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# CYTOGENETICAL AND BIOCHEMICAL ANALYSIS OF INTERSPECIFIC HYBRIDS BETWEEN *VIGNA RADIATA* AND *V. UMBELLATA*

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## ABSTRACT

**Interspecific hybrids were raised between *V. radiata* and *V. umbellata*. Both the species exhibit genotypic differences for crossability which can be exploited to have more number of vigorous hybrid seeds. The crossability percentage varied between 3.8 to 15.2 among different genotypes. The application of 4 ppm NAA was observed to enhance the foliage and pod set percentage. The hybrids were identified at the early stage by their mode of germination and twiny habit. Cytologically, the hybrids include an array of meiotic abnormalities ranging from non-pairing of chromosomes through the presence of univalents, chain and ring multivalents and laggards at anaphase-1. The codominant expression of species specific isoperoxidase at two development stages also characterized the interspecific hybrids.**

**Key words :** *V. radiata*, *V. umbellata*, interspecific hybrid, isoperoxidase, meiotic behaviour.

Interspecific hybridization between Asian pulses belonging to genus *vigna* has been attempted with varying degree of success thereby enlarging the gene pool and enhancing recombination between desired and diverse genotypes [1-4]. *V. radiata* (mungbean) characterized with dwarf erect, determinate/indeterminate, semiprostrate/tendrilous and profusely branched growth habit has several desirable traits such as large number of pods per cluster, more number of seeds per pod, early maturity, easily digestible proteins and suitability to drought conditions, but it is highly susceptible to mungbean yellow mosaic virus (MYMV) and other insect or pest diseases. However, *V. umbellata* (rice bean) with long pods and very bold seeds has high degree of resistance to MYMV, but it is known to be sensitive to whitefly (*Bemisia tabaci*) which is the vector for MYMV. Thus, its resistance operates through resistance to MYMV. Therefore, interspecific hybridization involving these species would be rewarding for introgression of desirable genes from one species to another. However, incompatibility between these species hampers the transfer of required genes and limits the potential for improved productivity and adaptation to different agroecological systems. The present attempt has been made to raise the

hybrids between these two species and to study their cytogenetical and biochemical characteristics.

## MATERIAL AND METHODS

Crosses were made between six genotypes of *V. radiata* (ML 505, ML 131, ML 267, ML611, MUG288 and MG125) and five of *V. umbellata* (RBL167, RBL93, RBL33, RBL140 and RBL141). Emasculation and pollination were carried out as given by Boiling *et al.* [5] with *V. radiata* as female parent. To enhance the crossability and pod setting percentage, 4ppm NAA (α-Naphthyl acetic acid) was applied to the pedicel of emasculated bud as well as after pollination twice a day atleast for ten days consecutively [6].

The meiotic analysis of the parents and hybrids was carried out by fixing the floral buds in 1:3 acetic alcohol (Carnoy's-1) from 6.30-7.30 a.m. The anthers were excised from flower buds and smeared in 3 per cent acetocarmine. Pollen fertility was determined using 2 per cent Iodine potassium iodide solution.

Isoperoxidase isozymes were studied at seedling and initiation of flowering stages in the parents and interspecific hybrids through starch get electrophoresis according to Smithies [7] and different bands were named, following Pawar and Gupta [8].

## RESULTS AND DISCUSSION

The crosses made between *V. radiata* and *V. umbellata* showed high degree of flower abscission both after emasculation and pollination which might be due to injury, shock or physiological changes. The pod set percentage varied from 3.8 per cent (MG125 × RBL33) to 15.2 per cent (ML505 × RBL93) (Table 1). Low crossability and pod set percentage may be attributed to genetic diversity between the parents as well as due to the inability of pollen tube to reach the stigma and style [9-10] or due to embryo abortion after fertilization [1,3]. The germination and survival percentage varied between 6.06 to 88.8 and 75 to 100, respectively. Three hybrid plants were identified in each of the crosses ML505 × RBL93, ML505 × RBL167 and ML131 × RBL93 followed by two in ML131 × RBL167 and ML267 × RBL93 and one each in ML267 × RBL140 and ML611 × RBL167 based on their twiny habit and intermediate mode of germination between epigeal (*V. radiata*) and hypogeal (*V. umbellata*) type.

### CYTOLOGICAL STUDIES

Meiosis was normal in both the parents with eleven bivalents at diakinesis/ metaphase 1. However, it was highly irregular in the interspecific hybrids with 2n=22

**Table 1. Crossability between *V. radiata* and *V. umbellata***

| Crosses | Total no. of flowers emasculated | Total no. of flowers pollinated | Pod set percentage | No. of mature pods | Mature seeds/ pollination (%) | No. of seeds sown | Germination percentage | Percentage of seedlings survived | Percentage of hybrid plant(s) |
|---|---|---|---|---|---|---|---|---|---|
| ML505 × RBL33 | 300 | 170 | 6.5 | 7 | 29.40 | 45 | 17.7 | 75.0 | 16.6 |
| ML505 × RBL93 | 250 | 145 | 15.2 | 16 | 43.44 | 50 | 8.3 | 100.0 | 60.0 |
| ML505 × RBL167 | 380 | 140 | 14.3 | 10 | 39.28 | 45 | 11.1 | 100.0 | 60.0 |
| ML131 × RBL33 | 200 | 95 | 5.3 | 1 | 5.26 | 5 | - | - | - |
| ML131 × RBL93 | 250 | 140 | 7.1 | 7 | 27.80 | 39 | 28.2 | 100.0 | 33.3 |
| ML131 × RBL167 | 180 | 100 | 13.0 | 8 | 33.00 | 33 | 6.1 | 100.0 | 100.0 |
| ML131 × RBL140 | 180 | 80 | 7.5 | 4 | 25.00 | 10 | 10.0 | 100.0 | - |
| ML131 × RBL141 | 135 | 65 | 6.2 | 1 | 4.60 | 3 | 66.6 | 100.0 | - |
| ML131 × RBL33 | 250 | 167 | 7.2 | 7 | 13.20 | 19 | 36.8 | 100.0 | - |
| ML267 × RBL93 | 290 | 130 | 7.6 | 6 | 13.80 | 18 | 16.6 | 100.0 | 66.6 |
| ML267 × RBL167 | 200 | 110 | 9.0 | 4 | 14.50 | 8 | 75.0 | 100.0 | - |
| ML267 × RBL140 | 150 | 75 | 9.3 | 3 | 22.60 | 15 | 26.7 | 100.0 | 25.0 |
| ML267 × RBL141 | 150 | 105 | 8.5 | 10 | 14.20 | 5 | 80.0 | 100.0 | - |
| ML611 × RBL33 | 250 | 142 | 5.6 | 1 | 4.30 | 3 | 33.3 | 100.0 | - |
| ML611 × RBL167 | 200 | 100 | 10.0 | 3 | 34.00 | 17 | 22.4 | 100.0 | 20.0 |
| ML611 × RBL141 | 270 | 75 | 4.0 | 1 | 12.00 | 3 | 33.3 | 100.0 | - |
| MUG288 × RBL33 | 300 | 150 | 4.0 | 2 | 10.60 | 8 | 50.0 | 100.0 | - |
| MUG288 × RBL93 | 225 | 116 | 13.7 | 5 | 36.60 | 22 | 59.5 | 76.9 | - |
| MG125 × RBL33 | 330 | 208 | 3.8 | 2 | 4.30 | 8 | 88.8 | 75.0 | - |
| MG125 × RBL167 | 360 | 185 | 6.3 | 8 | 21.05 | 20 | 25.0 | 100.0 | |

chromosomes. In different crosses, the average number of bivalents ranged from 1.64 to 4.12; univalents 13.2 to 18.2; trivalents 0.04 to 0.18 and quadrivalents from 0.04 to 0.14. The anomalous chromosome segregation also varied depending upon the number of bivalents per cell. Laggards at A-I were observed in ML505 × RBL93, ML505 ×RBL 167, ML131 × RBL93 and ML611 × RBL167 and 6-10 micronuclei were

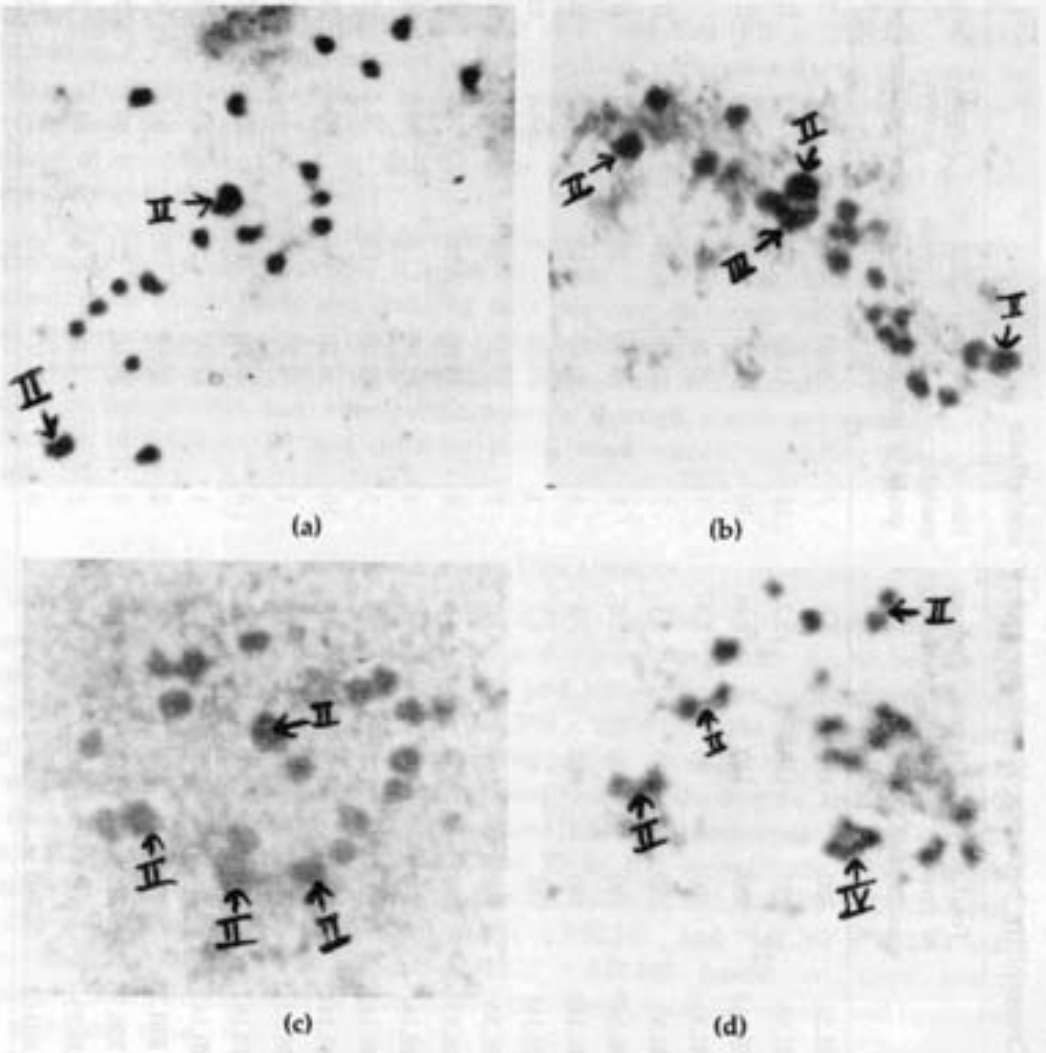


**Fig 1. (a-b): Multiple association of chromosomes in interspecific hybrids between *V. radiata* and *V. umbellata*. (a) 2II + 18I at MI in ML611 × RBL167; (b) 1III + 3II + 13I at MI in ML611 × RBL 167; (c) 4II + 14I at MI of ML505 × RBL93 and (d) 1IV + 3II + 12I at MI in ML505 × RBL33**

present in all the hybrids. Pollen fertility ranged from 13.7 to 17.7 per cent with maximum in ML131 × RBL167 (17.7%) followed by ML505 × RBL167 (16.3%) and minimum (13.7%) in ML267 × RBL93 (Figs. 1 (a-d); Table 2).

The meiotic analysis in $F_1$ revealed the presence of ring or chain configurations which suggest that these two species differ in their structural rearrangement of chromosomes through interchanges. Large number of univalents might be due to lack of homology, desynaptic/asynaptic behaviour or early disjunction of chromosomes at A-I which causes genetic sterility due to numerical imbalance of chromosomes. The formation of micronuclei and the failure of tetrad separation are the post-meiotic abnormalities responsible for high pollen sterility of hybrids [11, 12].

## BIOCHEMICAL STUDIES

Six anodal and two cathodal bands were observed for peroxidase at seedling stage, whereas at initiation of flowering, there were nine anodal and two cathodal bands. The hybrids were characterized by the presence/absence of isozyme bands in either or both the parents and presence of unique bands.

The interspecific hybrids between different cultivars of *V. radiata* × *V. umbellata* consisted of $A_1$ and $A_2$ anodal bands from female and male parent, respectively except for ML267 × RBL140, where $A_3$ was contributed by the male parent along with $A_4$ and $A_5$ from both the parents at the seedling stage. In the crosses, ML267 × RBL140, ML267 × RBL93 and ML131 × RBL93, $A_6$ and in ML611 × RBL167, $C_1$ was present as unique band at seedling stage. However, at the flowering stage, anodal bands $A_2$ and $A_3$ were contributed by both the parents in ML505 × RBL93, ML267 × RBL93, ML131 × RBL93 and ML131 × RBL 167. The novel bands $A_1$ and $A_7$ were observed in ML505 × RBL93, $A_7$ in ML505 × RBL33 and ML267 × RBL140; $C_1$, $A_3$ and $A_5$ in ML505 × RBL167 $A_2$, $A_4$ and $A_7$ in ML267 × RBL93 and $C_1$ in ML131 × RBL167. Similarly, ML131 × RBL93 and ML611 × RBL167 had $A_7$ and $A_2$ as unique bands, respectively. The rest of the bands were similar to either of the parents at both the developmental stages (Fig. 2a and b).

The cultivars of both the parents could also be differentiated by the presence/absence of peroxidase isozyme bands. In the mungbean genotypes, ML505, ML267, ML131 and ML611, $A_1$, $A_4$, $A_5$ and $C_2$ were the common bands at the seedling stage whereas $A_3$, an additional band was present in ML131. The ricebean genotypes RBL167, RBL33, RBL93, RBL140 had $C_2$ and $A_5$ as common bands with $A_3$ in RBL140 and $A_2$ and $A_4$ in RBL167, RBL93 and RBL33 as additional bands. At the initiation of flowering, these genotypes could be characterized by the presence of $A_8$, $A_9$ and $C_2$ common bands with $A_3$ in RBL93 and RBL33, $A_4$ in RBL167 and $A_2$ and $A_4$ in RBL140 as novel bands. The mungbean genotypes were characterized

**Table 2. Average chromosome configuration at diakinesis/metaphase 1 and chromosome distribution at A-I of interspecific hybrids of *V. radiata* x *V. umbellata***

| Cross | Number of cells | Frequency per cell at diakinesis/metaphase-I | | | | PMCs at anaphase-I showing | | | Pollen fertility (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | IV | III | II | I | Normal distribution | Laggards | Aberrant distribution | |
| ML505 x RBL33 | 25 | 0.04 | 0.08 | 1.64 | 18.32 | 16.67 | - | 83.33 | 14.1 |
| ML505 x RBL93 | 25 | 0.08 | 0.08 | 4.12 | 13.20 | 20.33 | 6.33 | 73.34 | 14.8 |
| ML505 x RBL167 | 22 | 0.14 | 0.18 | 2.86 | 15.18 | 30.62 | 10.20 | 59.18 | 16.3 |
| ML131 x RBL93 | 25 | 0.04 | 0.04 | 1.72 | 18.28 | 16.88 | 8.26 | 74.86 | 16.1 |
| ML267 x RBL93 | 28 | - | 0.04 | 3.00 | 15.89 | 13.35 | - | 86.65 | 13.7 |
| ML267 x RBL140 | 25 | 0.12 | 0.16 | 2.44 | 16.16 | 18.33 | - | 81.67 | 15.2 |
| ML131 x RBL167 | 26 | 0.04 | - | 3.38 | 15.08 | 25.49 | - | 74.51 | 17.7 |
| ML611 x RBL167 | 22 | - | - | 2.36 | 17.27 | 18.23 | 3.33 | 78.44 | 14.5 |

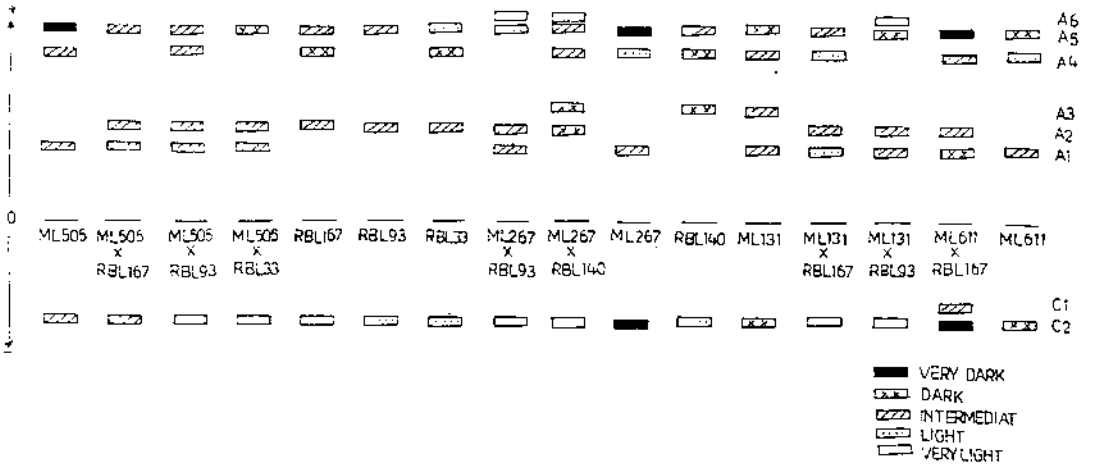


**Fig. 2(a): Zymograms showing peroxidase banding patterns at seedling stage**

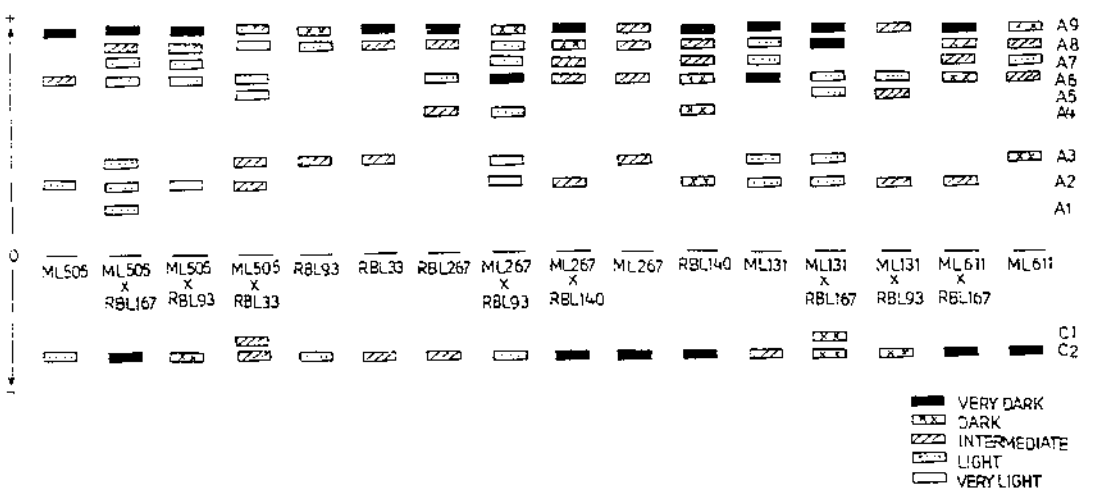


**Fig. 2(b): Zymograms showing peroxidase banding patterns at flowering stage**

by the presence of $A_3$ and $A_8$ in ML267, $A_2$ and $A_5$ in ML131, $A_2$ in ML505 and $A_3$, $A_7$ and $A_8$ in ML611 in addition to $A_6$, $A_9$ and $C_2$ as common bands at the flowering stage.

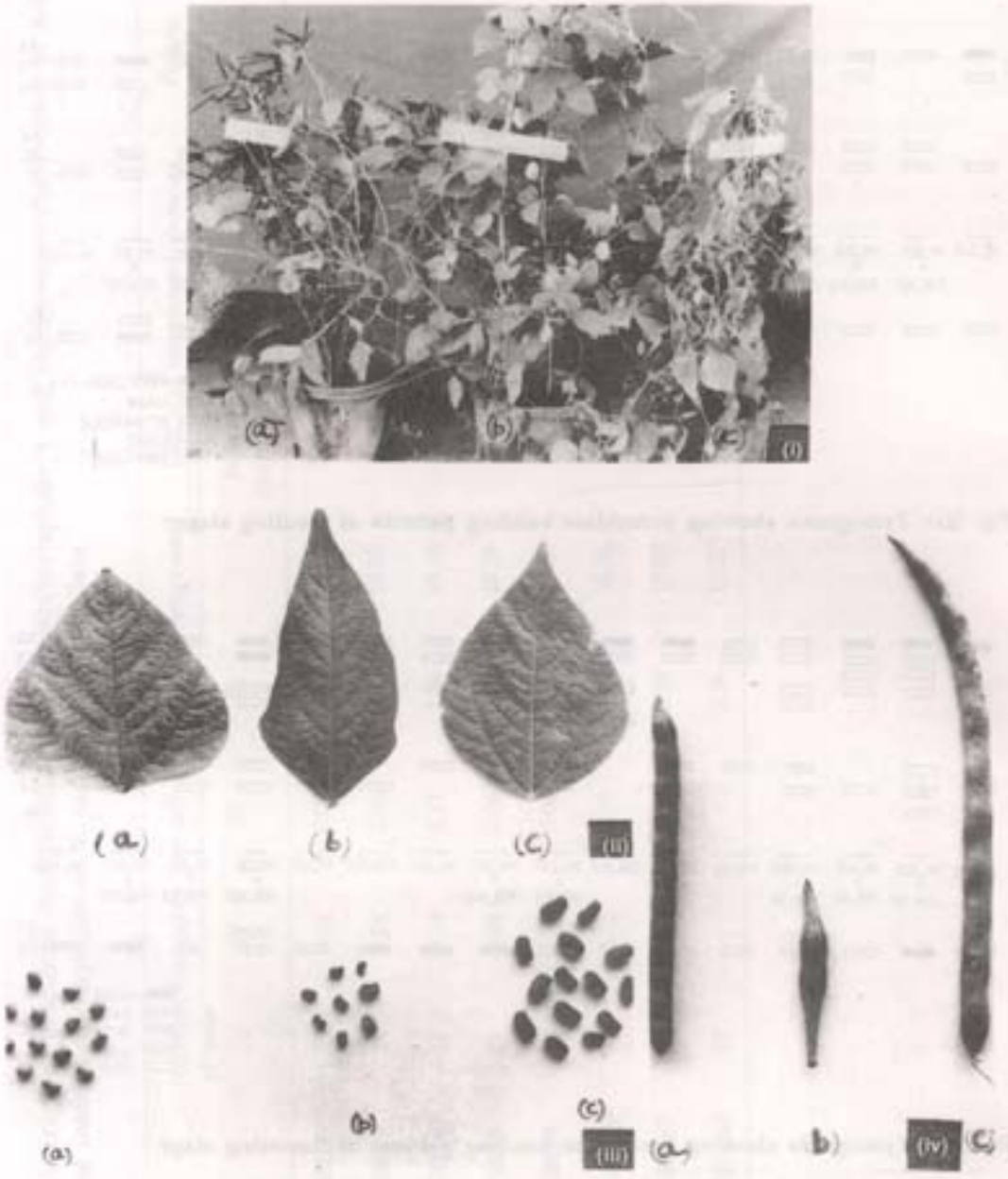


**Fig. 3(i-iv): Morphological characteristics of parents and the hybrids between *V. radiata* and *V. umbellata*. (i) Plants of (a) ML131; (b) ML131 × RBL93; (c) RBL93 (ii) Leaf morphology of (a) ML267; (b) ML267 × RBL 140; (c) RBL140; (iii) Seeds of (a) ML267; (b) ML267 × RBL93; (c) RBL93 and (iv) Pods of (a) ML267; (b) ML267 × RBL93; (c) RBL93**

The codominant expression of isoperoxidase isozymes, thus, could be used as a genetic marker to understand the mechanism of heterosis through complementation as well as to differentiate wide range of plant species as these markers exhibit species specific expression. It also offers a precise method for differentiating genotype/species than the morphological markers [13-16]. Thus, variation in banding pattern at different stages suggest differential gene expression for the identification of intra and interspecific hybrids as well as to understand the mechanism of heterosis through complementation.

Morphologically, the hybrid plants had similarity with either or both the parents. The plant height was either close to male parent or exceeding both the parents except in ML267 × RBL93. The number of primary branches per plant in hybrids was intermediate between two parents. Mean leaflet length was exceeding both parents except in ML131 × RBL93 (9.0 ± 0.39 cm) and ML267 × RBL93 (9.26 ± 0.40 cm). Whereas, leaflet width in the hybrids was intermediate between the two parents except ML611 × RBL167 (9.43 cm). The parents and the hybrids could also be characterized on the basis of leaf shape as it was broadly ovate in mungbean, ovate to ovate lenceolate in ricebean and ovate lenceolate in the hybrids. Mean leaflet petiole length was found either close to female or intermediate. Number of mature pods/plants, mean pod length and number of seeds/pod were least in the hybrids as compared to their parents (Fig. 3 i-iv).

The present study clearly demonstrated *V. radiata* and *V. umbellata* to be two distinct species, although their chromosome number is uniform 2n = 2x = 22. Based on cytological and pollen-pistil interaction studies [10], it was observed that both prezygotic and postzygotic barriers are operating which prevent the successful scoring of hybrids and flow of desirable genes from one species to another. Other barriers including hybrid sterility, breakdown, weak seedlings and chromosomal differentiation also restrict the gene flow between these species. However, it is easy to score interspecific hybrids in the field at seedling stage through morphological and isozyme markers. This will also enable the breeder to induce polyploidy or backcrossing with the desirable parents along with the use of growth regulators and embryo culture techniques to have sufficient number of hybrid seeds. It will allow free exchange of genetic material which can further be improved through progeny selection and intermating of desirable genotypes to enhance recombination to break undesirable linkages, thereby combining desirable attributes of both species with better yield potential.

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