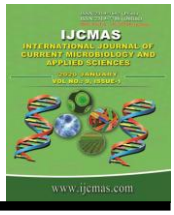
Int.J.Curr.Microbiol.App.Sci (2020) *9*(2): 268-276

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 2 (2020)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2020.902.034

Alien Introgression Studies Involving V*igna mungo* x *Vigna umbellata* Hybridization

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Keywords

Alien introgression, Urdbean, Ricebean, hybridization

Article Info

Accepted:
05 January 2020
Available Online:
10 February 2020

A B S T R A C T

Urdbean has narrow genetic base due to common ancestry of various superior genotypes, poor plant type and genetic vulnerability to biotic and abiotic stresses. The underutilized related specie, *V. umbellata* (ricebean), nutritive and resistant to most of the fungal pathogens, is speculated to be potential donor. Therefore, *V. mungo* and *V. umbellata* were crossed with a view to introgress alien genes. On inter-specific hybridization, the presence of pre-fertilization barriers and post-fertilization barriers were observed. As, hybrids exhibited reduced germination and sterility. The frequency of hybridization, radicle and plant production percentage among three cross combinations revealed genotype specific response between both the species.

Introduction

The grain legume, urdbean (*Vigna mungo* L. Hepper) also known as blackgram, black lentil, mungo bean, black *matpe* bean. It belongs to the sub-genus *Ceratotropis* domesticated from *V. mungo* var. *silvestris.* Low productivity in this crop is attributable to its narrow genetic base due to common ancestry, poor plant type, vulnerability to biotic and abiotic stresses and its cultivation in marginal land and harsh environmental conditions. It is susceptible to various fungal pathogens such as *Cercospora canescens*, *C. cruenta*, *Colletotrichum truncatum*,*Erysiphie polygoni* and Mungbean Yellow Mosaic Virus (MYMV) in high rainfall areas of North-Western Himalayas.

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, there is no other alternative, but to look for alien *Vigna* species to diversify and broaden the genetic base of cultivated germplasm. Introgression of alien genes from wild species would not only minimize the risks of biotic and abiotic stresses but will also make discernible yield advances and quality in the crop.

Pre-breeding practices such as inter-specific hybridization are required particularly involving those wild species carrying useful alien genes for improving yield, quality, biotic and abiotic stress resistance. The underutilized related species,*V. umbellata* (Thunb.) Ohwi and Ohasi (ricebean) with 2n=22 as of urdbean, has been found to be nutritive and resistant to most of the fungal pathogens such as; *Cercospora* leaf spots (Marappa, 2008), *Colletotrichum truncatum, Erysiphie polygoni* and MYMV. Therefore, it is a potential source for economically important traits along with biotic and abiotic stress tolerance, giving a window to breeders to broaden the narrowed genetic base of the crop. With this view the present study was undertaken to develop a new gene pool following *V. mungo* x *V. umbellata* hybridization to introgress genes for desirable traits from *V. umbellata*.

Materials and Methods

Three genotypes each of Urdbean (Him Mash-1, HPBU-111 and Palampur-93) and ricebean (RBL-6, PRR-1 and RBL-1) (Table 1) were utilized in the present study.

Table.1 Parentage/ source of genotypes used for inter-specific hybridization

Species		Genotype(s)	Source/parentage
Vigna mungo	1.	Him Mash-1	Pure line selection from local material of Himachal Pradesh by CSKHPKV, Palampur
	2.	HPBU-111	Pure line selection from local material of Himachal Pradesh by CSKHPKV, Palampur
	3.	Palampur-93	Pure line selection from local material of Himachal Pradesh by CSKHPKV, Palampur
Vigna umbellata	1.	RBL-1	Pure line selection from Rajasthan material by PAU, Ludhiana
	2.	RBL-6	Pure line selection from Rajasthan material by PAU, Ludhiana
	3.	PRR-1	Pureline selection from Jagdhar (Tehri) collections by G.B. Pant University, Pant Nagar

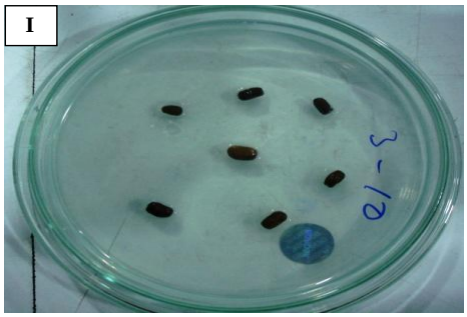
Crosses were attempted under glasshouse conditions between Him Mash-1x RBL-6, HPBU-111 x PRR-1 and Palampur-93 x RBL-1.

In order to synchronize flowering between *V. mungo* and *V. umbellata*, staggered sowings (23 and 17, respectively) were done at an interval of seven days. This was carried out to ensure sufficient availability of flower buds and pollen from *V. Mungo* and *V. umbellate*, respectively. F_1 seeds obtained from *V. mungo* x *V. umbellata* were germinated on sterilized salt solution (Modification of Sanders *et al.*, 1959) (Plate 1) in petri-plates. Ferric citrate was used instead of ferrous sulphate as in the original formula to have better keeping quality of the solution.

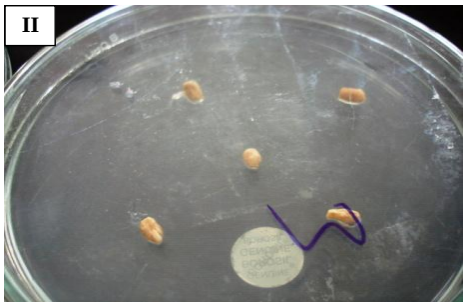
Four per cent sucrose and 0.70 per cent agar was also added to the medium. Before placing the F_1 seed on the medium they were surface sterilized with mercuric chloride followed bythree to four washingswith sterilized distilled water. Petri plates with sterile F_1 seeds were placed in incubator at 25±1ºC for four to five days. The sterilized salt solution was changed every day under sterile conditions.

On second day, seed coat of imbibed F_1 seeds were removed before transferring to fresh salt solution and allowed to develop for one or two days. Four to five days old seeds showing radical formation/seedling were treated with Bavistin and Dithane M45 (1g+2.5g/l) and transferred to sterile soil in paper cups. F_1 seedlings having fully developed cotyledonary leaves were transferred to pots kept under glasshouse conditions.

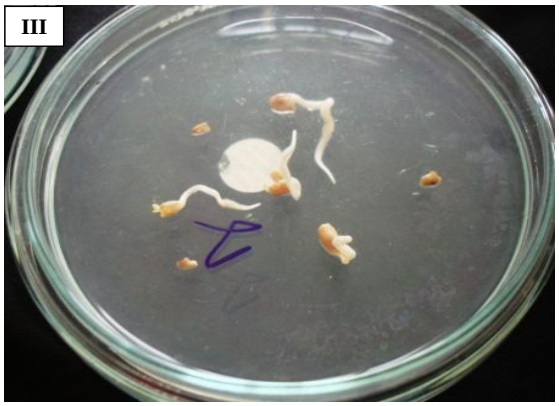
Plate.1 Protocol for germination of F_1 seeds in salt solution



Sub-cultured for two days



Sub-cultured for two days after removal of seed coat on third day



Radicle formation in responsive hybrid seed on fifth day

Results and Discussion

Wide or distant hybridization is mating between individuals of different species of same genus (inter-specific/intra-generic) or different genera (inter-generic). It provides a way to combine diverged genomes or introgression of few desirable genes into one genome by breaking species barrier(s). Thereby, bringing about changes in genotype and phenotype of the progenies.

The underutilized related specie *i.e.V. umbellata* (ricebean) has been found to be nutritive and resistant to most of the fungal pathogens. With a view to broaden the narrowed genetic base of urdbean study was carried out to introgress useful economic traits especially against biotic and abiotic stresses from *V. umbellata*.

Crosses were attempted with over more than 1000 pollinations under glass house conditions amid Him Mash-1x RBL-6, HPBU-111 x PRR-1 and Palampur-93 x RBL-1. It was observed that HPBU-111 x PRR-1 exhibited maximum crossability upto 36.79 per cent (Table 2) followed by Him Mash-1 x RBL-6 (32.72 per cent) and Palampur-93 x RBL-1 (18.01 per cent).

Similar crossability success rate were also reported by various research workers *viz;* Bharathi *et al.,* (2006) in *V. radiata* x *V. umbellata* with 29.63 per cent, 8.48 per cent in *V. radiata* x *V. trilobata*, 7.69 per cent in *V. radiata* x *V. aconitifolia*, Pandiyan *et al.,* (2012) in *V. radiata* x *V. trilobata* upto 10.25 per cent and Nwosu *et al.,* (2013) observed pod set as high as 40.8 per cent in *V. umguiculata* x *V umguiculata* var. spontanea.

Seeds obtained from crossed pods were germinated under *in-vitro* conditions in salt solution- a modification of Sanders *et al.,* (1959). It was observed that response of Him Mash-1 x RBL-6 on salt solution with respect to radicle formation was maximum (59.34 per cent) followed by Palampur-93 x RBL-1 (23.08 per cent) and HPBU-111 x PRR-1 (6.94 per cent) (Table 3).

Maximum number of hybrid plants produced was of Him Mash-1 x RBL-6 (8.65 per cent) while of Palampur-93 x RBL-1 and HPBU-111 x PRR-1 showed minimum (0.77 per cent and 0.46 per cent, respectively). Pandiyan *et al.,* (2012) also reported 34.21 per cent germination of *V. radiata* x *V. trilobata* hybrid.

The developing pods of parents and F_1's were also placed on MS media (Murashige and Skoog, 1962) with a view to achieve embryo formation. But none of them responded except HPBU-111 in which callus formation was observed (Table 4)

Table.2 Cross ability success rate in crosses of *V. mungo* x *V. umbellata*

Cross	Number of flowers pollinated	Number of pods set	Percentage of pod set (%)
Him Mash-1 x RBL-6	1620	530	32.72
HPBU-111 x PRR-1	1052	387	36.79
Palampur-93 x RBL-1	1216	219	18.01

Table.3 Hybrid plant production in *V. mungo* x *V. umbellata*

Cross	Seeds cultured	Seeds showing radicle formation	Per cent radicle formation	Number of inter-specific plantlets obtained	Per cent hybrid plants produced
Him Mash-1 x RBL-6	728	432	59.34	63	8.65
HPBU-111 x PRR-1	216	15	6.94	1	0.46
Palampur-93 x RBL-1	130	30	23.08	1	0.77

Table.4 Response of developing pods of parents and F_1's cultured on MS basal media

Cross	Number of developing pods cultured	Response
Him Mash-1	**10**	**-**
HPBU-111	**10**	**1***
Palampur-93	**10**	**-**
Him Mash-1 x RBL-6	**30**	**-**
HPBU-111 x PRR-1	**15**	**-**
Palampur-93 x RBL-1	**15**	**-**

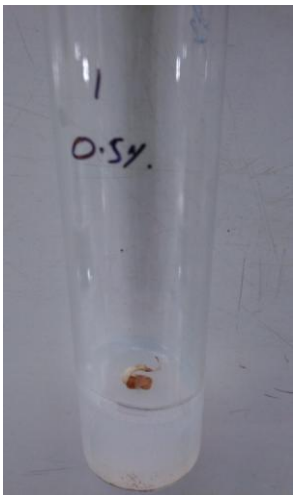
Table.5 Response to colchicine treatment in *V. mungo* x *V. umbellata* hybrid seeds

Cross	Seeds cultured		Response		Seedling formation	
	0.25 %	0.50%	0.25%	0.50%	0.25%	0.50%
Him Mash-1 x RBL-6	15	15	3	4	0	1
HPBU-111 x PRR-1	15	15	3	0	0	0
Palampur-93 x RBL-1	15	15	0	0	0	0

Callus formation

Colchicine treatment was also tried at 0.25 per cent and 0.50 per cent concentration on 15 F_1 seeds for 24 hours in each cross combination (Plate 6). To which three seeds responded by radicle formation each in Him Mash-1 x RBL-6 and HPBU-111 x PRR-1 at 0.25 per cent concentration (Table 5). While four seeds of Him Mash-1 x RBL-6 cross combination at 0.50 per cent out of which only in one case plantlet was produced (Plate 2).

Plate.2 Response to colchicine treatment in *V. mungo* x *V. umbellata* seeds cultured on MS media



Radicle formation in F_1

Seedling formation in F_1

Various morphological characters were recorded for the confirmation of hybridity in plants produced. True hybrids were observed to exhibit hypogeal mode of germination, stem color, flower color and inflorescense as of male parent (ricebean) while lanceolate leaves as of female parent. Since, there was sterility in the F_1's, the morphological characters *viz;* pod shape, pod pubescence, pod length, pod number and 100-seed weight could not be recorded and presented. F_1's exhibited hybrid breakdown at various growth stages *i.e*. seedling, vegetative and flowering stage. Some of the F_1's showed no flowering while others exhibited profuse flowering with pseudo or no pod formation (Plate 3). The F_1's germinated displayed pollen sterility (Plate 4) ranging from 0.00 to 5.47 per cent, a probable reason for no seed set in inter-specific hybrids.

Backcrosses were also attempted with an aim to have seed set, but there was high bud drop with no positive results. None of the hybrid exhibited fertility/ partial fertility inspite being the fact that both the species under study have the same chromosome number *i.e*. 2n= 22.

Plate.3 F_1's showing pseudo-pod formation

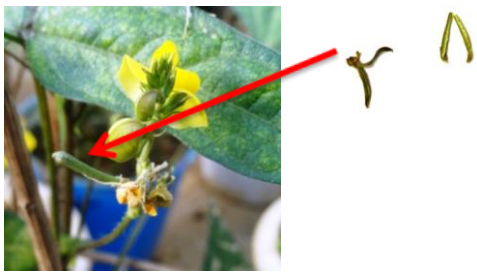
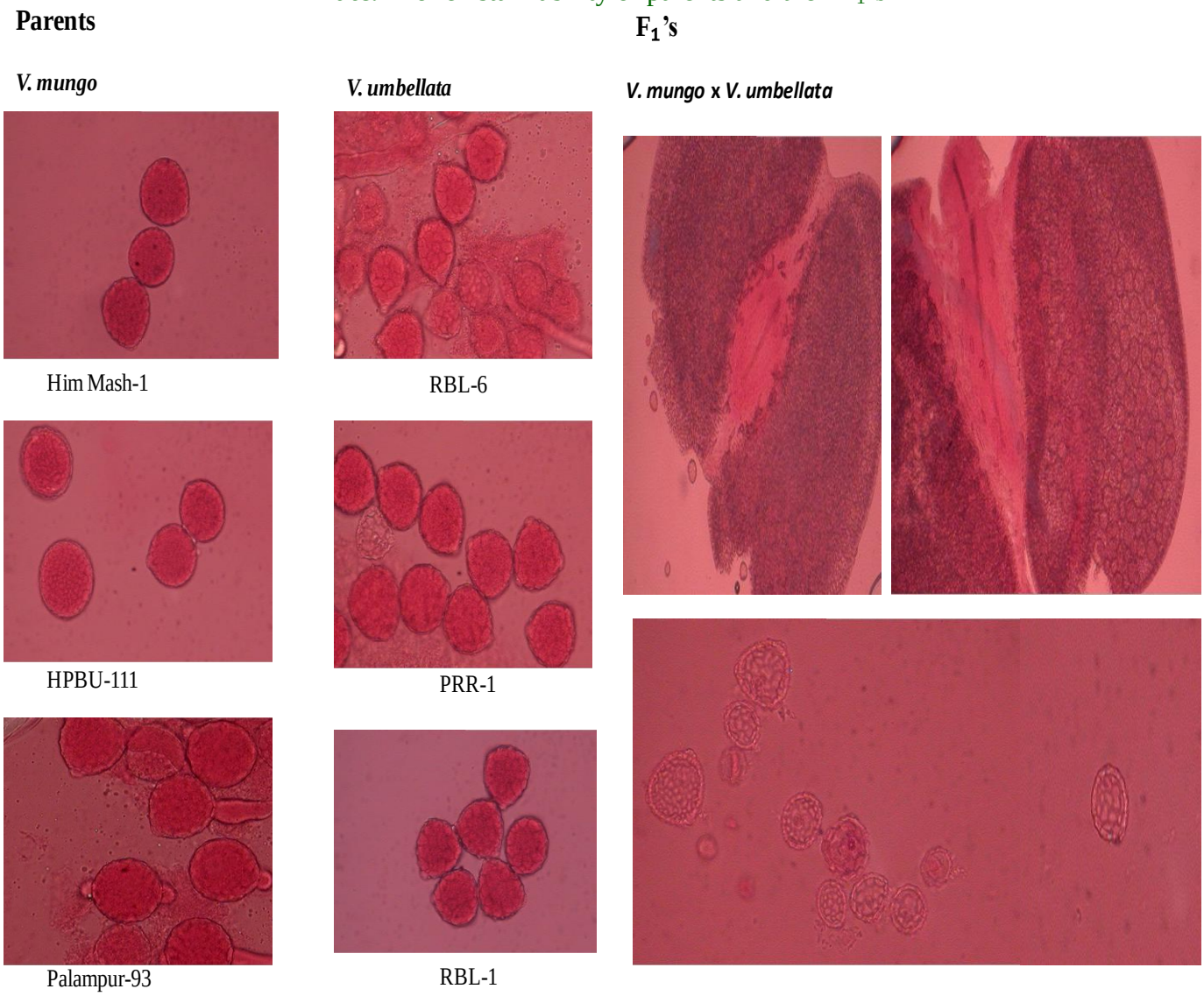


Plate.4 Pollen stain ability of parents and their F_1's



Thiyagu *et al*.(2008) observed low percentage of pod set (5.56%) in *V. mungo* x *V. umbellata* indicating the presence of reproductive barriers that renders introgression. They also found normal pollen grain germination on stigmatic surface but slow pollen tube growth in addition to structural abnormalities in stigmatic and stylar regions.

Pollen sterility was also reported in *V. radiata* x *V.umbellata* crosses by Pandiyan *et al.*, (2008) due to which viable F_2 segregants were not obtained by them as in present study. In *Vigna* crops, inability of pollen tube to germinate and penetrate stigma and style (Chowdhury and Chowdhury, 1977) and ovary (Gopinathan *et al.*, 1986), slow rate of pollen growth (Thiyagu *et al.*, 2008); are reported to be significant pre-fertilization barriers. Post-fertilization barriers of varying degrees have been reported in most of the inter-specific *Vigna* crosses (Gopinathan *et al.*, 1986; Bharithi *et al.*, 2006; Pandiyan *et al.*, 2010; Chaisan *et al.*, 2013; Basavaraja *et al.*, 2018; Bhanu *et al.*, 2018).

Thus, recovery of desirable recombinants is reduced, as hybrids exhibit varying levels of sterility (Rashid *et al.,* 2013), inviability, lethality and genotype specific response (Dhiman *et al.,* 2013).

Inter-specific hybridization revealed the presence of pre-fertilization barriers; confirmed by the frequency of pod set and post-fertilization barriers as F_1's exhibited reduced germination and sterility.

The frequency of inter-specific hybridization, radicle and plant production percentage, revealed the genotype specific response of both the species. Still other tissue culture techniques involving embryo culture and/or protoplast fusion could be tried for the development of fertile or partially fertile *V. mungo* x *V.umbellata* hybrid for the introgression of alien genes.

Acknowledgement

The authors are thankful to The Department of Science and Technology (DST), Ministry of Science and Technology, India for providing funds for the conduct of the study.

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How to cite this article:

Shayla Bindra, R.K. Mittal, V.K. Sood and Chaudhary. H.K. 2020. Alien Introgression Studies Involving V*igna mungo* x *Vigna umbellata* Hybridization. *Int.J.Curr.Microbiol.App.Sci.* 9(02): 268-276. doi: https://doi.org/10.20546/ijcmas.2020.902.034