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Research Article

Development of multiple disease resistant pre-breeding lines through interspecific hybridization between greengram (*Vigna radiata*) and ricebean (*Vigna umbellatta*)

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Abstract
The present study involves interspecific hybridization between ricebean (RBL 35) and six greengram varieties *viz*., CO6, CO7, CO8, VBN 2, VBN 3 and VBN 4. Successful interspecific hybrids were obtained from two crosses *viz*., CO 6 x RBL 35 and CO 8 x RBL 35. A total of 13 contrasting morphological characters among the parents were used for characterization of the interspecific hybrids. The traits *viz*., germination type, hypocotyl colour, leaf colour, terminal leaflet shape, growth pattern, inflorescence type, pod colour, pod pubescence, seed shape and hilum type were identified as dominant traits and were transmitted from ricebean, served as good indicators for earmarking true greengram x ricebean interspecific hybrids.The hybrids obtained from the cross CO 8 x RBL 35 were found to be completely sterile. However, the partial fertile hybrid with 5.60 per cent pollen fertility was obtained in the cross CO6 x RBL 35 with poor pod set. The seeds obtained from the above cross were advanced and found with an improvement of pollen fertility in F_6 generation (80 %).The progenies were screened for four major diseases in five different seasons and was found to be resistant to yellow mosaic, powdery mildew, leaf crinkle and *cercospora* leaf spot diseases. Hence, the pre - breeding lines developed in this study could be used as donor for introgression of resistant genes into greengram.

Keywords: Interspecific hybridization, greengram, ricebean, *Vigna radiata, V. umbellata*

INTRODUCTION

Among the *Vigna* species, greengram is the widely distributed and commonly cultivated crop. India is the largest producer of greengram, covering an area about 55.47 lakh hectares with production of 36.76 lakh tones and productivity of 663 kg/ha (Annual report, DPD, 2023-24). It is the third important pulse crop and serves as an excellent source of easily digestible proteins with low flatulence (Doughty and Walker, 1982). The cultivated species of *V. radiata* possess several desirable traits such as short duration, high yield and suitability for crop rotation. However, it also exhibits susceptibility to bruchids, yellow mosaic virus, leaf crinkle, powdery mildew and cercospora leaf spot diseases leads to heavy yield loss (War *et al.,* 2017; Pandey *et al*., 2018). Despite extensive screening of greengram germplasm, a stable resistance sources for the above biotic stresses have not been identified. Additionally, the available greengram germplasm possess the limited variability and low harvest index which impedes the efforts to enhance productivity. In order to address these limitations and to broaden the genetic base of cultivated greengram genotypes, there is a need to explore the transfer of alien genes from other related *Vigna* species.

Incorporating alien genes from wild species not only reduces the risks of biotic and abiotic stresses in greengram but also could lead to considerable improvement in productivity (Stalker, 1980; Kumar *et al*., 2011). Therefore, there is a clear need to enhance greengram through hybridization with wild species. Among the wild species studied, *Vigna umbellata* stands out for its resistance against bruchids, yellow mosaic virus, cercospora leaf spot, powdery mildew *etc*. (Pattanayak *et al*., 2019; Gayacharan *et al*., 2024). Moreover, it was recorded with the highest grain yield among the *Ceratotropis* spp. Therefore, interspecific hybridization could be effectively employed to transfer the desirable characteristics of ricebean to greengram.

Interspecific hybridization was attempted between greengram (*V. radiata*) and ricebean (*Vigna umbellata*), belonging to the tertiary gene pool of greengram by many workers. Bharathi *et al*. (2006), Chaisan *et al*. (2013) and Ujianto *et al*. (2019) reported successful hybridization when greengram was used as the female parent and ricebean as the male parent. This establishes that no pre fertilization barriers were encountered when greengram was used as the maternal parent. Pandiyan *et al*. (2008) and Satyan *et al*. (1982) have reported the sterility of the F_1 hybrids between greengram and ricebean. However, partial fertility and poor pod set was observed in the above crosses (Bhanu *et al*., 2017; Ludhat *et al*., 2021). The above workers have also reported that the interspecific hybrids developed between greengram and ricebean were found to have resistance against mungbean yellow mosaic disease. Therefore, interspecific hybridization could be effectively employed to transfer the biotic resistance to greengram.

MATERIALS AND METHODS

Plant material and hybridization: For interspecific hybridization, the greengram varieties *viz*., CO 6, CO 7, CO 8, VBN 2, VBN 3 and VBN 4 were raised in two staggered sowings at 15 days interval in a crossing block at Department of Pulses, Centre for Plant Breeding and Genetics, Coimbatore during *rabi,* 2021 -22 and *kharif,* 2022 along with ricebean variety RBL 35. Each cultivar was planted in a couple of rows of four-meter length with a spacing of 30 x 10 cm. All the prescribed package of practices was followed at appropriate time to establish a healthy crop. Hybridization was carried out using the method given by Boling *et al*. (1961). In the female parent (greengram cultivars), the flower buds that were expected to open the next day were selected for emasculation. Those flower buds were emasculated in the previous day evening (4.00 to 5.00 p.m.), prior to the day of crossing. In order to identify the emasculated flower buds, a small thread was tied around the pedicels of the emasculated bud. Pollination was done on the following morning (6.00 to 8.00 a.m.) with pollen grains collected from ricebean genotype. After the crossed pods attained full maturity, they were collected separately, labelled and stored. The number of flower buds crossed and the crossed pods obtained from each cross were counted in order to work out the per cent crossability. The crossed seeds were raised along with the parents during *rabi*, 2022-23. Per cent crossability, hybrid inviability and hybrid lethality were computed using the following formula.

$$\text{Per cent crossability} = \frac{\text{Number of pods set from crossed flower buds}}{\text{Number of flower buds crossed}} \times 100$$

$$\text{Inviability per cent} = \frac{\text{Number of ungerminated seeds}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Lethality per cent} = \frac{\text{Number of plants that did not survive}}{\text{Total number of seeds germinated}} \times 100$$

Morphological characterization: The contrasting traits observed among the parents were used to characterize the F_1 hybrids which includes germination type, hypocotyl colour, leaf colour, terminal leaflet shape, growth pattern, twinning tendency, inflorescence type, corolla colour, colour of keel petal, immature pod colour, pod pubescence, seed shape and hilum type.

Studies on pollen fertility: Iodine Potassium Iodide (I_2 – KI) staining technique was used to assess the status of pollen fertility in parents and hybrids. In order to estimate the pollen fertility, a flower bud was taken, placed on a microscopic slide, anthers were removed gently, smashed on the slide with one per cent I_2 – KI solution and covered with coverslip. Then the slide was observed under a light microscope. Swollen and stained pollen grains were counted as fertile one, whereas shrunken and unstained pollen grains were counted as sterile. In this way, pollen count was taken for three microscopic fields and the average of the three fields were used for computing per cent pollen fertility.

$$\text{Per cent pollen fertility} = \frac{\text{Number of stained pollen}}{\text{Total number of pollen studied}} \times 100$$

Advancement of generations and screening for biotic stress: The two F_2 plants of the cross CO 6 x RBL 35 were forwarded up to F_6 generation by following pedigree method during *kharif* 2023 to *rabi* 2024-25. The progenies were morphologically uniform and segregation of greengram plant type was not observed. The progenies were screened in five different seasons for yellow mosaic (0-9 scale), powdery mildew (0 – 5), leaf crinkle (%) and cercospora leaf spot (0 – 9) diseases (Annual report, *Kharif* pulses, 2024) along with susceptible greengram check (VBN 4) under field condition.

RESULTS AND DISCUSSION

Crossability, inviability and lethality: The pod set (crossability) ranged from 1.49 to 26.56 per cent (**Table 1**). The maximum pod set of 26.56 per cent was observed in the cross VBN 4 x RBL 35 and the minimum pod set of 1.49 per cent in the cross CO 6 x RBL 35. Similarly, varying degree of pod set percentages between greengram and ricebean cross were documented by Bhanu *et al*. (2017). The differences in pod set percentage may be attributed due to the combinations of parental cultivars selected from each species for interspecific hybridization (Chen *et al*., 1983). The inviability per cent among the

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Table 1. Impact of pre- and post-fertilization barriers in crosses of greengram and ricebean

Cross	Number of flowers crossed	Number of pod set	Pod set per cent	Number of hybrid seeds sown	Number of seeds germinated	Germination per cent	Inviability per cent	Unsurvived F_1	Hybrid lethality per cent	Survived true hybrids	Pollen fertility (%)
CO 6 x RBL 35	335	5	1.49	14	11	78.57	21.43	10	90.90	1	5.60
CO 7 x RBL 35	184	20	10.87	59	26	44.07	55.93	26	100.00	0	Nil
CO 8 x RBL 35	117	18	15.38	41	34	82.93	17.07	32	94.11	2	0.00
VBN 2 x RBL 35	352	9	2.56	18	6	33.33	66.67	6	100.00	0	Nil
VBN 3 x RBL 35	60	8	13.33	31	20	64.52	35.48	20	100.00	0	Nil
VBN 4 x RBL 35	64	17	26.56	55	43	78.18	21.82	43	100.00	0	Nil
Range	60 - 352	5 - 20	1.49 - 26.56	14.00 - 59.00	6.00 - 43.00	33.33 - 82.93	17.07 - 66.67	6 - 43	90.90 - 100.00		

crosses ranged from 17.07 to 66.67 per cent. Inviability refers to the inability of the hybrid seeds to germinate. This might be the result of small and shrivelled seeds which were developed due to poor embryo development (Chaisan *et al*., 2013). The cross, VBN 2 x RBL 35 was registered with the highest inviability per cent (66.67 %), whereas the cross, CO 8 x RBL 35 was recorded with the lowest inviability per cent (17.07 %).

Hybrid lethality refers to the death of hybrid seedlings after germination. The values of hybrid lethality varied from 90.90 (CO 6 x RBL 35) to 100.00 per cent in four crosses (**Table 1**). However, only three plants from two crosses *viz*., CO 6 x RBL 35 (1) and CO8 x RBL 35 (2) were survived and identified as true hybrid based on hypogeal germination, a characteristic germination of ricebean and also using SSR marker (CEDG 115) (**Fig.1**). Biswas and Dana (1975) also used hypogeal germination for hybrid confirmation of the hybrid derived from blackgram x ricebean cross. Similarly, Bhanu *et al*. (2017) used germination type and hypocotyl colour to confirm the true hybrid plants derived from greengram x ricebean cross. Among the two crosses, the plants in the CO 8 x RBL 35 were observed as completely sterile, whereas a plant in the cross, CO 6 x RBL 35 showed 5.60 per cent pollen fertility and hence observed with few pod set (**Table 1**).

Characterization of F_1 hybrids: The plants obtained in two interspecific crosses *viz*., CO 6 x RBL 35 and CO 8 x RBL 35 were characterized using 13 contrasting morphological traits identified between the parents (**Table 2 and Fig.2**). The hybrids obtained from the cross, CO 8 x RBL 35 could not be characterized beyond the flowering phase as they were completely sterile and no pod set was observed. Epigeal germination was observed in greengram, whereas in ricebean, the germination type was hypogeal. The F_1 plants exhibited hypogeal germination similar to that of male parent, ricebean. In greengram, the colour of hypocotyl in CO 6 and CO 8 was purple and green, respectively. Purple hypocotyl was observed in ricebean. In F_1 hybrids, hypocotyl colour was purple resembling the male parent. Colour of the leaf was green in both the greengram varieties, whereas it was light green in ricebean. Light green leaves were observed in the F_1 plants. Shape of the terminal leaflet was recorded as ovate in greengram and lanceolate in ricebean. However, the terminal leaflet shape of F_1 plants were observed as lanceolate. In greengram, growth pattern was observed as indeterminate in CO 6, whereas it was determinate in CO 8. Ricebean showed indeterminate growth pattern. In F_1

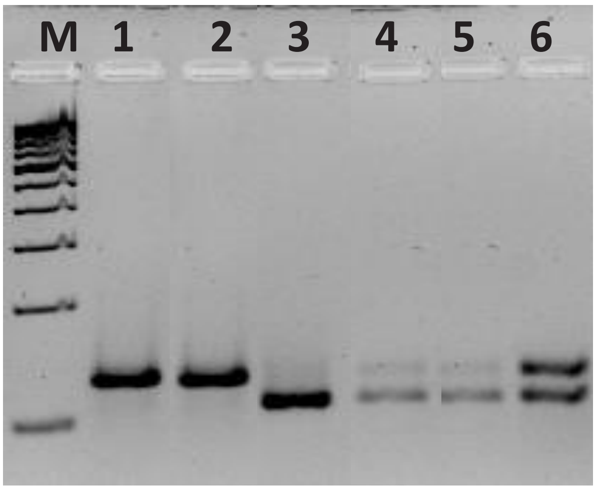

Fig.1. Confirmation of F_1 hybrids using SSR marker (CEDG115)

M: 1 kb ladder, 1: CO 8, 2: CO6, 3: RBL 35,
4: F1 (CO 8 x RBL 35), 5: F1 (CO 8 x RBL 35),
6: F1 (CO 6 x RBL 35)

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Table 2. Characterization of F_1 hybrids and parents for morphological traits and pollen fertility

S.No.	Characters	*Vigna radiata* (P1)	*Vigna umbellata* (RBL35) (P2)	Hybrids(F_1)	
				CO 6 x RBL 35	CO 8 x RBL 35
1.	Germination type	Epigeal	Hypogeal	Hypogeal	Hypogeal
2.	Hypocotyl colour	CO 6 - Purple CO 8 - Green	Purple	Purple	Purple
3.	Colour of leaf	Green	Light green	Light green	Light green
4.	Terminal leaflet shape	Ovate	Lanceolate	Lanceolate	Lanceolate
5.	Growth pattern	CO 6 - Indeterminate CO 8 - Determinate	Indeterminate	Indeterminate	Indeterminate
6.	Twining tendency	CO 6 - Absent CO 8 - Absent	Prominent	Absent	Absent
7.	Inflorescence type	Compound raceme	Simple raceme	Simple raceme	Simple raceme
8.	Corolla colour	Greenish yellow	Bright yellow	Yellowish green	Yellowish green
9.	Colour of keel petal	Grey	Translucent yellow	Translucent yellow	Translucent yellow
10.	Pod colour (Immature)	Green	Dark green	Dark green	Dark green
11.	Pod pubescence	Moderately pubescent	Glabrous	Glabrous	-
12.	Seed shape	Globular	Flat	Flat	-
13.	Hilum	Non concave	Concave	Concave	-
14.	Pollen fertility (%)	CO 6 – 97.12 CO 8 – 96.72	RBL 35 – 97.44	5.60	0.00

plants, the growth pattern was indeterminate resembling the male parent. Twining tendency was absent in both the greengram varieties, whereas it was pronounced in ricebean. Strikingly, it was absent in the F_1 plants. The type of inflorescence in greengram was observed as compound raceme, while it was documented as simple raceme in ricebean. The F_1 plants were detected with simple raceme type of inflorescence akin to that of ricebean. Greenish yellow corolla colour was noticed in greengram (CO 6 and CO 8) whereas the corolla colour in ricebean was observed as bright yellow. Interestingly, the colour of the corolla in F_1 plants was observed as yellowish green, representing the intermediate expression of both the parents. The colour of keel petal was recorded as grey colour in greengram. In contrast, it was translucent yellow in ricebean and F_1 plants.

Satyan *et al*. (1982) carried out hybridization between greengram (Jawahar-45) and ricebean (IC 156431). The expression of the characters *viz*., epicotyl colour, plant habit, leaflet margin and pigmentation of the standard petal and sepals was completely dominant. However, the expression of flower colour was intermediate. Similarly, Bindra *et al*. (2020) reported that germination type, stem colour, flower colour and inflorescence type of the true hybrid plants of blackgram x ricebean cross was similar to ricebean. The plants in both the crosses produced profuse flowers with no (CO 8 x RBL 35) or low pod set (CO 6 x RBL 35). In line with the present study, Pandiyan *et al*. (2008) [greengram and ricebean] and Bhardwaj *et al*. (2022) [blackgram, adzukibean and ricebean] reported no pod set in the F_1 hybrids derived from the interspecific crosses. Singh *et al*. (2013) also reported that partially fertile F_1 plants derived from blackgram x ricebean cross developed partially filled pods with three to four seeds per pod. Immature pod colour of greengram and ricebean was observed as green and dark green, respectively, while it was dark green in F_1 hybrid (CO 6 x RBL 35). Moderate pod pubescence was noticed in greengram. Ricebean was observed to have glabrous pods. Interestingly, pod pubescence was absent in F_1 hybrid (CO 6 x RBL 35). Seed shape was documented as globular in greengram, whereas flat in ricebean and F_1 hybrid (CO 6 x RBL 35). However, the size of the seeds obtained from the F_1 pod was notably larger than that of both the parents. In greengram, hilum was non-concave (aril was not prominent), while it was concave (deeply furrowed prominent aril) in ricebean. The seeds from the F_1 pod also with deeply furrowed prominent aril (concave hilum), akin to that of ricebean. Many traits found in the ricebean were dominant in the greengram x ricebean cross and hence could be used for confirmation of interspecific hybrids.

Pollen fertility studies: The pollen fertility of the parents varied between 96.72 and 97.44 per cent. The hybrids in the cross, CO 8 x RBL 35 exhibited 100 per cent pollen sterility, whereas the hybrid plant in the cross, CO 6 x RBL 35 was observed with 5.60 per cent pollen fertility which resulted in few pod set **(Fig. 3).** Bhanu *et al*. (2017) also reported similar range of pollen fertility (1.6 to 3.4 %) in the F_1 plant derived from greengram and ricebean cross.

a.Germination type

Epigeal germination (P_1) **Hypogeal germination (P_2)** **Hypogeal germination (F_1)**

b.Terminal leaflet shape

Ovate (P_1) **Lanceolate (P_2)** **Lanceolate (F_1)**

c. Corolla colour

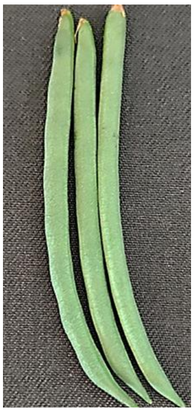
Grey (P_1) **Translucent yellow (P_2)** **Yellowish green (F_1)**

d. Colour of flower keel petal

Greenish yellow (P_1) **Bright yellow (P_2)** **Yellowish green (F_1)**

Fig.2. Characterization of parents and hybrid (P1: CO6; P2: RBL35)

e .Pod pubescence

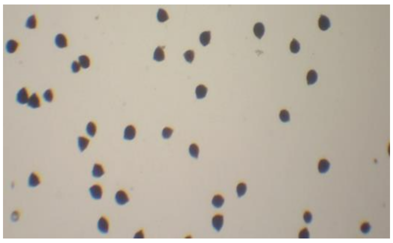




Moderately pubescent (P_1) Glabrous (P_2) Glabrous (F_1)

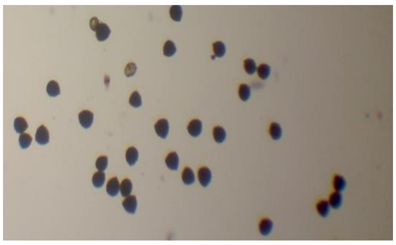
f. Hilum type

Not concave (P_1) Concave (P_2) Concave (F_1)

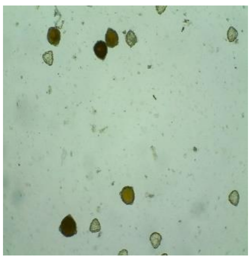
Fig.2. Characterization of parents and hybrid (P1: CO6; P2: RBL35)



CO 6



RBL 35



F_1 hybrid (CO 6 x RBL 35) ; 5.60 % pollen fertility

Fig.3. Evaluation of parents and hybrids for pollen fertility

Table 3. Screening of two hybrid progenies and parents for major diseases

Genotypes	Kharif 2023	Rabi 2023-24	Summer 2024	Kharif 2024	Rabi 2024-25
Yellow mosaic disease (0-9 scale) 0 – Free, 3- Moderatly Resistant, 7- Susceptible, 9- Highly Susceptible					
CO 6 x RBL 35/1	-	0	0	-	-
CO 6 x RBL 35/2	-	0	0	-	-
CO 6	-	3	3	-	-
RBL 35	-	0	0	-	-
VBN 4 (Sus. check)	-	7	9	-	-
Powdery mildew disease (0-5 scale) 0 – Free, 5 - Highly susceptible					
CO 6 x RBL 35/1	-	0	-	-	0
CO 6 x RBL 35/2	-	0	-	-	0
CO 6	-	5	-	-	5
RBL 35	-	0	-	-	0
VBN 4 (Sus. check)	-	5	-	-	5
Cercospora leaf spot disease (0-9 scale) 0 – Free, 9 - Highly susceptible					
CO 6 x RBL 35/1	-	0	-	-	-
CO 6 x RBL 35/2	-	0	-	-	-
CO 6	-	9	-	-	-
RBL 35	-	0	-	-	-
VBN 4 (Sus. check)	-	9	-	-	-
Leaf crinkle disease (%) 0 – free, 0.1 - 10.0 Resistant, 10.1 -20.0 Moderately Resistant					
CO 6 x RBL 35/1	0	0	0	0	0
CO 6 x RBL 35/2	0	0	0	0	0
CO 6	10	20	13	20	15
RBL 35	0	0	0	0	0
VBN 4 (Sus. check)	20	20	10	20	20

Note: (-) : symptoms not appeared

Low fertility in the hybrid plant could be due to abnormal pairing of chromosomes and unequal chromosome segregation during meiosis. However, in the present study the pollen fertility was improved from 5.60 per cent in F_1 to 80 .00 per cent in F_6 generation.

Backcross was attempted in the F_1 plants derived from the cross, CO 8 x RBL 35 (100 % sterile) using CO 8 as the male parent. But, no pod set was observed. Similarly, Pandiyan *et al*. (2008) also documented lack of pod set in the backcrosses involving hybrid plants derived from the cross of greengram and ricebean.

Screening of progenies for major diseases: The male parent RBL 35 (ricebean) was found to be free from yellow mosaic, powdery mildew, leaf crinkle and cercospora leaf spot diseases. The female parent CO 6 was moderately resistant to yellow mosaic disease but susceptible to other three diseases. Hence, the progenies of the cross CO 6 x RBL 35 were screened for the above diseases under field condition for five seasons. The results revealed that, the two progenies of the above cross were found to be free from yellow mosaic, powdery mildew, leaf crinkle and *cercospora* leaf spot diseases (0 scale) in all the generations/seasons (**Table 3**). The segregation for disease resistance level was not observed in the segregating generations.

To overcome the narrow genetic base of greengram, exploitation of novel genes from wild species becomes extremely important which acts as potential donors for both biotic and abiotic resistance. From the study, it could be recognized that most of the prominent ricebean characters *viz*.,germination type, hypocotyl colour, leaf colour, terminal leaflet shape, growth pattern, inflorescence type, pod colour, pod pubescence, seed shape and hilum type were identified as a dominant traits for determining the true hybridity of greengram x ricebean crosses.The two progenies with good pod set were developed from the cross CO6 x RBL 35 and was found to be resistant for four major diseases that are affecting greengram. Hence, pre - breeding lines developed in this study could be used as a potential donors for introgression of resistant genes into greengram.

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