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Introgression of disease resistance in urdbean (*Vigna mungo*) by involving its related species

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ABSTRACT

Cercospora leaf spots is one of the major foliar diseases of urdbean (*Vigna mungo* (L.) Hepper) causing a considerable yield loss. Screening of germplasm and induction of mutation has not yielded any source of resistance. Hence, an experiment was conducted at research farm of CSKHPKV Palampur, Himachal Pradesh during 2017–21 for introgression of disease resistance in urdbean. A total of 45 interspecific cross combinations were attempted between urdbean × moongbean [*Vigna radiata* (L.) R. Wilczek], urdbean × ricebean [*Vigna umbellata* (Thunb.) Ohwi and H. Ohashi] and urdbean × adzukibean [*Vigna angularis* (Willd.) Ohwi and H. Ohashi]. Only 12 interspecific crosses were successful. SSRs were used for confirmation of hybridity of the wide crosses. True F_1's were phenotyped for 13 morphological characteristics with respect to parental lines to study the trait expression. Most of the morphological characteristics of the interspecific F_1's were either intermediate to both the parents or resembled to their male parents. Here, we also report the interspecific wide hybridization in *Vigna* through bridge species crossing strategy where the F_1 of urdbean × moongbean was hybridized separately with ricebean and adzukibean**.** All the interspecific hybrids of urdbean and ricebean showed resistant reaction to *Cercospora* leaf spots.

Keywords: *Cercospora*, Hybridity, Interspecific, Morphological, *Vigna mungo*, *V. radiata*, *V. umbellata*

Urdbean [*Vigna mungo* (L). Hepper] is a self-pollinated diploid grain legume belonging to family Leguminoseae, domesticated from *V. mungo* var. silvestris (Lukoki *et al.* 1980). It has been reported to be originated in India with a secondary centre of origin in central Asia (Zeven and De Wet 1982). Urdbean is a rich source of dietary protein (25–28%), carbohydrates (62–65%), fibre (3.5–4.5%), ash (4.5–5.5%) and oil (0.5–1.5%), amino acids (lysine, methionine), vitamins (thiamine, niacin, riboflavin) and much needed iron and phosphorus (Gupta *et al.* 2020). One of the major constraints in achieving high yield of this crop in high rainfall areas of north-western Himalayas comprising Himachal Pradesh, Jammu and Kashmir and Uttarakhand is its susceptibility to a number of pathogens e.g. *Cercospora canescens, C. cruenta, Colletotrichum truncatum, Erysiphe polygoni* and urdbean yellow mosaic virus. *Cercospora* causes huge yield losses varying from 23–62% (Singh *et al.* 2011). Under palampur conditions, extensive screening of the germplasm and induced mutagenesis has not yielded any source of resistance to these pathogens. Thus, under the present circumstances, there is no other alternative left, but to look for alien *Vigna* species which can provide effective sources of resistance to this disease in varieties which are popular amongst farmers of the hill state.

Therefore, pre-breeding practices such as interspecific hybridization are required involving particularly those wild species that carry useful alien gene(s) for improving the crop (Pratap *et al.* 2021). The related underutilized species *V. umbellata* (Ricebean) and *V. angularis* (Adzukibean) have been found to be nutritive and resistant to most of the fungal pathogens of urdbean, giving a window to breeders to broaden the narrowed genetic base of the crop (Bindra *et al.* 2020). But the crossability of these *Vigna* species with urdbean is very low due the presence of pre- and post-fertilization barriers. Therefore, attempts were made to hybridize F_1 of *V. mungo* × *V. radiata* as a bridge species to overcome the difficulty of directly hybridizing these species. DNA markers along with morphological markers were also used to identify true F_1 plants from interspecific hybridization.

MATERIALS AND METHODS

The experimental material for interspecific hybridization studies included 5 cultivated urdbean [*Vigna mungo* (L). Hepper] genotypes (Him Mash-1, Palampur 93, HPBU 111,

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UG 218 and PDU1) used as female and 3 varieties each of ricebean [*Vigna umbellata* (Thunb.) Ohwi and H. Ohashi] (PRR-1, PRR-2 and VRB-3), moongbean [*Vigna radiata* (L.) R. Wilczek] (Suketi, SML-668 and ML-818) and adzukibean [*Vigna angularis* (Willd.) Ohwi and H. Ohashi] (HPU-51, IC-341983 and IC-341984) were used as male. During summer and rainy (*kharif)* season 2017, and summer 2018, staggered sowings were done at interval of 10 days to have synchronized flowering in the glasshouse of research farm of CSKHPKV Palampur, Himachal Pradesh. Emasculation of female parent(s) at plump bud stage was done in the evening (3:00–6:00 PM) followed by pollination in the next day morning (7:00–9:00 AM). Three immuno-suppressants i.e. gibberellic acid (GA_3), indole acetic acid (IAA) and Σ-amino caproic acid were used at 2 concentrations (500 ppm and 1000 ppm) about half an hour after pollination to prevent premature flower abscission and repeated for 3 consecutive days after pollination at an interval of 24 h. The immuno-suppressants were applied to cotton pad with the help of syringe at the base of the pedicel of the pollinated bud. The interspecific hybrid of urdbean × moongbean was also hybridized with ricebean and adzukibean using as a bridge to overcome the problem of directly hybridizing these *Vigna* species. A total of 14 morphological traits were recorded for the confirmation of hybridity of F_1's.

Molecular characterization: The parents as well as their F_1's were subjected to confirm hybridity through adzukibean specific SSR markers. 19 randomly chosen primers were screened for polymorphism. The samples for DNA extraction were collected at first and second trifoliate leaf stage and genomic DNA was isolated using CTAB method (Murray and Thompson 1980). The quantity and quality of DNA was estimated through electrophoresis using 0.8% agarose gel. The polymerase chain reaction was performed and amplifications were carried out in Eppendorf thermocycler. Amplification products were electrophoresed in 2% agarose gel (HIMEDIA) and stained with ethidium bromide (0.5 µg/ml). The gels were visualized and photographed using the Gel-Documentation Unit. From the amplified DNA of parental genotypes and their F_1's generated SSR marker profiles, the presence or absence of SSR bands was done manually. If both the bands of two parents, viz. P_1 and P_2 were present in F_1, it was designated as a true hybrid.

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. Day to day visual observation showed that most of the emasculated buds dropped 1–3 days after pollination and some aborted after about a week. Some of the crosses developed into pods without seeds and dropped, whereas a few crossed pods possessed shriveled to dimpled seeds with ruptured seed coat. Interspecific hybridization was successful between urdbean with ricebean and moongbean while that

Table 1 List of cross combinations attempted and successful cross pedigrees

Cross combination	F_1 seeds obtained	Number of plants obtained after germination
Palampur-93 × PRR-1	173	13
Palampur-93 × PRR-2	248	40
Palampur-93 × VRB-3	103	0
Him Mash-1 × PRR-1	193	19
Him Mash-1 × PRR-2	183	11
Him Mash-1 × VRB-3	235	34
HPBU-111 × PRR-1	83	0
HPBU-111 × PRR-2	78	0
HPBU-111 × VRB-3	0	0
UG-218 × PRR-1	40	0
UG-218 × PRR-2	64	0
UG-218 × VRB-3	0	0
PDU-1 × PRR-1	0	0
PDU-1 × PRR-2	216	17
PDU-1 × VRB-3	98	0
Palampur-93 × Suketi	226	30
Palampur-93 × SML-668	98	0
Palampur-93 × ML-818	0	0
Him Mash-1 × Suketi	166	10
Him Mash-1 × SML-668	128	0
Him Mash-1 × ML-818	205	18
HPBU-111 × Suketi	149	9
HPBU-111 × SML-668	99	0
HPBU-111 × ML-818	136	0
UG-218 × Suketi	235	39
UG-218 × SML-668	117	0
UG-218 × ML-818	91	0
PDU-1 × Suketi	125	0
PDU-1 × SML-668	210	27
PDU-1 × ML-818	0	0
Palampur-93 × HPU-51	0	0
Palampur-93 × IC-341983	0	0
Palampur-93 × IC-341984	0	0
Him Mash-1 × HPU-51	0	0
Him Mash-1 × IC-341983	0	0
Him Mash-1 × IC-341984	0	0
HPBU-111 × HPU-51	0	0
HPBU-111 × IC-341983	0	0
HPBU-111 × IC-341984	0	0
UG-218 × HPU-51	0	0
UG-218 × IC-341983	0	0
UG-218 × IC-341984	0	0
PDU-1 × HPU-51	0	0
PDU-1 × IC-341983	0	0
PDU-1 × IC-341984	0	0

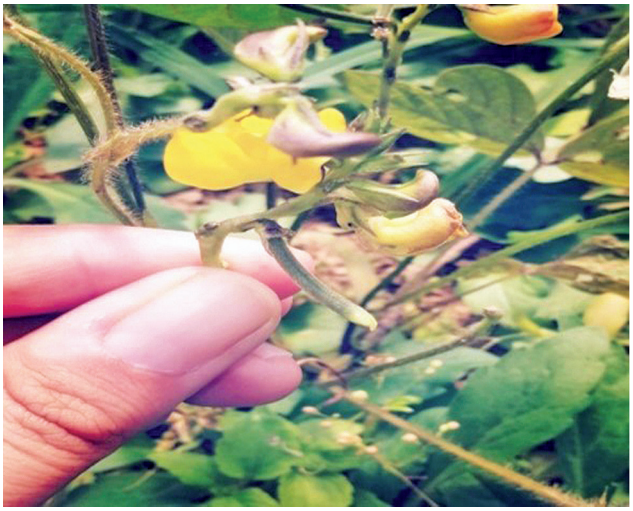

Fig 1 F_1's showing pseudo pod formation.

of urdbean with adzukibean yielded no pod set. Only 12 interspecific crosses were successful (6 each of urdbean × ricebean and urdbean × moongbean) (Table 1). The parents involved in interspecific hybridization showed differential genotypic response.

Confirmation of hybridity of interspecific hybrids

Various morphological characters were recorded on parents and F_1 hybrids between urdbean with moongbean and ricebean for the confirmation of hybridity. Most of the morphological characteristics of these true appearing F_1's were either intermediate to both the parents or resembled to their male parents indicating hybridity. Some of the F_1's between urdbean and ricebean showed no flowering, while some exhibited profuse flowering with pseudo or no pod formation (Fig 1). Some of the interspecific hybrids between urdbean and ricebean showed pollen sterility resulting in no seed set, so some morphological characters i.e. pod shape, pod pubescence, pod length, number of seeds per pod and 100-seed weight could not be recorded. Pollen fertility status of parents and F_1 hybrids in the interspecific crosses were studied under phase contrast microscope. Only fertile pollens took acetocarmine stain, while non-viable pollen grains did not. The reduced fertility of F_1 hybrids may be due to meiotic abnormalities leading to unequal distribution of chromosomes in about 50% of the cells (Lekhi *et al.* 2017).

Mode of germination: Mode of germination is a reliable morphological marker to confirm the hybridity of interspecific hybrids. Urdbean and moongbean have an epigeal mode of germination, whereas ricebean has hypogeal mode of germination. The interspecific hybrids of urdbean and ricebean showed hypogeal mode of germination similar to their male parent confirming their hybridity. Interspecific hybrids between urdbean and moongbean had an epigeal germination habit.

Stem colour: Stem colour of urdbean is green with purple base, ricebean stem colour is green with brown base and their hybrids showed purple green stem colour which was intermediate to both the parents. Some hybrids also showed green stem colour similar to the ricebean. Interspecific hybrids of the urdbean and moongbean showed green, purple and combination of green plus purple stem colours.

Leaf pubescence and leaf margin pubescence: Leaf pubescence and leaf margin pubescence was present in urdbean and moongbean and absent in ricebean, whereas it was present in all the interspecific hybrids between urdbean and ricebean, and urdbean and moongbean. Presence of pubescence is a desirable trait as, it is a mechanical barrier to insects and birds.

Leaf shape: Urdbean and moongbean have ovate leaf shape, whereas in ricebean it is lanceolate. Leaf shape of interspecific hybrids of urdbean and ricebean was lanceolate and ovate leaf shape was expressed by interspecific hybrids of urdbean and moongbean.

Stem pubescence: Stem pubescence was present in urdbean and moongbean, whereas it was partially present in ricebean. The F_1 hybrid plants of urdbean and ricebean showed stem pubescence sparsely and hybrids of urdbean and moongbean showed stem pubescence profusely.

Days to 50% flowering: Urdbean (43–49 days) and moongbean (37–41 days) have early 50% flowering as compared to ricebean (81–88 days). Interspecific hybrids of urdbean and ricebean were late in 50% flowering (55–65 days) compared to the female parent i.e. urdbean. Days to 50% flowering of interspecific hybrids between urdbean and moongbean was intermediate to both the parents (40–47 days).

Pollen stainability: Pollen stainability of parents was high (>90%). However, pollen stainability of hybrids

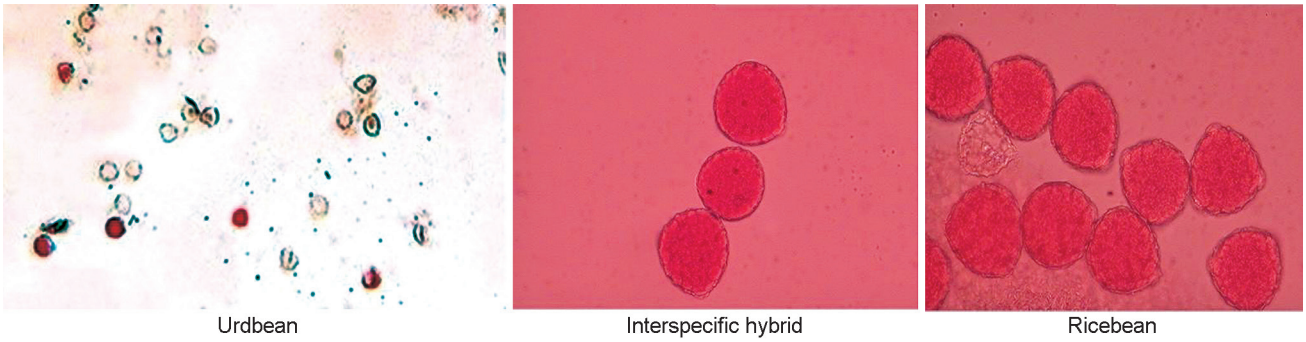

Fig 2 Pollen stainability of urdbean, ricebean and their interspecific hybrid.

between urdbean and ricebean was low ranging from 0–60%. Hybrids of urdbean and moongbean showed higher stainability (75–78%) as compared to hybrids of urdbean × ricebean (Fig 2).

Flower colour: Flower colour of urdbean, moongbean and ricebean were light yellow, light green and bright yellow respectively. The interspecific hybrids of urdbean and ricebean inherited the bright yellow flower colour of their male parent. The flower colour of hybrids of urdbean and moongbean was light yellow.

Plant height (cm): All the interspecific hybrids showed increased plant height which is a desirable trait. Height of urdbean genotypes ranged from 47.6–71.9 cm, ricebean 162.1–165.1 cm, moongbean 54.5–55.4 cm, interspecific hybrids of urdbean and ricebean 60.9–98.9 cm and urdbean and moongbean 49.7–68.2 cm.

Pod length (cm): Pods of urdbean were short (4.2–6.0 cm), whereas pods of moongbean (5.7–6.2 cm) and ricebean (7.3–9.2 cm) were long. The interspecific hybrids inherited the long pods of their respective male parent (5.1–6.2 cm).

Pod pubescence: Urdbean and moongbean showed presence of pod pubescence, whereas it was absent in ricebean. Pods of hybrids between urdbean and ricebean exhibited no pubescence, while pubescence was present in pods of interspecific hybrids of urdbean and moongbean.

Seeds per pod: Seeds per pod were less (3–4 seeds) in interspecific hybrids of urdbean and ricebean as compared to female (5–6 seeds) and male (7–8) seeds. The F_1 hybrids of urdbean and moongbean had 5–6 number of seeds per pod similar to their respective female parent. The F_1 seeds obtained from all cross combinations were small, wrinkled and shruken because of the poor development of the endosperm and embryo which might be due to incompatibility between the two parental genomes or due to the failure of embryo to reach maturity.

Testa colour: The colour of testa in urdbean is light black in colour, in moongbean it is green and in ricebean it varies from dark black to light brown in colour. The interspecific seeds of urdbean × ricebean were brown to dark brown in colour whereas light black colour was shown by interpecific hybrids of urdbean × moongbean.

100-seed weight: All the developed interspecific hybrids showed increase in 100-seed weight as compared to female parent. A small increase in100-seed weight of developed interspecific hybrids showed hybrid vigor over the female parent involved in interspecific hybridization. 100-seed weight of urdbean varies from 4.3–5.3 g, ricebean 7.2–7.6 g, moongbean 4.8–5.0 g. Interspecific hybrids of urdbean and ricebean and, urdbean and moongbean had 100-seed weight in the range of 5.1–6.3 g and 4.4–5.4 g respectively.

Confirmation of interspecific hybrids through SSR markers: Interspecific hybrids that showed morphological characteristics similar to their respective male parents were verified as true recombinants by using SSR markers. Out of 19 SSR markers used for parental polymorphism survey, 2 were found polymorphic between urdbean and ricebean, and only one marker was found polymorphic between urdbean and moongbean. These polymorphic markers were used for F_1 hybrid confirmation.

Hybridity of F_1 hybrids of *V. mungo* × *V. umbellata* combinations was confirmed using two primer pairs namely SSR 6581 and SSR 6255 polymorphic between parents and hybrids showed robust and reproducible bands. Hybridity of interspecific hybrids of *V. mungo* × *V. radiata* combinations was confirmed using one SSR primer i.e. VR0200. Bhanu *et al.* (2017) also confirmed the hybridity of *V. radiata* × *V. umbellata* interspecific hybrids using morphological and adzukibean specific SSR markers. Abbas *et al.* (2015) used SSR and RAPD markers to confirm the hybridity of interspecific hybrids between moongbean and urdbean.

Table 2 Reaction of parents and interspecific hybrids to *Cercospora* leaf spots disease

Recipient parent	Resistant (R)/ susceptible plant count	Donor parent	Resistant (R)/ susceptible plant count	F_1	Resistant (R)/ susceptible plant count
Urdbean/Ricebean					
Him Mash-1	5 S	VRB-3	5R	Him Mash-1 × VRB-3	4R
Palampur-93	5S	PRR-2	5R	Palampur-93 × PRR-2	4R
Palampur-93	5S	PRR-1	5R	Palampur-93 × PRR-1	4R
PDU-1	5S	PRR-2	5R	PDU-1 × PRR-2	4R
Him Mash-1	5S	PRR-1	5R	Him Mash-1 × PRR-1	4R
Him Mash-1	5S	PRR-2	5R	Him Mash-1 × PRR-2	4R
Urdbean/Moongbean					
Palampur-93	5S	Suketi	5S	Palampur-93 × Suketi	2S
Him Mash-1	5S	ML-818	5S	Him Mash-1 × ML-818	3S
Him Mash-1	5S	Suketi	5S	Him Mash-1 × Suketi	2S
PDU-1	5S	SML-668	5S	PDU-1 × SML-668	2S
HPBU-111	5S	ML-818	5S	HPBU-111 × ML-818	2S
UG-218	5S	Suketi	5S	UG-218 × Suketi	4S

Table 3 Use of F_1 of *V. mungo* × *V. radiata* as a bridge species

F_1 of cross combination	Buds emasculated and pollinated with *V. umbellata*	Pods harvested
Palampur-93 × Suketi	31	4
Him Mash × ML-818	28	6
Him Mash-1 × Suketi	33	3
PDU-1 × SML-668	38	7
HPBU-111 × ML-818	29	2
UG-218 × Suketi	34	5
	Buds emasculated and pollinated with *V. angularis*	
Palampur-93 × Suketi	26	0
Him Mash × ML-818	31	0
Him Mash-1 × Suketi	29	0
PDU-1 × SML-668	35	0
HPBU-111 × ML-818	30	0
UG-218 × Suketi	24	0

Disease reaction to Cercospora leaf spots under natural field conditions: The screening of interspecific hybrids had been carried out under natural field conditions along with parents by using 0–9 scale given by Mayee and Datar (1986). Urdbean and moongbean genotypes showed susceptible disease reaction whereas, ricebean genotypes gave resistant reaction. All the interspecific hybrids between *V. mungo* × *V. umbellata* showed resistant disease reaction. Interspecific hybrids of *V. mungo* × *V. radiata* showed susceptible disease reaction (Table 2).

Use of interspecific hybrid as a bridge species: F_1 of *V. mungo* × *V. radiata* were hybridized with ricebean and adzukibean with an aim to use it as bridge species and to have seed set, but there was high bud drop with less positive results (Table 3). There was no pod set when interspecific hybrids of urdbean × moongbean were pollinated with *V. angularis*. With pollen of ricebean, there were empty pods and seeds developed were so shriveled that these could not germinate under normal conditions as well on salt solution and MS medium.

The present study reveals the operation of pre- and post-fertilization barriers such as slow pollen tube development, no germination of pollen grains, delay in pollen tube entry into ovules, high abscission rate of crossed flowers within four days after pollination, hybrid lethality and hybrid inviability. Even though the fertilization barriers were predominant, it was possible to recover some interspecific hybrids. Morphological characters along with molecular markers are effective in confirmation of hybridity. These hybrids need further evaluation in future segregating generations for yield and disease resistance.

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