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Interspecific Hybridization between Mungbean (*Vigna radiata*) and Rice Bean (*Vigna umbellata*) for Crossability and Related Parameters

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Abstract

Understanding the feasibility of interspecific hybridization between mungbean and ricebean is a first step to introgress mungbean yellow mosaic virus (MYMV) resistant genes to cultivated mungbean varieties from ricebean which is highly resistant to MYMV. Twenty eight interspecific hybridization involving four mungbean (*Vigna radiata*) and seven rice bean (*Vigna umbellata*) varieties were attempted to study crossability and other related parameters. Among twenty eight interspecific crosses attempted sixteen crosses were successful in setting pods. Of these sixteen interspecific crosses, KKM-3 × KBR-1 was found better with high crossability per cent, high hybrid pollen fertility, high seed germination percentage, lower hybrid lethality and lower hybrid breakdown was observed, suggesting that among all the cross combinations attempted this cross combination is a potential source for obtaining new gene combinations. The other two crosses which were next better combinations were KKM-3 × RBL-6 and KKM-3 × BRBM 127.

Keywords: Interspecific hybridization, mungbean, rice bean, crossability

The genus *Vigna* subgenus *Ceratotropis* consists of 21 species which are distributed across wide regions of Asia. Among these, mungbean (*Vigna radiata*), rice bean (*Vigna umbellata*), black gram (*Vigna mungo*) and adzuki bean (*Vigna angularis*) are economically important and were domesticated in India. *Vigna radiata* (L.) Wilczek, commonly known as greengram or mungbean is the most widely distributed species among the six Asiatic wild *Vigna* accessions. Mungbean seed contains about 24 per cent protein. The protein is comparatively rich in lysine, the amino acid that is deficient in cereal grains. Mungbean seeds are rich in minerals like calcium (132 mg/100 g of seeds), iron (6.74 mg), magnesium (189 mg), phosphorus (367 mg) and potassium (1246 mg) and vitamins like ascorbic acid (4.8 mg), thiamine (0.621 mg), riboflavin (0.233 mg), niacin (2.251 mg) and pantothenic acid (1.910 mg) (Haytowitz and Matthews, 1986). The cultivated species *V. radiata* has desirable characters like short cycle duration, high yield, amenability for crop rotation and undesirable characters like susceptibility to bruchids and yellow mosaic virus, the latter provoking 100 per cent yield loss on severely affected plants. As the intraspecific hybridization has yielded limited results therefore there is a need to improve the greengram by hybridization with wild species. Rice bean [*Vigna umbellata* (Thunb.) Ohwi & Ohashi] is native of South and South East Asia. As a cultigen, rice bean occurs in India, Burma, Malaysia, China, Korea, Indonesia and Philippines. Rice bean plant is generally resistant to the common leguminous diseases and resistant genetic stocks have been identified for powdery mildew, damping off and bacterial leaf spot. The very striking feature of rice bean is that it is free from MYMV disease (Anon., 2003).

Hence, transfer of resistant genes for MYMV from rice bean to mungbean may be a possible means to develop MYMV resistant mungbean varieties. In this direction, to understand the crossability of rice bean and mungbean, their hybrid viability, expression and stabilization of resistant genes in interspecific hybrids are very essential. In order to produce new gene combination for yield and yield attributing characters interspecific hybridization can serve as one of the crop improvement tools. In this paper the interspecific crossability between mungbean (*Vigna radiata*) and rice bean (*Vigna umbellata*) and study of F_1 hybrids through morphological and pollen fertility is reported.

Material and Methods

Interspecific hybridization involving selected mungbean and rice bean was carried out during Summer-2017 and *kharif*-2017 at the crossing blocks of AICRN on Potential Crops Scheme, Main Agricultural Research Station, Hebbal and GKVK, UAS, Bangalore. The hybridization included four green gram varieties *viz*., KKM-3, LG-572, PS-16 and BGG-5 which are agronomically superior and well adopted varieties but susceptible to MYMV were used as female parents wherein seven rice bean lines *viz*., RBL-35, EC-181771, KBR-1, EC-108873, RBL-6, IC-521148 and BRBM-127 which are highly resistant to MYMV were used as male parents. The F_1s generated from the above mentioned crosses were sown in two rows along with one row of male and female parents and observations were recorded on the F_1s generated to study different parameters related to crossability indices such as :

1. Crossabilty per cent $= \frac{\text{Number of pods formed}}{\text{Number of successful crosses}} \times 100$

2. Seed germination per cent $= \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$

3. Hybrid lethality per cent $= \frac{\text{Numberof plantsdied}}{\text{Numberof seedsgerminated}} \times 100$

4. Hybrid breakdown $= \frac{\text{Hybrid plants died}}{\text{Number of plants raised}} \times 100$

Pollen fertility analysis was carried out by the acetocarmine squash technique. Flower buds of appropriate size of hybrids were fixed in 3:1 ethyl alcohol : acetic acid mixture for 24 hours. The buds were then transferred to 70 per cent ethyl alcohol and stored at 4 °C till use. The anthers were squashed with 1 per cent acetocarmine stain on a microscopic slide. The slides were examined under microscope at a magnification of 40X. Round well filled and deeply stained pollen grains were counted as fertile and shriveled and lightly stained were counted as sterile. Pollen fertility was expressed in percentage as given below.

5. Pollen fertility per cent $= \frac{\text{Number of fertile pollen grains with deep stain}}{\text{Total number of pollen grains observed}} \times 100$

Results and Discusion

Twenty eight interspecific crosses were attempted by involving four selected genotypes of *Vigna radiata* as female parentsand seven genotypes of *Vigna umbellata* as male parents. Though, crossability barriers were predominant in the *Vigna* species, it was possible to recover interspecific hybrids in the crosses made between *Vigna radiata* and *Vigna umbellata*. Among the twenty eight interspecific crosses attempted between *Vigna radiata* and *Vigna umbellata*, only sixteen crosses produced the pods successfully and the other crosses did not produce any pods (Table I).

In the successful interspecific crosses effected, the highest crossability per cent was recorded in the crosses KKM-3 × KBR-1 (14.43) followed by KKM-3 × RBL-6 (13.01) and KKM-3 × BRBM 127 (11.76). This suggests that the parents of these three interspecific cross combinations may be more usefull for transfer of desirable genes across the two divergent species and to broaden the genetic base of interspecific hybrids. This kind of successful interspecific hybridization between mungbean and rice bean have also been reported by George *et al*. (2010) and Basavaraja (2015). Moderate crossability per cent was recorded in the crosses KKM-3 × IC-521148 (9.75) and KKM-3 × RBL-35 (8.04) which may imply that parents of these cross combinations might be originated from diverse secondary gene pool. These cross combinations may be of secondary importance in the wide hybridization programme. Present results are in agreement with those of earlier results by Kamaludin *et al*. (2008), Pandiyan *et al*. (2012) and Basavaraja (2015). Lower crossability per cent was recorded in the crosses LG-572 × KBR-1 (6.89) followed by KKM-3 x EC 108873 (6.52), BGG-5 x IC-521148 (6.41), PS-16 × RBL-6 (5.56), BGG-5 × KBR-1 (5.56), KKM-3 x EC 181771 (5.56), PS-16 x BRBM-127 (4.81), LG-572 × RBL-35 (4.68), PS-16 x KBR-1 (3.45), LG-572 x IC 521148 (3.22) and PS-

TABLE I

Number of crosses attempted, successful crosses, pods produced and other related hybridization parameters in the interspecific crosses of ricebean and mungbean

Cross combination	No of emasculated flowers	No of emasculated flower drop	Percent of emasculated flower drop	No of crosses attempted	No of flowers with stigma breakdown	Percent of flowers with stigma breakdown	No of sucessful crosses	No of crossed pods produced
KKM-3 × RBL-35	148	27	18.24	121	20	16.52	112	9
KKM-3 × KBR-1	122	14	11.47	104	7	6.73	97	14
KKM-3 × BRBM-127	136	24	17.64	112	44	39.28	68	8
KKM-3 ×EC-181771	141	32	22.69	109	37	33.94	72	4
KKM-3 × EC108873	127	27	21.25	100	8	8	92	6
KKM-3 × RBL-6	182	42	23.07	140	17	12.14	123	16
KKM-3 × IC-521148	148	28	18.91	120	38	31.66	82	8
LG-572 × RBL-35	112	29	25.89	83	19	22.89	64	3
LG-572 × KBR-1	88	16	18.18	72	14	19.44	58	4
LG-572 × BRBM-127	107	21	19.62	86	33	38.37	53	0
LG-572 × EC-181771	141	28	19.85	113	40	30.07	73	0
LG-572 × EC 108873	136	20	14.7	116	54	46.55	62	0
LG-572 × RBL-6	143	31	21.67	112	19	16.96	93	3
LG-572 × IC-521148	129	23	17.82	106	44	41.5	72	4
PS-16 x RBL-35	152	31	20.39	121	64	52.89	57	0
PS-16 x KBR-1	133	29	21.8	104	29	27.88	75	3
PS-16 x BRBM-127	142	36	25.35	106	34	32.07	72	4
PS-16 x EC-181771	98	11	11.22	77	34	44.15	43	0
PS-16 x EC 108873	118	22	18.64	96	35	36.45	61	0
PS-16 xRBL-6	105	19	18.09	86	23	26.74	53	0
PS-16 x IC-521148	142	21	14.78	121	13	10.74	108	6
BGG-5 x RBL-35	116	18	15.51	98	30	30.61	68	0
BGG-5 xKBR-1	161	21	13.04	148	56	37.83	92	6
BGG-5 x BRBM-127	142	31	21.83	111	26	23.42	85	0
BGG-5 xEC-181771	136	19	13.97	117	44	37.6	73	0
BGG-5 x EC 108873	128	21	16.4	107	42	39.25	75	0
BGG-5 x RBL-6	138	26	18.84	112	25	22.32	87	0
BGG-5 x IC-521148	142	28	19.71	114	36	31.25	78	5

16 x EC 108873 (2.94) (Table II) suggesting the involvement of fertilization barriers in these cross combinations indicating the least preference for the parents for use in interspecific hybridization of mungbean × rice bean and parents. These cross combinations might have been originated from the tertiary gene pool so it is difficult to recover successful crosses by conventional crossing program and requires tissue culture techniques. Similar results in the past were obtained by Pandiyan *et al*. (2012) and Basavaraja (2015).

Hybrid pollen fertility per cent was highest in the cross KKM-3 × BRBM 127 (83.68) and KKM-3 × KBR-1 (82.63). However, moderate hybrid pollen fertility per cent was noticed in the cross KKM-3 × RBL-35 (78.91) followed by KKM-3 × RBL-6 (72.77) and KKM-3 × IC-521148 (69.11). On the other hand, lower pollen fertility per cent was noticed in the cross KKM-3 x EC 181771 (61.11) followed by KKM-3 x EC 108873 (58.00), BGG-5 x IC-521148 (56.43), PS-16 x KBR-1 (55.00), BGG-5 × KBR-1 (51.77), LG-572 × KBR-1 (43.50), PS-16 x BRBM-127 (41.68), LG-572 × RBL-35 (40.22), LG-572 x IC 521148 (35.00), PS-16 x EC 108873 (28.18) and PS-16 × RBL-6 (25.66).The hybrid seed germination per cent noticed was higher in the crosses PS-16 x KBR-1 (47.37), KKM-3 × IC-521148 (44.44), KKM-3 x EC 181771 (42.30) and LG-572 × KBR-1 (40.91). The moderate

TABLE II

Crossability, pollen fertility, germination, hybrid lethality and hybrid breakdown percentage in interspecific hybrids of mung bean × rice bean

Cross combination	NSC	PS	CB (%)	PF (%)	NSS	NSG	GN (%)	NPD	HL (%)	HI	HBD (%)
KKM-3 × RBL-35	112	9	8.04	78.91	52	18	34.62	5	27.78	2	11.11
KKM-3 × KBR-1	97	14	14.43	82.63	64	23	35.94	4	17.39	3	13.04
KKM-3 × BRBM-127	68	8	11.76	83.68	34	12	35.29	2	16.67	1	8.33
KKM-3 ×EC-181771	72	4	5.56	61.11	26	11	42.30	3	27.27	1	9.09
KKM-3 × EC108873	92	6	6.52	58.00	38	13	34.21	2	15.38	2	15.38
KKM-3 × RBL-6	123	16	13.00	72.77	68	20	29.41	6	30.00	3	15.00
KKM-3 × IC-521148	82	8	9.75	69.11	27	12	44.44	4	33.33	3	25.00
LG-572 × RBL-35	64	3	4.68	40.22	18	7	38.88	2	28.57	1	14.28
LG-572 × KBR-1	58	4	6.89	43.50	22	9	40.91	3	33.33	3	33.33
LG-572 × IC-521148	93	3	3.22	35.00	14	3	21.43	1	33.33	1	33.33
PS-16 × KBR-1	87	3	3.45	55.00	19	7	47.37	2	28.57	1	14.28
PS-16 × EC-108873	68	2	2.94	28.18	10	2	20.00	1	50.00	0	0.00
PS-16 × RBL-35	72	4	5.56	25.66	27	8	29.63	2	25.00	1	12.50
PS-16 × BRBM-127	104	5	4.81	41.68	28	11	39.29	2	18.18	1	9.09
BGG-5 × KBR-1	108	6	5.56	51.77	22	7	31.82	1	14.28	1	14.28
BGG-5 × IC-521148	78	5	6.41	56.43	17	4	25.53	1	25.00	1	25.00

NSC- No of successful crosses, PS- Pod set, CB- Crossability, PF- Pollen fertility, NSS- No. of seeds sown, NSG- No. of seeds germinated, GN- Germination, NPD- No. of plants died, HL- Hybrid lethality, HI- Hybrid inviability and HBD- Hybrid break down.

hybrid seed germination per cent was recorded in the crosses PS-16 x BRBM-127 (39.29) followed by LG-572 × RBL-35 (38.88), KKM-3 × KBR-1 (35.94), KKM-3 × BRBM 127 (35.29), KKM-3 × RBL-35 (34.62), KKM-3 x EC 108873 (34.21) and BGG-5 × KBR-1 (31.82). The lower hybrid seed germination per cent was noticed in the crosses PS-16 × RBL-6 (29.63), KKM-3 × RBL-6 (29.41), BGG-5 x IC-521148 (25.53), LG-572 x IC 521148 (21.43) and PS-16 x EC 108873 (20.00). The lowest hybrid lethality per cent noticed was in the cross BGG-5 × KBR-1 (14.28) followed by KKM-3 x EC 108873 (15.38), KKM-3 × BRBM 127 (16.67), KKM-3 × KBR-1 (17.39) and PS-16 x BRBM-127 (18.18). The moderate hybrid lethality per cent was recorded in the crosses PS-16 × RBL-6 and BGG-5 x IC-521148 showing 25.00 per cent hybrid lethality followed by KKM-3 x EC 181771 (27.27), KKM-3 × RBL-35 (27.78), PS-16 x KBR-1 (28.57), LG-572 x RBL-35 (28.57) and KKM-3 × RBL-6 (30.00). The higher hybrid lethality per cent was recorded in the crosses PS-16 x EC 108873 (50.00) followed by LG-572 x IC 521148, LG-572 × KBR-1 and KKM-3 × IC-521148 exhibiting 33.33 per cent hybrid lethality. No hybrid breakdown was observed in the cross PS-16 x EC 108873. The Lowest hybrid breakdown per cent was noticed in the crosses KKM-3 × BRBM 127 (8.33) followed by KKM-3 x EC 181771 (9.09), PS-16 x BRBM-127 (9.09), KKM-3 × RBL-35 (11.11), PS-16 × RBL-6 (12.5), KKM-3 × KBR-1 (13.04), LG-572 x RBL-35 (14.28), PS-16 x KBR-1 (14.28), BGG-5 × KBR-1 (14.28), KKM-3 × RBL-6 (15.00) and KKM-3 x EC 108873 (15.38). The moderate hybrid breakdown per cent was recorded in the crosses BGG-5 x IC-521148 and KKM-3 × IC-521148 exhibiting 25.00 per cent. The highest hybrid breakdown per cent was recorded in the crosses LG-572 x IC 521148 and LG-572 × KBR-1 with 33.33 per cent (Table II).

These results on interspecific hybridization between mungbean and ricebean revealed the existence of high hybrid pollen fertility coupled with high seed germination percentage, lower hybrid lethality and lower hybrid breakdown as observed in the cross KKM-3 x KBR-1. This suggests that there may not be any fertilization barriers in this cross and hence might have resulted in production of successful crossed pods thus making it a good source for introgression of desirable genes. The cross KKM-3 x KBR-1 exhibited high hybrid pollen fertility coupled with moderate germination percent,low hybrid lethality and low hybrid breakdown suggesting the least occurrence of fertilization barriers in this cross and hence serve as potential source for introgression of desirable genes. Cross combinations such as KKM-3 x RBL-35 and KKM-3 x IC521148 exhibiting moderate hybrid pollen fertility coupled with moderate to high seed germination percentage, low to high hybrid lethality and low to high hybrid breakdown inspite of existence of fertilization barriers confers its utility in introgression. These findings are in accordance with Pandiyan *et al*. (2012) and Basavaraja (2015). Low hybrid pollen fertility coupled with moderate to low seed germination percentage, low hybrid lethality and low hybrid breakdown was observed in crosses such as KKM-3 x EC 108873, BGG-5 x KBR-1, PS-16 x KBR-1 and LG-572 x RBL-35 suggesting the predominance of fertilization barriers but $_{F1}$s of these cross combinations exhibited very less hybrid lethality and hybrid breakdown. In all other remaining cross combinations low hybrid pollen fertility coupled with moderate to low seed germination percentage, moderate to low hybrid lethality and moderate to low hybrid breakdown were observed which suggested that parents of these cross combinations might be having distant lineage from their original ancestor or species. The occurrences of prezygotic barriers do not limit the cross and postzygotic barriers may restrict the successful gene exchange. Viable seed may give rise to weak F_1 individuals or develop into vigorous F_1 individuals. The F_1 hybrids that survive to sexual maturity may display varying degrees of sterility. Hybrid breakdown in advanced generations is manifested as weak, abnormal, or sterile progeny. These results are in accordance with the earlier reports of Kamaludin *et al*. (2008), Pandiyan *et al*. (2012) and Basavaraja (2015).

TABLE III

Morphological and quantitative characters of F_1 interspecific hybrids of V. radiata (♀) *x* V. umbellata (♂) *and their parents.*

Characters	*V.radiata* (♀)	*V. umbellata* (♂)	F_1 hybrids
Colour of stem during seedling stage	Greenish	Greenish	Light greenish
Primary leaves	Long-obovate	Linear-lanceolate	Rhomboid or obovate/ bi lobed
Growth form	Erect, determinate growth habit	Erect to suberect; main stem shows indeterminate growth	Sub erect, in determinate growth habit
Stem hairiness	Glabrous	Hairy	Hairy
Leaf shape	Broad	Lanceolate	Intermediate
Anther colour	Pale yellow	Yellow	Yellow
Pod hairiness	Hairy	Glabrous	Hairy
Seed lusture	Dull	Shiny	Shiny
Leaf hairiness	Glabrous	Hairy	Hairy
Length of petiole (cm)	3.2	2.4	1.6
Length and breadth of primary leaf (cm)	6.2×3.2	5.4×1.4	2.3×1.4
Plant height (cm)	Dec-38	22-62	16-Aug
Length and breadth of middle leaflet	12.3×5.8	11.6×7.5	5-8×2-3
Pod length (cm)	16-Jun	18-Sep	10-May
Inflorescence Length (cm)	8-Feb	10-Mar	4-Feb
Fruit type	Linear pod	Linear pod	Cylindrical pod
Seed colour	Green/brown	Green/red/yellow	Dark green
Seed size (mm)	2.5-4×2.1-3	3.6-5×1.8-2	1.8×1.2

Even though F_1 hybrids resembled like female parent *i.e.,* greengram, for major morphological and quantitative characters F_1 interspecific hybrids was found to be intermediate between their parents V. radiata (♀) and V. umbellata (♂) which has been listed in Table III. Among which leafy hairiness which has been transferred from ricebean to greengram may serve as a mechanical barrier in inducing resistance to viral disease such as Mungbean Yellow Mosaic Virus (MYMV).

The results of the present study revealed that, all the F_1s generated by interspecific crosses resembled female parent *i.e.,* greegram and all cross combinations involving KKM-3 as a parent was successful in producing crossed pods suggesting its better utility in interspecific hybridization. KKM-3 × KBR-1 would be a better cross combination for introgression of any important characters from ricebean to greengram and obtaining new gene combinations because of its high crossability per cent, high hybrid pollen fertility, high seed germination percentage, lower hybrid lethality and lower hybrid breakdown among all the cross combinations attempted. The other two crosses which were next better combinations were KKM-3 × RBL-6 and KKM-3 × BRBM 127.

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