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Crossability Studies Among Various Species of Genus *Vigna*

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ABSTRACT

A total of 30 inter-specific crosses of *Vigna mungo* x *V. umbellata* (urdbean x ricebean) and *V. mungo* x *V. angularis* (urdbean x adzukibean) were attempted to study the crossability of urdbean with ricebean and adzukibean. Out of 30 cross combinations, 12 cross combinations of *V. mungo* x *V. umbellata* were successful. Day-to-day visual observations showed that most of the emasculated buds dropped the very next day after pollination and some aborted after about a week. Some of the crosses developed into pods without seeds and dropped. Pod formation involving *V. angularis* as one of the parents in inter-specific hybridization was not very successful as there was no pod formation and buds dropped after 1-3 days of pollination. Among the crosses of *V. mungo* x *V. umbellata*, the crossability of Palampur-93 x PRR-2 was highest (14.18%) followed by Him Mash-1 x VRB-3 (13.64%) and PDU-1 x PRR-2 (13.05%). Interspecific hybridization was performed with the objective to transfer disease resistance in urdbean. The study indicated that different kinds of pre and post fertilization barriers are responsible for complete sterility to low fertility. The genotypes showing a substantially high percent of crossability may be utilized for genetic improvement of urdbean.

Keywords**:** Inter-specific hybridization, crosses, urdbean ricebean and adzukibean

Introduction

Urdbean [*Vigna mungo* (L.) Hepper], 2n=2x=22 popularly known as blackgram or mash, is the fourth most important food legume of India, belongs to family Leguminoseae and subfamily Papilionaceae, with its wild progenitor, *V. mungo* var. *silverstris* (Bhareti et al. 2011). It has been reported to be originated in India with a secondary center of origin in central Asia (Pratap and Kumar 2011). It is a short-duration pulse crop and self-pollinated grain legume grown in many parts of India.

Food legumes are a vital source of protein, especially for the poor who often cannot afford animal products. Urdbean occupies an important position due to its high seed protein (25-26%), carbohydrates (60%), fat (1.5%), minerals (high amount of iron and phosphorus), amino acids and vitamins and ability to restore the soil fertility through symbiotic nitrogen fixation (Malik, 1994: Harmankaya et al. 2016). Despite the huge benefits of urdbean, it is grown in 2.5 million hectares of area in India and produces about 1.5 million tonnes of urdbean annually with an average productivity of 400 kg per hectare (Anonymous, 2019). India is the largest producer as well as consumer of urdbean with major growing states, are Maharashtra, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Rajasthan, Karnataka and Himachal Pradesh. In Himachal Pradesh, its cultivation is mainly confined to low and mid hills, and is popularly grown as an intercrop with maize as well as a monocrop. However, its yield is low compared to other grain legumes. The low productivity in this crop is attributable to its narrow genetic base due to common ancestry of various superior genotypes, poor plant type, vulnerability to abiotic and biotic stresses and their cultivation in marginal and harsh environments (Ali et al. 2006; Sert and Ceyhan, 2012). It is susceptible to various leaf spotting pathogens such as *Cercospora canescens, C. cruenta, Colletotrichum truncatum* and *Erysiphe polygoni* in high rainfall areas in the mid

hills of North-Western Himalayas resulting a 40-60% reduction in grain yield.

Extensive screening of the germplasm collections of this species has not yielded any source of resistance to these pathogens. Induced mutagenesis for the induction of resistance using *in vivo* and *in vitro* techniques has also not been successful. Thus under present circumstances, there is no other alternative, but to look for alien *Vigna* species that can provide effective sources of resistance for introgression and other desirable traits to *V. mungo*. Inter-specific hybridization forms the major part of crop improvement. But in many cases, it may be desirable (for useful traits), or even necessary (in cases where there is minimal variability), to cross individuals belonging to two different species (inter-specific hybridization) or genera (inter-generic hybridization). The related species i.e. *V. angularis* (adzukibean) and *V. umbellata* (ricebean) have been found to be nutritive having high content of resistant starch, vitamins, amino acids, fibers, desirable fatty acids i.e. linoleic and linolenic acid and offers more protein and resistant to most of the fungal pathogen of urdbean. Therefore, the present study has been undertaken to study the crossability of *V. mungo* with various *Vigna* species.

Materials and Methods

For the present investigation, a total of 11 different varieties / genotypes i.e. 5 each of urdbean (Him Mash-1, HPBU-111, Palampur-93, UG-218 and PDU-1) were taken as female and 3 each of ricebean (PRR-1, PRR-2 and VRB-3) and adzukibean (HPU-51, IC-341983 and IC-341948) taken as male were used to study the crossability relationship (Table 1). The Experimental Farm is situated at 32°8′ N latitude and 76°3′ E longitude and at 1290 m above mean sea level, representing mid-hill zone of Himachal Pradesh (Zone II) characterized by humid sub-temperate climate with high rainfall (2500 mm) having acidic soil with pH ranging between 5.0 to 5.6. During summer and *Kharif* 2017 and 2018, staggered sowing was done at interval of 10 days starting from 15th February to 31st July to have synchronized flowering in the glasshouse of Department of Genetics and Plant Breeding. The crossing was performed from 15th May to 15th October. Emasculation of female parent(s) at plump bud stage was done in the evening (3:00 to 6:00 P.M.) followed by pollination in the next day morning (7:00 to 9:00 A.M.). Three immuno-suppressants i.e. gibberellic acid (GA_3), indole acetic acid (IAA) and amino caproic acid were used at two concentrations (500 ppm and 1000 ppm) about half an hour after pollination to prevent premature flower abscission (Fig.1). This was repeated for three consecutive days after pollination at an interval of 24 hours. A total of 30 interspecific crosses i.e. 15 each of *Vigna mungo* x *V. umbellata* (urdbean x ricebean) and *V. mungo* x *V. angularis* (urdbean x adzukibean) were attempted. Observations were recorded on number of buds pollinated and number of pods harvested to calculate the crossability percentage.

Crossability percentage was calculated as follows:

$$\text{Crossability percentage (\%)} = \frac{\text{Number of crossed pod set}}{\text{Total number of urdbean buds pollinated}} \times 100$$

Stastical Analysis

Since the data were in percent and lying beyond the range of 0 to 30% or 30 to 70% or 70 to 100%, hence it was subjected to arc sine transformation (Gomez and Gomez 1984). The analysis of variance was based on transformed data and original mean values were used to compare the results. In Microsoft Excel, arc sine transformation of percentage data was done by using the following formula:

=DEGREES[ASIN{SQRT(cell/100)}]

Simple t-test

To test whether the mean difference of crossability, simple t-test was performed as:

$$\text{Student'st-test} = \frac{\bar{X}_d}{SE(X_d)} \text{ (at n-1 df)}$$

where

$\bar{X}_d$ = mean-difference between two sets of related samples

$SE(X_d)$ = Standard error of mean difference

n = Number of related samples

Results and Discussion

An interspecific hybridization is a promising tool to transfer the desirable traits and to widen the gene pool of any crop. However, wide crosses are not always successful because of the existence of pre and post fertilization barriers that are operative at various stages of development and also various incompatibility barriers limit the potential for recombining the important characters for improving production and adaptation. The present investigation was carried out with the objective to study the crossability relationship of urdbean with ricebean and adzukibean.

A better understanding of the crossability relationship among the species had been helpful in opting methods for making successful crosses (Bhanu et al. 2018). Day-to-day visual observation showed that most of the emasculated buds dropped the very next day after pollination and some aborted after about a week. Pod formation involving *V. angularis* as one of the parents in inter-specific hybridization was not very successful. There was no pod formation and buds dropped after 1-3 days of pollination. The absence of seed set and abscission of crossed flowers within 72 hours from pollination demonstrate that the first barrier responsible for complete sterility is the delay in pollen tube entry into the ovules because of the difference in length of the style of species involved.

Varying degrees of success in interspecific hybridization was also reported by various workers *viz.* Ahn and Hartman (1977), Chen et al. (1983), Mittal et al. (2005, 2008, 2010) and Bhanu et al. (2018) owing to reproductive obstructions between the species involved in interspecific hybridization. The range of crossability percentage was observed to be 0-14.19% (Table 2). The analysis of results revealed that cross combinations Palampur-93 x PRR-2, Him Mash-1 x VRB-3, PDU-1 x PRR-2, Palampur-93 x PRR-1, Him Mash-1 x PRR-1 and Him Mash-1 x PRR-2 were found to be significantly superior over other cross combinations in *V. mungo* x *V. umbellata* hybridization in terms of crossability. Among the crosses of *V. mungo* x *V. umbellata*, the crossability of Palampur-93 x PRR-2 was highest (14.19%) followed by Him Mash-1 x VRB-3 (13.66%) and PDU-1 X PRR-2 (13.06%) (Fig.2). Crosses having a high crossability percentage were considered as successful crosses suggesting the parents of these cross combinations are ideal for the transfer of useful genes from one species to another species. Similar crossability success was also reported by Bhanu et al. (2018) 16.27% in *V. mungo* x *V. umbellata* and 37.50% in *V. mungo* x *V. radiata*; Lekhi et al. (2017) in *V. mungo* x *V. radiata* with 24.10%; Bharathi et al. (2006) in *V. radiata* x *V. umbellata* with 29.63 percent, *V. radiata* x *V. trilobata* with 8.48%, *V. radiata* x *V. aconitifolia* with 7.69%. The percent crossability among different cross combinations varied from species to species may be due to wide variation in the genetic architecture of the species involved in interspecific hybridization. Mittal et al. (2005) and Dhiman et al. (2013) also observed differential responses of the genotypes of urdbean and ricebean involved in interspecific hybridization. In the present study, cross combinations HPBU-111 x PRR-1 (5.37%), HPBU-111 x PRR-2 (4.96%), UG-218 x PRR-1 (2.65%), UG-218 x PRR-2 (4.70%) exhibited low crossability rates and there was no pod set in cross combination HPBU-111 x VRB-3, UG-218 x VRB-3 in urdbean x ricebean hybridization. Present results are in agree with the findings of Thiyagu et al. (2008) who observed a low percentage of pod set (5.56%) in *V. mungo* x *V. umbellata* indicating the presence of reproductive barriers that renders the introgression difficult. They also found normal pollen grain germination on the stigmatic surface but slow pollen tube growth in addition to structural abnormalities in stigmatic and stylar regions. In some crosses, there is abscission of young fruits between 3 to 30 days after pollination, which might be due to failure of endosperm nuclei to divide or the delayed endosperm nuclear divisions leading to embryo abortion (Bhanu et al. 2018). So, crossability between the species is a prerequisite for gene transfer through interspecific hybridization. Some of the pods which were formed were without seed or had shrivelled seeds with a ruptured seed coat. Crosses, where HPBU-111 is used as one of the parents, had more number of empty pods. F_1 seeds developed were of two types (i) highly shriveled, minute, brown colored (ii) bold, comparatively brown colored but very weak as compared to selfed ones (Fig.3). The number of seeds per pod in the inter-specific hybrids varied from 1-4. Sehrawat et al. (2016) also reported that the number of F_1 seeds per pod in interspecific crosses between urdbean and ricebean varied from 1 to 4. The F_1 seeds obtained from all cross combinations were small, wrinkled and shrunken. The F_1 seeds were small in size and shriveled because of the poor development of the endosperm and embryo which might be due to incompatibility between the two parental genomes or due to the failure of the embryo to reach maturity (Rashid et al. 1987).

Conclusions

The present study reveals the operation of pre fertilization barriers such as slow pollen tube development, no germination of pollen grains, delay in pollen tube entry into ovules and high abscission rate of crossed flowers within four days after pollination. Even though the fertilization barriers were predominant, some inter-specific hybrids were produced. The parents involved in interspecific hybridization showed differential genotypic response, which indicates the use of more genotypes and a large number of crosses should be attempted to get more F_1 plants. The crosses showing a substantially high percent of crossability an be utilized for genetic improvement of urdbean.

Table 1. Parentage/source of genotypes used in interspecific hybridization.

Species	Variety(s)	Source/Parentage
Vigna mungo	Palampur-93	Pureline selection from the local material of Himachal Pradesh collected by CSK HPKV, Palampur
	Him Mash-1	DPU 91-5 x Mash 338
	HPBU-111	Pureline selection from the local material of Himachal Pradesh collected by CSK HPKV, Palampur
	UG-218	IIPR Kanpur
	PDU-I	Selection from IC-8219
V. umbellata	PRR-1	Pureline selection from Jagdhar (Tehri) collection by GB Pant University by Pantnagar
	PRR-2	UUHF, Ranichauri
	VRB-3	Selection from heterogenous sample of accession IC538080
V. angularis	HPU-51	Pureline selection from the local material of Himachal Pradesh collected by CSK HPKV, Palampur
	IC-341983	Indigenous collection
	IC-341984	Indigenous collection

Table 2. Pod set and cross ability percentage in *V. mungo* and *V. umbellata* crosses.

No.	Cross Combination	Buds Emasculated and Pollinated	Crossed Pods Formed	F_1 Seeds Obtained	Cross Ability Percentage
1	Palampur-93 x PRR-1	369	44	173	11.92**
2	Palampur-93 x PRR-2	444	63	248	14.19**
3	Palampur-93 x VRB-3	343	27	103	7.87
4	Him Mash-1 x PRR-1	400	49	193	12.25**
5	Him Mash-1 x PRR-2	391	47	183	12.02**
6	Him Mash-1 x VRB-3	432	59	235	13.66**
7	HPBU-111 x PRR-1	409	22	83	5.37
8	HPBU-111 x PRR-2	403	20	78	4.96
9	HPBU-111 x VRB-3	366	0	0	0.00
10	UG-218 x PRR-1	377	10	40	2.65
11	UG-218 x PRR-2	382	17	64	4.70
12	UG-218 x VRB-3	351	0	0	0.00
13	PDU-1 x PRR-1	356	0	0	0.00
14	PDU-1 x PRR-2	421	55	216	13.06**
15	PDU-1 x VRB-3	339	26	98	7.67
	Mean ± SE				13.77 ± 2.09

** Significant at 1% level of significance

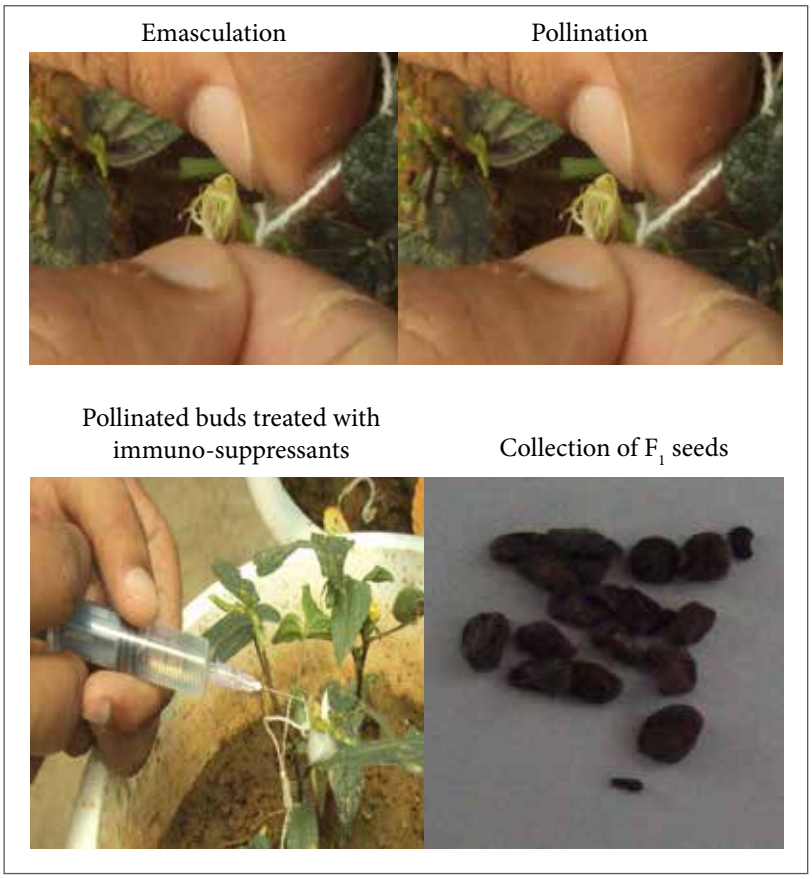

Figure 1. Interspecific hybridization. (Original)

Figure 2. Interspecific pods. (Original)

Figure 3. Interspecific seeds. (Original)

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