



## Effects of different growth media on germinability of interspecific hybrids between *Vigna mungo* and *V. umbellata*

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### Abstract

A total of 15 interspecific crosses involving five genotypes of urdbean (*Vigna mungo*) and three genotypes of ricebean (*V. umbellata*) were attempted to study the effects of different growth media on hybrid germination. Since the seeds obtained from the interspecific crosses did not germinate under normal soil conditions so attempts were made to grow them on various media i.e. Salt solution, Half and full strength MS medium, MS basal medium and Gamborg B5 media. Seeds were able to germinate only on the salt solution. F<sub>1</sub> seeds obtained from cross Him Mash-1 x VRB-3 showed maximum response on salt solution with respect to radicle formation (44.60 per cent) followed by Palampur-93 x PRR-2 (38.30 per cent) & Him Mash-1 x PRR-1 (23.89 per cent). Cross Palampur-93 x PRR-2 (17.02 per cent) produced maximum number of plantlets followed by Him Mash-1 x VRB-3 (15.32 per cent). The study indicated that different kinds of post fertilization barriers such as retarded embryo & endosperm development, production of inviable & shriveled seed, inability of seed to form radicle and yellowing of plantlets are responsible for complete lethality. The crosses showing substantially high per cent of germination can be utilized for genetic improvement of urdbean.

**Key words:** Interspecific hybridization, germinability, *V. mungo*, *V. umbellata*.

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 *Leguminosae* and subfamily *Papilionaceae*, with its wild progenitor *V. mungo* var. *silvestris* (Bhareti *et al.* 2011). Center of genetic diversity for urdbean is found in India with its secondary center of origin in Central Asia (Zeven and De Wet 1982). It is a short duration pulse crop and self pollinated grain legume grown in many parts of India.

Food legumes are a good protein source mainly for poor's who often cannot afford animal based 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 for restoration of soil fertility by symbiotic nitrogen fixation (Malik, 1994). Despite huge benefits of urdbean, it is grown in 2.5 million hectare of area in India and produces about 1.5 million tonnes of urdbean annually with an average productivity of 400 kg per hectare (Anonymous, 2018). 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 intercrop with maize as well as a monocrop. However, its yield is low compared to other grain legumes. 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 its cultivation in marginal and harsh environment (Ali *et al.* 2006). 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 in 40-60 per cent reduction in grain yield (Singh *et al.* 1978).

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 which can provide

effective sources of resistance against biotic & abiotic stresses and other desirable traits. Therefore, pre-breeding practices such as inter-specific hybridization are required involving particularly those species that carry useful alien genes for improving yield, quality, biotic and abiotic stress resistance. The related species *V. umbellata* (ricebean) has been found to be nutritive and resistant to most of the fungal pathogens of urdbean. The seeds obtained from the inter specific crosses showed no germination under normal soil conditions, attempts were made to grow them on various media. Therefore, the present study has been undertaken to study the efficacy of different growth media on germination of interspecific hybrids of *V. mungo* with *V. umbellata*.

### Materials and Methods

For the present investigation, a total of eight different genotypes i.e. five of urdbean (Him Mash-1, HPBU-111, Palampur-93, UG-218 & PDU-1) taken as female and three of ricebean (PRR-1, PRR-2 & VRB-3) taken as male were used to study the germinability of their hybrids. During summer & Kharif 2017 & summer 2018, staggered sowings were done at interval of 10 days starting from 15<sup>th</sup> February to 31<sup>st</sup> July to have synchronized flowering in the glasshouse of Department of Genetics & Plant Breeding, COA, CSKHPKV Palampur situated at 32°8' N latitude and 76°3' E longitude and at 1290.8m above mean sea level representing mid-hill zone of Himachal Pradesh, characterized by humid sub-temperate climate with high rainfall (2500 mm) having acidic soil with pH ranging between 5.0 to 5.6. Crossing was performed from 15<sup>th</sup> April to 15<sup>th</sup> October of 2017 & 15<sup>th</sup> April to 30<sup>th</sup> June of 2018. 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<sub>3</sub>), indole acetic acid (IAA) and Σ-amino caproic acid were used at two concentrations (500 ppm & 1000 ppm) about half an hour after pollination to prevent premature flower abscission. This was repeated for three consecutive days after pollination at an interval of 24 hours. A total of 15 interspecific crosses of *Vigna mungo* x *V. umbellata* (urdbean x ricebean) were attempted. The seeds obtained from the interspecific crosses were grown on various media i.e. Salt solution (Sander *et al.* 1959), Half and full strength MS medium, MS basal medium and Gamborg B5 media to study the response of different growth media on germinability of F<sub>1</sub> seeds. F<sub>1</sub> seeds were surface sterilized with 0.02 per cent mercuric chloride for two minutes, washed three to four times in sterilized distilled water and placed in petri-

plates with sterilized salt solution, MS medium (Half & Full Strength) & Gamborg B5 media under aseptic conditions. Petri plates with sterile F<sub>1</sub> seeds were placed in incubator at 25±1°C for four to five days. The sterilized salt solution was changed every day under sterile conditions. On second transfer on fresh salt solution seed coat of imbibed F<sub>1</sub> seeds were removed and allowed to develop on salt solution for one or two days. Four to five days old seeds showing radicle formation/seedling were transferred to paper cups having mixture of sand + cocopeat + vermicompost. Data were recorded with respect to:

- Total seeds cultured
  - Number of seeds showing radicle formation
  - Number of interspecific plantlets obtained
- Per cent radicle formation (Germination Percentage) was calculated as follows:

$$\text{Per cent radicle formation} = \frac{\text{Number of seeds showing radicle formation} \times 100}{\text{Total seeds cultured}}$$

Per cent hybrid plants obtained was calculated as follows:

$$\text{Per cent hybrid plants obtained} = \frac{\text{Number of interspecific plantlets obtained} \times 100}{\text{Total seeds cultured}}$$

### Statistical Analysis

Since the data were in percent and lying beyond the range of 0 to 30 per cent or 30 to 70 per cent or 70 to 100 per cent, 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 per cent data was done by using the following formula:

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

### Simple t-test

To test whether the mean difference of radicle and hybrid plant production in the programme, simple t-test was performed as:

$$\text{Student's T-test} = \frac{\bar{X}_d}{\text{SE}(\bar{X}_d)} \text{ at } (n-1) \text{ df}$$

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

SE( $\bar{X}_d$ ) = Standard error of mean difference

n = Number of related samples

## Results and Discussion

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 effects of different growth media on germination of interspecific seeds and germinability percentage of interspecific seeds of urdbean with ricebean. There is high rate of abscission of young fruits between 3 to 30 days after pollination suggesting the presence of post fertilization barriers. Some of pods harvested had no seed or had very minute seeds. The number of seeds per pod in the interspecific hybrids varied from 1 to 4. Similar results on number of seeds per pod were reported by Gosal and Bajaj (1983) in the cross between *Vigna radiata* x *V. mungo*. Sehrawat *et al.* (2016) reported that number of F<sub>1</sub> seeds per pod in interspecific crosses between genotypes of urdbean and ricebean varied from 1 to 4. The F<sub>1</sub> seeds obtained from all cross combinations were small in size and shriveled because of the poor development of the endosperm and embryo which is due to incompatibility between the two parental genomes (Rashid *et al.* 1987). Generally the hybrid seeds from interspecific hybridization were shriveled or partially filled and empty as was reported by earlier workers (Biswas and Dana 1975). Since the seeds obtained from the interspecific crosses under study did not germinate under normal soil conditions so attempts were made to grow them on various media i.e. Salt solution, Half and full strength MS medium, MS basal medium and Gamborg B5 media. Successful results were only obtained on the salt solution, in rest of medium seeds of interspecific crosses showed no germination (Table 1). Similar results were obtained by Mittal *et al.* (2005, 2008) in interspecific crosses between urdbean and ricebean. The present study reveals the operation of post fertilization barriers such as retarded embryo & endosperm development, production of inviable & shriveled seeds, inability of seed to form radicle and yellowing of plantlets. Cross combination Him Mash-1 x VRB-3 (68.47 per cent) showed highest percentage of shriveled seeds followed by Him Mash-1 x PRR-2 (60.59 per cent) & Palampur-93 x PRR-2 (59.57 per cent). For number of very shriveled seeds formation, HPBU-111 x PRR-2 (46.15 per cent) gave highest value followed by UG-218 x PRR-2 (37.25 per cent) & UG-218 x PRR-1 (37.04 per cent). Cross Him Mash-1 x

PRR-1 (41.67 per cent) records highest percentage of minute seeds formation followed by cross Palampur-93 x VRB-3 (32.22 per cent) & PDU-1 x PRR-2 (31.03 per cent). Seeds of interspecific cross HPBU-111 x PRR-1 (97.14 per cent) gave highest value with respect to no radicle formation followed by HPBU-111 x PRR-2 (96.92 per cent) & UG-218 x PRR-1 (96.30 per cent). Cross combination Him Mash-1 x VRB-3 (35.59 per cent) showed maximum number of yellowing of plantlets followed by cross Palampur-93 x PRR-1 (29.79 per cent) & Him Mash-1 x PRR-1 (19.44 per cent) (Table 2). Post-fertilization barriers of varying degrees have also been reported in the interspecific *Vigna* crosses by Gopinathan *et al.* (1986); Bharathi *et al.* (2006); Pandiyan *et al.* (2010); Chaisan *et al.* (2013). Cross combinations UG-218 x PRR-1 (33.33 per cent), PDU-1 x PRR-2 (10.84 per cent), HPBU-111 x PRR-2 (9.23 per cent), PDU-1 x VRB-3 (30.54 per cent) & HPBU-111 x PRR-2 (1.54 per cent) gave least percentage with respect to number of shriveled, very shriveled & minute seeds formation, seeds showing no radicle formation and number of plants showed yellowing after germination. These cross combinations will be successfully used for transfer of genes from ricebean to urdbean.

Even though crossability barriers were predominant, it was possible to recover interspecific hybrids. The range of per cent radicle & per cent hybrid plant production was observed to be 0-44.60 per cent & 0-17.02 per cent respectively in urdbean x ricebean hybridization. The analysis of results revealed that cross combinations Palampur-93 x PRR-2, Him Mash-1 x VRB-3, PDU-1 x PRR-2, Him Mash-1 x PRR-1 & Him Mash-1 x PRR-2 were found to be significantly superior over other remaining cross combinations with respect to per cent radicle formation. F<sub>1</sub> seeds of cross Him Mash-1 x VRB-3 showed maximum response on salt solution with respect to radicle formation (44.60 per cent) followed by cross combination Palampur-93 x PRR-2 (38.30 per cent) & Him Mash-1 x PRR-1 (23.89 per cent) (Table 3). As per the results, the 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-2 & Him Mash-1 x PRR-1 were significantly superior over other remaining cross combination with respect to per cent hybrid plants obtained. Maximum number of hybrid plantlets produced was of cross Palampur-93 x PRR-2 (17.02 per cent) followed by Him Mash-1 x VRB-3 (15.32 per cent). Some crosses showed only radicle formation but no hybrid plant production. Present results are in concordance with the findings of Bindra *et al.* (2020), they reported germination percentage upto 59.34

per cent in *V. mungo* x *V. umbellata* hybridization whereas, Basavaraja *et al.* (2018) found germination percentage of 36.84 per cent in interspecific crosses of mungbean & ricebean. Pandiyan *et al.* (2010) noted germination percentage ranged from 0-60.00 per cent in interspecific crosses of *V. radiata* with 13 wild *Vigna* species. Further Pandiyan *et al.* (2012) reported hybrid germination upto 34.21 per cent in *V. radiata* x *V. trilobata* crosses, Lekhi *et al.* (2017) found germination percentage in the range of 0- 30.56 per cent in interspecific crosses of urdbean and mungbean. Some of the F<sub>1</sub> seeds did not imbibe, some showed distorted cotyledons, poor root development whereas

in some cases roots developed but died before shoot formation so success rate in germination was low. Similar results are also reported by Mittal *et al.* (2005) in interspecific crosses of urdbean & ricebean. The parents involved in interspecific hybridization showed differential genotypic response which indicates the use of more number of genotypes and large number of crosses should be attempted to get more F<sub>1</sub> plants. Differential genotypic response of parents involved in interspecific hybridization also reported by Bindra *et al.* (2020). The crosses showing substantially high per cent of germination can be utilized for genetic improvement of urdbean.

**Table 1. Response of different growth media on germinability of F<sub>1</sub> seeds of urdbean and ricebean**

Sr. No.	Media used	Seeds cultured	Seeds germinated
1.	Autoclaved soil	30	0
2.	Salt solution	30	16
3.	MS-Full strength	30	0
4.	MS- Half strength	30	0
5.	Gamborg's B5 Media	30	0
6.	MS Basal Media (MS salts + B <sub>5</sub> vitamins)	30	0

**Table 2. Classification of F<sub>1</sub> seeds of interspecific crosses of urdbean x ricebean**

S.N.	Name of cross	Total seeds of cross	No. of shriveled seeds	Per cent of shriveled seeds	No. of very shriveled seeds	Per cent very shriveled seeds	No. of minute seeds	Per cent minute seeds	No. of seeds showed radicle formation	Per cent seeds showed no radicle formation	No. of plants showed yellowing after germination	Per cent plant showed yellowing
1.	Palampur-93 x PRR-2	235	140	59.57	58	24.68	37	15.74	145	61.70	70	29.79
2.	Him Mash-1 x VRB-3	222	152	68.47	34	15.32	36	16.22	142	63.96	79	35.59
3.	PDU-1 x PRR-2	203	118	58.13	22	10.84	63	31.03	167	82.27	30	14.78
4.	Palampur-93 x PRR-1	160	87	54.38	43	26.88	30	18.75	138	86.25	17	10.63
5.	Palampur-93 x VRB-3	90	51	56.67	10	11.11	29	32.22	85	94.44	5	5.56
6.	Him Mash-1 x PRR-1	180	75	41.67	30	16.67	75	41.67	138	76.67	35	19.44
7.	Him Mash-1 x PRR-2	170	103	60.59	28	16.47	39	22.94	136	80.00	25	14.71
8.	HPBU-111 x VRB-3	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
9.	HPBU-111 x PRR-1	70	32	45.71	20	28.57	18	25.71	68	97.14	2	2.86
10.	HPBU-111 x PRR-2	65	29	44.62	30	46.15	6	9.23	63	96.92	1	1.54
11.	UG-218 x PRR-1	27	9	33.33	10	37.04	8	29.63	26	96.30	1	3.70
12.	UG-218 x PRR-2	51	25	49.02	19	37.25	7	13.73	47	92.16	4	7.84
13.	UG-218 x VRB-3	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
14.	PDU-1 x VRB-3	85	41	48.24	18	21.18	26	30.59	26	30.59	0	0.00
15.	PDU-1 x PRR-1	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00



**Table 3. Per cent radicle formation and hybrid plants production in interspecific crosses of urdbean & ricebean**

Sr. No.	Cross	Number of Seeds Cultured in salt solution	Number of Seeds showing radicle formation	Per cent radicle formation	Number of interspecific plantlets obtained	Per cent hybrid plants obtained
1.	Palampur-93 x PRR-2	235	90	38.30 **	40	17.02 **
2.	Him Mash-1 x VRB-3	222	99	44.60 **	34	15.32 **
3.	PDU-1 x PRR-2	203	36	17.73 **	17	8.37 **
4.	Palampur-93 x PRR-1	160	22	13.75	13	8.13 **
5.	Palampur-93 x VRB-3	90	5	5.56	0	0.00
6.	Him Mash-1 x PRR-1	180	42	23.89 **	19	10.56 **
7.	Him Mash-1 x PRR-2	170	34	20.00 *	11	6.47 *
8.	HPBU-111 x VRB-3	0	0	0.00	0	0.00
9.	HPBU-111 x PRR-1	70	2	2.86	0	0.00
10.	HPBU-111 x PRR-2	65	2	3.08	0	0.00
11.	UG-218 x PRR-1	27	1	3.70	0	0.00
12.	UG-218 x PRR-2	51	4	7.84	0	0.00
13.	UG-218 x VRB-3	0	0	0.00	0	0.00
14.	PDU-1 x VRB-3	85	5	5.88	1	1.18
15.	PDU-1 x PRR-1	0	0	0.00	0	0.00
	TOTAL	1558	342		134	

P≤0.01; \*\* ; P≤0.05; \* = significantly positive; Mean=17.17, SE±=3.28 for per cent radicle formation

P≤0.01; \*\* ; P≤0.05; \* = significantly positive; Mean=8.05, SE±=2.43 for per cent hybrid plants obtained

## Conclusion

The present study revealed the operation of post fertilization barriers such as retarded embryo & endosperm development, production of inviable & shriveled seeds, inability of seed to form radicle and yellowing of plantlets. Even though the fertilization barriers were predominant, some interspecific hybrids were produced. Salt solution was most efficient growth media for germinability. F<sub>1</sub> seeds of cross Him Mash-1 x VRB-3 showed maximum response on salt solution

with respect to radicle formation. Cross Palampur-93 x PRR-2 produced maximum number of hybrid plantlets. The parents involved in interspecific hybridization showed differential genotypic response which indicates the use of more number of genotypes and large number of crosses should be attempted to get more F<sub>1</sub> plants. The crosses showing substantially high per cent of germination can be utilized for genetic improvement of urdbean.

## References

- Ali M, Gupta S, Singh BB and Kumar S. 2006. Role of plant introduction in varietal development of pulses in India. *Indian Journal of Plant Genetic Resources* **19**: 346-352.
- Anonymous. 2018. Data on pulses IIPR Kanpur. <http://iipr.res.in/e-pulse-data-book.html>
- Basavaraja T, Murthy N, Kumar VL and Mallikarjun K. 2018. Studies on cross compatibility in interspecific crosses of *Vigna radiata* x *Vigna umbellata* species. *Legume Research* DOI:10.18805/LR-3974.
- Bharathi A, Selvaraj KSV, Veerabathiran P and Laksh BS. 2006. Crossability barriers in mungbean (*Vigna radiata* (L.) Wilczek): with its wild relatives. *Indian Journal of Crop Science* **1**: 120-124.
- Bhareti P, Singh DP and Khulbe RK. 2011. Genetic variability and association analysis of advanced lines and cultivars following intervarietal and interspecific crosses in blackgram. *Crop Improvement* **38**: 67-70.
- Bindra S, Mittal RK, Sood VK and Chaudhary HK. 2020. Alien Introgression studies involving *Vigna mungo* x *Vigna umbellata* hybridization. *International Journal of Current Microbiology and Applied Sciences* **9** (02): 268-276.
- Biswas MR and S Dana. 1975. Blackgram x ricebean cross. *Cytologia* **40**: 787-795.
- Chaisan T, Somta P, Srinives P, Chanprame S, Kaveeta R, Dumrongkittikule S. 2013. Development of tetraploid plants from an interspecific hybrid between mungbean (*Vigna radiata*) and ricebean (*Vigna umbellata*). *Journal of Crop Science and Biotechnology* **16**: 45-51.
- Gomez KA and Gomez AA. 1984. Problem data. In: *Statistical procedures for Agricultural Research*. Wiley-Interscience Publication, John Wiley & Sons, Singapore. p272-315.
- Gopinathan MC, Babu CR and Shivanna KR. 1986. Interspecific hybridization between ricebean (*Vigna umbellata*) and its wild relative (*V. minima*), fertility sterility relationships. *Euphytica* **35**: 1017-1022.
- Gosal SS and Bajaj YPS. 1983. Interspecific hybridization between *Vigna mungo* and *Vigna radiata* through embryo culture. *Euphytica* **32**: 129-137.
- Lekhi P, Gill RK, Kaur S and Bains TS. 2017. Generation of interspecific hybrids for introgression of mungbean yellow mosaic virus resistance in *Vigna radiata* (L.) Wilczek. *Legume Research* **41** (4):526-531.
- Malik BA. 1994. Grain legumes. In: *Crop Production* (Ed.): MS Nazir. National Book Foundation, Islamabad. p 301.
- Mittal RK, Katna G and Sood BC. 2005. Interspecific hybridization in the genus *Vigna*. In: *Proceedings of Fourth International Food Legumes Research*, Oct.2005, New Delhi.
- Mittal RK, Sood BC, Sharma R and Katna G. 2008. Interspecific hybridization and DNA based polymorphism in urdbean (*V. mungo*), ricbean (*V. umbellata*) and adzukibean (*V. angularis*). Abst. The Third Asian Chromosome Colloquium, *Chromosome Science* **11**: 48.
- Pandiyan M, Senthil N, Suresh R, Chakravarthy N, Packiraj D and Jagadeesh S. 2012. Interspecific hybridization of *Vigna radiata* x *V. trilobata*. *Wudpecker Journal of Agricultural Research* **1**: 233-234.
- Pandiyan M, Senthil N, Ramamoorthi N, Muthiah AR, Tomooka N, Duncan V and Jayaraj T. 2010. Interspecific hybridization of *Vigna radiata* x 13 wild *Vigna* species for developing MYMV donar. *Electronic Journal of Plant Breeding* **1**: 600-610.
- Rashid KA, Smartt J and Haq N. 1987. Hybridization in the genus *Vigna*. In: *Mungbean*, Proceeding of the Second International Symposium, AVRDC, Shanhua, Taiwan p 205-214.
- Sanders MR, Franzke CJ and Ross JG. 1959. Influence of environmental factors on origin of colchicine-induced true-breeding diploid mutants in sorghum. *American Journal of Botany* **46**: 119-125.
- Sehrawat N, Yadav M, Bhat KV, Sairam RK and Jaiwal PK. 2016. Introgression of mungbean yellow mosaic virus resistance in *Vigna mungo* (L.) Hepper and purity testing of F<sub>1</sub> hybrids using SSRs. *Turkish Journal of Agriculture and Forestry* **40**: 95-100.
- Singh BM, Sood AK and Saharan GS. 1978. Occurrence of leaf spot and blight of blackgram caused by *Colletotrichum dematium*. *Indian Phytopathology* **31**: 100-101.
- Zeven AC and De Wet JMJ. 1982. *Dictionary of cultivated plants and their regions of diversity*. Centre for Agricultural Publication and Documentation, Wageningen.