Indian J. Pl. Genet. Resources 3 (1) : 46-60, 1990

INTERSPECIFIC HYBRIDIZATION AND CROSSABILITY STUDIES AMONG CULTIVATED AND CLOSELY RELATED WILD *Vigna* SPECIES IN INDIAN GENE CENTRE

B. Audi Lakshmi[1] and K.P.S. Chandel

National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi - 110 012

Interspecific crosses among different cultivated and wild species of genus Vigna *were attempted involving* mung *bean (*V. radiata *(L) var.* radiata *Wilczek),* V. radiata *(L) var.* sublobata *(Roxb.) Verdc.,* urad *bean (*V. mungo *(L) Hepper) and wild progenitor* V. mungo *(L) var.* silvestris *Lukoki Marechal and Otoul. The reciprocal crosses were simultaneously attempted in each cases and vice-versa. Several other crosses between* V. radiata *var.* sublobata *and* V. trilobata Ait. *and also with rice bean (*V. umbellata (Thunb.) Ohwi and Ohashi *were performed. Successful crosses were established between mung bean with its wild putative progenitor form* V. radiata *var.* sublobata. *Fertile F_1 hybrids were also successfully produced from crosses involving urad bean with its closely allied species* V. mungo *var.* silvestris. *Interestingly,* mung *bean when used as a female parent in the cross combinations involving var.* sublobata *and var.* silvestris *independently, exhibited considerable cross compatibility. The results clearly suggest that above allied taxa fall into the primary genepools and do not possess genetic barriers.* V. umbellata *exhibited cross incompatibility barriers when crossed with* V. radiata *var.* sublobata. *The reasons for failure of crosses have been critically analysed deriving evidential support from several other investigations. The paper presents a comprehensive review on the interspecific hybridization to identify closely related genepools of Asiatic species of* Vigna *based on crossability studies.*

Interspecific hybridization has played significantly important role in the speciation and evolution of several flowering plants in nature. At the sametimes, experimental hybridization has been used to study the nature and extent of isolating barriers operating between species, such studies also provide evidences for understanding the phylogenetic relationships (Ahn and Hartmann, 1978). Further interspecific crosses among cultivated and wild progenitors and weedy races are important for the understanding of the extent of crossability in order to ascertain the success of gene transfer from wild to the advance cultivars. It is well recognised fact that weedy races have served as reservoirs of germplasm periodically transferring/exchanging genes into the crop under conditions that would most favour increase in variability, heterozygosity and heterosis (Harlan, 1975). Weedy races have played immensely valuable role in the evolution of domesticated plants.

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[1] Scientist, National Research Centre for Sorghum, Rajendranagar, Hyderabad, 500 030

However, once isolated from cultivated forms have no mechanism to transfer genes even occasionally unless helped by man under controlled environments. Similar situation exists among genus *Vigna* sub section 'Ceratotropies', where wild occurring closely related species possess useful genes, that could easily be transferred from them to the cultivated species, once our knowledge about their relationships and mechanism for the exchange of genes is well understood. Unfortunately there has been regrettable lack of consistency among taxonomists in naming such species and forms, the expedient of naming them as subspecies has created considerable confusion (Smartt, 1979; 1990). The *Vigna* species group provides such an example. Infact this problem could be avoided by adoption of a constant practice in the naming of conspecific wild and cultivated forms. This perhaps could be most helpful in further investigations. The present study is an attempt to highlight the results of the interspecific hybridization and crossability studies among *mung*, *urad*, rice bean and their well authenticated wild putative progenitor species/forms.

MATERIALS AND METHODS

Interspecific hybridization and crossability studies were attempted between *mung* bean (*Vigna radiata* (L) Wilczek) and its wild progenitor species *V. radiata* var. *sublobata* Verdc.; also between *urad* bean (*V. mungo* (L) Hepper) and its wild progenitor species *V. mungo* var. *silvestris* Lukoki Marechal and Otoul. Reciprocal crosses among above species were also attempted. Besides, interspecific crosses were made between *V. trilobata* and *V. umbellata* independently with *V. radiata* var. *sublobata* Verdc. For above mentioned species crosses, two accessions each in *mung* bean (Plm 586 and Plm 124), *V. radiata* var. *sublobata* (Plx 270 and Plx 274), *urad* bean (Plu 261 and Plu 274) and four accessions of wild *V. mungo* (L) var. *silvestris* (Plx 298, Plx 409, Plx 413 and Plx 416) were utilized. Besides, one accession each in *V. umbellata* and *V. trilobata* was involved in the cross with *V. radiata* var. *sublobata*. The material was grown in the field at NBPGR farm at Pusa Campus, New Delhi. Each accession was planted in two rows, each 2 m long, 60 cm apart with 15 cm distance between plants. Interculture operations and prophylactic measures were adequately adapted to grow a good healthy crop. To avoid any risk, different species were also grown in pot culture under controlled green house conditions. The emasculation was carried out during the month of September between 3 to 5 PM and pollination was carried out next morning between 8 to 9 AM The details of the material, main distinguishing features of each species and details of crosses etc., are given in Table 1 and Table 2, respectively. Approximately 250 crosses were attempted in all combinations among six cultivated and wild *Vigna* species.

RESULTS AND DISCUSSION

Among the 250 crosses attempted between cultivated species of *mung* bean, *urad* bean and their wild ancestral forms *V. radiata* var. *sublobata* and *V. mungo* var. *silvestris* respectively, involving several accessions resulted in variable success. The crosses between *V. umbellata* and *V. radiata* var. *sublobata* and between *V. trilobata* success showed only in latter case.

Table 1. Major differences between important cultivated and allied wild *Vigna* species

S. No.	Characters	Mung bean (*V. radiata*)	Wild Progenitor (*V. radiata* var *sublobata*)	Urad bean (*V. mungo*)	Wild progenitor (*V. mungo* var. *silvestris*)	Moth bean (*V. aconitifolia*)	Rice bean (*V. umbellata*)	*V. trilobata*
1.	Distribution/ Occurrence	Widely cultivated in India	Natural habitats, Widely spread in Himalayas, Northern plains and peninsular India	Widely cultivated in India	Wild, natural, Disturbed habitats in Poona-Khandla region, Western Ghats	Widely cutlivated north western India, central India and Decan Plateau	Mainly cultivated North-Eastern India Sikkim, W. Bengal, Bihar and Orissa	Semi wild, wide spread in northern plains, central India
2.	Plant/type growth habit	Dwarf, erect, determinate, branched, vigourous	Erect indeterminate, long internodes vigorous branched	Dwarf, erect, semi prostrate to indeterminate type, viny	Thin stemmed tendrillous forms. condensed inter-form exist	Erect viny, semi prostrate to procumbent forms, branched node, branched	Erect to viny indeterminate forms branched. profusely	Erect to semi-viny cultigen forms Vigourous forms diffused
3.	(a) Leaflet shape	Entire, broad	Small, elongated	Variable shape	Small to moderately large	Small moderately lobed to highly dissected	Large, broad moderately lobed	Small, deeply lobed
	(b) Post cotyledondery leaf	Sessile	Sessile	Sessile	Sessile	Petiolate	Petiolate	Petiolate
4.	Seedling Pigmentation	Green	Green	Purplish	Purple	Purple	Purple	Nonpigmented
5.	Stipule (shape/ size)	Broad, peltate	Broad peltate	Linear lanceolate	Linear lanceolate	Moderately broad	Broad lanceolate	Small peltate
6.	Pod size (cm.)	4.0 - 16.0	4.0 - 6.0	4.0 - 8.0	2.8 - 4.2	3.0 - 4.5	7.5 - 14.0	2.5 - 4.5
7.	Pod shape	Straight, sickle shaped, cuneate	Straight, slightly cuneate	Slightly curved, cuneate	Slightly curved cuneate	Small, slightly curved, cuneate	Pod straight or curved, cylinderical	Small, straight or slightly curved
8.	Pod colour	Brown, dark tan	Black, straw coloured	Brown, tan	Straw coloured	Straw coloured, brown	Brown or straw coloured	Brown or straw coloured
9.	Pods/peduncle	4 - 8	4 - 8 Sometimes only 1	4 - 8	4 - 6 Generally in cluster	3 - 5	3 -11	2 - 6,
10.	Orientation of pods on peduncle	Asecnding suberect	Ascending suberect	Usually borne horizantally or facing upward	Usually borne horizontally deflexed type	Ascending	Asceding suberect, dropping	Usually ascending, borne horizontally
11.	Pod dehiscence	Delayed shattering	Violent shattering	Delayed shattering	Violent shattering	Delayed shattering in cutigen and violent in wild forms	Delayed shattering in cultigen and violent in wild forms	Highly violent shattering

12.	**Peduncle size**	Small to moderately long	Small to moderately long	Moderately long	Long	Moderate	Moderate	Very long
13.	**Pubescence**	Smooth or mildly hairy	Hairy plant type, pods pubescent	Smooth pod	Hairy pods	Plants non hairy, pods mildy hairy	Pod glabrous	Glabrescent leaves and pods
14.	**Seeds/pod**	8 - 16	8 - 14	6 - 8	10 - 14	6 - 8	8 - 12	8 - 12
15.	**Seed colour (S)**	Yellow, green brown and chocolate	Dark brown, tan	Green, black, greyish mottled	Dark brown, tan	Green, greyish	Yellow, green, red, brown, black, chocolate (very variable)	Brown, blackish
16.	**SEM seed coat cellular structure**	Elongated, rectangular cells arranged in parallel rows	Very much elongated, rectangular arranged in parallel rows; similar to cultigen	Moderate in size, hexagonal cells, cup shaped	Moderate hexagonal cells, cup shaped, similar to cultigen	Epidermal cells Irregular	Seed surface smooth epidermal cells arranged in network	Epidermal cells hexagonal, cup shaped
17.	**Hilum shape and structure**	Linear, flatish not raised	Linear, flastih not raised	Deeply furrowed, concave raised and arilate	Deeply furrowed, concave, raised arilate	Linear, wedge shaped arilate	Prominent, raised rectangular in shape set on one side	Semi raised hilum, broad
18.	**Seed germination habit**	Epigeal	Epigeal	Epigeal	Epigeal	Epigeal	Hypogeal	Epigeal
19.	**Protein (%)**							
	range	20.4 - 26.90	15.7 - 22.0	21.0 - 28.80	26.00	22.5	19.0 - 25.0	14.8 - 18.5
	Average (%)	(23.6)		(23.6)			(25.0)	
20.	**C DNA content (pg.)**	2.67	*	2.83	*	*	2.84	2.60
21.	**Y-glytaml methionine and its sulfoxide**	Absent	Absent	Present	Present	*	*	*
22.	**Y-glutamyl-S methyle Cystine and its sulfoxide**	Present	Present	Absent	Absent	*	*	*
23.	**Presence/absence Amino acids**	Methionine (1.14)	Methionine (1.18)	Methionine (2.00)	Methionine (1.65)	*	Methionine (1.18)	*

* Information not available

Cross I. *V. radiata* x *V. radiata* var. *sublobata*.

The above cross was fully compatible. The two accessions of *mung* bean (Plm 586 and Plm 124) crossed freely with var. *sublobata* (Plx 274). The fertile hybrids were obtained in above cross. The initiation of pod set among pollinated buds took place in 64 per cent cases, while 48 per cent pods reached the full maturity stage with well developed pods/seeds. The harvested seeds possessed 100 per cent viability. The pollen fertility of F_1 hybrid was as high as 90 per cent. The other strain of mung bean (Plm 124) crossed very well with var. *sublobata* (Plx 274) and in both the crosses, hybrids were fully fertile and vigorous, being twiny. Leaves were like *V. radiata* while pods and seeds resembled more like *V. radiata* var. *sublobata*. However, cross involving mung bean with wild var. *sublobata* (Plx 270) was unsuccessful. The cross combination of *mung* bean, *urad* bean and their wild forms exhibited pod initiation only in 16 per cent cases, out of which only 40 per cent reached maturity.

Cross II. *V. radiata* var. *sublobata* x *V. radiata* (Reciprocal cross).

In the reciprocal cross involving var. *sublobata*, the wild progenitor forms (Plx 274 and Plx 270) as female parent and cultivated *mung* bean (Plm 586), 24 per cent pods were initiated, 22 per cent reached maturity while remaining 78 per cent pods dropped after 6-10 days of initiation. Four seeds obtained from this cross were shrivelled and did not germinate.

Cross III. *V. radiata* x *V. mungo* var. *silvestris*

Mung bean accession Plm 586 was crossed with var. *silvestris* (Plx 409, Plx 298 and Plm 416). Out of buds emasculated, 23 per cent pods initiated but only 11 per cent reached maturity yielding 4 shrivelled seeds. Remaining buds dropped after 8-11 days of initiation.

Cross IV. *V. mungo* var. *silvestris* x *V. radiata* (Reciprocal cross).

Pollination of var. *silvestris* using four wild type accessions (Plx 298, Plx 409, Plx 413 and Plx 416) did not result in pod initiation due to cross incompatibility. It clearly indicated that perhaps some genetic barriers existed between these wild putative ancestral forms of urad and mung bean.

Cross V. *V. mungo* x *V. mungo* var. *silvestris*

Two well identified and authenticated *urad* bean accessions (Plu 261 and Plu 274) were crossed with var. *silvestris* (Plx 298 and Plx 409) as pollen parent.

(i) *V. mungo* (Plu 274) x *V. mungo* var. *silvestris* (Plx 298)

(ii) *V. mungo* (Plu 261) x *V. mungo* var. *silvestris* (Plx 409)

The crosses were fully compatible and fertile F_1 hybrids were successfully established showing that post zygotic isolating mechanisms were not operative between these two species and there is free exchange of genes. The hybrids were more vigorous and early flowering than both the parents. Plants were viny with narrow lanceolate leaves like that of var. *silvestris* while pods and flower colour resembled like *urad* bean.

Cross VI. *V. mungo* var. *silvestris* x *V. mungo* (Reciprocal).

In this reciprocal cross, 18 per cent pods were initiated, but only about 25 per cent of these reached maturity. Matured pods yielded few shrivelled unfilled seeds which did not germinate possibly due to embryo inviability.

Cross VII. *V. mungo* x *V. radiata* var. *sublobata.*

This cross was not attempted earlier due to taxonomic and nomenclatural confusion that existed before. The F_1 hybrid from the cross (Plu 261 x Plx 270) was more like female parent in plant habit, leaf shape and branching pattern. It possessed small pale yellow flowers and very short, condensed internodes. The pollen was highly sterile (upto 74%); bud initiation took place but no seed setting was observed. The crossability results showed that cross incompatibility exists between *urad* bean (*V. mungo*) and wild progenitor from *V. radiata* var. *sublobata.*

Cross VIII. *V. radiata* var. *sublobata* x *V. mungo.* (Reciprocal).

When var. *sublobata* buds were pollinated from pollen obtained from cultivated accessions of *urad* bean, 3 per cent pod initiation took place but none of the pods reached maturity.

Cross IX. *V. radiata* var. *sublobata* x *V. mungo* var. *silvestris*.

The above cross involved both the wild putative progenitor forms of *mung* and *urad* beans respectively. Two wild accessions of var. *sublobata,* Plx 270 (Chandigarh) and Plx 274 (Rishikesh) were crossed as female parents with var. *silvestris* (Plx 409, Plx 298 and Plx 416) as pollen parent. Among this group, pod initiation was observed only in the cross Plx 270 x Plx 409. 18 pollinations were attempted, out of which in 33 per cent cases, pods were initiated, while only 5-8 per cent pods attained maturity and five seeds were obtained giving rise to sterile hybrids. The F_1 hybrid possessed intermediate stipules of moderate width, the flower colour and leaves were similar to that of var. *sublobata.* Interesting results were obtained in the reciprocal crosses involving var. *silvestris* x var. *sublobata.* The var. *silvestris* (Plx 409 and Plx 298) were crossed with var. *sublobata* (Plx 270 and Plx 274). Out of 28 buds pollinated, 23 pods initiated and 23 per cent pods matured yielding to fully ripened seeds. The F_1 hybrids had twiny plant habit, intermediate plant type and flower colour and were observed to be sterile.

Cross X. *V. mungo* var. *silvestris* x *V. radiata* var. *sublobata* (Reciprocal).

In this unique reciprocal cross between two wild putative progenitor forms of *urad* and *mung* beans respectively four distinct forms were hybridized as female parent with two distinct forms of var. *sublobata* as pollen parent Table 2. The pod initiation was not observed in most of the crosses except in a cross involving Plx 409 x Plx 274. In this cross, pollen fertility was found to be very low resulting in complete sterility.

Cross XI. *V. radiata* var. *sublobata* x *V. trilobata*

The cross between wild putative progenitor of mung bean (*V. radiata* var. *sublobata*) with the wild occurring types *V. trilobata* was very interesting. In this

Table 2. Interspecific crosses among cutlivated and wild Asiatic *Vigna* species in India

Cross	Buds pollinated	Pods initiated	No. of days after pods dropped	No. of pods matured	No. of seeds obtained	Germination %	(F1 (Hybrids) Pollen Fertility %	(F1 (Hybrids) Pod setting
I *V. radiata* x *V. radiata* var. *sublobata*								
Plm 586 x Plx 274	25	16	2 pods dropped in 12 days	12	28	100	90%	Fertile
Plm 586 x Plx 270	14	0	1 " " " 8 "	0	–	–	–	–
Plm 124 x Plx 274	16	4	1 " " " 4 "	2	2	100	80-90	Fertile
Plm 124 x Plx 270	2	–	1 " " " 4 "	–	–	–	–	
Plm 1290 x Plx 274	10	2	2	7	100	90		Fertile
II *V. radiata* var. *sublobata* x *V. radiata* (Reciprocal)								
Plx 270 x Plm 586	22	3	2 pods dropped in 8 days 1 " " " 6 days					
Plx 274 x Plm 586	20	6	2 " " " 10 days 2 " " " 8 days	2	4	0	–	–
Plx 270 x Plm 124	10	–		–				
Plx 274 x Plm 124	10	–		–				
III *V. radiata* x *V. mungo* var. *silvestris*								
Plm 586 x Plx 409	28	6	1 pod dropped in 10 days 3 " " " 8 days 1 " " " 7 days	1	4 shrivelled seeds	0	–	–
Plm 586 x Plx 298	6	2	2 " " " 11 days	–				
Plm 586 x Plx 416	5	2	1 " " " 9 days					
IV *V. mungo* var. *silvestris* x *V. radiata*								
Plx 298 x Plm 586	15	–						
Plx 238 x Plm 124	10	–						
Plx 409 x Plm 586	12	–						
Plx 413 x Plm 586	8	–						
Plx 413 x Plm 124	8	–						
Plx 416 x Plm 586	18	–						
Plx 416 x Plm 124	16							

(Table Continued)

(Continued from Page 52)

V. *mungo* x V. *mungo* var. *silvestris*								
Plu 261 x Plx 298	20	2	–	2	6	66	90-95	Fertile
Plu 268 x Plx 409	18	2	–	2	4	100	90	Fertile
Plu 274 x Plx 298	16	4	–	4	12	100	90	Fertile
VI. V. *mungo* var. *silvestris* x V. *mungo* (Reciprocal)								
Plx 298 x Plu 274	15	2	2 dropped in 6 days	–	–	–	–	–
Plx 298 x Plu 261	6	4	3 " " 9 "	1	2 shrivelled seeds	0	–	–
Plx 409 x Plu 261	10	–		–	–	–	0	–
VII. V. *mungo* x V. *radiata* var. *sublobata*								
Plu 261 x Plx 270	20	2	1 dropped in 12 days	1	2	50	50-60	Sterile
Plu 261 x Plx 274	8	–						
Plu 274 x Plx 270	8	1	1 dropped in 7 days	–				
Plu 274 x Plx 274	10	–						
VIII. V. *radiata* var. *sublobata* x V. *mungo* (Reciprocal)								
Plx 270 x Plu 261	15	2	2 dropped in 10 days					
Plx 274 x Plu 261	20	–						
Plx 274 x Plu 274	15	–						
IX. V. *radiata* var. *sublobata* x V. *mungo* var. *silvestris*								
Plx 270 x Plx 409	20	6	1 dropped in 13 days " " " 14 days	3	2 bold + 3 shrivelled seeds	50	40	Sterile
Plx 270 x Plx 298	13		" " " 15 days					
Plx 270 x Plx 416	10							
Plx 274 x Plx 409	14							
Plx 274 x Plx 298	13							
X. V. *mungo* var. *silvestris* x V. *radiata* var. *sublobata* (Reciprocal)								
Plx 409 x Plx 270	15	–	–	–	–	–	–	
Plx 409 x Plx 274	15	4	–	4	5 bold seeds 2 shrivelled seeds	28	30-40%	Sterile
Plx 298 x Plx 270	13			–	–			
Plx 298 x Plx 274	12			–	–			
XI. V. *radiata* var. *sublobata* x V. *Trilobata*								
Plx 274 x I.W. 2488	42	5	–	28	10	95	–	–
XII. V. *umbellata* x V. *radiata* var. *sublobata*								
Plx 274 x I.C. 17656	39	5	–	2	2 shrivelled seeds	–	–	–

cross, out of the 42 buds pollinated after emasculation, only in 12 per cent cases pod initiation was observed. Out of this 69 per cent pods matured yielding 10 well developed seeds. The cross was unique in the sense that both the wild species occur sympatrically in the natural/disturbed habitats and such situation can possibly allow introgression of genes from these two or more allied species. The success of the cross resulting in the well developed hybrid seeds, suggests that these wild species constitute the same genepool. This cross has not been attempted earlier.

Cross XII. *V. umbellata* x *V. radiata* var. *sublabata.*

In all, 39 buds were emasculated and pollinated, 15 per cent pods initiated, however, only 5 per cent pods matured resulting in shrivelled immature seeds. In reciprocal cross, 28 per cent pods were initiated without yielding any mature seeds. This showed that cross was incompatible and cross ability barriers existed between above species. Also that var. *sublobata* is not related to *V. umbellata.* The later appears to have evolved independently from its wild occurring putative ancestral forms.

The cytological studies of the F_1 hybrids of the crosses, *Vigna radiata* x *V. radiata* var. *sublobata*; *Vigna radiata* var. *sublobata* x *V. mungo* var. *silvestris; Vigna mungo* x *V. mungo* var. *silvestris*; *Vigna mungo* x *V. radiata* var. *sublobata* were performed. The studies included pachytene and diakinesis analysis.

In the first cross, out of the five seeds obtained, all germinated very well. However, only 3 hybrid plants survived till maturity. The F_1 hybrid resembled female parent in plant habit, leaf shape, size and flower size, while pod and seed characters resembled with pollen parent (var. *sublobata*). The hybrids from above crosses showed 80-90 per cent pollen fertility and fertile pods were obtained. The pachytene and diakinesis showed complete pairing with eleven normal bivalents. The perfect chromosome pairing between *V. radiata* with *V. radiata* var. *sublobata* clearly suggested their close affinity and phylogentic relationships.

The flowering buds of the F_1 hybrids between a cross involving urad bean (*V. mungo* (L) Hepper) x *V. mungo* var. *silvestris* Lukoki, Marechal and Otoul were fixed in acetic alcohol and the meiotic studies were undertaken. In two respective hybrids produced from two independent cross combinations, the pollen fertility ranged from 80-90 per cent and 95-98 per cent respectively. The pachytene showed perfect chromosomal homology and complete pairing with eleven bivalents. Dikenesis confirmed association of 11 pairs of chromosomes. However, majority of cells showed some kind of loose association near nucleoli in 4-5 bivalents. The F_2 populations were raised through the seeds obtained from F_1 raised plants. The segregating populations (H 67, H 68, H 69, H 70, H 71, H 72 and H 73) exhibited considerable variations giving rise to very valuable recombinations for yield components, seed size, colour and resistance/tolerance to yellow mosaic virus disease.

The hybrid (*V. mungo* x *V. radiata* var. *sublobata*) was sterile with 50% average pollen fertility. The cytology of the meiosis showed incomplete pairing between all the eleven bivalents, showing loops in pachytene and 2-4 univalents in many cells at diakinesis. A tetravalent was also observed in one cell. In the cross involving two wild putative progenitors var. *sublobata* and var. *silvestris* of *mung* and *urad* beans respectively, the hybrids were highly sterile. The pollen fertility was low and ranged between 30-40 per cent. Meiotic studies showed loops and incomplete pairing at dikinesis. Very few cells showed 11 bivalents. Most of the cells possessed 2-5 univalents.

Interspecific hybridization among several cultivated species of *Vigna* have been attempted earlier by several workers in the past two decades. However, the real situation remained hazy and blurred due to the confused nomenclature of the Indian cultigens and taxonomy of several wild occurring species and their forms continued to be eluding the correct scientific status. This fact is amply reinforced by the excellent review on interspecific hybridization (Smartt, 1979 and 1990). It was emphasized that considerable difficulty is encountered while discussing interspecific hybridization due to lack of satisfactory definition of the species. No experimental studies were reported of hybridization with wild species earlier (Smartt, 1971). This study reports the results of interspecific hyrbridization and crossability studies between well known cultivated species/forms and several well identified and authenticated wild species (putative progenitor forms) occurring in the natural and disturbed habitats in Indian Gene Centre.

A very comprehensive crossing programme involving 250 crosses was attempted between different species of cultivated and wild *Vigna* species comprising several accessions. The crosses between *V. radiata* x *V. radiata* var. *sublobata* was successful while reciprocal cross did not succeed. The cross involving *V. radiata* with *V. mungo* var. *silvestris* also did not yield fruitful result. Similarly, cross between two wild putative progenitor forms var. *sublobata* and var. *silvestris* resulted in sterile hybrids. The two cross combinations involving *V. mungo* var. *silvestris* as male parent and *V. mungo* as female parent, were fully compatible and fertile F_1 hybrids giving rise to useful recombinants were established. The cross established the close relationships between two species. However, reciprocal cross did not result, as matured pods yielded few shrivelled unfilled seeds which did not germinate. The cytological studies involving the meiotic homology and segregation behaviours showed perfect pairing and complete homology forming 11 bivalents at pachytene and diakinesis.

Apart from our own studies, interspecific crosses between *V. mungo* and *V. mungo* var. *silvestris* were made (Dwivedi and Singh, 1985 a). The cross was found fully compatible and F_1, F_2 and F_3 generations were produced. The recombinants possessing resistance to *mung* bean yellow mosaic virus were recovered. The resistance was reported to be digenic and controlled by recessive genes. The hybrid between *V. mungo* x *V. radiata* var. *sublobata* and cross involving *V. radiata* var. *radiata* x var. *silvestris* gave rise to sterile hybrids showing incomplete pairing between the chromosomal complements of the species involved in the crosses. The pollen fertility being very low and formation of loops at dikinesis. The hybrid break down resulted from the embryo inviability and non homology or lack of complete pairing or due to irregular meiosis. Sterile hybrids were earlier reported in cross involving *V. radiata* x *V. mungo* (Dana, 1966). In above cross, meiosis was observed to be irregular in the hybrids and 11 bivalents were noted in 50 per cent of the cells. Similarly wild var. *sublobata* crossed very well with *V. radiata* and fertile hybrids

V. mungo, produced only shrivelled seeds (Dana, 1975). In earlier reports, the production of hybrid between *V. radiata* x *V. mungo* was reported (Sen and Ghosh, 1960). Cytogenetic differentiation apparently appeared to have occurred between the two cultigen species *mung* and *urad* beans (De and Krishnan, 1966). The above authors had based their conclusions on the assumption that *P. sublobatus* (*V. radiata* var. *sublobata* Verdc.) was the common ancestral form of both the domesticated species (de Candolle, 1886; Baker, 1879; Prain, 1897; Vavilov, 1951; Zeven and Zhukovskij, 1975), which has been disproved recently (Lukoki *et al.*, 1980; Chandel, 1984; Chandel *et al.*, 1984).

In a similar studies involving interspecific crosses, one of our wild forms of *Vigna radiata*. var *sublobata* (Plx 270,) was crossed independently with *mung* and *urad* beans (Biswas and Dana, 1945). It was reported that all the three species and their hybrids possessed 2n = 22 chromosomes. Cytogenetic evidences of closeness of *V. radiata* var. *sublobata* with *V. radiata* was also provided (Karmakar and Dana, 1987). The hybrids of *V. radiata* x *V. radiata* var. *sublobata* possessed 78 to 90 per cent fertile pollen and produced many well developed seeds. Completely paired eleven bivalents at pachytene and equal separation of chromosomes in most of the cells at later stages in the hybrid showed that cross was fully compatible and var. *sublobata* is the close progenitor of *mung* bean. However, when Plx 270 was crossed with *V. mungo*, it exhibited 26-88 per cent pollen fertility resulting only in two shrivelled seeds. Closely paired bivalents were noted in most of the pachytene cells in F_1 hybrid. Duplication and deficiency loops were observed in few cases.

Interspecific hybridization between four cultigen species were also reported (Ahn and Hartmann, 1975; 1978). *V. radiata* produced fertile hybrids as a female parent with *urad* bean (*V. mungo*), rice bean (*V. umbellata*) and adzuki bean (*V. angularis*). However, reciprocal crosses were reported to be non-viable. A hybrid between *V. umbellata* x *V. angularis* was also reported. The meiotic studies of F_1 hybrid suggested occurrence of chromosomal structural differences between *V. radiata* and other species. In the above interspecific hybridization studies, after pod initiation in most of the crosses, embryo aborted before reaching maturity stage except in case of pollination of *mung* bean by *urad* bean and rice bean, the embryo abortion was not evident. As is the case in the present investigations, embryo culture was followed by loss of all seedlings in the seedling stage, while seedlings which survived remained weak and those which grew well produced hybrid plants with complete or partial sterility. The results indicate similar patterns in the unsuccessful crosses.

The differential behaviour of varieties/genotypes in interspecific hybridization and crossability studies emphasized clearly the need for involving more than one genotypes. Further, it may be seen from the results that reciprocal differences do exist in such interspecific crosses, the manifestation of which can be noted from embryo abortion, embryo invigorousity and seedling mortality. In this only few reciprocal crosses were successful. It is primarily due to the fact that unidirectional success is a common phenomenon in all crosses between any two species. It is believed that cytoplasmic differentiation is assumed to have played the primary role in speciation of several *Vigna* species.

In this study, a unique interspecific cross was attempted between *V. radiata* var. *sublobata* x *V. trilobata*. The cross was quite successful as out of the 42 buds pollinated, 10 well developed seeds were obtained. It suggests that gene exchange can occur between these two wild species in the nature and *V. trilobata* together with var. *sublobata* and *V. radiata* constitute the secondary gene pool. However, when *V. umbellata* was crossed as female parent with var. *sublobata* as pollen parent, the cross was not successful. Incompatibility and crossability barrier resulted in the embryo abortion and inviability. Possibly *V. umbellata* has evolved independently. Earlier, crosses between *V. radiata* x *V. umbellata* (Dana, 1966 b), with *V. trilobata* (Dana, 1966 c) and also between *V. radiata* x *V. mungo* (Dana, 1966 d) were made. Successful hybridization between *V. angularis* x *V. radiata* and *V. radiata* x *V. umbellata* were reported (Megumi Sawa, 1974). It was suggested that differentiation among *V. radiata* and *V. mungo* was less apparent (Ahn and Hartmann, 1978). According to them, some gene exchange appears to be possible between *V. radiata* and *V. mungo* and also between *V. umbellata* and *V. angularis*. However, the later species represented a closed genepools as *V. radiata* produced sterile hybrids between both the above species. The fertilization barriers were encountered in crosses involving *V. minima* and *V. umbellata*. The delay and absence of divisions in the endosperm and the failure of embryo to divide were considered as the post fertilization barriers responsible for somatoplastic sterility in normal crosses which yielded a few hybrid seeds. *V. minima* was considered to constitute genepool of the rice bean.

It has been observed in the present investigations that when closely related wild and cultivated species/forms were crossed, the hybrids produced were frequently viable and fertile in F_1 and subsequent generations such as crosses of *mung* bean with var. *sublobata* and that of urad bean with var. *silvestris*. It provides clear evidence that two forms (wild and cultivated) are conspecific, although much nomenclatural and taxonomic confusion has continued to prevail; and due to which such forms have been assigned different specific names. The success of presumed interspecific hybridization frequently prompts a re-examination of the taxonomic status of the hybridizating parents, where these forms produce hybrids with little or no immediate reduction of fertility and follow normal patterns of segregation. In case of genus *Vigna*, this has been a frequent case, when such forms have been confused for correct taxonomic status.

In such cases, one parent is an highly evolved domesticated species (*mung*, *urad*, *moth* or rice bean) and the other is a wild plant. There is a strong presumption that both are descendent from a common ancestral form, part of which was then domesticated, thus they are conspecific (Smartt, 1979). It is reasoned that very nature of the evolutionary mechanism, the generation of morphological and other variations of discontinuous nature between populations which enables the recognition of species/subspecies is a continuing process. It is argued (Smartt, 1979) that theoretically, a population having reached the stage of effective isolation, would then proceed to generate internally further discontinuities, if appropriate selection pressure is able to operate. Another, confusing factor in the situation arises from differences in taxonomic practice in the naming of conspecific wild and cultivated forms.

The interspecific hybridization and crossability studies among genus *Vigna* involving (i) only cultivated species; (ii) where one parent is cultivated and another is wild and (iii) where both the parents are wild has brought out the facts that failure of interspecific hybrids among *Vigna* could be attributed to several events and processes. In those crosses where hybrid was completely pollen sterile, the cytological causes underlying sterility in the hybrid encompasses array of meiotic abnormalities ranging from no pairing, presence of univalents, chain and ring multivalents and sometime anaphase bridges to the formation of micronuclei during microsporogensis. Several earlier reports as well as present investigations amply suggest that failure to establish F_1 hybrid could occur due to failure of foreign pollen to germinate on female stigma, pollen tubes may fail to penetrate the styler tissue, pollen nuclei may fail to effect the double fertilization. Often it is observed that fertilized egg or zygote may fail to develop and subsequently may abort. In some cases, endosperm may fail to develop and induce abortion of a potentially viable embryo. In some cases suspensor fail to function normally and produce a late abortion (Ahn and Hartmann, 1978). Apparently, normal seed may develop, as is the case in the several interspecific crosses in the present study, but later fail to germinate or produce a viable mature plant. Further, even if established, generally fail to reproduce sexually. If reproduction occurs poorly, viable segregants may not be able to produce offspring. Also in the segregation populations, progeny breakdown can occur (Chawdhary and Chawdhary, 1977).

Chromosomal aberrations in such interspecific crosses, as evident from occurrence of duplication, deficiency and inversions may occur resulting in disruption of normal process of sexual cycle (Dana, 1966 a, b, c). The presence of chain and ring multivalents at dikinesis not only confirms the structural hybridity of the F_1 hybrid, but also suggests that small interchanges and inversions played a significant role in the speciation within the genus *Vigna* (Gopinathan and Babu, 1986). It is also considered that reduction in fertility in all cases of interspecific hybridization provides useful confirmation of authenticity of a successful cross. It is well accepted that a low level of failure in emasculation technique is probably inevitable as indicated by above researchers. In this context, a valuable list of genetic markers for use in authentication of the interspecific hybrids between *V. radiata* x *V. umbellata* is important (Chen *et al.*, 1978). The authenticated specimens and the critical analysis of the data will eventually prove beneficial to the evolutionists, cytogeneticist and the plant breeders particularly interested in genus *Vigna*.

ACKNOWLEDGEMENTS

Authors sincerely thank Dr. R.K. Arora, ex-Head, Division of Plant Exploration and Collection, NBPGR, New Delhi for his help in collection of germplasm of *Vigna* species.

REFERENCES

Ahn, Chang Soon and R.W. Hartmann, 1975. Interspecific hybridization between *mung* bean (*Vigna radiata* (L) Wilczek) and adzuki bean (*V. angularis* (Willd.) Ohwi and Ohashi. *J. American Soc. Hort. Sci* **103** : 3 - 6.

Ahn, Chang Soon and R.W. Hartmann, 1978. Interspecific hybridization among four species of the *genes Vigna* Savi. The Ist International mung bean symp. AVRDC, 240 - 252 p.

Baker, J.C., 1879. Leguminosae *In* Flora of British India II, : 200 - 207, Revised Ed. L. Reeves and Co. Ltd. Ash Ford, Kent.

Biswas, M.R. and S. Dana, 1975. Pytogenetic status of *Chaseolus sublobatus, J. Cytol. Genet.* 2nd All India Congress Supp. 39.

Chen, M.C., Baker, R.B. and S. Honmac 1983. Interspecific crossability among four species of *Vigna* food legumes. *Euphytica* **32** : 925 - 937.

Chandel, K.P.S., 1984. Role of wild *Vigna* species in the evolution and improvement of *mung* (*Vigna radiata* (L) Wilczek) and urad bean (*V. mungo L) Hepper) Annals Agric. Res.* **5** : 98 - 111.

Chandel, K.P.S., R.N. Lester and R.J. Starling, 1984. The wild ancestors of urad and mung beans (*Vigna mungo* (L) Hepper and *V. radiata* (L) Wilczek). *Bot. J. Linn. Soc.* **89** : 85 - 96.

Chowdhury, R.K. and J.B. Chowdhury, 1977. *V. mungo* (L) : Intergeneric hybridization between *V. mungo* (L) Hepper and *Phaseolus calcaratus* Raxb. *Indian J. agric Sci.* **47** : 117 - 121.

Dana, S., 1966 a. Species cross between *Phaseoles aureus* Roxb. and *P. trilobus* Ait. *Cytologia* **31** : 176 - 187.

Dana, S., 1966 b. Interspecific hybrid between *Phaseoles mungo* L x *P. trilobus* Ait. *J. Cytology & Cytogenetics 1* : 61 - 66.

Dana, S., 1966 c. Cross between *Phaseolus aureus* Roxb. *and P. ricciardianus* Ten. *Genetica Iberica* **18** : 141 - 156.

Dana, S., 1966 d. The cross between *Phaseolus aureus* Roxb. and *P. mungo* L. *Genetica* **37** : 259 - 274.

de Candolle, Alphonse, 1886. Origin of Cultivated Plants. Hafner Publishing Company, Newyork and London. pp-1-468 (345-347).

De, D.P. and R. Krishnan, 1966. Cytological studies of the hybrid *Phaseolus aureus x P. mungo. Genetica* **37** : 588 - 600.

Harlan, J.R. 1975. *Crops and Man.* Madison, Wisconsin, American Society of Agronomy and Crop Science Society of America, 295 pp.

Gopinathan, M.C., C.R. Babu and K.R. Shivanna, 1986. Interspecific hybridization between rice bean (*V. umbellata*) and its wild relatives (*V. minima*) : fertility - sterility relationships. *Euphytica* **35** : 1-6.

Karmakar, P.C. and S. Dana. 1987. Cytogenetic identification of *Vigna sublobata* collection. *The Nucleus* **30** (1, 2) : 47 - 53.

Lukoki, L., R. Marechal and E. Otoul, 1980. Les ancestres sauvages des haricots cultivees; *Vigna radiata* (L) Wilczek et. *V. mungo* (L) Hepper. *Bull. Jard. Bot. Nat. Belg.* **50** : 385 - 391.

Megumi Sawa, 1974. On the interspecific hybridization between the Adzuki bean (*Phaseoles angularis* (Willd.) W.F. Wight and the green gram (*P. radiatus* L). II. On the characteristics as amphidiploid in C_1 generation from the cross green gram x rice bean (*P. calcaratus* Roxb.). *Japan J. Breed* **24** : 282 - 86.

Prain, D. 1897. Some Additional Leguminosae, *J. Asiatic Soc. Bengal* **66** : 421 - 25.

Sen. N.K. and Ghosh, A.K., 1961. Genetic studies in green gram. *Indian J. Genet.* **19** : 220 - 227.

Smartt, J., 1979. Interspecific hybridization in the grain legumes - a review. *Eco. Bot.* **33** : 323 - 337.

Smartt. J., 1990. Grain Legumes - Evolution and Genetic Resources, Cambridge Univesity Press. Cambridge, New York, 337 pp.

Vavilov, N.I., 1951. Origin, Variation, Immunity and Breeding of Cultivated Plants. *Chron. Bot.* **13** : 1 - 364.

Zeven, A.C. and P.M. Zhukovsky 1975. Dictionary of Cultivated Plants and their Centres of Diversity. Centre for Agricultural Publishing and Documentation, Wageningen. pp. 1 - 219.