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Interspecific Hybridization in Direct and Reciprocal Crosses of *Vigna radiata* and *Vigna aconitifolia*

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Abstract

The experiment was conducted with an objective to develop superior segregants for yield components coupled with pest and disease resistance, the interspecific hybridization was attempted between mungbean (*V. radiata*) and *Vigna aconitifolia*. In direct and reciprocal crosses, the pod set percentages was high (13.27) in direct crosses when compared with reciprocal crosses which were recorded 8.97 only. The observation for parents and both direct and reciprocal cross combinations were recorded. The germination percentage was more (98%) *Vigna radiata* when compared with *Vigna aconitifolia* with 45 percentage. But there is no much variation in germination percentage of the direct and reciprocal cross of *V. radiate* x *Vigna aconitifolia* and *Vigna aconitifolia* x *V. radiata* were recorded 48% and 50% respectively. The hybrid leathality percentage was high (58.33) in *V. radiata* x *Vigna aconitifolia* where as in *Vigna aconitifolia* x *V. radiata* was 40.00. The hybrid break down percentage was less (41.67) in *V.radiata* x *Vigna aconitifolia* while in reciprocal cross of *Vigna aconitifolia* x *V. radiata* with more (60.00 percent). The huge number (80) of seedlings attained maturity in the in *V. radiata* and but only 29 seedlings in *Vigna aconitifolia* and the same character for the both direct and reciprocal crosses of 7 and 2 percent in *V. radiata* x *Vigna aconitifolia* and *Vigna aconitifolia* x *V. radiata* respectively. The pollen fertility percentage of parent *V. radiata* was 85.25 and 65.85percent in *Vigna aconitifolia* and F1 hybrids of *V. radiata* x *Vigna aconitifolia* was more (53.50) and *Vigna aconitifolia* x *V. radiata* recorded less (35.80 percent).

The quantitative traits such as plant height, number of branches per plant, days to fifty percent flowering, length of branch, number of clusters per branch, number of clusters per plant, number of pods per plant, pod length, number of seeds per pod, hundred seed weight, dry matter production and seed yield exhibited high per se performance in the direct cross *V. radiata* x *Vigna aconitifolia* like parent *Vigna radiata* except days to full maturity. For all the traits in the cross of *Vigna radiata* x *Vigna aconitifolia* the skewness was positive indicating that predominance of dominant alleles. Hence it is concluded that the direct cross of *Vigna radiata* x *Vigna aconitifolia* performance was good when compared with indirect cross indicating that better segregants can be obtained in the direct cross which will be useful in development of green gram genotypes with biotic resistance.

Keywords: Interspecific hybridization; *Vigna radiate*; *Vigna aconitifolia;* Crossability; Seeds germination; Hybrid leathality (%); % of hybrid break down; Pollen fertility; F1 and F2 generations

Introduction

Vigna radiata (L.) Wilczek, commonly known as green gram or mungbean is the most widely distributed species among the six Asiatic Wild *Vigna accessions*. It is one of the predominant sources of protein and certain essential amino acids like lysine and tryptophan in vegetarian diets. The basic reason for limited success had been due to the limited variability prevailed among the parents used for hybridization in most of the studies. There had been always possibility of improving the crop by incorporating wild genes to the cultivated species. Stepwise utilization of primary, secondary and tertiary gene pools of this crop can result in tremendous improvement in yield. It is essential to attempt interspecific crosses and to develop viable hybrids. These hybrids need to be critically evaluated as such and in the segregating generations for improvement in yield and yield components.

The introgressed materials developed through wide crosses can also contribute as genetic reservoirs for novel genes apart from contributing to the improvement of yield and yield components. With a view to evaluate for attempting interspecific hybridization to generate segregants for better yield, this study was taken up keeping the objectives in mind such as to generate variability through interspecific hybridization involving *Vigna radiata* with species in secondary and tertiary gene pools and to compare the variability created for yield and yield components among segregants generated

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through interspecific hybridization.

Materials and Methods

The materials consists of the genotype of greengram (*Vigna radiata*) (VRM (Gg) 1) and one species *Vigna aconitifolia*. The mungbean was used as female parent and male parent is *Vigna aconitifolia* and vice versa for wide hybridization.

Vigna radiata and *Vigna aconitifolia* were raised during Rabi 2012-2013 in a crossing block. The direct and reciprocal crosses were effected as per the method suggested by Boling [1] for hybridization.

The number of plants survived over germinated seeds were taken to assess the leathality of F1 hybrids.

Hybrid lethality(%) = (No. of plants died / No. of seeds germinated) X 100

The set seeds (F1) from the above mentioned crosses were sown in two rows along with one row of male and female parents with spacing of 45 x 30 cm during rabi season. The following quantitative traits such as plant height (cm), Number of branches per plant, length of branches (cm), days to 50 percent flowering, number of clusters per branch, number of clusters per plant, number of pods per plant, pod length (cm), number of seeds per pod, hundred seed weight (g), grain yield per plant (g), dry matter production and days to maturity were studied for all F1 hybrids and their parents.

The pollen fertility analysis was carried out at the time of flowering in the parents and their hybrids by acetocarmine staining technique.

$$\text{Pollen fertility} = \frac{\text{No. of viable pollen}}{\text{Total no. of pollen observed}} \times 100$$

The seeds from individual F1 plants along with their parents were collected separately and they were sown as progeny rows during Kharif 2013. Observations for the quantitative traits as that of F1 generation except the traits *viz*., days to 50 percent flowering and days to full maturity were recorded. The descriptive parameters such as mean, range, SE, SD, skewness and kurtosis were computed.

Results

The result of this parental crosses pertaining to crushability related characters, pollen fertility, mean performance of F1s and F2 morphology characters studies were carried out and revealed. The successful direct and reciprocal crosses between *V. radiata* and *Vigna aconitifolia* species and the results were obtained for the following observations namely number of flower emasculated, number of flower crossed, pod set and crossability percentage presented in Table 1. In this direct cross a sum of 135 flowers were emasculated and 113 flowered crossed from which 15 numbers of pods obtained with 13.27 pod set percentage. In this reciprocal cross, a total of 110 flowers were emasculated and 78 flowered were crossed from which 7 numbers of pods obtained with 8.97 pod set percentage.

In Table 2, the following observations namely number of seeds germinated, hybrid leathality percentage, percentage of hybrid break down, percentage of germination and number of seedlings attained maturity in the parents and both direct and reciprocal cross combinations were recorded. A sum of 100 seeds from each parent, 50 seeds from both direct and reciprocal crosses namely *V.radiata* x *Vigna aconitifolia* and *Vigna aconitifolia* x *V.radiata* were used for test respectively. In the parents and crosses, 98 seeds were germinated in *V.radiata* while 45 seeds were germinated in *Vigna aconitifolia*. In direct cross 12 seeds were germinated while in the reciprocal cross only 5 seeds were germinated. The hybrid leathality percentage of 58.33 recorded in *V.radiata* x *Vigna aconitifolia* where as in *Vigna aconitifolia* x *V.radiata* it was 40.00.

In the direct cross of *V. radiata* x *Vigna aconitifolia*, the hybrid break down percentage was 41.67 while in reciprocal cross of *Vigna aconitifolia* x *V. radiata*, recorded 60.00 percent.. The germination percentage of 98 and 78 was recorded in *V. radiata* and *Vigna aconitifolia* parents respectively. In the direct cross of *V.radiata* x *Vigna aconitifolia* and in the reciprocal cross *Vigna aconitifolia* x *V. radiata*, observed germination percent of 48.00 and 50.0 respetively. Eighty number of seedlings attained maturity in *V.radiata* and 29 in *Vigna aconitifolia* and for the same character *V. radiata* x *Vigna aconitifolia* and *Vigna aconitifolia* x *V. radiata* recorded 7 and 2 respectively.

V. radiata and *Vigna aconitifolia* was recorded the pollen fertility percentage of 85.25 and 65.85 percent respectively and F1 hybrids of interspecific crosses for *V. radiata* x *Vigna aconitifolia* was observed 53.50 percent and *Vigna aconitifolia* x *V. radiata* recorded 35.80 percent Table 3.

The mean performance of parents and hybrids both direct and reciprocal crosses of *V. radiata* and *Vigna aconitifolia* and cross *Vigna aconitifolia* x *V.radiata* and *Vigna aconitifolia* x *V. radiata* for various 13 characters like plant height (cm), branch length (cm), bays to fifty percent flowering, number of cluster per branch, number of cluster per plant, number of pods per plant, pod length, hundred grain weight, single plant yield, dry matter production and days to complete maturity were recorded Table 4.

In, *V. radiata* registered highest mean value with plant height of 47.2 while the *Vigna aconitifolia* with plant height of 38.2. In the direct cross, the hybrid plant of *V. radiata* x *Vigna aconitifolia* was found to be taller with the height of 28.5 where as in the reciprocal crosses the *Vigna aconitifolia* x *V. radiata* found to be shorter with the height of 19.3.

The parental species *Vigna aconitifolia* possessed more (5.0) number of branches *V. radiata* while recorded (3.0). The hybrid of the direct cross *V. radiata* x *Vigna aconitifolia* registered 3.0 numbers of branches. The hybrid of the reciprocal cross *Vigna aconitifolia* x *V. radiata* registered 2.0 number of branches per plant.

V. radiata possessed lengthiest branch branch length of 40.5 while the parent *Vigna aconitifolia* was recorded with branch length of 32.0. The hybrids of the cross *V. radiata* x *Vigna aconitifolia* registered the maximum branch length of 20.0 as compared to reciprocal crosses *Vigna aconitifolia* x *V.radiata* recorded 13.5 cm.

The parental species *Vigna aconitifolia* was the earliest to flower with 35 days closely followed by *V. radiata* with 38 days. The indirect cross *Vigna aconitifolia* x *V. radiata* registered early flowering (32.0 days) followed by *V. radiata* x *Vigna aconitifolia* (33.0 days).

Table 1: The crosses, pod set and crossability percentage of *Vigna radiata* x *Vigna acconitifolia* and *Vigna acconitifolia* x *V. radiata*.

Parents	No. of flowers emasculated	No. of flowers crossed	No.of crossed pods obtained (Pod set)	pod set %
V. radiata x *Vigna acconitifolia*	135	113	15	13.27
Vigna acconitifolia x V. radiata	110	78	7.0	8.97

Table 2: Germination percentage of parents and F1 hybrids for direct and reciprocal crosses.

Parents and Crosses	No. of crossed seeds obtained	No. of seeds germinated	Hybrid lethality (%)	Hybrid break down (%)	Germination (%)	No. of seedlings attained maturity
V.radiata	100	98			98	80
Vigna acconitifolia	100	45			45	29
V. radiata x *Vigna acconitifolia*	25	12	58.33	41.67	48.0	7
Vigna acconitifolia x *V.radiata*	10	5	40.00	60.00	50.0	2

Table 3: Pollen fertility percentage of parents and F_1 hybrids of interspecific crosses for *Wild Vigna accessions.*

Parents and Hybrids	Pollen fertility (%)
V. radiata	85.25
V. acconitifolia	65.85
V. radiata x *V. acconitifolia*	53.50
V. acconitifolia x *V. Radiata*	35.80

Table 5: Mean performance of the yield contributing characters among F2 families of interspecific crosses.

Crosses	NOB	BRL	NCB	NOC	NPP	HSW	SPY
V. radiata **x** *V. acconitifolia*	2.50	22.00	4.00	9.12	28.85	1.98	3.53
V. acconitifolia x *V.radiata*	2.43	21.83	3.86	8.20	27.85	1.70	3.26
Mean	**2.46**	**21.91**	**3.93**	**8.66**	**28.35**	**1.84**	**3.39**

The parent *Vigna aconitifolia* registered the highest value of 8.0 for number of clusters per branch but the other parent *V. radiata* recorded clusters per branch of 4.0. In the cross *V. radiata* x *Vigna aconitifolia* possessed highest number of clusters per branch of 3.0 while *Vigna aconitifolia* x *V. radiata*, registered highest value of one cluster per branch in the reciprocal crosses.

The numbers of clusters per plant was observed in the parent *V. radiata* was 12.0 while other parent *Vigna aconitifolia* was 13.0. The direct cross *V. radiata* x *Vigna aconitifolia* recorded the value of 10.00 for this trait. The reciprocal cross *Vigna aconitifolia* x *V.radiata*. hybrid registered with the value of 5.00 for this trait.

Highest number of pods per plant recorded by *Vigna aconitifolia* with the value of 50.0 followed by *V. radiata* with the value of 48.0. In the direct cross *V. radiata* x *Vigna aconitifolia* registered more number of pods 25.00, in case of hybrid of reciprocal crosses *Vigna aconitifolia* x *V. radiata* observed highest value of 15.00.

The parent *V. radiata* recorded the pod length of 9.80 but other parent *Vigna aconitifolia* with pod length of 6.30. In the direct cross *V. radiata* x *Vigna aconitifolia* has recorded the pod length of 7.5 cm and reciprocal cross *Vigna aconitifolia* x *V. radiata* registered with the pod length of 5.5.

More number of seeds per pod observed in the parental species *V. radiata*, with value of 10.0 while *Vigna aconitifolia* recorded only 6.0 seeds per pod. The hybrid of the cross *V. radiata* x *Vigna aconitifolia* recorded (6.0) while the reciprocal hybrid *Vigna aconitifolia* x *V. radiata* exhibited the value of 5.00 for this trait.

The parental species of *V. radiata* registering 3.8 for trait hundred seed weight. *Vigna aconitifolia* recorded the value of 1.8 for this trait. In the direct cross hybrid *V. radiata* x *Vigna aconitifolia* recorded 1.8 when *Vigna aconitifolia* x *V.radiata* exhibited the value of 1.5 for this trait.

The parent *V. radiata* registered more single plant yield of 4.85 and other parent *Vigna aconitifolia* registered with the yield of 2.8. In the direct cross, the value of 1.8 recorded by the hybrid *V. radiata* x *Vigna hainiana*. In the reciprocal *Vigna aconitifolia* x *V. radiata* registered the value of 1.5.

V. radiata registered the highest value of dry matter production of 24.5 and *Vigna aconitifolia* registered 8.5 in the parents. In the direct crosses the value of 5.0 recorded by the hybrids *V. radiata* x *Vigna aconitifolia* in the reciprocal crosses *Vigna aconitifolia* x *V. radiata* revealed the value of 5.0.

For days to full maturity, *Vigna aconitifolia* was the parent to mature earliest at 60 days followed by *V. radiata* in 65 days. The hybrid of the reciprocal cross *Vigna aconitifolia* x *V. radiata* was earliest with 60 days. The hybrid of the cross *V. radiata* x *Vigna aconitifolia* was 65

Table 4: Mean performance of parents and hybrids both direct and reciprocal crosses.

Parents and crosses	PHT	NOB	BRL	DFF	NCB	NOC	NPP	POL	NSP	HSW	SPY	DMP	DFM
V. radiata	47.2	3.0	40.5	38.0	4.0	12.0	48.0	9.80	10.0	3.8	4.85	24.5	65.0
Vigna aconitifolia	38.2	5.0	32.0	35.0	8.0	13.0	50.0	6.3	6.0	1.8	2.8	8.5	60.0
V. radiata x *V. acconitifolia*	28.5	3.0	20.0	32.0	3.0	10.0	25.0	7.5	6.0	1.8	1.8	5.0	65.0
V. acconitifolia **x** *V. radiata*	19.3	2.0	13.5	33.0	1.0	5.0	15.0	5.5	5.0	1.5	1.5	5.0	60.0

PHT: Plant Height (cm); BRL: Branch Length (cm); DFF: Days To Fifty Percent Flowering; NCB: Number of Cluster per Branch; NOC: Number of Cluster per Plant; NPP: Number of Pods per Plant; POL: Pod Length; HSW: Hundred Grain Weight; SPY: Single Plant Yield; DMP: Dry Matter Production; DFM: Days To Full Maturity.

Table 6: Skewness and Kurtosis of yield contributing characters among F2 families of interspecific crosses.

Crosses	NOB		BRL		NCB		NOC		NPP		HSW		SPY	
	Skewness	Kurtosis	Skewness	Kurtosis	Skewness	Kurtosis	Skewness	Kurtosis	Skewness	Kurtosis	Skewness	Kurtosis	Skewness	Kurtosis
V. radiata x *V. acconitifolia*	0.48	-0.88	-0.65	-0.19	1.45	2.00	0.38	1.15	1.35	0.28	0.29	-0.35	0.67	-1.75
V. acconitifolia **x** *V. radiata*	0.58	-1.11	0.61	-0.57	0.28	-0.85	0.98	0.39	0.58	-0.82	-0.48	0.00	-0.48	0.00
Mean	**0.53**	**-0.99**	**-0.02**	**-0.38**	**0.86**	**0.57**	**0.68**	**0.77**	**0.96**	**-0.27**	**-0.09**	**0.17**	**0.09**	**-0.87**

days similar to female parent.

Mean performance of different yield contributing characters of *Vigna radiata* and *Vigna aconitifolia* cross in F2 segregants of both direct and reciprocal crosses was presented in Table 5. The number of branches per plant ranged from 2.43 (*Vigna aconitifolia* x *V.radiata*) to 2.50 (*V.radiata* x Vign aconitifolia) with mean value of 2.46.

The mean value for the length of branches per plant varied from 21.83 to 22.00 with over all mean of 21.91 for F2 segregants. The cross combination *V. radiata* x *Vigna aconitifolia* has exhibited in the direct and reciprocal cross combination with mean value of 22.00 and 21.83 respectively.

Number of clusters per branch, the number of clusters per branch ranged from 4.00 (*V.radiata* x *Vigna aconitifolia*) to 3.86 (*Vigna aconitifolia* x *V. radiata*) with the overall mean of 3.93.

The number of clusters per plant ranged from 8.20 (*Vigna aconitifolia* x *V. radiata*) to 9.12 (*V.radiata* x *Vigna aconitifolia*) with over all mean of 8.66 among the crosses.

The range for number of pods per plant was found to be between 27.85 (*Vigna aconitifolia* x *V.radiata*) to 28.85 (*V. radiata* x V. Vigna acconitifolia) with the overall mean value of 28.35.

The hundred seed weight ranged from 1.70 (*Vigna aconitifolia* x *V. radiata*) to 1.98 g (*V. radiata* x *Vigna aconitifolia*) with the overall mean of 1.84 g for F2 segregants.

The seed yield ranged from 3.26g (*Vigna aconitifolia* x *V. radiata*) to 3.53 g (*V. radiata* x *Vigna aconitifolia*) with a overall mean of 3.39g for F2 segregants.

For most of the direct as well as reciprocal crosses, the F2 progenies revealed high per se performance and also exhibited high variability for seven yield contributing traits. For all the traits in majority of the crosses, the skewness was positive indicating that predominance of dominant alleles Table 6.

The frequency distribution of the F2 segregants for seven traits that are significantly correlated with single plant yield namely, number of branches per plant, length of branch, number of clusters per branch, number of clusters per plant, number of pods per plant and hundred seed weight.

Discussion

In the present investigation with an objective to transfer useful traits from the *Vigna aconitifolia* (wild) and into *Vigna radiata* (greengram), the interspecific hybridization was attempted. The extent of crossability, fertility of hybrids and possibility of obtaining superior recombinants in the F2 generation through recombination of genomes were studied. The wild relatives of greengram possess desirable genes for many yield components coupled with resistance to bruchids and Mung bean Yellow Mosaic Virus (MYMV). Transferring of these genes into cultivated species, could result in development of high yielding resistant types. The use of wild species in greengram improvement programme was difficult because of some problems encountered in obtaining successful F1 hybrids due to crossability barriers. In spite of these difficulties, wide hybridization between *V. radiata* and its wild relative was successfully accomplished by many workers [2-18]. Crossability is a pre-requisite for gene transfer in wide hybridization. An understanding of crossability relationship among the species had been helpful not only in choosing methods for producing F1 hybrids, but also in tracing phylogenic relationship among species.

In the present study, successful pod set was observed in interspecific crosses with *Vigna radiata* either as ovule or pollen parent. This result is in agreement with the reports of Ahuja and Singh [19], Parida and Singh [20], Gopinathan et al., [21], Egawa et al., [22], Mendioro and Ramirez [23], Pandiyan et al., [6,24,8,9,25,11] Kathikeyan et al., [12,13], Sudha et al., [26,15] Devina Serum et al., [27], Premkumar et al., [17], Mariyammal et al., [28].

The pollen fertility percentage of parent *V. radiata* was 90.55 and 91.26 percent in *Vigna aconitifolia* and F1 hybrids of interspecific crosses for *V. radiata* x *Vigna aconitifolia* was 78.26 and *Vigna aconitifolia* x *V. radiata* recorded 65.26 percent Table 3.

In the direct cross the hybrid break down percentage was 18.74 recorded in *V. radiata* x *Vigna aconitifolia* while in reciprocal cross of 31.82 percent recorded in the cross *Vigna aconitifolia* x *V. radiata*. Similar observations on hybrid leathality and inviability were noticed in interspecific crosses involving different Wild *Vigna accessions* by AL- Yasiri and Corijne [29], Chen et al[30], , Adinarayanamurthy et al., [31], Pandiyan et al., [6-11] Kathikeyan et al., [13], Sudha et al., [14,15], Premkumar et al., [17], Mariyammal et al., [18]., Ganeshram [4] and Renganayaki [2]. Stebbins [32] had attributed the hybrid weakness, inviability, lethality and sterility as mechanisms of nature for maintaining the integrity of related species.

In general, the pollen fertility in the direct crosses was higher as compared to their corresponding reciprocal crosses which indicated that the approach using the cultivated species as a female parent is likely to generate better hybrids and segregants. Similar result was reported by various authors for differential pollen fertility among interspecific crosses of Wild *Vigna accessions* (Pandae et al., [3], Mendioro and Ramirez, [23], Ravi et al., [33], Anandabaskaran and Rangasamy, [34], Subramanian and Muthiah [5], Monika et al.,

[35]. Pandiyan et al., [6-11] Kathikeyan et al., [12,13], Sudha et al., [14,15,26], Premkumar et al., [17], Mariyammal et al., [18], and Sidhu and Satija [36]). Among the crosses the pollen fertility was highest in the cross *V. radiata* x *Vigna aconitifolia* and this observation support the view of Pandae et al., [3], Pandiyan et al,. [6-11], Mendioro and Ramirez [37] *Vigna aconitifolia* is the probable progenitor for *V. radiate*.

The range of pollen fertility observed in direct and reciprocal crosses was high enough to obtain sufficient viable F2 segregants.

The primary criterion used for the evaluation of hybrids was the per se performance for different traits. In the present study, in the direct cross exhibited high mean value for important traits *viz*., plant height, number of branches per plant, length of branch, number of clusters per branch, number of clusters per plant, number of pods per plant, pod length, number seeds per pod, hundred seed weight, single plant yield and dry matter production. The reciprocal cross exhibited less mean performance which is good value for day to fully maturity traits.

The hybrid of the direct cross *V. radiata* x *Vigna aconitifolia* recorded high per se performance for almost all the characters. Hence the segregants that could be recovered from these promising lines might serve as better breeding base for improvement of yield and yield components. Such promising interspecific hybrids were also reported by, Subramaninan and Muthiah [5] and Ganeshram [4], Pandiyan et al., [6-11].

For most of the direct as well as reciprocal crosses, the F2 progenies revealed high per se performance and also exhibited high variability for seven yield contributing traits. In majority of the crosses, the skewness was positive for all the traits indicating that predominance of dominant alleles as opined by Fisher et al. [38] and Darbeshwar Roy [39] Pandiyan et al., [6-11]. In this situation selection for traits in the early generation will not be fixable hence selections in later generations or by adopting modified breeding procedures such as intermating the segregants followed by recurrent selection may shift the gene action towards additive effects. Since sterility factors will gradually reduce over generations in case of interspecific crosses and more recombined populations will be available for selection, the effecting selection in the later generation will be more effective.

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