Comparative Study of Crossability Behavior in Intra-specific and Inter-specific Crosses of *Vigna radiata* and *Vigna mungo*

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ABSTRACT

Background: The basic plant material consisted of 9 mungbean and 5 urdbean genotypes. 42 different crosses were made comprising 13 *Vigna radiata* × *V. radiata*, 4 *V. radiata* × *V. mungo* and 25 *V. radiata* × *V. mungo* crosses. Varying degree of success has been achieved in obtaining intraspecific and interspecific crosses having desired variability for yield and yield contributing traits along with MYMV resistance.

Methods: The present investigation was done at Agricultural Research Farm, Banaras Hindu University, Varanasi, during *Kharif*, 2019 and S*ummer*, 2020. 42 intra-specific and inter-specific cross combinations were initially examined for their crossability. Out of these, six promising crosses with higher crossability percent were further analyzed for hybrid pollen fertility, hybrid lethality and, evaluation of F_1 and parents for different yield traits.

Result: Crossability per cent found highest in intraspecific cross HUM 2 × IPM 02-3 (41.86%) in mungbean while, in urdbean cross NDU 1 × R3/12 (41.38%). In interspecific crosses, the maximum crossability per cent was found in SKAU M 365 × R3/28 (34.92%). Two mungbean genotypes (HUM 2 and HUM 26) and two urdbean genotypes (R3/12 and R3/28) can be further utilized for genetic improvement through inter-specific hybridization as there cross combinations are better performing on the basis of yield attributes along with MYMV resistance.

Key words: Crossability, Hybrid breakdown, Inter-specific hybridization, Mungbean, MYMV resistance, Urdbean.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) Wilczek; 2*n*=2*x*=22] is a short duration pulse crop with low input requirements having high worldwide demand. It is also an outstanding rotation crop in rice-based agricultural system (Bhanu *et al*., 2018). 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) disease leads to significant yield losses of upto 85% (Haq *et al*., 2010). The other cultivated *Vigna* species, urdbean [*Vigna mungo* (L.) Hepper; 2*n*=2*x* =22], which is also a self-pollinating pulse crop. It possess non shattering pods with synchronous maturity, more clusters per plant, pods with large seeds and comparatively more durable resistance to MYMV (Singh, 1990). Breeding for resistant varieties is one of the most suitable strategies against biotic stresses (Karthikeyan *et al*., 2012).

The gene pool of a cultivated species could be widened through intra-specific and inter-specific hybridization in order to produce such crop varieties that integrate high yield with disease and pest resistance. Effectiveness of distant hybridization and subsequent foreign gene transfer is mostly determined by pollination behaviour, frequency of pollination, ploidy status of the species involved in the cross and the employment of efficient techniques. Many wild *Vigna* species are not crossable with their cultivated equivalents due to pre- and post-fertilization obstacles and, therefore, are useless for crop improvement (Tyagi and Chawla, 1999). When such hybridization is attempted, crossing barriers are often encountered. Keeping these aspects in consideration, current investigation was carried out to know the crossability barriers existing among the two *Vigna* species, belonging to mungbean and urdbean, through inter-specific and intra-specific hybridization with the aim to combine the yield and yield attributing traits along with the MYMV resistance.

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MATERIALS AND METHODS

Plant material for present experiment consisted of nine mungbean and five urdbean genotypes. Initially, 42 different crosses were made comprising of 13 mungbean × mungbean, four urdbean × urdbean and 25 mungbean × urdbean crosses (Supplementary Table 1). All crosses were attempted during *Kharif* 2019 and evaluated in the summer of 2020. The experiment was conducted at the Agricultural Research Farm, Banaras Hindu University, Varanasi, Uttar Pradesh, India. On the basis of higher crossability percent and number of cross pod produced (Supplementary Table 1), six cross combinations were further evaluated. Details of the parents involved in these six crosses of mungbean and urdbean have been presented in Table 1.

Observations were made on the number of buds emasculated, flower drop, per cent emasculated flower drop, number of buds pollinated, number of flowers with stigma breakdown, per cent of flowers with stigma breakdown, number of successful crosses, number of cross pod produced and crossability per cent (Supplementary Table 1). At physiological maturity, crosses were harvested and the crossability per cent obtained from the formulae *i.e.*,

$$\frac{\text{Number of cross pods produced}}{\text{Number of buds pollinated}} \times 100$$

The pollen fertility testing was done on the parents and their hybrids during blooming using acetocarmine staining methodology.

Pollen fertility per cent =

$$\frac{\text{Number of viable pollen}}{\text{Total number of pollen observed}} \times 100$$

Similarly, Hybrid lethality (%)=

$$\frac{\text{Number of plants died}}{\text{Number of seeds germinated}} \times 100$$

During *summer* 2020, parents and F_1 s were sown in two rows with one row of male and female parents with spacing of 45 × 30 cm in two replications under randomized block design (RBD). Five plants were randomly chosen from each of the parents and F_1 to record data on ten quantitative traits, *viz.*, days to 50 per cent flowering, days to maturity, plant height (cm), number of primary branches per plant, number of clusters per plant, number of pods per plant, pod length (cm), number of seeds per pod, 100-seed weight (g) and seed yield per plant (g).

RESULTS AND DISCUSSION

Inter-specific hybridization has been limited in many crops due to presence of certain pre- and post-fertilization barriers, but in pulse crops such as mungbean and urdbean, interspecific hybridization has proved successful (Pandiyan *et al.*, 2010). The current investigation sought to obtain better recombinants amongst six intra-specific and inter-specific crosses of *V. radiata* and *V. mungo* in terms of yield and MYMV resistance.

Crossability percent of intra-specific and inter-specific crosses

The higher crossability attained in intra-specific crosses in comparison to inter-specific crosses depict absence of external barriers impeding cross pollination (Supplementary Table 1). Though, the possible causes of hybrid failure and breakdown of interspecific crosses in *Vigna* are poorly understood (Chen *et al.*, 1983), several factors including

Table 1: Details of the mungbean and urdbean genotypes used in the present study.

Name of genotype	Pedigree	Centre	MYMV disease reaction	Characteristics
Mungbean				
HUM 2	Selection from TVCM 3	I. Ag. Sc., BHU, Varanasi	Susceptible	Tall plants (~58 cm), more number of pod, high yielding, 3.4 g/ 100 seeds
HUM 26	(HUM 4 × HUM 14) × BDYR 1	I. Ag. Sc., BHU, Varanasi	Susceptible	Medium in height (~47 cm), large seeded (4.5 g/100 seeds), seeds attractive green, high yielding
IPM 02-3	IPM 99-125 × Pusa Bold 2	ICAR-IIPR, Kanpur	Resistant	Medium to tall (~54 cm in height), 4.2 g/100 seeds
SKAU M 365	SL-44 × TM 98-3	SKAU, Kashmir	Susceptible	Medium height (48.5 cm), long size pod, bold shining green seeds, seed size (4.6 g/100 seeds)
Urdbean				
NDU 1	G 65 × UPM 79-3-4	ANDUAT, Ayodhya	Susceptible	Days to maturity (78-80 days), compact plant type, Suitable for round the season growing
Mash 338	VG 201 × PDU 3	PAU, Ludhiana	Resistant	Suitable for kharif, dwarf and short duration variety, each pod having bold 6 to 7 seeds
R3/28	-	AICRP on MULLaRP	Resistant	Suitable for summer season, compact plant type, bold seed (3.3 g/100 seed)
R3/12	-	AICRP on MULLaRP	Resistant	Suitable for summer season, bold seed (3.4 g/100 seed)

Supplementary Table 1: The crosses, pod set and crossability percent of intra-specific (M × M; U × U) and inter-specific (M × U) crosses.

Cross combination	Number of emasculated buds	Flowers drop	Per cent emasculated flower drop	Number of buds pollinated	Number of flowers with stigma breakdown	Percent of flowers with stigma breakdown	Number of successful crosses	Number of cross pod produced	Crossability per cent (%)
***Vigna radiata* × *Vigna radiata* (M × M)**									
HUM 1 × IPM 02-3	40	15	37.5	25	14	56.00	11	4.00	16.0
HUM 1 × IPM 205-7	42	14	33.33	28	13	46.43	15	6.00	21.43
HUM 2 × IPM 205-7	45	19	42.22	26	10	38.46	16	8.00	30.77
HUM 2 × IPM02-3	54	11	20.37	43	7	16.28	36	18.0	41.86
HUM 23 × IPM 205-7	43	21	48.84	22	11	50.00	11	6.00	27.27
HUM 23 × IPM02-3	48	19	39.58	29	14	48.28	15	5.00	17.24
HUM 26 × IPM02-3	58	12	20.69	46	8	17.39	38	17.0	36.96
HUM 26 × IPM205-7	56	18	32.14	38	16	42.11	22	8.00	21.05
HUM 6 × IPM 205-7	36	18	50	18	9	50.00	9.0	3.00	16.67
HUM 6 × IPM02-3	44	17	38.64	27	11	40.74	16	4.00	14.81
HUM 7 × IPM 205-7	50	28	56	22	12	54.55	10	5.00	22.73
HUM 7 × IPM02-3	55	27	49.09	28	14	50.00	14	7.00	25.0
SKAUM 365 × IPM02-3	56	23	41.07	33	14	42.42	19	9.00	27.27
***Vigna radiata* × *Vigna Mungo* (M × U)**									
HUM 1 × Mash338	41	15	36.59	26	12	46.15	14	5	19.23
HUM 1 × PU31	56	20	35.71	36	16	44.44	20	6	16.67
HUM 1 × R3/12	52	15	28.85	37	17	45.95	20	6	16.22
HUM 1 × R3/28	42	15	35.71	27	13	48.15	14	5	18.52
HUM 2 × Mash338	60	18	30	42	14	33.33	28	9	21.43
HUM 2 × PU31	55	15	27.27	40	17	42.5	23	8	20.0
HUM 2 × R3/12	60	22	36.67	38	16	42.11	22	8	21.05
HUM 2 × R3/28	65	19	29.23	46	9	19.56	37	16	34.78
HUM 23 × Mash338	40	17	42.5	23	15	65.22	8	4	17.39
HUM 23 × R3/12	44	19	43.18	25	14	56.00	11	4	16.0
HUM 23 × R3/28	42	18	42.86	24	16	66.67	8	3	12.5
HUM 26 × Mash338	54	25	46.3	29	14	48.28	15	5	17.24
HUM 26 × PU31	40	18	45	22	9	40.91	13	4	18.18
HUM 26 × R3/28	44	19	43.18	25	11	44.00	14	5	20.0
HUM 6 × Mash338	39	19	48.72	20	8	40	12	5	25.0
HUM 6 × PU31	37	17	45.95	20	8	40	12	5	25.0
HUM 6 × R3/12	38	20	52.63	18	11	61.11	7	4	22.22
HUM 6 × R3/28	42	22	52.38	20	12	60	8	4	20.0

Supplementary Table 1: Continue....

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HUM 7 × Mash338	54	23	42.59	31	15	48.39	16	6	19.35
HUM 7 × PU31	50	22	44	28	13	46.43	15	5	17.86
HUM 7 × R3/12	54	25	46.3	29	14	48.28	15	4	13.79
HUM 7 × R3/28	52	21	40.38	31	17	54.84	14	5	16.13
SKAUM 365 × Mash338	54	29	53.7	25	11	44	14	7	28.0
SKAUM 365 × PU31	66	32	48.48	34	12	35.29	22	9	26.47
SKAUM 365 × R3/28	75	12	16	63	13	20.63	50	22	34.92
***Vigna mungo × Vigna mungo* (U × U)**									
NDU1 × Mash338	74	17	22.97	57	12	21.05	45	22	38.6
NDU1 × PU31	48	24	50	24	13	54.17	11	7	29.17
NDU1 × R3/12	72	14	19.44	58	9	15.52	49	24	41.38
NDU1 × R3/28	52	28	53.85	24	11	45.83	13	8	33.33

specific cross combination, genetic divergence and environment factors can be the major reasons which influence the hybridization process leading to reduced success rate in case of interspecific crosses. However, the crossability barriers are pre-dominant, but still there is possibility to recover interspecific hybrids. Bhanu *et al.* (2018) reported higher crossability percent in crosses between *V. radiata* × *V. umbellata* and *V. mungo* × *V. umbellata*. Moreover, crossability is genotype dependent, which leads to cross-incompatibility in some particular combinations while, other cross-combinations may be comparatively more successful (Rashid *et al.*, 1988).

Germination and pollen fertility of parents and F_1 hybrids (inter-specific and intra-specific crosses)

Parents and the six promising crosses were utilized to determine number of seeds per parent, number of seeds germinated, seed germination per cent, number of seedlings matured, hybrid lethality per cent, hybrid in-viability, hybrid breakdown per cent and pollen fertility per cent (Table 2). In the intra-specific crosses of mungbean (HUM 2 × IPM 02-3 and HUM 26 × IPM 02-3), moderate pollen fertility (42.21% - 39.12%) coupled with moderate seed germination percent (85-80%) were observed. Whereas, the two urdbean crosses (NDU 1 × Mash 338 and NDU 1 × R3/12) exhibited moderate pollen fertility (38.22-38.12%) and low germination (60-50%). These two urdbean × urdbean crosses revealed 8.33% and 10% hybrid breakdown coupled with, 33.33% and 40% hybrid lethality, as well. Similarly, the two inter-specific crosses (SKAUM 365 × R3/28 and HUM 2 × R3/28) revealed low hybrid pollen fertility of 37.24% and 31.29% as well as, moderate and low seed germination percent of 75% and 40%, respectively (Table 2).

Present study revealed minimum occurrence of fertilization barriers in the above mentioned crosses which might have resulted in the production of higher number of successful cross pods (Supplementary Table 1). Results have been similarly interpreted by Basavaraj *et al.* (2019) who produced a cross between *V. radiata* and *V. umbellata* and discovered certain post and pre-fertilization obstacle, prohibiting cross pollination based on crossability percent, pod set per cent, seed germination per cent and hybrid lethality. On the contrary, Mahalingam and Manivannan (2021) discovered the presence of many pre and post zygotic crossability impediments between wild and cultivated species.

Yield performance of parents and F_1 hybrids (inter-specific and intra-specific crosses)

Results on yield and its component traits in parents and the six crosses have been presented in Table 3. Compared to the parents, hybrid showed high *per se* performance in the urdbean × urdbean intra-specific cross, NDU 1 × R3/12 manifested by the traits, *viz.*, number of clusters per plant (19), number of pods per plant (69) and seed yield per plant (14.12 g) while, in the mungbean × mungbean intra-specific cross, HUM 26 × IPM 02-3, recorded as many as 45 pods per plant with highest yield (17.95 g) among the crosses

Table 2: Parents and six F_1 hybrids germination and pollen fertility per cent.

Cross and parent	No. of seeds per parent	No. of seeds germinated	Seed germination per cent (%)	Number of seedlings matured	Hybrid lethality (%)	Hybrid In-viability	Hybrid breakdown per cent (%)	Pollen fertility (%)
HUM 2	40	37	92.5	35	-	-	-	91.08
HUM 26	40	38	95.0	37	-	-	-	89.98
IPM 02-3	40	39	97.5	35	-	-	-	89.14
SKAUM 365	40	37	92.5	34	-	-	-	91.12
NDU 1	40	39	97.5	38	-	-	-	88.23
Mash 338	40	40	100.0	38	-	-	-	88.12
R3/12	40	39	97.5	37	-	-	-	87.12
R3/28	40	38	95.0	36	-	-	-	88.65
HUM 2 × IPM 02-3	20	17	85.0	14	17.65	1	5.88	42.21
HUM 26 × IPM 02-3	20	16	80.0	11	31.25	2	12.5	39.12
SKAUM 365 × R3/28	20	15	75.0	9	40.00	2	13.33	37.24
HUM 2 × R3/28	20	8	40.0	6	25.00	2	25.0	31.29
NDU 1 × Mash 338	20	12	60.0	8	33.33	1	8.33	38.22
NDU 1 × R3/12	20	10	50.0	6	40.00	1	10.0	38.12

Table 3: The average performance of the yield attributing traits among the parents and six F_1 hybrids (interspecific and intraspecific crosses).

Parent/cross	DFF	DM	PH (cm)	NPB	NCPP	NPP	PL (cm)	SPP	100 SW (gm)	SYPP (gm)
HUM 2	30.50	65.25	57.0	2.30	11.60	36.0	7.77	9.20	3.35	11.08
HUM 26	31.75	64.50	56.0	2.20	10.70	39.0	7.88	9.12	4.35	14.98
IPM 02-3	31.0	66.0	56.22	2.95	9.82	48.0	8.22	9.12	4.23	17.73
SKAUM 365	34.25	68.67	52.35	3.45	9.25	34.0	8.12	9.23	4.50	12.15
NDU 1	43.75	79.0	22.22	3.10	9.15	40.0	3.12	5.80	3.60	7.95
Mash 338	42.25	76.50	19.22	2.70	10.25	42.0	4.10	6.20	3.90	10.12
R3/12	41.50	77.75	22.65	3.40	12.65	44.0	4.12	5.90	3.40	8.65
R3/28	42.75	77.50	24.22	3.55	11.10	43.0	4.22	6.22	3.40	8.54
HUM 2 × IPM 02-3	28.0	63.0	58.60	3.20	13.50	42.0	8.44	9.48	3.95	15.64
HUM 26 × IPM 02-3	29.0	63.0	54.20	3.0	14.31	45.0	8.65	9.55	4.37	17.95
SKAUM 365 × R3/28	34.0	71.0	60.20	3.65	16.0	46.0	8.00	7.95	4.25	14.95
HUM 2 × R3/28	32.0	70.0	62.10	3.67	13.80	47.0	7.50	7.89	3.60	13.12
NDU 1 × Mash 338	40.0	76.0	24.0	3.50	16.0	63.0	4.25	6.0	3.70	13.95
NDU 1 × R3/12	39.0	76.0	24.0	3.70	19.0	69.0	4.35	5.90	3.50	14.12

DFF= Days to 50% flowering; DM= Days to maturity; P= Plant height (cm); NPB= Number of primary branches per plant; NCPP= Number of clusters per plant; NPP= Number of pods per plant; PL= Pod length; SPP= Seeds per pod; 100-SW= 100 Seed weight (g); SYPP= Seed yield per plant (g).

attempted. In the mungbean × urdbean inter-specific cross, SKAUM 365 × R3/28, have recorded 16 clusters per plant, 46 pods per plant and 14.95 g of seed yield per plant. It was observed from Table 3 that different quantitative traits were contributing differently in inter and intra-specific crosses. Successful inter-specific hybridization between mungbean and urdbean to obtain transgressive segregants for different yield traits have been reported by many workers (Singh *et al.*, 1996; Pathak *et al.*, 2015; Singh *et al.*, 2020).

CONCLUSION

In the present study, 42 crosses have been made and despite crossability constraints which were prevalent, a few inter-specific and intra-specific hybrids with intermediate characters of both the parents along with MYMV resistance were obtained. On the basis of pollen fertility, germination per cent, hybrid lethality, hybrid in-viability and hybrid breakdown per cent, three crosses *viz.*, HUM 2 × IPM 02-3, SKAU M 365 × R3/28 and NDU 1 × R3/12 showed higher crossability. Among the crosses, two M × M crosses (HUM 26 × IPM 02-3 and HUM 2 × IPM 02-3) have recorded highest seed yield per plant whereas, one inter-specific cross between M × U *i.e.*, HUM 2 × R3/28 is third highest yielder. These three crosses have been recommended to be used in *Vigna* crop improvement program. Since, these crosses have positive contribution of many desirable yield and yield contributing traits along with MYMV resistance.

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