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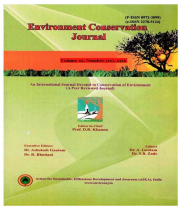

Development of interspecific hybrids between urdbean & mungbean

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ABSTRACT

Interspecific hybridization was performed between five genotypes of urdbean & three genotypes of mungbean to check the crossability relationship. Thirteen cross combinations of urdbean x mungbean were successfully developed. Interspecific seeds showed no germination under soil conditions so various growth media were used for germination. Interspecific seeds germinated only on the salt solution. F_1 seeds of cross UG-218 x Suketi exhibited highest crossability and showed maximum response on salt solution. The study put emphasis on the different kinds of fertilization barriers. In future genetic improvement studies can be carried out with the genotypes showing substantially high percent of crossability.

Introduction

Protein is an important source of individual diet which is mostly taken form milk, eggs and pulses. The fourth most important food legume in India is Urdbean [*Vigna mungo* (L.) Hepper] also known as blackgram, black lentil, mash, mungo bean belongs to family *Leguminoseae* and subfamily *Papilionaceae*, domesticated from *V. mungo* var. *silverstris* (Bhareti *et al*., 2011). Urdbean is short duration, self-pollinated crop and found in most of parts of India. India is center of genetic diversity for urdbean with its secondary center of origin in Central Asia (Singh *et al*., 2016).

Urdbean is valuable source of protein, minerals and amino acids like lysine and methionine, as well as vitamins like niacin, riboflavin and thiamine, as well as phosphorus and iron (Gill *et al*., 2017). Plant parts are used as fodder for animals and green manuring. India is the world's greatest producer and consumer of urdbean, producing 2.93 million tonnes per year from 4.49 million hectares of land, with an average productivity of 500 kg per hectare (Anonymous, 2019). In Himachal Pradesh, it is mainly cultivated on low and mid hills & grown as intercrop with maize as well as a monocrop. However, yield potential of urdbean is low as compared to other grain legumes. Narrow genetic base of urdbean often results in low yield and productivity due to poor plant type, cultivation in marginal and harsh environment, common ancestry of various superior genotypes and its vulnerability to abiotic and biotic stresses viz., Cercospora leaf spots (*Cercospora canescens, C. cruenta*), anthracnose (*Colletotrichum truncatum*), powdery mildew (*Erysiphe polygoni*), and Mung Bean Mosaic Viruses (Ali *et al.,* 2006). The related species *V. radiata* (mungbean) has been found to be nutritive, easily digestible & early maturity as compared to urdbean. As urdbean is self-pollinated crop to get its better understanding of crossability relationship among the species is helpful in chosing methods for making successful crosses. Interspecific seeds developed shows no germination under normal soil condition due to which different media composition were attempted in the present

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investigation to check the efficacy of growth media's on germination of interspecific seeds and for studying the crossability of *V. mungo* with *V. radiata*.

Material and Methods

For the study, a total eight different genotypes i.e. five of blackgram (HPBU-111, Him Mash-1, Palampur-93, UG-218 & PDU-1) taken as female and three of mungbean (Suketi, SML-668 & ML-818) taken as male were used to study the crossability relationship & germinability of their hybrids (Table 1).

Table 1: List of genotypes along with their sources used in the study.

Species	Genotype	Source
Urdbean	Palampur-93	CSK HPKV, Palampur
	Him Mash-1	DPU 91-5 x Mash 338
	HPBU-111	CSK HPKV, Palampur
	UG-218	IIPR Kanpur
	PDU-I	Selection from IC-8219
Mungbean	Suketi	CSKHPKV Palampur
	SML-668	Selection from AVRDC material
	ML-818	5145/87 x ML 267

During summer & *Kharif* 2017 & summer 2018, staggered sowings were done at interval of 10 days starting from 15th March to 31st July to have synchronized flowering in the glasshouse of Department of Genetics & Plant Breeding, COA, CSKHPKV Palampur located at an elevation of 1,290 m above mean sea level with geographical co-ordinates of $36^0$6'N latitude and $76^0$3'E longitude representing the mid-hill zone of Himachal Pradesh and is characterized by humid sub-temperate climate with high rainfall (2,500 mm per annum). Crossing was performed from 15th April to 15th October of 2017 & 15th April to 30th June of 2018. In evening, the emasculation of female parent(s) at plump bud stage was done (3:00 – 5:30 P.M.) followed by pollination in morning (6:00 to 8:00 A.M.). Three immuno- suppressants i.e. giberellic acid (GA_3), 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. Fifteen interspecific crosses of urdbean x mungbean were attempted. Observations on number of buds pollinated and number of pods harvested were recorded to calculate the crossability percentage. The seeds obtained from the interspecific crosses were grown on various media i.e. Salt solution (Sander *et al*., 1959), Gamborg B5 and Half & full strength MS medium to study the response of different growth media on germinability of F_1 seeds. Under aseptic conditions, F_1 seeds were surface sterilized with 0.02 % 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. Petri plates with sterile F_1 seeds were placed in incubator at 25±1ºC for four to five days and salt solution was changed every day under sterile conditions. On second transfer on fresh salt solution seed coat of imbibed F_1 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 which were then transferred to field and glasshouse after the development of cotyledonary leaves (Figure 1). Data were recorded with respect to:

- Number of buds pollinated
- Number of pods harvested
- Total seeds harvested of each cross
- Total seeds cultured
- Number of seeds showing radicle formation
- Number of interspecific plantlets obtained

Crossability percentage was calculated as follows:

$$\text{Crossability percentage (\%)} = \frac{\text{Number of crossed pods set}}{\text{Total number of urdbean buds pollinated}} \times 100$$

% radicle formation (Germination Percentage) & % hybrid plants obtained was calculated as follows:

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

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

Simple t-test

Simple t-test was performed to test the mean difference of radicle and hybrid plant production in the study.

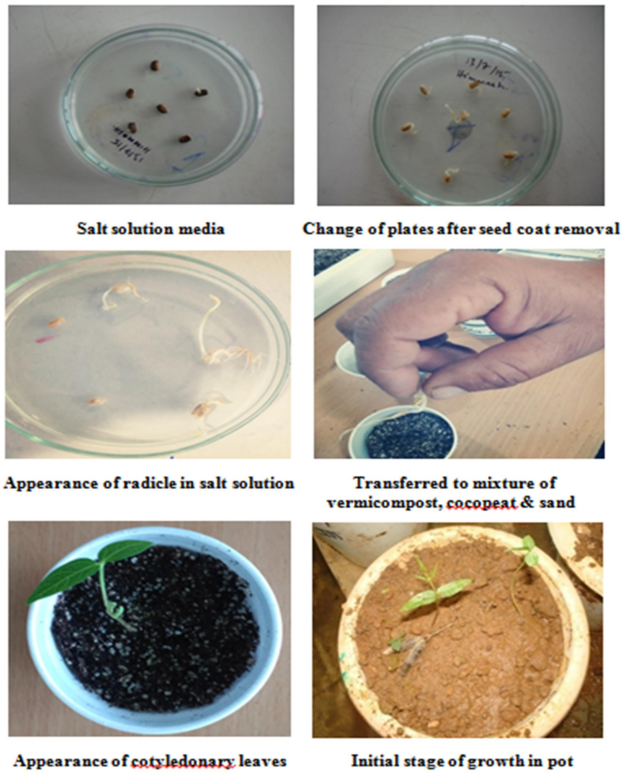

Figure 1: Germination of F_1 seeds

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 study was taken with the objective to study the crossability relationship between urdbean & mungbean and to study the efficacy of different growth media on germination of interspecific seeds. There is high incidence of abscission of crossed flowers within 72 hours from pollination & young fruits dropping between 3 to 30 days after pollination implying the presence of fertilization barriers. Some of pods harvested had no seed or had very minute seeds. The range of crossability percentage was observed to be 0-19.64 % in case of urdbean x mungbean hybridization. The analysis of results revealed that cross combinations UG-218 x Suketi, Palampur-93 x Suketi, Him Mash-1 x ML-818, HPBU-111 x Suketi & PDU-1 x SML-668 were found to be significantly superior over other crosses. Interspecific cross combination UG-218 x Suketi exhibited highest crossability (19.64 %) followed by Palampur-93 x Suketi (19.25 %) and Him Mash-1 x ML-818 (18.54 % in case of *V.mungo* x *V. radiata* hybridization (Table 2). Crosses having high crossability percentage were considered as successful crosses suggesting the parents of these cross combinations are ideal for transfer of useful genes from one species to another species. Similar results in relation to crossability were also reported by various research workers viz; Bhanu *et al*., (2018) in *V. mungo* x *V. umbellata* with 16.27 % & in *V. mungo* x *V. radiata* with 37.50 % and Lekhi *et al*., (2017) in *V. mungo* x *V. radiata* with 5.50 to 24.10 %. The percent crossability among different sets of crosses varies from species to species due to substantial heterogeneity in the genetic architecture of species involved in interspecific hybridization, resulting in differences in cross compatibility. Some of the pods which were formed were without seed or had shriveled seeds with ruptured seed coat. In the crosses which had HPBU-111 as one of the parent had large number of empty pods. F_1 seeds developed were of two types, viz. highly shriveled, minute, brown coloured and the second was bold and comparatively brown coloured but very weak as compared to self-ones. The number of seeds per pod in the interspecific hybrids varied from 1-4. The results are in agreement with the earlier studies of Sehrawat *et al*. (2016a) for number of F_1 seeds per pod in interspecific crosses of urdbean and ricebean. The F_1 seeds obtained from all cross combinations were small, wrinkled and shrunken (Figure 2). The F_1 seeds 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 or due to the failure of embryo to reach maturity (Rashid *et al*., 1987). Even though crossability barriers were present, few interspecific hybrids were produced. The seeds obtained from the interspecific crosses under study shows no

germination under normal soil conditions so attempts were made to grow them on various media. To study the response of different growth media on germination of interspecific seeds, 30 seeds comprising of all the cross combinations of urdbean & mungbean were put on different growth media i.e. Salt solution, Gamborg B5 media and

Table 2: Pod set & Crossability percentage in *V. mungo* and *V. radiata* crosses

SN	Cross Combination	Number of buds emasculated & pollinated	Number of pods harvested	Total Seeds Collected	Crossability percentage
1.	UG-218 x Suketi	331	65	235	19.64**
2.	Palampur-93 x Suketi	322	62	226	19.25**
3.	Him Mash-1 x ML-818	302	56	205	18.54**
4.	Him Mash-1 x Suketi	314	39	166	12.42
5.	Him Mash-1 x SML-668	307	22	128	7.17
6.	HPBU-111 x Suketi	309	41	149	13.27*
7.	HPBU-111 x SML-668	313	17	99	5.43
8.	HPBU-111 x ML-818	310	29	136	9.35
9.	Palampur-93 x ML-818	304	0	0	0.00
10.	Palampur-93 x SML-668	319	16	98	5.02
11.	UG-218 x SML-668	312	20	117	6.41
12.	UG-218 x ML-818	315	15	91	4.76
13.	PDU-1 x Suketi	318	26	125	8.18
14.	PDU-1 x SML-668	316	50	210	15.82**
15.	PDU-1 x ML-818	306	0	0	0.00

****, * = significantly positive at 1 & 5 % level of significance; Mean=16.46, SE± =2.12**

Figure 2: Interspecific F_1 pods & seeds

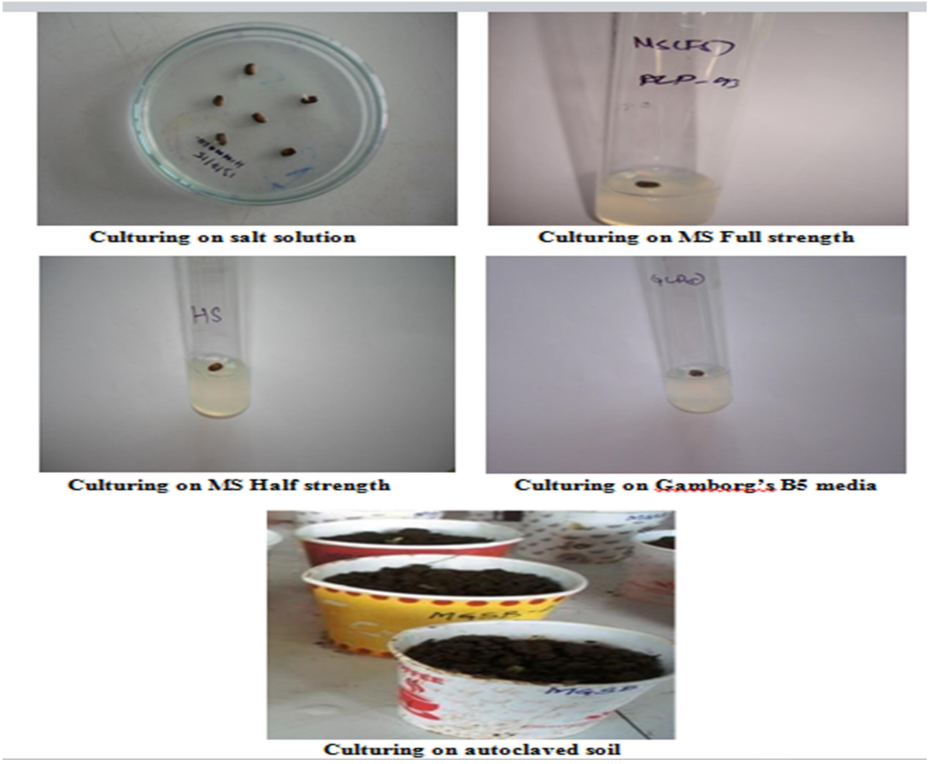

Figure 3: Germination of interspecific F_1 seeds in different media.

Half & full strength MS medium (Figure 3). Successful results were only obtained on the salt solution, in rest of media's seeds of interspecific crosses of urdbean and mungbean showed no germination (Table 3). Mittal *et al*. during 2005 and 2008 obtained similar results in interspecific crosses between urdbean and ricebean. The range of % radicle & hybrid plant production were found to be 0-25.56 % & 0-17.49 % respectively. The analysis of results revealed that cross combinations UG-218 x Suketi, Palampur-93 x Suketi, Him Mash-1 x ML-818 & PDU-1 x SML-668 were found to be significantly superior for % radicle formation. As per the present results, the response of cross combination UG-218 x Suketi (25.56 %) was maximum with respect to radicle formation followed by cross Palampur-93 x Suketi (19.63 %) & PDU-1 x SML-668 (19.20 %) respectively. Cross combinations UG-218 x Suketi, Palampur-93 x Suketi, Him Mash-1 x ML-818, Him Mash-1 x Suketi, HPBU-111 x Suketi & PDU-1 x SML-668m were significantly superior for % hybrid plants obtained. Radical formation & hybrid plantlet formation in F_1 seeds obtained highest in cross formed between UG-218 x Suketi (17.49 %) followed by Palampur- 93 x Suketi (14.02 %) (Table 4). The results are in agreement with the findings of Bindra *et al*., (2020), they reported germination percentage upto 59.34 % in *V. mungo* x *V. umbellata* hybridization whereas, Basavaraja *et al*., (2018) found germination percentage of 36.84 % in interspecific crosses between *V. radiata* & *V. umbellata*, Lekhi *et al*., (2017) noted germination percentage upto 30.56 % in interspecific crosses of urdbean and mungbean. Some of the F_1 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. Mittal *et al*., (2005) reported similar results 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_1 plants. Differential genotypic response of parents involved in interspecific hybridization also reported by Mittal *et al*. (2005).

Table 3: Response of different growth media + Autoclaved Soil on germination of interspecific F_1 seeds of urdbean and mungbean

SN	Media used	Seeds cultured	Seeds germinated
1.	Autoclaved soil	30	0
2.	Salt solution (Sanders et al. 1959)	30	11
3.	MS-Full strength	30	0
4.	MS- Half strength	30	0
5.	Gamborg's B5 Media	30	0

Table 4: % radicle formation and hybrid plants production in interspecific crosses of urdbean & mungbean

SN	Name of Cross	Seeds Cultured (No.)	Seeds showing radicle formation (No.)	Radicle formation (%)	Interspecific plantlets obtained (No.)	Hybrid plants obtained (%)
1.	UG-218 x Suketi	223	57	25.56**	39	17.49**
2.	Palampur-93 x Suketi	214	42	19.63**	30	14.02**
3.	Him Mash-1 x ML-818	193	30	15.54*	18	9.33**
4.	Him Mash-1 x Suketi	154	18	11.69	10	6.49*
5.	Him Mash-1 x SML-668	116	10	8.62	0	0.00
6.	HPBU-111 x Suketi	137	18	13.14	9	6.60*
7.	HPBU-111 x SML-668	87	5	5.75	0	0.00
8.	HPBU-111 x ML-818	124	11	8.87	0	0.00
9.	Palampur-93 x ML-818	0	0	0.00	0	0.00
10.	Palampur-93x SML-668	86	5	5.81	0	0.00
11.	UG-218 x SML-668	105	7	6.67	0	0.00
12.	UG-218 x ML-818	79	3	3.80	0	0.00
13.	PDU-1 x Suketi	113	11	9.82	0	0.00
14.	PDU-1 x SML-668	198	38	19.20**	27	13.64**
15.	PDU-1 x ML-818	0	0	0	0	0.00

****, * = significantly positive at 1 & 5 % level of significance; Mean=16.92, SE± =2.23 for % radicle formation**
****, * = significantly positive at 1 & 5 % level of significance; Mean=7.72, SE± =2.61 for % hybrid plants obtained**

Conclusion

The study put emphasis on the different kinds of fertilization barriers that are responsible for complete sterility to low fertility. Even though the fertilization barriers were predominant, some interspecific hybrids were produced. Salt solution was most efficient growth media for germinability. 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_1 plants. In future genetic improvement studies can be carried out with the genotypes showing substantially high percent of crossability.

Conflict of interest

The authors declare that they have no conflict of interest.

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