Induced High Yielding Amphiploid of *Vigna radiata* × *Vigna mungo*

Kalpana Pande, S. S. Raghuvanshi and Dhan Prakash¹

Plant Genetics Unit, Botany Department, Lucknow University,
Lucknow-226007, U. P., India

Accepted November 24, 1989

The Indian subcontinent is apparently the area of great dependence on pulses. Due to high population pressure, the per capita availability of pulses in India is only about 40 g/capita/day as against the FAO/WHO recommended intake of 104 g/capita/day for a balanced diet. Inspite of efforts of plant breeders break through in pulse yield has not been achieved.

Vigna radiata L. Wilczek and *V. mungo* L. Hopper are two important pulses used as major source of protein to supplement cereal diet. They also enrich the soil fertility through biological nitrogen fixation. Both species are short term crops and are grown in rainy season. Though *V. radiata* possesses long pods but it lacks seed dormancy and is susceptible to yellow mosaic virus. On the other hand *V. mungo* var. T₉ possesses several desirable traits as resistance to YMV and seed dormancy but is not a good yielder. Therefore, a programme of interspecific hybridization between *V. radiata* variety T₄₄ as female parent and *V. mungo* variety T₉ as male parent followed by polyploidy was initiated for the combination of desired characters of both of these species into one variety.

The present communication deals with the performance of interspecific F₁ hybrid (2x) and amphiploid (4x) in C₁ and subsequent generations as compared to their diploid progenitors.

Materials and methods

The seeds of *V. radiata* var. T₄₄ and *V. mungo* var. T₉ obtained from C. S. A. University of Agriculture and Technology, Kanpur (U.P.) were sown at Lucknow (India) soil and climatic conditions. *V. mungo* was sown 15 days earlier than *V. radiata* to get synchronised flowering. The flower buds of *V. radiata* which were expected to bloom next day, were emasculated. Stigmas were examined by the hand lens and contaminated flowers were rejected. Emasculated flowers were covered with bags to check the cross-pollination. Next day early in the morning, pollens of *V. mungo* were dusted on the stigmas of *V. radiata* to facilitate pollination.

The mature hybrid pods were collected and seeds were sown to raise F₁ generation. The F₁ seedlings were divided into two lots: one lot was permitted to grow to investigate the morphology of diploid F₁ hybrid, while the shoot tips of the young seedlings of the second lot were treated with 0.2% aqueous solution of colchicine to raise amphiploid. The morphological data of the raw amphiploid (4x) were taken at maturity and compared with F₁ hybrid (2x) and the diploid progenitors. Data were subjected to statistical analysis (AM ± SE and heterosis estimates). Performance of amphiploid was further investigated in C₂ and C₃ generations.

For the assay of nutritional and antinutritional factors the dried seeds were powdered to pass through a 40-mesh screen and defatted with *n*-hexane (Soxhlet) for 6 hrs. The total protein (% N × 6.25) was determined by the Microkjeldahl method. Trypsin inhibitor activity was assayed in the defatted seed meals as described by SMITH *et al.* (1980).

¹ Protein Chemistry Laboratory, National Botanical Research Institute, Lucknow-226001, India.

Results and discussion

The hybrid seeds were green and wrinkled and could be clearly distinguished from the smooth and rounded seeds of both the parents. The hybrid was intermediate between female (Fig. 1) and male (Fig. 2) parents. The stem colour of the hybrid was purple and leaves were narrow which show its resemblance with *V. mungo* in contrast of *V. radiata* which had green stem and broad leaves. However, the flower shape shows its resemblance with *V. radiata*. The yellowness of flower colours of the parents and the F_1 hybrid could be arranged as *Vigna mungo* > F_1 hybrid > *Vigna radiata*. The F_1 hybrid was smaller (Table 1) than the parents. In spite of large number of flowers the diploid hybrid did not produce any pod. The

Table 1. $AM \pm SE$ values of different agronomic traits of diploid progenitors, F_1 hybrid (2x), C_1 , C_2 and C_3 amphiploids (4x)

Characters	<i>V. radiata</i>	<i>V. mungo</i>	F_1 hybrid	Amphiploid		
				C_1	C_2	C_3
Plant height (cm)	31.2 $\pm$ 0.93	27.9 $\pm$ 0.55	27.6 $\pm$ 0.65	37.8 $\pm$ 1.01	39.8 $\pm$ 0.63	43.4 $\pm$ 1.88
Nodes/plant	9.2 $\pm$ 0.83	8.6 $\pm$ 0.64	10.2 $\pm$ 0.37	15.2 $\pm$ 0.37	11.6 $\pm$ 0.40	11.8 $\pm$ 0.91
Branches/plant	1.6 $\pm$ 0.54	1.8 $\pm$ 0.21	3.2 $\pm$ 0.31	8.6 $\pm$ 0.74	7.6 $\pm$ 0.92	7.4 $\pm$ 0.92
Internodal length (cm)	2.4 $\pm$ 0.12	1.9 $\pm$ 0.08	1.9 $\pm$ 0.03	1.8 $\pm$ 0.08	3.1 $\pm$ 0.17	3.5 $\pm$ 0.21
Leaves/plant	8.2 $\pm$ 0.58	10.8 $\pm$ 0.81	11.2 $\pm$ 0.64	20.8 $\pm$ 2.77	15.2 $\pm$ 1.77	23.6 $\pm$ 2.61
Pods/plant	11.8 $\pm$ 0.63	21.1 $\pm$ 2.08	nil	2.2 $\pm$ 0.37	11.4 $\pm$ 1.21	31.4 $\pm$ 6.39
Flowers/plant	36.2 $\pm$ 2.20	38.2 $\pm$ 1.83	66.2 $\pm$ 3.15	112.3 $\pm$ 7.87	104.2 $\pm$ 6.31	108.6 $\pm$ 4.20
Pod length (cm)	7.2 $\pm$ 0.10	4.0 $\pm$ 0.08	nil	3.1 $\pm$ 0.16	3.9 $\pm$ 0.12	5.3 $\pm$ 0.52
Seeds/pod	10.6 $\pm$ 0.67	3.6 $\pm$ 0.42	nil	2.2 $\pm$ 0.21	2.8 $\pm$ 0.23	4.6 $\pm$ 0.78
Yield/plant (g)	3.46 $\pm$ 0.27	2.88 $\pm$ 0.12	nil	0.14 $\pm$ 0.03	1.39 $\pm$ 0.27	5.26 $\pm$ 0.78
Pollen fertility (%)	97.1 $\pm$ 0.49	96.3 $\pm$ 0.37	4.4 $\pm$ 0.46	81.3 $\pm$ 1.94	82.5 $\pm$ 1.67	88.6 $\pm$ 1.31

Table 2. Test of significance (*t*-test) for some morphological traits in diploid parents and C_3 amphiploid

Source of variation	Plant height (cm)	Branches/plant	Pods/plant	Yield plant (g)
1. C_3 amphiploid <i>V. radiata</i>	4.21**	5.01**	6.29**	2.12*
2. C_3 amphiploid <i>V. mungo</i>	8.07**	5.09**	2.87**	2.55*

* Significant at 5%.

** Significant at 1%.

sterility may probably be manifested in the male part, as the pollen fertility of hybrid was very low (4.4%) as compared to the male and female parents (96.3 and 97.1%, respectively). Similar sterility was reported by Smartt (1970) in reciprocal crosses between *P. vulgaris*, *P. lunatus* and *P. acutifolius* and Prendota *et al.* (1883) in *P. acutifolius* $\times$ *P. vulgaris*, whereas in latter report there was partial restoration of fertility after chromosomal doubling of the F_1 hybrid (2x).

The amphiploid (4x) in present studies showed characteristic gigantism in various traits viz., plant height, leaf size, flower and seed over the F_1 hybrid and its diploid progenitors. This is in accordance with the findings of Sawa (1974) in *Phaseolus* and Dutta and Roy (1974) in *Luffa*. The C_2 amphiploid (Fig. 5) was taller than the parents and F_1 hybrid. The C_2 (Fig. 7) and C_3 amphiploid showed gradual increase in height. The leaves of C_1 amphiploid

Figs. 1-6. 1, *Vigna radiata* variety T₄₄ (female parent). 2, *Vigna mungo* variety T₉ (male parent). 3, F₁ hybrid plant (2x). 4, amphiploid plant (4x) in C₁ generation. 5, amphiploid plant in C₂ generation. 6, high yielding amphiploid in C₃ generation.

were thick and dark green in colour and resembled its male parent in shape. In respect to the pod length and number of seeds/pod also the amphiploid was very close to its male parent but inferior than female parent, whereas seeds were brownish-black, bolder and heavier than either of the parents. The number of pods in the raw amphiploid (2.2 ± 0.37) was very low, however, in C_2 (11.4 ± 1.21) and C_3 (31.4 ± 6.39) generations there was tremendous increase resulting in high yield (Table 1).

The test of significance *t*-test was applied to observe differences between the mean of both parents and C_3 amphiploid for different morphological traits which were found to be significant (Table 2).

The heterosis estimates of different agronomic traits of diploid hybrid and amphiploid over superior parent (SP) and mid parent (MP) are presented in Table 3. The F_1 hybrid showed negative heterosis for height and internodal length, while C_2 and C_3 generations of amphiploid exhibited high heterosis over superior parent. Number of nodes, leaves and branches per plant also revealed significant positive heterosis in both hybrid and amphiploids as compared to the diploid progenitors. Number of pods/plant showed negative magnitude in C_1 (-89.62%) and C_2 (-46.22%) generations inspite of exceptionally very high heterosis

Table 3. Heterosis estimates (%) in F_1 hybrid (*V. radiata* $\times$ *V. mungo*), C_1 , C_2 , and C_3 amphiploids over superior parent (SP) and mid parent (MP) for various agronomic traits

Characters	F_1 hybrid		C_1		C_2		C_3	
	SP	MP	SP	MP	SP	MP	SP	MP
Plant height (cm)	-11.53	-6.44	21.15	28.14	27.56	34.91	39.10	46.87
Nodes/Plant	10.87	14.61	65.22	70.78	26.04	30.34	28.26	32.58
Branches/plant	77.78	88.23	377.77	405.88	322.22	347.06	311.11	335.29
Internodal length (cm)	-17.39	-9.52	-21.74	-14.28	34.78	47.62	47.83	61.90
Leaves/plant	3.70	17.89	92.59	118.94	40.74	60.00	118.52	148.42
Pods/plant	nil	nil	-89.62	-86.67	-46.22	-30.91	48.11	90.30
Flowers/plant	73.29	77.95	193.98	201.88	172.77	180.11	184.29	191.93
Pod length (cm)	nil	nil	-56.94	-44.64	-45.83	-30.36	-27.78	-7.14
Seeds/pod	nil	nil	-80.19	-69.01	-73.58	-60.56	-56.60	-35.21
Yield/plant (g)	nil	nil	-95.89	-95.52	-59.82	-56.15	52.02	65.93
Pollen fertility	-95.29	-95.55	-16.52	-16.01	-15.29	-14.82	-9.03	-8.52

for number of flowers/plant in both hybrid and amphiploid. However, C_3 amphiploid (48.11%) exhibited high positive heterosis over superior parent. Pod length and number of seeds/pod showed negative heterosis in all the generations of amphiploid. Negative heterosis was observed for seed yield/plant in C_1 (-95.89%) and C_2 (-59.82%) generations, however, C_3 (52.02%) amphiploid showed high positive heterosis over superior parent. The heterosis is the function of genetic diversity of the parents (Moll *et al.* 1962, Tsotsis 1972).

The protein contents of the male parent (*V. mungo*) and female parent (*V. radiata*) were 21.2 and 19.3%, respectively. The amphiploid of C_3 generation showed 20.8% protein contents very close to both parents. However, the protein contents were appreciably high in the amphiploid C_4 generation (27.4%). The trypsin inhibitor activity (TIA) in the male and female parents were 14.5 and 18.5 mg/g sample, respectively. In the amphiploid C_3 and C_4 generations TIA was 21.0 and 13.0 mg/g, respectively. The lower TIA and higher protein contents of the C_4 amphiploid generation showed its higher nutritional value as compared to those of original parents.

From present studies it may be concluded that interspecific hybrid of *V. radiata* and *V. mungo* after colchicine treatment was superior in yield and C_4 generation was found to be appreciably high in nutritional factors as well.

Summary

Interspecific F_1 hybrid ($2x$) between *V. radiata* var. T_{44} and *V. mungo* var. T_0 showed intermediate morphological characters of both the parents and low pollen fertility (4.4%). In spite of profuse flowering there was no pod formation. However, on the contrary, the amphiploid ($4x$) obtained with the colchicine treatment of the F_1 hybrid seedling exhibited gigantism in most of the characters and restoration of pollen fertility (81.3%) and seed fertility. The amphiploid showed negative heterosis for various agronomic traits in C_1 and C_2 generation. Tremendous increase in the number of pods/plant resulting in 52.02% heterosis in yield over its superior parent was observed in C_3 generation. While C_4 generation of amphiploid besides higher yield was also better for its nutritional value.

Key words

Vigna radiata, *V. mungo*, interspecific hybrid, amphiploid, heterosis, protein and trypsin inhibitor activity.

Acknowledgments

Financial support from University Grants Commission, New Delhi, is gratefully acknowledged by one of the authors (KP).

References

- Dutta, B. and Roy, R. P. 1976. Cytogenetic studies in an experimental amphiploid in *Luffa*. *Cytologia* **29**(1): 15–25.
- Moll, R. H., Salhuana, W. H. and Robinson H. F. 1962. Heterosis and genetic diversity in variety crosses of maize. *Crop Sci.* **2**: 197–198.
- Prendota, K., Baudoin, J. P. and Marechal, R. 1983. Fertile allopolyploid from the cross *P. acutifolius* × *P. vulgaris*. *Bull. Rech. Agron. Gembloux* **17** (2): 177–190.
- Sawa, M. 1974. Interspecific hybridization of *P. vulgaris* W. F. Wight and *P. radiatus* II. Characteristic of C_1 amphiploid of *P. radiatus* × *P. calcaratus* Roxb. *Japanese Journal of Breeding (Ikushugaku Zasshi)* **24** (6): 282–286.
- Smartt, J. 1970. Interspecific hybridization between cultivated American species of the genus *Phaseolus*. *Euphytica* **19**: 480–489.
- Smith, C., Megen van W., Twaalhoven, L. and Hitchcock, C. 1980. The determination of trypsin inhibitor levels in food stuff. *J. Sci. Food Agric.* **31**: 341–350.
- Tsotsis, B. 1972. Objectives of industry breeders to make efficient and significant advances in the future. *Proc. Ann. Corn Sorghum Res. Conf.* **27**: 93–107.
-