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Crossability Studies of Interspecific Hybridization among Vigna Species

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Abstract

A total of 80 interspecific crosses i.e., 36 each of *V. radiata* × *V. umbellata* (*V. radiata* as female) and *V. mungo* × *V. umbellata* (*V. mungo* as female) and 08 crosses of *V. radiata* × *V. mungo* (*V. radiata* as female) were attempted to study the crossability relationship among these three Vigna species. Among the crosses of *V. radiata* × *V. umbellata* the crossability was observed highest in HUM 12 × RBL 9 (16.27%) followed by HUM 12 × RBL 9 (15.78%). In case of *V. mungo* × *V. umbellata,* the maximum crossability of 11.36% was noticed in cross, Mash 338 × RBL 9. For *V. radiata* × *V. mungo,* the highest crossability was visualized in hybrid, ML 1464 × Mash 338 (37.5%). The study indicated that different kinds of pre and post fertilization barriers are responsible for complete sterility to low fertility. RBL 1 and RBL 9 gnotypes of ricebean showing substantially high percent of crossability and better seed set with different cultivars of mungbean and blackgram may be utilized for genetic improvement of the mungbean and blackgram.

Keywords: Interspecific hybridization; Crossability; *V. radiata; V. mungo; V. umbellata*

Introduction

Legumes are next to cereals in terms of their economic and nutritional importance as human food. They also play an important role in maintaining soil fertility and sustainability of production systems. Among several pulses grown mungbean and blackgram are one of the important grain legumes grown throughout the year. Being a short duration crop they can be a better option for enhancing the pulse production of pulses. However, the total production and productivity of mungbean is affected by a number of biotic and abiotic factors. Among biotic factors, Mungbean Yellow Mosaic Virus (MYMV) transmitted through whitefly, i.e., *Bemesia tabaci* is a major constraint to the cultivation of grain legumes in India, particularly mungbean and blackgram. The weather parameters play a vital role in survival and multiplication of white fly and influence MYMV outbreak during monsoon season. Management of this disease is only possible by the way of reducing the whitefly population using insecticides which are ineffective under severe infestations making complete destruction due to virus. Therefore, development and use of virus resistant cultivars turns out to be the most effective and economical strategy against MYMV [1]. Basic reason for limited success had been due to the limited variability prevailed among the mungbean and blackgram genotypes used for hybridization in most of the studies.

Interspecific hybridization plays a significant role in alien gene introgression and is the probable option for transferring the desirable genes of qualitative and quantitative characters in mungbean and blackgram. Ricebean [*V. umbellata* (Thunb.) Ohwi and Ohashi], a long duration (90-120 days) minor legume which is genetically close to mungbean and blackgram (all three species 2n = 2x = 22) possess resistance for MYMV, CLS, bruchids, and powdery mildew. Successful hybridization primarily depends on the intercrossing potential/ crossability of the parents involved as well as development of the hybrid embryos including fertility of the F_1 hybrids and their derivatives. In interspecific crosses of food legumes failure of interspecific hybridization due to embryo degeneration is common [2,3]. Interspecific hybridization among mungbean, blackgram and ricebean with varying degree of success has been reported. Keeping this in view, the present piece of investigation was initiated to study the crossability relationship among three Vigna species viz. *V. radiata* (mungbean), *V. mungo* (blackgram) and *V. umbellata* (ricebean).

Materials and Methods

For the present experiment, a total of six diverse genotypes/ varieties, each of mungbean viz. Pusa 0672, ML 1464, SML 1455,

HUM 12, KM 2241, TM 96-2, ricebean namely RBL 1, RBL 6, RBL 9, RBL 33, RBL 140, RBL 141 and blackgram viz. Mash 338, Mash 114, Co 5, Palampur 93, Shekhar 2 and T 9 were selected. The experimental material was planted in crossing block in cemented pots at two different dates of sowing of 10 days intervals (August 10 and August 20, 2014) at Agricultural Research Farm, Banaras Hindu University Varanasi during Kharif season, 2014 [4-6]. Buds of optimum size of the female parent were emasculated the day before anthesis (1600 to 1800 HRS) and pollinated in the next morning (0600 to 0800 HRS). 8 to 12 flowers per plant per day were emasculated besides picking the self-pollinated flowers/ pods to avoid any severe load. Hybridization technique using hand emasculation and pollination was followed [7]. A total of 80 interspecific crosses i.e. 36 each of *V. radiata* × *V. umbellata* (*V. radiata* as female) and *V. mungo* × *V. umbellata* (*V. mungo* as female) and 08 crosses of *V. radiata* × *V. mungo* (*V. radiata* as female) were accomplished. Observations were recorded on the number of buds emasculated, pollinated, pod initiated and matured pods harvested. Percent pod setting was obtained from [Number of pods set/ Number of buds pollinated] × 100. Percent ovule fertility was calculated [Total No. of developed seed/ Total No. of ovule scar] × 100. Meteorological observations were taken from the Meteorological Unit, Department of Agronomy, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Results and Discussion

Introgression of desirable gene into cultivated species could lead to development of high yielding varieties coupled with resistance for biotic and abiotic stresses. Close relatives of mungbean and blackgram have been used in the breeding programme. However, recovery of successful hybrid is difficult due to crossability barriers. In spite of these technical hitches, interspecific hybridization among Vigna speices has been successfully accomplished by many workers [8,9-12]. Further, in any interspecific hybridization, crossability is the pre-requisite for gene transfer. A better understanding of crossability relationship among the species had been helpful in opting methods for making successful crosses and also in drawing the phylogenetic relationship among species. *V. umbellata* possessing many desirable components coupled with resistance to MYMV, CLS and bruchids and powdery mildew can be useful in developing high yielding resistant varieties of mungbean and blackgram by transferring these genes into the cultivated species. The present investigation was carried out attempting interspecific hybridization with an objective to transfer useful traits from the *V. mungo and V. umbellata* into *V. radiata* and *V. umbellata* into *V. mungo*. The extent to crossability and ovule fertility was studied. The result of crosses pertaining to crossability and ovule fertility are furnished in (Table 1).

Table 1: Pod set, Crossability Percentage and Ovule Fertility Percentage among Vigna Species.

Sl. No.	Cross	No. of Buds Emasculated (1)	No. of buds pollinated (2)	No. of Buds Fertilized (3)	No. of Pods Harvested (4)	Crossability Percentage (%) [(2)/(4) × 100]	Ovule Fertility (%)	Remarks
Vigna Radiata × Vigna Umbellata								
1	TM 96-2 × RBL 1	30	22	5	1	4.54	33.33	Viable Dimpled Seeds
2	TM 96-2 × RBL 6	25	18	0	0	0.00	0	Absence of Seed set and Abscission of Crossed Flowers
3	TM 96-2 × RBL 9	32	22	5	1	4.54	40.00	Viable Dimpled Seeds
4	TM 96-2 × RBL 33	75	60	4	2	3.33	42.85	Viable Dimpled Seeds
5	TM 96-2 × RBL 140	32	26	3	1	3.84	25.00	Tiny, Dimpled Seeds
6	TM 96-2 × RBL 141	35	28	4	1	3.57	28.57	Tiny, Dimpled Seeds
7	Pusa 0672 × RBL 1	45	34	9	5	14.70	26.67	Viable Dimpled Seeds
8	Pusa 0672 × RBL 6	65	56	8	5	8.92	15.62	Viable Dimpled Seeds
9	Pusa 0672 × RBL 9	50	41	11	5	12.19	44.12	Tiny, Dimpled Seeds
10	Pusa 0672 × RBL 33	22	16	3	0	0.00	0	Abscission of Young Fruits
11	Pusa 0672 × RBL 140	24	16	0	0	0.00	0	Absence of Seed Set and Abscission of Crossed Flowers
12	Pusa 0672 × RBL 141	32	26	4	1	3.84	14.28	Viable Dimpled Seeds

13	KM 2241 × RBL 1	22	18	3	0	0.00	0	Abscission of Young Fruits
14	KM 2241 × RBL 6	24	18	0	0	0.00	0	Absence of Seed set and Abscission of Crossed Flowers
15	KM 2241 × RBL 9	30	24	4	1	4.10	44.44	Viable Dimpled Seeds
16	KM 2241 × RBL 33	45	42	8	3	7.14	30.77	Viable Dimpled Seeds
17	KM 2241 × RBL 140	22	17	3	0	0.00	0	Abscission of Young Fruits
18	KM 2241 × RBL 141	40	35	5	1	2.85	36.37	Viable Dimpled Seeds
19	ML 1464 × RBL 1	45	42	9	4	9.52	64.70	Viable Dimpled Seeds
20	ML 1464 × RBL 6	45	42	9	5	11.90	47.05	Tiny, Dimpled Seeds
21	ML 1464 × RBL 9	42	36	8	4	11.11	55.55	Viable Dimpled Seeds
22	ML 1464 × RBL 33	45	38	6	3	7.89	20.00	Tiny, Dimpled Seeds
23	ML 1464 × RBL 140	18	15	0	0	0.00	0	Absence of Seed Set and Abscission of Crossed Flowers
24	ML 1464 × RBL 141	20	16	0	0	0.00	0	Absence of Seed Set and Abscission of Crossed Flowers
25	HUM 12 × RBL 1	56	43	12	7	16.27	70.00	Viable Dimpled Seeds
26	HUM 12 × RBL 6	35	28	10	4	14.28	44.44	Viable Dimpled Seeds
27	HUM 12 × RBL 9	50	38	10	6	15.78	66.67	Viable Dimpled Seeds
28	HUM 12 × RBL 33	35	24	7	2	8.33	40.00	Tiny, Dimpled Seeds
29	HUM 12 × RBL 140	30	20	1	0	0.00	0	Abscission of Young Fruits
30	HUM 12 × RBL 141	25	18	0	0	0.00	0	Absence of Seed Set and Abscission of Crossed Flowers
31	SML 1455 × RBL 1	40	32	8	4	12.50	55.55	Viable Dimpled Seeds
32	SML 1455 × RBL 6	40	34	6	3	8.82	28.57	Tiny, Dimpled Seeds
33	SML 1455 × RBL 9	55	41	11	5	12.19	47.37	Viable Dimpled Seeds
34	SML 1455 × RBL 33	28	21	0	0	0.00	0	Absence of Seed Set and Abscission of Crossed Flowers
35	SML 1455 × RBL 140	24	16	2	0	0.00	0	Abscission of young Fruits
36	SML 1455 × RBL 141	28	21	2	0	0.00	0	Abscission of Young Fruits
Vigna Mungo × Vigna Umbellata								
37	Mash 338 × RBL 1	60	41	9	5	11.11	60.00	Viable Dimpled Seeds
38	Mash 338 × RBL 6	30	18	5	1	5.55	40.00	Tiny, Dimpled Seeds

39	Mash 338 × RBL 9	60	44	14	5	11.36	50.00	Viable Dimpled Seeds
40	Mash 338 × RBL 33	44	35	6	2	5.71	33.33	Tiny, Dimpled Seeds
41	Mash 338 × RBL 140	28	18	0	0	0.00	0	Absence of Seed Set and Abscission of Crossed Flowers
42	Mash 338 × RBL 141	42	34	2	2	5.88	40.00	Tiny, Dimpled Seeds
43	Mash 114 × RBL 1	45	38	9	4	10.52	20.00	Viable Dimpled Seeds
44	Mash 114 × RBL 6	30	21	4	0	0.00	0	Abscission of Young Fruits
45	Mash 114 × RBL 9	44	37	8	4	10.81	55.55	Viable Dimpled Seeds
46	Mash 114 × RBL 33	25	18	3	1	5.55	25.00	Tiny, Dimpled Seeds
47	Mash 114 × RBL 140	46	36	8	3	8.33	25.00	Tiny, Dimpled Seeds
48	Mash 114 × RBL141	42	32	6	2	6.25	42.86	Viable Dimpled Seeds
49	T 9 × RBL 1	38	28	6	2	7.14	66.67	Tiny, Dimpled Seeds
50	T 9 × RBL 6	52	42	9	3	7.14	20.00	Tiny, Dimpled Seeds
51	T 9 × RBL 9	34	22	4	1	4.54	25.00	Viable Dimpled Seeds
52	T 9 × RBL 33	58	48	7	4	8.33	20.00	Tiny, Dimpled Seeds
53	T 9 × RBL 140	32	18	3	0	0.00	0	Absence of Seed Set and Abscission of Crossed Flowers
54	T 9 × RBL 141	25	18	2	0	0.00	0	Abscission of Young Fruits
55	Shekhar 2 × RBL 1	38	24	7	2	8.33	54.55	Viable Dimpled Seeds
56	Shekhar 2 × RBL 6	34	28	6	2	7.14	50	Tiny, Dimpled Seeds
57	Shekhar 2 × RBL 9	24	16	5	0	0.00	0	Abscission of Young Fruits
58	Shekhar 2 × RBL 33	44	36	8	3	8.33	19.81	Tiny, Dimpled Seeds
59	Shekhar 2 × RBL 140	34	25	7	3	8.50	17.98	Tiny, Dimpled Seeds
60	Shekhar 2 × RBL 141	50	39	11	4	10.25	29.41	Tiny, Dimpled Seeds
61	Co 5 × RBL 1	32	22	5	0	0.00	0	Abscission of Young Fruits
62	Co 5 × RBL 6	20	12	3	0	0.00	0	Abscission of Young Fruits
63	Co 5 × RBL 9	35	26	6	0	0.00	0	Abscission of Young Fruits
64	Co 5 × RBL 33	24	13	4	1	7.69	25	Tiny, Dimpled Seeds
65	Co 5 × RBL 140	20	14	3	1	7.14	25	Tiny, Dimpled Seeds
66	Co 5 × RBL 141	25	16	0	0	0.00	0	Absence of Seed Set and Abscission of Crossed Flowers

67	Palampur 93 × RBL 1	68	55	14	5	9.09	33.33	Viable Dimpled Seeds
68	Palampur 93 × RBL 6	35	26	7	1	3.84	0	Viable Dimpled Seeds
69	Palampur 93 × RBL 9	34	25	8	1	4.00	33.33	Viable Good Seeds
70	Palampur 93 × RBL 33	26	19	4	1	5.26	25.00	Tiny, Dimpled Seeds
71	Palampur 93 × RBL 140	25	14	0	0	0.00	0	Absence of Seed Set and Abscission of Crossed Flowers
72	Palampur 93 × RBL 141	24	15	3	0	0.00	0	Abscission of Young Fruits
Vigna Radiata × Vigna Mungo								
73	SML 1455 × Mash 338	36	30	13	7	23.33	76.71	Viable Shrivelled Seeds
74	SML 1455 × Mash 114	37	30	12	6	20.00	81.8	Viable Shrivelled Seeds
75	HUM 12 × Mash 338	40	30	17	11	36.66	85.18	Viable Shrivelled Seeds
76	HUM 12 × Mash 114	36	28	13	8	28.57	73.71	Viable Shrivelled Seeds
77	ML 1464 × Mash 338	50	32	18	12	37.50	75.00	Viable Shrivelled Seeds
78	ML 1464 × Mash 114	42	20	13	5	25.00	74.35	Viable Shrivelled Seeds
79	Pusa 0672 × Mash 338	56	34	15	9	26.47	62.07	Viable Shrivelled Seeds
80	Pusa 0672 × Mash 114	55	35	17	9	25.71	59.68	Viable Shrivelled Seeds

Even though crossability barriers were predominant, it was possible to recover interspecific hybrids. The crossability of *Vigna radiata × Vigna umbellata* and *Vigna radiata × Vigna mungo* was successful only when Vigna radiata was used as female and of *Vigna mungo × Vigna umbellata* when *Vigna mungo* was used as female. The percent crossability among different sets of crosses varied from species to species. The differences in pod setting among different set of crosses might be because of wide variation in their genetic architecture leading to differences in cross compatibility. In *V. radiata × V. umbellata*, best combination recorded was HUM 12 × RBL 9 and HUM 12 × RBL 9 with the highest pods set percentage viz., 16.27% and 15.78% respectively. In *V. mungo × V. umbellata,* the maximum crossability of 11.36% was noticed in cross, Mash 338 × RBL 9. Similar crossability success were also reported in *V. radiata × V. umbellata* (29.63%), *V. radiata × V. trilobata* (8.48%), *V. radiata × V. aconitifolia* (7.69%) [13] and in *V. radiata × V. trilobata* (10.25%) [14]. similarly, highest pod set of 40.8% was observed in *V. unguiculata × V. unguiculata* var. spontanea [15].

Further, inter-specific hybrids involving three cultivars of urdbean (PDU-1, Palampur-93 and UG-2018) and six of ricebean (Naini, BRS-1, BRS-2, PRR-1, PRR-9301 and Local) exhibited differential response of crossability involving different genotypes [16,17]. The timings of anthesis (between 0500 to 0900 HRS), dehiscence of anthers (10 to 14 hours before anthesis) and receptivity of the stigmas (from the time of anthesis up to 6 to 8 hours after anthesis) were identical for the parental species. The length of style was different in the three species- it was 19 mm in *V. umbellata*, 23 mm in *V. radiata* and 21 mm in *V. mungo*. There are no external barriers, which prevent cross-pollination between *V. radiata* and *V. umbellata,* and *V. mungo × V. umbellata*, because the timing of anthesis, dehiscence of anthers and receptivity of the stigma are identical for both the parental species. Normal pollen germination in both selfed and cross flowers shows that the stigma does not act as barrier.

Absence of seed set and abscission of crossed flowers within 72 hours from pollination in crosses *V. radiata × V. umbellata* (TM 96-2 × RBL 6; Pusa 0672 × RBL 140; KM 2241 × RBL 6; ML 1464 × RBL 140; ML 1464 × RBL 141; HUM 12 × RBL 141 and SML 1455 × RBL 33) and V. mungo × V. umbellata (Mash 338 × RBL 140; Co 5 × RBL 141 and Palampur 93 × RBL 140) demonstrate that the first barrier responsible for complete sterility is the delay in pollen tube entry in to the ovules. This might be expected because of the difference in the length of style of three species. Such barriers are known in many other interspecific crosses as well [13,18,19]. In addition, relatively more number of crosses of *V. radiata × V. umbellata* showed high abscission of crossed flowers than *V. mungo × V. umbellata* which further supports that the difference in length of style is responsible for complete sterility.Pre fertilization barriers are absent in the interspecific crosses *V. umbellata × V. radiata* and *V. umbellata × V. mungo* as evident from normal pollen tube growth in both selfed and crossed flowers and low abscission rate of crosses flower within 72 hours from pollination.

However, in *V. radiata × V. mungo*, the highest crossability was visualized in hybrid, ML 1464 × Mash 338 (37.5%). The relatively high number of pods harvested for *V. radiata × V. mungo* suggests that there were no barriers in crossing of these two species for the parental cultivars used. However, barriers were observed in embryogenesis as both inviable and viable seeds were produced, but completely inviable seeds in the reciprocal cross, *V. mungo × V. radiata.* The reciprocal difference in crossability of *V. radiata and V. mungo* suggests interaction between genic and cytoplasmic factors [20], which may be the cause of hybrid embryo degeneration when *V. mungo* is used as the female parent [2,21]. The high rate of abscission of young fruits between 3 to 30 days after pollination and low seed set in crosses of *V. radiata × V. umbellata, V. mungo × V. umbellata* and *V. radiata × V. mungo* are suggestive for the presence of post fertilization barriers. The failure of endosperm nuclei to divide or the delayed endosperm nuclear divisions is responsible for abortion of embryo and the subsequent abscission of young fruits in the interspecific crosses. The failure of embryo to reach maturity might be the probable cause of the production of shrivelled seeds from these crosses.

These Crossability barriers between the cultigen and its wild relative constitute somatoplastic sterility [22]. Such sterility barriers have been recorded in the interspecific crosses between Phaseolus lunatus × Phaseolus vulgaris [23]. No differences in pod set between the parental cultivars were found when V. radiata or *V. mungo* were used as the female parent. However, significant differences in numbers of seed set were obtained for the interspecific cross *V. radiata × V. umbellata* and *V. mungo × V. umbellata.* The difference between the *V. umbellata* cultivars as the pollen parents was highly significant. Based on the percentage pod set and ovule fertility, out of the six ricebean genotypes used, RBL 1 and RBL 9 showed substantially high percent of crossability and better seed set with different cultivars of mungbean and blackgram suggesting that these two genotypes may be utilized for genetic improvement of these crops.

Conclusion

Different kinds of pre and post fertilization barriers are responsible for complete sterility to low fertility. Despite this, novel genes and alleles from exotic germplasm and related species must be exploited and accordingly hybridization should be utilized to create a wide genetic variation for breeding programs in the Vigna species. Significant progress has been made in basic techniques of tissue culture and in development of techniques to transfer genes from more distantly related taxa. The application of embryo rescue, ovary and ovule culture, chromosome doubling and induced chromosomal exchanges through tissue culture techniques holds considerable promise for the development of new cultivars incorporating genes from wide species.

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