genotypes through interspecific hybridization between *Vigna radiata* var VBN (Gg) 2, *Vigna radiata* var VBN (Gg) 3 and *Vigna mungo* var. Mash 114.

MATERIALS AND METHODS

Parental materials

The present experiments were carried out at the National Pulses Research Centre (NPRC), Tamil Nadu Agricultural University, Vamban, Pudukkottai during the year 2016-2018. The experimental material comprising of two distinguished cultivated species *viz*., *Vigna radiata* var. VBN (Gg)2, VBN (Gg) 3 and *Vigna mungo* var. Mash 114. *Vigna radiata* var. VBN (Gg)2 is a high yielding variety with tri lobbed leaves and shiny green colour seeds having susceptibility to Mungbean Yellow Mosaic Virus (MYMV) disease whereas, VBN (Gg)3 is a high yielding variety with ovate leaves and green colour dull seeds but highly susceptible to MYMV disease. *Vigna mungo* var. Mash 114 a derivative from wild progenitor *Vigna mungo* spp. *silvestris* is highly resistant to MYMV disease with determinate plant type and synchronized maturity having a lanceolate leaves and black colour seeds.

Screening for yellow mosaic virus symptoms

The three *vigna* species (*Vigna radiata* var. VBN (Gg)2, VBN (Gg) 3 and *Vigna mungo* var. Mash 114 were evaluated for their MYMV disease resistance during *Rabi* 2015-16 season following the infector row method, where in two test rows alternating with one infector row of the susceptible variety (CO 5 blackgram). The plants were maintained properly by providing row to row and plant to plant spacing of 30 cm and 10 cm respectively. No insecticides were sprayed in order to maintain the natural whitefly population in the experimental field. The scoring of test materials was done only when the 80% of infector rows showed MYMV disease incidence. The rating scale suggested by Singh and Singh (1988) was adopted as given below:

Scale	Percentage of plant foliage affected	Reaction
1	Mottling of leaves covering 0.1 to 5.0% of the leaf area	Resistant (R)
3	Mottling of leaves covering 5.1 to 10.0% of the leaf area	Moderately resistant (MR)
5	Mottling and yellow discolouration of 10.1to 25.0% of the leaf area	Moderately susceptible (MS)
7	Mottling and yellow discolouration of 25.1 to 50.0% of the leaf area	Susceptible (S)
9	Severe yellow mottling on more than 50.0% and up to 100% of the leaf area	Highly Susceptible (HS)

Based upon the MYMV disease score, the greengram plants were classified into five categories *viz*., resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS).

Hybridization of MYMV susceptible and resistant *vigna* species

Vigna mungo var. Mash 114 has been used as donor parent (male) for MYMV disease resistance while the susceptible *Vigna radiata* var. VBN (Gg)2 and VBN (Gg) 3 were taken as female parents for hybridization. The crossing block consisted of four rows of female parent and two rows of male parent spaced at 50 × 30 cm during *Summer* 2016. The emasculation and pollination was done on the same day following bud pollination technique. Hybridization was effected during morning 7-9.30 am. The hybridized seeds were collected and dried for F_1 evaluation.

Evaluation of F_1 hybrid for MYMV resistance

The interspecific F_1 hybrids developed were evaluated during *Kharif* 2016. The F_1 seeds were planted at a spacing of 50 × 50 cm on either side of F_1s parental lines were raised. F_1s were evaluated for days to 1st flowering, days to maturity, plant height (cm), number of branches/plant, number of clusters/plant, number of pods/clusters, pod length (cm), number of seeds/pod, number of pods/plant, single plant yield (g), pollen fertility (%), pod setting percentage and MYMV resistance.

Evaluation of F_2 and F_3 population for yield parameters and MYMV resistance

F_2 and F_3 populations developed were evaluated during *Rabi* 2016-17 and summer 2017 respectively. The F_2 and F_3 seeds were planted at a normal spacing of 30 × 10 cm. On either side of F_2 and F_3 populations MYMV susceptible blackgram variety CO 5 was raised as infector row. The F_2 and F_3 populations were evaluated for days to 1st flowering, days

to maturity, plant height (cm), number of branche/plant, number of clusters/plant, number of pods/clusters, pod length (cm), number of seeds/pod, number of pods/plant, single plant yield (g) and MYMV resistance. Phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %) were calculated by the method suggested by Burton (1952). Heritability (h^2) in broad sense was calculated as suggested by Lush (1940) and expressed in percentage. Genetic advance and genetic advance as per cent of mean was estimated by the method formulated by Johnson *et al*. (1955).

RESULTS AND DISCUSSION

Screening of *Vigna* species for MYMV symptoms

The field evaluation of three different *Vigna* species under infector row method, the *vigna radiata* var. VBN (Gg) 2 and VBN (Gg) 3 showed moderately susceptible and susceptible reaction with a disease score of 5 and 7 respectively to mungbean yellow mosaic virus (MYMV) disease. Whereas the *vigna mungo* var. Mash 114 was free (0 score) from MYMV disease. The F_1 interspecific hybrid plants were also free from MYMV disease (0 score). Similarly the only survived F_2 plant also free from MYMV disease (Table 3). In F_3 generation, 23 single plants were found to be free from MYMV disease.

Cross compatibility and performance of F_1 hybrid

Crosses between species of the same or different genera have contributed immensely to crop improvement, gene and genome mapping, understanding of chromosome behaviour and evolution in crops like rice, wheat, maize, sugarcane, cotton, tomato, *etc* (Sharma, 1995). Stalker (1980) elaborated the gaps between hybridization and utilization, along with approaches for the utilization of wild species in food legumes. However, it is well recognized that gene transfer through wide crosses is a long and tedious process, due to lack of homology between chromosomes of participating species in the crosses, pre and post zygotic

Table 1: Pod set, crossability, pollen fertility and germination percentage of parents and F_1 hybrids of interspecific cross of *Vigna radiata* × *Vigna mungo*.

Parents and hybrids	Number of buds pollinated	Pod set	Crossability (%)	No. of seeds obtained	No.of plants germinated	Germination (%)	No. of plants till maturity	Pollen fertility (%)	No. of true F_1 plants
Vigna radiata var. VBN (Gg)2 × *Vigna mungo* var. Mash	120	15	12.50	20	8	40.0	7	42.5	04
Vigna radiata var. VBN (Gg)3 × *Vigna mungo* var. Mash	74	03	4.05	5	0	0	0	0	0

Table 2: Morphological features of F_1 hybrid plant between *Vigna radiata* var. VBN (Gg)2 × *Vigna mungo* var. Mash 114.

Character		Observation		
		F_1	*Vigna radiata* var. VBN (Gg)2	*Vigna mungo* var. Mash 114
Plant type	:	Determinate	Indeterminate	Determinate
Stem Colour	:	Purple	Purple	Purple
Hairiness of stem	:	Present	Present	Present
Leaf shape	:	Basel leaves were shallow lobbed and top leaves were lanceolate	Lobbed	Lanceolate
Leaf colour	:	Green	Green	Green
Petiole colour	:	Greenish purple	Greenish purple	Purple
Immature pod colour	:	Green	Green	Green
Matured pod colour	:	Black	Black	Black
Pod length	:	4.5 cm	8.2 cm	3.0 cm
Seed colour	:	Black	Green	Black
Days to 1st flowering	:	36 days	40	36
Days to maturity	:	69 days	70	65
Plant height	:	58 cm	75 cm	52 cm
Number of seeds per pod	:	3-4	11-12	5-6
MYMV score	:	0	5	0

crossability barriers between wild and cultivated species. In the present investigation, fifteen and three crossed pods were harvested from 120 and 74 flower buds that were pollinated in VBN (Gg) 2 × Mash 114 and VBN (Gg) 3 × Mash 114 crosses with a crossability percentage of 12.50 and 4.05 respectively. The hybrid germination of 40.0% and hybrid pollen fertility of 42.5% was recorded in VBN (Gg) 2 × Mash 114 cross combination (Table 1).

Table 3: Mean performance of parents, F_1 and F_2 generation.

Parent / Generation	Days to 1st flowering	Days to maturity	Plant height (cm)	Number of branches / plant	Number of clusters / plant	Number of pods / clusters	Pod length (cm)	Number of seeds / pod	Number of pods / plant	Single plant yield (g.)	MY MV
Vigna radiata 2 var. VBN (Gg)	40	70	75	3	12	5	8.2	11-12	51	12.2	5
Vigna mungo 36 var. Mash 114	65	52	3	8	4	3.0	5-6	56	10.5	0	
F_1-1	32	65	46	3	0	0	0	0	0	0	0
F_1-2	36	70	45	4	0	0	0	0	0	0	0
F_1-3	35	68	44	3	0	0	0	0	0	0	0
F_1-4	36	69	58	3	13	5	4.5	3-4	12	2.5	0
F_2	36	70	65	4	15	4	7.3	8	29	9.5	0

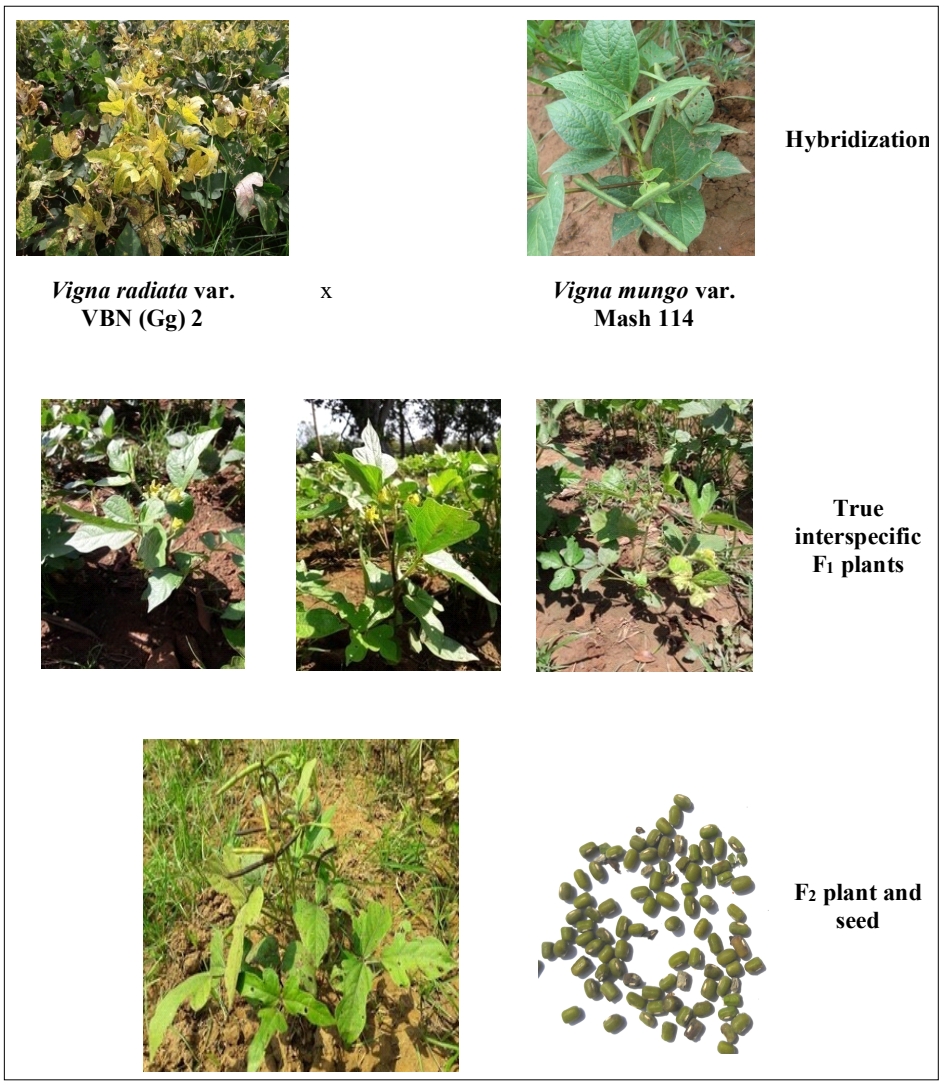

Fig 1: Generation and evaluation of *Vigna radiata* var. VBN (Gg) 2 × *Vigna mungo* var. Mash 114 interspecific cross combination.

In *Vigna radiata* var. VBN (Gg) 2 × *Vigna mungo* var. Mash 114 cross combination, among the 20 crossed seeds sown during *kharif* 2016 only eight plants have germinated. Most interestingly, four plants were found to be a true interspecific F_1 hybrid plants. Among the four true interspecific F_1 hybrid plants three were sterile and one was partially sterile with pollen fertility of 42.5%. Phenotypically the F_1 plants are intermediate in expression. The F_1 plant had the greengram features of shallow lobbed leaf shape and flowering characters. Determinate plant type, pod length (4.5cm), seed colour (black), days to 1st flowering (36 days), days to maturity (69 days), number of seeds per pod (3-4) were similar to blackgram features (Table 2).

In VBN (Gg) 3 × Mash 114 cross combination, among the five crossed seeds sown none of them have germinated (Table 1). The observed failure of germination of the hybrid seeds have resulted from the loss of seed viability which could have been caused by series of factors such as poor seed preservation technique, unfavourable germination condition and the presence of lethal alleles in hybrid genotype (Saccardo *et al*., 1992). Egawa (1988) reported that all the 56 seeds from a *V. mungo* × *V. radiata* cross and 5 seeds from a *V. umbellata* × *V. radiata* cross failed to germinate. The reasons for failures of interspecific crosses in *Vigna* are poorly understood (Chen *et al*., 1983), several factors such as specific cross combination, genetic divergence and environment have influenced the low success rate. Furthermore, pre and post fertilization barriers such as pollen pistil incompatibility (Chowdhury and Chowdhury, 1977), embryo abortion (Ahn and Hartmann, 1977; Fatokun *et al*., 1997) and hybrid failure and breakdown (Chen *et al*., 1983) have also been responsible for the low success rates and failure of crosses.

A number of studies undertaken on crossability among different *Vigna* species have been reviewed by Dana and Karmakar (1990) and Singh (1990). Most reports indicate that *V. radiata* produced successful hybrids as seed parent with *V. mungo*, *V. umbellata* and *V. angularis*, although their reciprocal cross hybrids were not viable. However, by using sequential embryo rescue methods, the reciprocal hybrids



Fig 2: Variations in the F_3 generation.

between *V. mungo* and *V. radiata* could be successfully produced (Gosal and Bajaj, 1983; Verma and Singh, 1986). *V. mungo* was also successfully crossed with *V. delzelliana* (Chavan *et al*., 1966), *V. glabrescens* (Dana, 1968) and *V. trilobata* (Dana, 1966).

Chances of success can be increased through *in vitro* pollination and fertilization followed by embryo rescue technique (Fatokun and Singh, 1987; Chen *et al*., 1977). Pollination methods such as cut style and style grafting can also be employed alongside the bud pollination technique (Van Tuyl and De Jeu, 1997).

The results of crossability in the cross VBN (Gg) 2 × Mash 114 is in agreement with the findings of different interspecific crosses of Blakeslee (1945), Burton (1952), Bhatnagar *et al*. (1974), Ahuja and Singh (1977), Chen (1989), Ganeshram (1993). The average pollen fertility in F_1 hybrid plants were reported by Stebbins (1958) and Rego *et al*., (2000).

In the present study, the four F_1 hybrid plants between VBN (Gg) 2 × Mash 114 were free from MYMV disease and the single plant of F_2 generation was also free from MYMV infestation (Table 3). Results of MYMV reaction of F_1 and F_2 generation clearly showed that, there is a great scope for the development of MYMV resistant cultivars of greengram through greengram × blackgram interspecific hybridization.

Performance of F_2 and F_3 population

In the F_2 population of *Vigna radiata* var. VBN (Gg) 2 × *Vigna mungo* var. Mash 114 cross combination only one plant survived. The F_2 plant had the greengram feature of long pod (7.3 cm) and blackgram feature of determinate plant type with single plant yield of 9.5 grams (Table 3 and Fig 1). The hidden variability has been observed in F_3 generation for all the quantitative traits studied. In the present study, wider range of variability has been recorded for plant height (30 to 89 cm), number of branches per plant (1-7 branches),

Table 4: Performance of F_3 individuals of interspecific cross between *Vigna radiata* var. VBN (Gg) 2 × *Vigna mungo* var. Mash 114.

Plant. No	Plant height (cm)	Number of branches / plant	No. of clusters / plant	Number of pods / cluster	Pod length (cm)	Number of seeds / pod	Number of pods / plant	100 seed weight (g.)	Single plant yield (g.)	MY MV
1	59	7	14	2	6.5	8	20	3.5	4.6	0
2	59	4	37	4	8.2	10	120	3.8	41.3	0
3	63	6	70	3	7.5	8	161	4.5	49.8	0
4	59	4	34	4	7.2	8	83	4.1	25.3	0
5	51	6	27	3	8.4	9	74	3.9	21.7	1
6	46	3	21	2	5.4	6	30	3.2	4.7	3
7	45	1	6	2	8.2	9	13	3.6	3.5	0
8	-	-	-	-	-	-	-	-	-	0
9	35	6	4	1	5.3	6	2	-	0.75	1
10	70	4	38	3	8.2	9	105	4.6	41.4	0
11	50	4	29	3	6.2	7	63	3.3	12.3	0
12	51	2	11	3	7.1	8	31	3.8	8.3	0
13	67	4	4	3	5.3	6	5	-	-	5
14	89	4	27	3	6.3	6	43	4.1	8.7	0
15	-	-	-	-	-	-	-	-	-	0
16	58	4	46	3	7.4	10	88	4.7	37.5	0
17	72	4	41	3	7.2	8	104	3.2	23.6	0
18	66	6	54	3	7.7	10	116	3.9	49.5	0
19	72	4	27	2	6.3	6	32	3.5	5.6	1
20	47	0	5	2	5.2	5	7	-	1.86	0
21	58	5	31	3	7.4	9	73	4.7	25.8	0
22	66	2	15	3	6.5	5	42	3.6	6.5	0
23	60	5	38	3	7	7	74	4.8	22.6	0
24	63	6	31	3	8.3	9	59	3.4	15.1	3
25	69	7	28	3	8.3	10	72	3.8	25.3	0
26	43	4	8	2	5.5	3	8	-	0.82	3
27	-	-	-	-	-	-	-	-	-	0
28	46	5	3	3	7.2	4	5	-	0.85	1
29	65	7	33	3	7.3	8	68	3.7	18.2	0
30	53	6	37	3	6.5	7	78	3.7	18.2	0
31	30	1	2	1	5.1	4	2	-	0.31	0

Table 5: Variability parameters among the F_3 interspecific derivatives between greengram and blackgram for yield and yield component traits.

Trait	Mean	Range		PCV	GCV	h^2	GAM	Skewness	Kurtosis
		Min	Max	(%)	(%)	(%)	(%)		
Plant height (cm)	57.57	30.00	89.00	21.35	19.93	87.15	50.47	0.01	0.27
Number of branches/plant	4.32	1.00	7.00	42.43	40.69	91.96	95.05	-0.55	-0.29
No. of clusters/plant	25.75	2.00	70.00	64.84	64.76	99.75	133.90	0.42	-0.09
Number of pods/cluster	2.71	1.00	4.00	25.83	14.29	30.61	173.82	-0.79	0.64
Pod length (cm)	56.36	2.00	161.00	73.60	73.28	99.12	152.97	0.46	-0.45
Number of seeds/pod	7.32	3.00	10.00	26.60	26.56	99.74	54.93	-0.43	-0.73
Number of pods/plant	6.88	5.10	8.40	15.11	7.55	25.00	124.47	-0.25	-1.09
100 seed weight (g)	3.79	3.20	4.80	12.38	8.34	45.45	56.09	0.86	-0.33
Single plant yield (g)	16.95	0.50	49.80	90.00	89.97	99.91	185.57	0.75	-0.55

number of clusters per plant (2-70 clusters), number of pods per cluster (1-4), pod length (5.1 to 8.4 cm), number of seeds per pod (3-10 seeds), pods per plant (2-161 pods) and single plant yield (0.31 to 49.8 grams). Among the 31 F_3 single plants, 23 plants were free from MYMV disease infection (Table 4 and Fig 2).

Variability parameters in F_3 generation

In the present investigation of F_3 interspecific progenies between greengram and blackgram, all the traits studied have recorded more variability among the progenies. The traits *viz*., number of branches per plant, number of clusters per plant, number of pods per plant, pod length and seed yield per plant were recorded high PCV and GCV among the interspecific progenies of F_3 generation (Table 5). The traits *viz*., plant height and number of pods per clusters were recorded high PCV along with moderate GCV. The traits number of seeds per pod and 100-seed weight were recorded moderate PCV and low GCV. High heritability was recorded by the traits *viz*., plant height (87.15%), number of branches per plant (91.96%), number of clusters per plant (99.75%), number of pods per plant (99.12%), pod length (99.74%) and seed yield per plant (99.91%). Moderate heritability percentage was recorded by number of pods per cluster (30.61%) and 100-seed weight (45.45%). Low heritability percentage was recorded by the trait number of seeds per pod (25.00%). In case of genetic advance as per cent of mean all the nine traits were recorded higher values among the interspecific derivatives of greengram and blackgram. In case of skewness and kurtosis all the traits studied among the interspecific derivatives were showed mesokurtic nature and no skewness has been shown. High heritability coupled with high genetic advance indicated that most likely the heritability is due to additive genetic effects and the selection may be effective. This also suggested that the variation in the environment played relatively limited role in influencing the inheritance of these characters and thus the response to selection would be higher.

CONCLUSION

The success in the improvement of cultivated variety for yield, quality, resistance to biotic and abiotic resistance largely depends upon the natural variability present in the population. Through hybridization among the selected genotypes, it is possible to reshuffle desired characteristics provided the segregating generations contain large variability. Selection for quantitative characters is generally taken up in the early segregating generations mostly from F_2 generation onwards. For characters like grain yield the selection is continued till the material become homozygous. Because, such characters are controlled by large number of genes and huge number of population has to be raised for making the selection effective. This is not always true, because the effective selection was known to restricted by close linkages between desirable and undesirable component characters and these undesirable linkages delay the utilization of full recombination potential (Mike, 1985). Present investigation also revealed that, in F_2 generation only one plant was survived with poor yield and the hidden variability was released only in F_3 generation. Interspecific hybridization between *Vigna radiata* and *Vigna mungo* the variability for yield and yield component traits and MYMV resistance can be expected in F_3 generation and the selection pressure can be applied till F_8 or F_9 generation in order to attain homozygous breeding line.

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