RESEARCH ARTICLE Legume Research- An International Journal, Volume 45 Issue 12: 1484-1489 (December 2022)

Crossability Barriers in Interspecific Hybridization of Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] with Other *Vigna* Species

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10.18805/LR-4277

ABSTRACT

Background: Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] is a multipurpose grain legume of Mid-Himalayan region mainly cultivated for food, fodder, green manure and has emerged as a good alternative to other pulse crops such as blackgram and greengram which do not flourish in this region due to their susceptibility to cold temperature stress. It is well reported that the nutritional value of ricebean is higher as compared to many other legumes of the *Vigna* family and has some superior qualities greater than greengram, blackgram and cowpea. It is also resistance to drought, diseases and pests specially the storage pests during growth period and possesses high percentage of seed viability. Despite having all the favourable traits, it is not much popular among the farmers due to the late maturity and indeterminate growth habit. Instead, farmers prefer other crops which fit easily into their cropping pattern and are easy to harvest. A little genetic improvement with respect to maturity and growth habit can revive its cultivation and show great results in its production as a valuable crop. Thus, the present investigation was formulated to introgress desired traits from mash and adzukibean into otherwise high yielding ricebean genotypes using inter-specific hybridization.

Methods: The present investigation involves the inter-specific hybridization among three *Vigna* species *viz*, ricebean (*Vigna umbellata),* blackgram (*Vigna mungo)* and adzukibean (*Vigna angularis)*. In the year 2017, six genotypes of ricebean (RBHP-36, RBHP-38, RBHP-43, RBHP-61, RBHP-107 and RBHP-108) were crossed with two genotypes of blackgram (HimMash-1 and Palampur-93) and one genotype of adzukibean (HPU-51) in glasshouse conditions.

Result: The study revealed that successful crosses were possible only between ricebean and blackgram. All the Inter-specific crosses showed very low pod set percentage ranging from 0-4% and F_1 germination percentage ranging from 20-42%. Pod set percentage and pods harvested varied with combinations of two parental cultivars of each species for most of the inter-specific hybrids. The successful pod set was observed in 16 out of 36 inter-specific crosses. Highest crossability was observed in blackgram and ricebean crosses. Crossing of adzukibean with ricebean showed poor or no pod set among the entire cross combinations which are attributed to early embryo abortion and degeneration during embryogenesis.

Key words: Inter-specific hybridization, Synchronous maturity.

INTRODUCTION

Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] is a multipurpose grain legume of Mid-Himalayan region mainly cultivated for food, fodder, green manure and has emerged as a good alternative to other pulse crops such as blackgram and greengram which do not flourish in this region due to their susceptibility to cold temperature stress. It is a warm season annual legume mainly grown in East Asia (Chen *et al*., 2016). It is an annual pulse of high nutritional status which has remained in general, neglected for its improvement by breeding either by hybridization or other methods. Legumes are significant source of carbohydrates and largest producer and consumer of legumes in the world are in India. It well reported that the nutritional value of rice bean is higher as compared to many other legumes of the *Vigna* family and has some superior qualities greater than greengram, blackgram and cowpea. It is also resistance to drought, pests and diseases during growth period, synchronising habit of pod maturity, resistance to attack of storage pests and a high percentage of seed viability.

Despite having all the favourable traits; it is not much popular among the farmers due to the late maturity and indeterminate growth habit. Since variability for these traits is very low in this crop, inter-specific hybridization of ricebean with other *Vigna* species for the incorporation of earliness,

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How to cite this article: Bhardwaj, N., Kapoor, T. and Sharma, P. (2022). Crossability Barriers in Interspecific Hybridization of Ricebean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] with Other *Vigna* Species. Legume Research. 45(12): 1484-1489. DOI: 10.18805/LR-4277.

Submitted: 05-11-2019 **Accepted:** 01-09-2020 **Online:** 12-03-2021

synchronous maturity and non-shattering can lead to its improvement. Thus, in the present study inter-specific hybridization was performed between ricebean, adzukibean, mash and mung to introgress desired traits into otherwise high yielding ricebean genotypes.

MATERIALS AND METHODS

Sowing plan

Eight ricebean (*Vigna umbellata*), two urdbean (*Vigna mungo*) and one adzukibean (*Vigna angularis*) genotype(s) were sown during the year 2015 in the glasshouse, Department of Organic Agriculture, CSK HPKV, Palampur, Himachal Pradesh (Table 1). Staggered sowing was done at seven days interval starting from first week of November to first week of December, 2015. The accessions of ricebean were sown twenty days prior to each sowing date of urdbean and adzukibean so as to synchronize flowering.

Crossing plan

Inter-specific crosses among ricebean (*V. umbellata*), urdbean (*V. mungo*) and adzukibean (*V. angularis*) were attempted under glasshouse conditions by performing emasculation from 3-5 PM and pollination at 8-9 AM upon flowering.

Data recording pertaining to development of F_1 hybrids

Data were recorded with respect to:
Number of flower buds emasculated and pollinated.
Number of mature pods obtained.
Number of mature seeds obtained.

Sowing of F_1 hybrids

F_1 hybrids were evaluated during *kharif,* 2016 in the Experimental Farm, Department of Organic Agriculture, Palampur. The inter-specific hybrids along with their parents were raised in pots containing mixture of soil, sand and vermi-compost in 2:1:1, in a Completely Randomized Design (CRD) with unequal replications as number of F_1 seeds varied for different crosses (Image 1).

Recording of observation

Data was recorded for various morphological traits on five randomly taken plants for each F_1 progeny and parent.

Molecular characterization of F_1 hybrids

The parents as well as their F_1's were subjected to confirm hybridity through SSR markers using standard protocol. Forty four randomly chosen primers were screened, out of which six were found polymorphic (details of SSR primers is given in Table 2). The experiment was carried out in the molecular Lab of the Department of Genetics and Plant Breeding, CSK HPKV, Palampur, Himachal Pradesh.

RESULTS AND DISCUSSION

Generation of inter and intra-specific hybrids and crossability studies

Since the main objective of the present study was to incorporate earliness and determinate habit into *Vigna umbellatta*; the donor parents used were genotypes from *Vigna mungo* and *Vigna angularis*. Thus, crosses of six genotypes of *Vigna umbellate* (RBHP-36, RBHP-38, RBHP-43, RBHP-61, RBHP-107 and RBHP-108) with *Vigna mungo* (Him Mash-1 and Palampur-93) and *Vigna angularis* (HPU-51) were attempted with the objective to transfer genes for earliness and determinate trait, under glasshouse conditions in the year 2015.

The crossability percentage (Table 3a and 3b) ranged from 0-4%. In the present study the crossability was possible only among the genotypes of *Vigna umbellata* and *Vigna mungo*. Differences in percentage of pods harvested were highly significant among the crosses indicating presence of reproductive barriers that renders introgression difficult (Thiyagu *et al*., 2008). The cross combinations RBHP-36 × Him Mash-1, RBHP-107 × Him Mash-1 and RBHP-108 × Him Mash-1 resulted in highest pod set percentage. It was observed that pod set percentage was maximum when ricebean was used as female parent as compared to the crosses where urdbean was used as female parent. In most of the cross combination the pod set percentage was 0% when urdbean was taken as female. In remaining crosses the pods developed normally but the seeds obtained were wrinkled.

Table 1: Parentage/source of genotypes used for inter-specific hybridization.

Species	Genotype(s)	Source/parentage
Vigna umbellata	RBHP-36	CSKHPKV, Palampur
	RBHP-38	CSKHPKV, Palampur
	RBHP-43	CSKHPKV, Palampur
	RBHP-61	CSKHPKV, Palampur
	RBHP-107	CSKHPKV, Palampur
	RBHP-108	CSKHPKV, Palampur
Vigna mungo	Him Mash-1	CSKHPKV, Palampur
	Palampur-93	CSKHPKV, Palampur
Vigna angularis	HPU-51	CSKHPKV, Palampur

Image 1: Interspecific Hybrids of ricebean and urdbean.

The F_1's showed very low percentage of seed germination (Table 4) ranging from 20 to 42.85 per cent. The germination percentage was recorded highest one in the cross RBHP-61 × Palampur-93 (42.85%) while lowest one in the cross Palampur-93 × RBHP-38 (20%).

Reciprocal crosses among *Vigna umbellata* and *Vigna mungo* showed lowest pod set percentage ranging from 0 to 3 per cent. The reciprocal difference in crossability between ricebean and urdbean suggests interaction between genic and cytoplasmic factors (Stebbins, 1958). This interaction may be the cause of hybrid embryo degeneration when *Vigna mungo* is used as the female parent (Ahn and Hartmann, 1977). There are no external barriers, which prevent cross-pollination between

Table 2: List of ricebean SSR primer sequences used in the present study.

Primers	Forward sequence	Reverse sequence	Annealing temperature
CEDG127	GGTTAGCATCTGAGCTTCTTCGTC	CTCCTCACTTGGTCTGAAACTC	64°
CEDG018	AGCGTGTTTGTGGTGATAGC	ACACAGGAACGAACAAACCC	55°
CEDG150	GAAGGGAATGAAAATGAAACCC	GTTCAATCCATTCAGTCTCC	55°
CEDG214	CACTCACTGCAAAGAGCAAC	CTACCTATCTGAGGGACAC	54°
CEDAAG002	GCAGCAACGCACAGTTTCATGG	GCAAAACTTTTCACCGGTACGACC	65°
CEDG204	CCTTGGTTGGAGCAGCAGC	CACAGACACCCTCGCGATG	57°
CEDG043	AGGATTGTGGTTGGTGCATG	ACTATTTCCAACCTGCTGGG	55°
CEDG021	GCAGAATTTTAGCCACCGAG	AAAGGATGCGAGAGTGTAGC	55°
CEDG084	ATCAACTGAGGAGCATCATCGA	CAACATTTCAACCTTGGGACAG	57°
CEDG015	CCCGATGAACGCTAATGCTG	CGCCAAAGGAAACGCAGAAC	57°
CEDG026	TCAGCAATCACTCATGTGGG	TGGGACAAACCTCATGGTTG	55°
CEDG073	CCCCGAAATTCCCCTACAC	AACACCCGCCTCTTTCTCC	55°
CEDG008	AGGCGAGGTTTCGTTTCAAG	GCCCATATTTTTACGCCCAC	54°
CEDG286	CGAGCAGAACACTGATCATG	CCTCTTAGAGGTCATTGCTC	55°
CEDG294	CACCTTCTTAATCTCTTCACC	GGGTTTCTCTTAATTCATTGAGTC	58°
CEDG232	GATGACCAAGGTAACGTG	GGACAGATCCAAAACGTG	49°
CEDG071	GGTCCATTGAGACGGATCGAG	TCCCACCTCAGCGGAATCC	59°
CEDG253	CACTTCCATGATGACTCACC	CACCCTTCTTTATCCTCTTCG	56°
CEDG090	ATAAGTAGAAATTGGTTCAAATG	GGTTCGTTAAAGTAACTTTTAAT	53°
CEDG044	TCAGCAACCTTGCATTGCAG	TTTCCCGTCACTCTTCTAGG	53°
CEDG141	CCAGGCATCCATGATGACC	GAAGTTGTTGGTAATGGTTGCCTC	60°
CEDG178	CGGAAGAAGAACGCAGAGTG	GCATCAACAAGGACTTCTGC	58°
CEDG118	AACCCAACCAACCCTTGTGGTAAG	GCTGGAATCATAATACCGCCTTGT	66°
CEDG154	GTCCTTGTTTTCCTCTCCATGG	CATCAGCTGTTCAACACCCTGTG	63°
CEDG037	GAAGAAGAACCCTACCACAG	CACCAAAAACGTTCCCTCAG	55°
CEDG195	GAGGGTCTCCACTTTTGAAACCC	GATACTAAGGCTTTCTCCACCCAC	66°
CEDG134	CTCCGTGTTGAAACAATGACG	GGTCTTTCTGATCTACGAACTTG	59°
CEDG104	TATGGCCCGAGCAAACCTTG	CCGTTCGGTCTTCGGTTGAA	57°
CEDG050	GGCAGAATCGTACAAGTG	GTCAGATTCTCGCTTGCATG	52°
CEDG087	CCTCCTTGAAATTCTCCTTGA	CCTCTTGTGAACCTTGGGACAG	59°
CEDG305	GCAGCTTCACATGCATAGTAC	GAACTTAACTTGGGTTGTCTGC	62°
CEDG018	AGCGTGTTTGTGGTGATAGC	ACACAGGAACGAACAAACCC	55°
CEDG204	CCTTGGTTGGAGCAGCAGC	CACAGACACCCTCGCGATG	57°
CEDC016	ACTCTTGTCAATTGTCCAGG	TAACTTGTCACTGGAAAGGC	53°
CEDG003	CCACTTTCTCTTGACTTTGC	GACCAAAGTGAAGCCAAGAG	59°
CEDG011	CCCAACCAAAGCGTTTTG	CTTCTAGACTCTGAGCACTG	57°
CEDG024	CATCTTCCTCACCTGCATTC	TTTGGTGAAGATGACAGCCC	55°
CEDG029	GATTGCTTTTAGCAGAGGGC	GAAGAAACCCATCTCGATCC	55°
CEDG041	GCTGCATCTCTATTCTCTGG	GCCAACTAGCCTAATCAG	57°
X1	GTGCAGCCACTACATGAATG	GAAGTTGACACTCATCCACC	55°
X2	AGGCGAGGTTTCGTTTCAAG	GCCCATATTTTTACGCCCAG	55°
X3	CCCGATGAACGCTAATGCTG	CGCCAAAGGAAACGCAGAAC	60°
X4	CATCTTCCTCACCTGCATTC	TTTGGTGAAGATGACAGCCC	58°
X6	GATTGCTTTTAGCAGAGGGC	GAAGAAACCCATCTCGATCC	60°

Vigna umbellata and *Vigna mungo* because the timing of anthesis, dehiscence of anthers and receptivity of the stigma are identical for both the parental species. Absence of seed set and abscission of crossed flowers within four days from pollination in crosses *Vigna mungo* × *Vigna umbellata* indicates that complete sterility is the result of delay in pollen tube entry into the ovule. This may be due to difference in length of style of *Vigna umbellata* and *Vigna mungo* which leads to inability of pollen tube to germinate and penetrate stigma and style (Chowdhury and Chowdhury, 1977) and ovary (Gopinathan *et al*., 1986) and slow rate of pollen growth (Thiyagu *et al*., 2008). These factors are reported to be significant pre-fertilization barriers.

Table 3(a): Number of buds pollinated and pod set (%) in *V. umbellata* × *V. mungo* hybrids.

Hybrid combinations	Number of flower emasculated and pollinated	Number of mature putative hybrid pods	Per cent pod set
***V. umbellata* × *V. mungo* (Him Mash-1)**			
RBHP-36 × Him Mash-1	100	4	4.00
Him Mash-1 × RBHP-36	100	0	0.00
RBHP-38 × Him Mash-1	100	3	3.00
Him Mash-1 × RBHP-38	100	3	3.00
RBHP-43 × Him Mash-1	100	3	3.00
Him Mash-1 × RBHP-43	100	0	0.00
RBHP-61 × Him Mash-1	100	0	0.00
Him Mash-1 × RBHP-61	100	0	0.00
RBHP-107 × Him Mash-1	100	4	4.00
Him Mash-1 × RBHP-107	100	3	3.00
RBHP-108 × Him Mash-1	100	4	4.00
Him Mash-1 × RBHP-108	100	0	0.00
***V. umbellata* × *V. mungo* (Palampur-93)**			
RBHP-36 × Palampur-93	100	3	3.00
Palampur-93 × RBHP-36	100	3	3.00
RBHP-38 × Palampur-93	100	3	3.00
Palampur-93 × RBHP-38	100	2	2.00
RBHP-43 × Palampur-93	100	2	2.00
Palampur-93 × RBHP-43	100	2	2.00
RBHP-61 × Palampur-93	100	3	3.00
Palampur-93 × RBHP-61	100	3	3.00
RBHP-107 × Palampur-93	100	0	0.00
Palampur-93 × RBHP-107	100	0	0.00
RBHP-108 × Palampur-93	100	0	0.00
Palampur-93 × RBHP-108	100	0	0.00

Table 3(b): Number of buds pollinated and pod set (%) in *V. umbellata* × *V. angularis* hybrids.

Hybrid combinations	Number of flower bud emasculated and pollinated	Number of mature pod harvested	Numberof mature putative hybrid seed
***V. umbellata* × *V. angularis* (HPU-51)**			
RBHP-36 × HPU-51	100	0	0.00
HPU-51 × RBHP-36	100	0	0.00
RBHP-38 × HPU-51	100	0	0.00
HPU-51 × RBHP-38	100	0	0.00
RBHP-43 × HPU-51	100	0	0.00
HPU-51 × RBHP-43	100	0	0.00
RBHP-61 × HPU-51	100	0	0.00
HPU-51 × RBHP-61	100	0	0.00
RBHP-107 × HPU-51	100	0	0.00
HPU-51 × RBHP-107	100	0	0.00
RBHP-108 × HPU-51	100	0	0.00
HPU-51 × RBHP-108	100	0	0.00

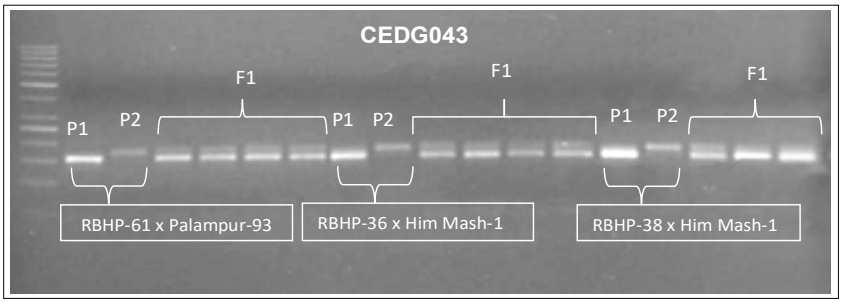

Plate 1: Hybridity of inter-specific crosses at molecular level.

Table 4: Germination percentage of inter-specific hybrids.

Hybrid combinations	Total number of F_1 seed sown	Number of seeds germinated	Germination of hybrid (%) seeds
RBHP-36 × Palampur-93	5	2	40.00
Palampur-93 × RBHP-36	9	3	33.33
RBHP-38 × Palampur-93	6	2	33.33
Palampur-93 × RBHP-38	5	1	20.00
RBHP-43 × Palampur-93	4	1	25.00
Palampur-93 × RBHP-43	7	2	28.57
RBHP-61 × Palampur-93	7	3	42.85
Palampur-93 × RBHP-61	5	2	40.00
Him Mash-1 × RBHP-36	8	3	37.50
RBHP-38 × Him Mash-1	5	2	40.00
Him Mash-1 × RBHP-38	4	1	25.00
RBHP-43 × Him Mash-1	6	2	33.33
RBHP-107 × Him Mash-1	7	2	28.57
Him Mash-1 × RBHP-107	4	1	25.00
RBHP-108 × Him Mash-1	6	2	33.33

The failure of endosperm nuclei to divide or the delayed endosperm nuclear divisions are responsible for abortion of embryo and the subsequent abscission of young fruits in the interspecific crosses. The production of shrivelled seeds from these crosses is probably associated with the failure of embryo to reach maturity. Such sterility barriers have also been recorded in the interspecific crosses between *Vigna radiata* × *Vigna umbellata* by Bharathi *et al*., 2006.

Inter-specific hybridization possesses the presence of pre-fertilization barriers confirmed by the frequency of pod set and post-fertilization barriers as F_1's exhibited reduced germination and sterility. The frequency of inter-specific hybridization and radical and plant production percentage revealed the genotype specific response of both the species (Shayla, 2016). Thus, recovery of desirable recombinants is reduced, as hybrids exhibit varying levels of sterility (Rashid *et al*., 2013), inviability, lethality and genotype specific response (Dhiman *et al*., 2013). These different kinds of pre and post fertilization barriers are also responsible for complete sterility to low fertility in the back crosses involving the F_1 hybrid and both its parents.

Confirmation of inter-specific hybrids through SSR markers

Out of 70 SSR markers used for parental polymorphism survey, 8 were found polymorphic between different parents for inter-specific hybrids. These polymorphic markers were used for F_1 hybrid confirmation. F_1 hybrids were confirmed using two primer pairs namely CEDG043 and CEDG037 polymorphic between parents and hybrids. Both these primers showed robust and reproducible bands as shown in Plate 1. All the inter-specific hybrids were true hybrids as confirmed by molecular marker analysis. Similar result was observed by Chaisan *et al*., (2013) who confirmed hybridity of inter-specific hybrids between mungbean and ricebean. They screened forty random amplified polymorphic DNA (RAPD) primers for polymorphism among the parents and finally choose two specific primers for testing of hybridity. All putative F_1 hybrids were confirmed as the inter-specific hybrids. To observe their fertility, some of the hybrid seedlings were transplanted. The hybrid produced flowers profusely but failed to set pods. To overcome the sterility, plants were induced to become tetraploid by colchicine treatment *in vitro*. Nandini *et al*. (2020) also used SSR markers to detect water use efficiency in parental lines. Similarly Prine Lekhie *et al*. (2018) also attempted interspecific hybridization in *Vigna radiata* and *V. mungo* genotypes. The purity of hybrids were tested through microsatellite markers. All the F1 plants gave resistant reaction to mungbean yellow mosaic virus (MYMV) indicating the introgression of resistance gene(s) from *V. mungo* to *V. radiata*.

Sterlity in inter-specific crosses is the result of various pre and post fertilization barriers (Nishant Bhanu *et al*., 2018). Though very little success has been achieved till date in this field but it is necessary to create a wide genetic variation for breeding programs through interspecific hybridization specially in the crops like ricebean where genetic variability is low but the production potential is very high. Alternative methods like embryo rescue, ovary and ovule culture and chromosome doubling holds considerable promise for the development of new cultivars incorporating genes from wide species.

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