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Shailja Sharma
Department of Crop Improvement, COA, CSKHPKV Palampur, Himachal Pradesh, India

RK Mittal
Department of Crop Improvement, COA, CSKHPKV Palampur, Himachal Pradesh, India

VK Sood
Department of Crop Improvement, COA, CSKHPKV Palampur, Himachal Pradesh, India

Sanchit Thakur
Department of Crop Improvement, COA, CSKHPKV Palampur, Himachal Pradesh, India

Corresponding Author:
Shailja Sharma
Department of Crop Improvement, COA, CSKHPKV Palampur, Himachal Pradesh, India

Studies on efficacy of various *in vivo* and *in vitro* techniques on crossability and pod setting percentage in *Vigna mungo* x *V. umbellata* hybridization

Shailja Sharma, RK Mittal, VK Sood and Sanchit Thakur

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Abstract
The present investigation was carried out with objective to study the efficacy of various *in vitro* and *in vivo* techniques for the success of interspecific hybridization between urdbean and ricebean. Interspecific crosses were attempted between three genotypes of urdbean i.e. HPBU-111, Him Mash-1 & Palampur-93 and three genotypes of ricebean i.e. PRR-1, PRR-2 & VRB-3.The study of effect of immuno-suppressants revealed that amino caproic acid at 1000 ppm was found to be the most efficient immuno-suppressant in all the three combination for achieving maximum per cent of pod set. Four minutes of UV irradiation treatment to pollen gave maximum pod setting in Palampur-93 x PRR-2 and Him Mash-1 x VRB-3. The present study revealed the presence of pre-fertilization barriers in interspecific crosses between urdbean & ricebean and were confirmed by the low frequency of pod set. Both the parents involved in interspecific hybridization shows differential genotypic response which indicates the use of more number of genotypes of urdbean and ricebean.

Keywords: Urdbean, ricebean, immuno-suppressants, UV irradiation, interspecific hybridization

Introduction
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 *Leguminoseae* and subfamily *Papilionaceae*, with its wild progenitor, *V. mungo* var. *silverstris* (Bhareti *et al*. 2011) [5]. It has been believed to have originated in India and secondary centre of origin in central Asia (Pratap and Kumar 2011) [14]. It is a short duration pulse crop and self pollinated grain legume grown in many parts of India. 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.
India is the largest producer as well as consumer of urdbean produces about 1.5 million tonnes of urdbean annually from about 3.25 million hectare of area with an average productivity of 4 quintal per hectare (Anonymous, 2018) [4]. Major growing states of urdbean are Maharashtra, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh, Rajasthan, Karnataka and Himachal Pradesh. 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 their cultivation in marginal and harsh environment (Ali *et al*. 2006) [3]. It is susceptible to various leaf spotting pathogens such as *Cercospora canescens*, *Cercospora cruenta*, *Colletotrichum truncatum* and *Erysiphe polygoni* in high rainfall areas in the mid hills of North Western Himalayas resulting in drastic reduction in grain yield.
Stepwise utilization of primary, secondary and tertiary gene pools of urdbean can result in tremendous improvement in yield. (Pandiyan *et al*. 2010) [13]. The use of underutilized related species i.e. *V. umbellata* (Ricebean) is more desirable for introgression breeding due to no linkage drag of undesirable traits such as pod dehiscence and it is widely consumed by human than the other wild species (Watanasit and Pichitporn 1996) [17]. These species were found in Western Ghats, Eastern Ghats and Northern Western Himalayas, which act as a potential source of resistance to diseases such as *Cercospora* leaf spots, anthracnose, powdery mildew and mung bean yellow mosaic virus (MYMV) and to insect pest such as bruchids (Monika *et al*. 2005) [12].

For the first time, interspecific hybrids of *V. mungo* x *V. umbellata* were classified as a partially compatible cross by Al- Yasiri and Coyne (1966) [2] in which pods collapse in early stages of development and the reciprocal cross as incompatible in which no pods set. Ahn and Hartman (1978) [1] successfully obtained wide hybrids of urdbean and ricebean but found it a very difficult combination to produce. Varying degree of success in interspecific hybrids of *V. mungo* x *V. umbellata* has been reported by various workers viz. Chowdhury and Chowdhury (1977) [7], Ahn and Hartmann (1978) [1], Chen *et al*. (1983) [6], Rashid *et al*. (1987) [15] and Mittal *et al*. (2005, 2008 and 2010) [9, 10, 11].

There are certain pre and post fertilization barriers that hamper wide hybridization and seed development. Therefore, the present investigation had been carried out with objective to study the efficacy of various *in vitro* and *in vivo* techniques for the success of interspecific hybridization between urdbean and ricebean.

Material and Methods

The experimental material for present investigation included three genotypes of urdbean i.e. HPBU-111, Him Mash-1 & Palampur-93 and three genotypes of ricebean i.e. PRR-1, PRR-2 & VRB-3. During summer & *Kharif* 2017 & 2018, staggered sowing of urdbean and ricebean genotypes were done at interval of 10 days starting from 15th March to 31st July to have synchronized flowering in the glasshouse of Department of Crop Improvement. The interspecific hybridization work was conducted between urdbean as female and ricebean as male. Crossing was performed from 15th May to 15th October. 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.).

Application of immuno- suppressants

Three immuno- suppressants i.e. giberellic acid (GA_3), indole acetic acid (IAA) and amino caproic acid were used at two concentrations (500 ppm & 1000 ppm). The immuno-suppressants were applied to cotton pad with the help of syringe at the base of pedicel of the pollinated bud 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.

Use of irradiated pollen

Mature pollen of male parent (ricebean) were treated with UV light (0.5 x 10^4 erg/cm^2 /sec) under laminar air flow for 1 min, 2 min, 3 min, 4 min and 5 minutes. These treated pollen were used to pollinate the emasculated immature buds.

Embryo rescue

Since, the developing F_1 s aborted/dropped so, embryo rescue technique was undertaken. The embryos were dissected out, surface sterilized with 70% alcohol followed by three to four washing in autoclaved sterile distilled water and culturing on half strength and full strength MS media under laminar air flow. In half strength the constituent of major and minor salt was reduced to half with same volume.

Results

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 *in vitro* and *in vivo* techniques on the crossability and pod setting percentage.

Effect of immuno – suppressant on crossability and pod setting percentage

Three immuno – suppressants GA_3 (500, 1000 ppm), amino caprioc acid (500, 1000 ppm) and IAA (500, 1000 ppm) were sprayed in the morning and evening daily on female parent at the premeiotic stage for 15 days. These chemicals were used on assumption that an active principle may be synthesized in the leaf which causes or control hybrid embryo abortion and this incompatibility reaction may be analogous to an "immune- response". This immune response could perhaps, be suppressed by the use of immuno – suppressants.

When the female parents in the cross combinations viz. Palampur-93 x PRR-2, Him Mash-1 x VRB-3 and HPBU-111 x PRR-1 were subjected to GA_3 (500 ppm) treatment, Him Mash-1 x VRB-3 showed highest pod set per cent (30.00) followed by Palampur-93 x PRR-2 and HPBU-111 x PRR-1 i.e. 23.3 per cent and 20.00 per cent respectively but when GA_3 concentration was increased to 1000 ppm highest pod set per cent was found in Palampur-93 x PRR-2 (16.70 per cent) followed by HPBU-111 x PRR-1 and Him Mash-1 x VRB-3 i.e. 10.00 per cent and 6.70 per cent, respectively.

Palampur-93 x PRR-1, HPBU-111 x PRR-1 and Him Mash-1 x VRB-3 when treated with amino caproic acid (500 ppm) and IAA (500 ppm), highest percentage of pod set was observed in Palampur-93 x PRR-2 (23.33, 36.67 per cent) followed by HPBU-111 x PRR-1 (13.33, 26.67 per cent) and Him Mash-1 x VRB-3 (10.00, 20.00 per cent), respectively but when the concentration of both the immuno – suppressant was increased to 1000 ppm, amino caproic acid showed highest pod set percentage in Him Mash-1 x VRB-3 (43.33 per cent) followed by Palampur-93 x PRR-2 (33.33 per cent) and HPBU-111 x PRR-1 (26.67 per cent). In case of IAA, highest pod set was observed in Palampur-93 x PRR-2 (16.67 per cent) followed by Him Mash-1 x VRB-3 (10.00 per cent) and HPBU-111 x PRR-1 (6.67 per cent). Therefore, amino caproic acid (1000 ppm) showed significant results among all the three crosses with highest pod setting percentage in Him Mash-1 x VRB-3 (43.33). Shrivastava and Chawla (1993) [16] reported that treatments of GA_3 significantly increased the pod set and pod harvest by 20 per cent and 34 per cent respectively in *V. mungo* x *V. unguiculata*. Further, Kaushal *et al*. (1988) [8] also found successful results by application of amino caproic acid in cross between *V. mungo* x *V. angularis*.

Table 1: Effect of immuno –suppressants on pod set of *V. mungo* x V. umbellata crosses

Immuno – suppressants	Concentration (ppm)	Cross Combination	Buds emasculate	Pod set	Pod set (%)
GA_3	500	Palampur-93 x PRR-2	30	7	23.30
		Him Mash-1 x VRB-3	30	9	30.00
		HPBU-111 x PRR-1	30	6	20.00
	1000	Palampur-93 x PRR-2	30	5	16.70
		Him Mash-1 x VRB-3	30	2	6.70
		HPBU-111 x PRR-1	30	3	10.00
Amino caproic acid	500	Palampur-93 x PRR-2	30	7	23.33
		Him Mash-1 x VRB-3	30	3	10.00
		HPBU-111 x PRR-1	30	4	13.33
	1000	Palampur-93 x PRR-2	30	10	33.33
		Him Mash-1 x VRB-3	30	13	43.33
		HPBU-111 x PRR-1	30	8	26.67
IAA	500	Palampur-93 x PRR-2	30	11	36.67
		Him Mash-1 x VRB-3	30	6	20.00
		HPBU-111 x PRR-1	30	8	26.67
	1000	Palampur-93 x PRR-2	30	5	16.67
		Him Mash-1 x VRB-3	30	3	10.00
		HPBU-111 x PRR-1	30	2	6.67

Effect of irradiated pollen on crossability

The pollen of male parent (ricebean) was exposed to Ultra-Violet (UV) light at 0.5 x 10^4 erg/cm^2 /sec intensity. Pollen was irradiated for different time intervals ranging from 1,2,3,4 and 5minutes and used to pollinate the female parent after exposure to UV light. The effects of using UV- irradiated pollen of ricebean are shown in Table-2.

Significant pod set (20%) were observed when pollen of PRR-2 and VRB-3 were irradiated for three minutes, in cross combinations Palampur-93 x PRR-2 and Him Mash-1 x VRB-3. Kaushal *et al*. (1988) [8] observed significant improvement in pod formation by using UV irradiated pollen on *V. mungo* x *V. angularis*.

Table 2: Effect of UV irradiated pollen of ricebean for pod setting of blackgram

Irradiation Time (min)	Cross Combination	Buds Pollinated	Pod Set	Pod Set (%)
1	Palampur-93 x PRR-2	10	1	10
	Him Mash-1 x VRB-3	10	1	10
	HPBU-111 x PRR-1	10	0	0
2	Palampur-93 x PRR-2	10	0	0
	Him Mash-1 x VRB-3	10	1	10
	HPBU-111 x PRR-1	10	0	0
3	Palampur-93 x PRR-2	10	1	10
	Him Mash-1 x VRB-3	10	[1]	10
	HPBU-111 x PRR-1	10	1	10
4	Palampur-93 x PRR-2	10	2*	20
	Him Mash-1 x VRB-3	10	2*	20
	HPBU-111 x PRR-1	10	1	10
5	Palampur-93 x PRR-2	10	0	0
	Him Mash-1 x VRB-3	10	0	0
	HPBU-111 x PRR-1	10	0	0

Embryo Rescue

Developing F_1 embryos were cultured on full strength and half strength MS media but no success was obtained in any one of the cross combination.

Conclusion

The present study revealed the presence of pre-fertilization barriers in interspecific crosses between urdbean and ricebean and were confirmed by the low frequency of pod set. Amino caproic acid at 1000 ppm was found to be the most efficient immuno- suppressant in all the three combination for achieving maximum per cent of pod set. Four minutes of UV irradiation treatment to pollen gave maximum pod setting in Palampur-93 x PRR-2 and Him Mash-1 x VRB-3. Both the parents involved in interspecific hybridization shows differential genotypic response which indicates the use of more number of genotypes of urdbean and ricebean.

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