# INTERSPECIFIC HYBRIDIZATION IN THE GENUS *VIGNA*

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ABSTRACT

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Interspecific hybridization among four species of Asian food legumes, namely, *Vigna radiata* (L.) Wilzcek (mungbean), *V. mungo* (L.) Hepper (black gram), *V. umbellata* (Thunb.) Ohwi and Ohashi (rice bean) and *V. angularis* (Wild.) Ohwi and Ohashi (adzuki bean) was investigated. Pod-set and percentage of pods harvested varied with interspecific cross combinations and cultivars used. An increase in the number of $F_1$ seeds was possible in the cross, *V. radiata* x *V. umbellata*, through the use of intraspecific hybrids as parents. An interspecific hybrid of *V. mungo* x *V. angularis* was also accomplished by the use of intraspecific hybrids as parents. Except for the cross between *V. umbellata* and *V. angularis*, all other reciprocal interspecific crosses were unsuccessful.

Hybrid seeds were obtained from the following crosses: *V. radiata* x *V. mungo*, *V. radiata* x *V. umbellata* and *V. mungo* x *V. angularis*. Hybrid plants were obtained using embryo culture from the following crosses: *V. radiata* x *V. angularis*, *V. umbellata* x *V. angularis* and *V. angularis* x *V. umbellata*. A reciprocal hybrid of the interspecific

cross between *V. umbellata* and *V. angularis* was successfully made.

Growth and lethality of interspecific hybrid seedlings were influenced by the genotypes of the parental species. Parnetal genotypes also affected the fertility of interspecific hybrids which ranged from partially fertile to completely sterile depending upon the interspecific cross combinations.

Three chemicals, E-amino-n-caproic acid (EACA), gentisic acid and L-lysine, were used to increase the success of making interspecific crosses in the genus *Vigna*. The EACA as a foliar spray was the most effective chemical in producing of interspecific hybrids between *V. radiata* and *V. umbellata*. Daily foliar applications of EACA to the plants of the maternal parent, *V. radiata*, for 14 days prior to hybridization with *V. umbellata* significantly increased the frequency of hybrid embryos (22%) and the numbers of $F_1$ seeds (from 2 to 9 times). Concentrations of 100 to 1000 mg EACA/1 were the most effective.

Other techniques were also used to facilitate the success of the interspecific cross, *V. radiata* x *V. umbellata*. Number of $F_1$ seeds was increased when pods were allowed to mature with part of the pedicels were severed 10-14 days after pollination and when they were cultured *in vitro*. The number of $F_1$ seeds increased by 9 and 5 times for partial detached and detached pods, respectively. Partial and complete removal of leaves from the maternal plant 4 days after pollination also increased the number of $F_1$ seeds from 7- to 10-fold as compared to the control. "Side-grafting" between two species and the use of *V. radiata* var. *sublobata* as a species bridge were effective in reducing embryo abortion and increasing the numbers of $F_1$ seeds by 11 and 45 times, respectively. Hybrid sterility was circumvented by doubling the chromosome number. Triploid plants (amphidiploid x *V. radiata*) were successfully obtained by using embryo culture. Variation in morphological characteristics and fertility was observed in the progenies by selfing the backcross progenies. Similar observations were made in the progenies resulting from the second backcrossing.

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Guidance Committee:

Section I, II and III are segments of related thesis research information condensed into formats suited and intended for publication in *Euphytica* (Section I and III) and in the *Journal of the American Society for Horticultural Science* (Section II).

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

SECTION I

LIST OF FIGURES

Figure | Page

SECTION I



SECTION III

# SECTION I

## INFLUENCE OF PARENTAL CULTIVAR AND PARENTAL HETEROZYGOSITY ON INTERSPECIFIC CROSSABILITY AMONG FOUR SPECIES OF VIGNA

SUMMARY

Interspecific crossability among four species of *Vigna*, namely, *V. radiata* (mungbean), *V. mungo* (black gram), *V. umbellata* (rice bean) and *V. angularis* (adzuki bean), was investigated. Pod-set and percentages of pods harvested varied with the combinations of two parental cultivars of each species for most of the interspecific hybrid crosses. The use of intraspecific hybrids as parents was slightly superior to the use of cultivars. A remarkable increase in viable seed production was found for the interspecific cross, *V. radiata* x *V. umbellata*, by using intraspecific hybrid as parents. Furthermore, a successful interspecific hybrid of *V. mungo* x *V. angularis* was accomplished through the use of intraspecific hybrid parents. Reciprocal cross differences were common in all the interspecific combinations. Except for the cross between *V. umbellata* and *V. angularis*, all other reciprocal interspecific crosses were unsuccessful.

Viable seeds were obtained from the interspecific crosses of *V. radiata* x *V. mungo*, *V. radiata* x *V. umbellata* and *V. mungo* x *V. angularis*. Hybrid plants were obtained from cultured embryos for the interspecific crosses of *V. radiata* x *V. angularis*, *V. umbellata* x *V. angularis* and *V. angularis* x *V. umbellata*. A successful reciprocal hybrid of the interspecific cross

between V. umbellata and V. angularis is reported.

Growth and lethality of the interspecific hybrid seedlings were influenced by the genotypes of both parental species. Parental genotypes also affected the fertility of interspecific hybrids. Complete hybrid sterility was found in the interspecific crosses of V. radiata x V. umbellata, V. radiata x V. angularis and V. mungo x V. angularis, while reduced fertility was observed for the interspecific hybrids of V. radiata x V. mungo, V. umbellata x V. angularis and V. angularis x V. umbellata.

# INTRODUCTION

Interspecific hybridization is a useful tool in plant breeding to create new genetic variation. Interest in interspecific hybridization has gained impetus as improved hybridization techniques increased the possibilities of successful crosses (Sanchez-Monge & Garcia-Olmedo, 1977).

The four oriental species of food legumes, *Vigna radiata* (L.) Wilzcek (mungbean), *V. mungo* (L.) Hepper (black gram), *V. umbellata* (Thunb.) Ohwi and Ohashi (rice bean) and *V. angularis* (Wild.) Ohwi and Ohashi (adzuki bean), are important pulse crops in parts of Asia and Africa (Rachie & Roberts, 1974). These species are distinguished from other food legumes by their Asian origin and their yellow flowers and were recently separated from *Phaseolus* by Verdicourt (1970).

Varying degrees of success in interspecific hybridization of *Vigna* have been reported (Dana, 1966, 1967; De & Krishnan, 1966; Al-Yasiri & Coyne, 1966; Sawa, 1973; Biswas & Dana, 1975; Chen et al., 1977; Chowdhury & Chowdhury, 1977; Ahn & Hartmann, 1977, 1978a, 1978b). The degree of success was influenced by the parental cultivars used and the parental heterozygosity. Honma and Heeckt (1958, 1959), using different cultivars and intraspecific hybrids, were successful in obtaining the interspecific hybrids of _P. vulgaris_ and _P. lunatus_ and _P. coccineus_ x _P. lunatus_. Mok et al., (1978) reported that the genotypes of the parents appear to be essential in generating interspecific hybrids. By using different parental genotypes, they were able to obtain the hybrids of _P. vulgaris_ x _P. lunatus_, _P. vulgaris_ x _P. acutifolius_ and _P. acutifolius_ x _P. vulgaris_ through the aid of embryo culture. The rate of growth and final size of these hybrid embryos seemed to be influenced by the genotypes of both parents. In other studies, Taira et al., (1978) reported that the _Triticum turgidum_ (wheat) and _Secale cereale_ (rye) parental genotypes exerted a definite effect on the frequency of normal embryo formation and seed-set of wheat-rye crosses. Parental genotypes do play an important role in the success of interspecific hybridization.

The causes of failures of interspecific crosses in food legumes are not fully understood. In some cases, the pollen tubes are unable to penetrate the stigma and style (Chowdhury & Chowdhury, 1977). In other cases, fertilization occurs, but embryo abortion takes place during embryogenesis (Honma, 1956; Al-Yasiri & Coyne, 1966; Ahn & Hartmann, 1977, 1978a, 1978b). The failure of interspecific hybridization due to embryo degeneration is common in interspecific crosses of food legumes (Ahn

& Hartmann, 1977; Chen et al., 1977). Moreover, interspecific hybrids obtained are often completely sterile or only partially fertile, while relatively few are fertile. This investigation reports the effects of parental cultivars on interspecific crossability, describes the interspecific hybrid plants derived from four *Vigna* species, and compares the use of homozygous with heterozygous parents for interspecific crossability.

## MATERIALS AND METHODS

Plant materials. Two parental cultivars each of *V. radiata* (Tainan #1, M865), *V. mungo* (T-9, NI208), *V. umbellata* (HK, S-91) and *V. angularis* (Chien Shien, KS#210) were used for hybridization (Table 1). In general, the two selected parents of each species were diverse in their origins and phenotypes. All possible combinations, including reciprocals, were made between four species. Intraspecific hybrids were produced by crossing the two cultivars of each species to obtain heterozygous parents for the interspecific crosses.

Cultural conditions. Plants were grown under glass-house conditions. During winter, temperatures were adjusted to 26$^{o}$C (day) and 18$^{o}$C (night) with a photoperiod of 14 hr light supplied by high intensity metal-halide lamps (10-12 klx). Two plants were grown in a 20 cm pot containing "Peat Lite Mix". A total of eight plants of each parental cultivar were used for the female. All parental cultivars including intraspecific $F_1$ were planted at the same time. Several plantings were made of the male parents to assure adequate pollen. Plants were fertilized with 3 g/pot of Peter's fertilizer (20N-20P-20K) biweekly. A short-day photoperiod was used for *V. umbellata* to induce flowering.

Table 1. Description of four *Vigna* species used as parents for interspecific hybridization.

| Species | Common name | Cultivar name | AVRDC[1] Accession No. | Origin |
|---|---|---|---|---|
| *V. radiata* | Mungbean | Tainan #1 | 2013 | Taiwan |
| | | M865 | 1837 | India |
| *V. mungo* | Black gram | T-9 | 3115 | India |
| | | NI208 | - | Zaire |
| *V. umbellata* | Rice bean | HK | 4006 | Hong Kong |
| | | S-91 | 4065 | Philippines |
| *V. angularis* | Adzuki bean | Chien Shien | 5124 | Japan |
| | | KS#210 | 5122 | Taiwan |

[1]Asian Vegetable Research and Development Center (AVRDC). Shanhua, Taiwan.

Crossing methods. Flowers used as female parents were emasculated the day before anthesis and pollinated immediately. The hybridization technique used was similar to that described by Boling et al., (1961). For the same interspecific cross, pollinations were made on both parental cultivars over the same period of time so that comparisons could be made between parental cultivars for crossability. A minimum of 100 pollinations were attempted in all possible 60 cross-combinations. Self-pollinated pods were removed daily from the plants to eliminate competition.

Data collection and analysis. Data were taken on the number of flowers pollinated, number of pods set and number of mature pods harvested. Flowers which abscised within 24 hr were excluded on the basis of mechanical damage. Therefore, the number hybridization included those flowers which opened normally the day after pollination. Pod-setting was recorded on the fourth day after pollination.

Mature pods were harvested individually and the number of pods and seeds recorded. The 'hybrid' seeds were germinated in petri dishes to determine their viability. Germinating seedlings were transplanted to Jiffy-7 pots. The lethality of seedlings was recorded. Morphological characteristics of the 'hybrid' plants were

observed and compared with the parental species. Evidence of hybridity was determined from their morphological features. Genetic markers for germination habit, epicotyl color, petiole length of the primary leaves and lobing of leaflets were used as indicators of hybridity. Pollen viability was estimated from the percentage of plump pollen grains that stained darkly and uniformly from an $I_2$-KI solution (Johansen, 1940). A minimum of 400 pollen grains were counted from each of five flowers per 'hybrid' plant.

Embryo culture was attempted on interspecific crosses which did not produce viable seeds from cross-pollinations. Immature embryos from 14 to 18 day old pods were excised and cultured on an artificial medium described by Linsmaier and Skoog (1965). The resulting seedlings were transplanted to Jiffy-7 pots and gradually acclimatized to glasshouse conditions.

The effects of parental cultivar on crossability was tested by chi-square for percent pod-set, percent pods harvested, number of viable hybrid seeds, reciprocal cross difference, and homozygous versus heterozygous parents.

## RESULTS AND DISCUSSION

Crossability of V. radiata and V. mungo. Interspecific hybridization was successful only when *V. radiata* was used as the female parent. The overall percentage for pods harvested was 85 and 75% for *V. radiata* x *V. mungo* and *V. mungo* x *V. radiata*, respectively (Table 2). Percent pods harvested was generally high which suggested fertilization was successful in order to obtain pod-set between these two species. The chi-square test for reciprocal cross difference in percent pods harvested was not significant (Table 2).

In the *V. radiata* x *V. mungo* crosses, differences in percent pods harvested were highly significant among the crosses and between the two cultivars of *V. radiata*. The best combination was Tainan #1 x T-9 which produced the highest percentage of pods harvested (92%). The percentage of pods harvested was higher on cultivar Tainan #1 than on M865; i.e., 91 and 62%, respectively (Table 2). There was no significant difference between the cultivars of the pollen parent for the number of pods harvested as well as between the use of cultivars and an intraspecific hybrid as parents for the species cross. The set-pods developed normally to maturity. Two kinds of hybrid seeds were obtained; viz., partially

Table 2. Crossability for the interspecific cross of *V. radiata* (M) and *V. mungo* (B).

| Parents[1] Female | Male | No. flowers crossed | Pods harvested (%) | Total seeds obtained | Viable seeds (%) | Viable seeds No./attempt |
|---|---|---|---|---|---|---|
| M1 | B1 | 125 | 92.0 | 949 | 87.7 | 6.7 |
| M1 | B2 | 140 | 90.7 | 617 | 79.1 | 3.5 |
| Mean | | 133 | 91.0 | 783 | 84.3 | 5.1 |
| M2 | B1 | 136 | 55.9 | 793 | 88.0 | 5.1 |
| M2 | B2 | 145 | 67.6 | 968 | 73.6 | 4.9 |
| Mean | | 141 | 61.7 | 881 | 80.0 | 5.0 |
| (M1xM2) | (B2xB1) | 146 | 91.1 | 1132 | 80.7 | 6.9 |
| B1 | M1 | 243 | 60.1 | 431 | 0 | 0 |
| B1 | M2 | 203 | 52.1 | 228 | 0 | 0 |
| Mean | | 223 | 56.5 | 330 | 0 | 0 |
| B2 | M1 | 148 | 82.4 | 546 | 0 | 0 |
| B2 | M2 | 156 | 85.9 | 568 | 0 | 0 |
| Mean | | 152 | 84.2 | 557 | 0 | 0 |
| (B2xB1) | (M1xM2) | 203 | 82.3 | 664 | 0 | 0 |

| Comparison | % pods harvested | Comparison | % pods harvested |
|---|---|---|---|
| Within M x B | **[2] | Within B x M | ** |
| between M | * | between B | * |
| between B | ns | between M | ns |
| cultivars vs $F_1$s | ns | cultivars vs $F_1$s | ns |
| M x B vs B x M | | | |
| cultivar x cultivar | ns | | |
| $F_1$ x $F_1$ | ns | | |

[1] M1 = Tainan #1, M2 = M865, B1 = T-9, B2 = NI 208.

[2] Significant at 5% (*) or 1% (**); ns = not significant by chi-square test.

shrivelled and ruptured seeds. The partially shrivelled seeds were found to be viable by germination; while the ruptured seeds were inviable. There were no significant differences in number of viable seeds per attempt among the different combinations of parental cultivars.

Number of pods harvested varied significantly according to the parental cultivars when _V. mungo_ was used as the female parent. The cultivar NI208 was superior to T-9 for crossability as indicated by the higher percentages of pods harvested and numbers of seeds produced (Table 2). Early pod development appeared normal, but later aborted. Two types of seeds, shrivelled and empty, were obtained in the crosses of _V. mungo_ x _V. radiata_ which were all inviable.

The relatively high number of pods harvested would suggest that apparently there are no barriers in crossing of these two species for the parental cultivars used. However, there were barriers to seed development which produced inviable and viable seeds for the cross of _V. radiata_ x _V. mungo_, but completely inviable seeds in the reciprocal cross, _V. mungo_ x _V. radiata_. Attempts to culture immature hybrid embryos of _V. mungo_ x _V. radiata_ failed to produce any seedlings.

The 15-day-old hybrid embryos were of adequate size (3.4 mm), but 10 of 21 cultured embryos formed only callus, and all others failed to grow. The reciprocal difference in crossability of _V. radiata_ and _V. mungo_ suggests interaction between genic and cytoplasmic factors (Stebbins, 1958). This interaction may be the cause of hybrid embryo degeneration when _V. mungo_ is used as the female parent (Ahn & Hartmann, 1977).

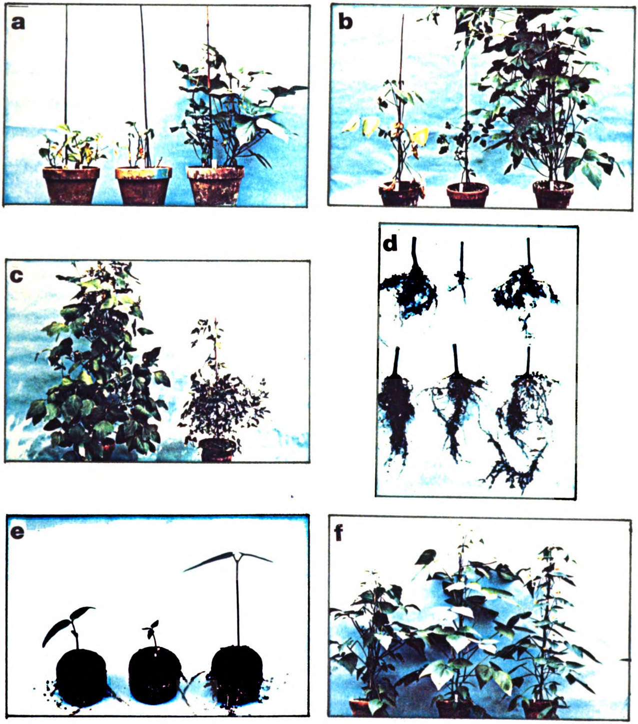
_Growth and morphology of V. radiata x V. mungo hybrids_. The hybrid plants differed according to the parental cultivars used. Hybrid lethality was high when the cross combinations involved NI208 of _V. mungo_. The $F_1$ plants of Tainan #1 x NI208 and M865 x NI208 grew slowly and poorly with small crinkled leaves (Table 3, Fig. 1-a,b,c). Hybrid plants of Tainan #1 x NI208 died at various stages of growth development and never reached the flowering stage. The hybrids from the cross of Tainan #1 x T-9 grew normally at early stages but suddenly lost their vigor before or upon flowering and eventually died. The root systems of Tainan #1 x T-9 plants were observed to grow slowly and eventually succumb to root rot (Fig. 1-d). The hybrid of M865 x T-9 was the superior among the five cross combinations. The plants displayed hybrid vigor in the seedling stage which extended throughout their life cycle. Hybrid plants

Table 3. Effects of parental cultivar on the growth and fertility of interspecific hybrids of *V. radiata* x *V. mungo*.

| Interspecific hybrid cross | Germination (%) | Plant Height[1] (cm) | Leaf size (LxW, cm) Terminal leaflet | Leaf size (LxW, cm) Lateral leaflet | Pollen stain-ability (%) |
|---|---|---|---|---|---|
| Tainan #1 x T-9 | 82 | 38.0 | 12.0 x 8.2 | 11.5 x 7.6 | 17.3 |
| Tainan #1 x NI208 | 100 | 34.2 | 5.0 x 3.5 | 4.2 x 2.9 | - |
| M865 x T-9 | 100 | 41.9 | 15.6 x 11.6 | 14.9 x 10.5 | 46.7 |
| M865 x NI208 | 92 | 25.1 | 6.6 x 4.3 | 5.6 x 3.9 | 31.3 |
| (Tainan # 1 x M865) x (NI 208 x T-9) | 88 | 53.5 | 17.0 x 12.4 | 16.6 x 11.4 | 23.3 |

[1]Planted on June 5; plant height was measured 30 days after planting.


Figure 1. Interspecific hybrids of *V. radiata* x *V. mungo* and *V. radiata* x V. *umbellata*. 1-a: Hybrid plants of Tainan #1 x T-9 (left), Tainan #1 x NI208 (middle) and (Tainan #1 x M865) x (NI208 x T-9). 1-b: Hybrid plants of Tainan #1 x T-9 (left), M865 x NI208 (middle) and M865 x T-9. 1-c: Hybrid plants of M865 x T-9 (left) and M865 x NI208. 1-d: Root systems, top: Tainan #1 (left), Tainan #1 x T-9 (middle) and T-9; bottom: M865 (left), M865 x T-9 (middle) and T-9. 1-e: Seedlings of *V. radiata* (Tainan #1, left), *V. umbellata* (HK, right) and their interspecific hybrid (middle) showing the germination habits. 1-f: Plants of *V. radiata* (Tainan #1, left), *V. umbellata* (HK, right) and their interspecific hybrid (middle).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derived from two heterozygous intraspecific hybrids of two parental species; e.g., (Tainan #1 x M865) x (NI208 x T-9), had a range of vigor from weak like the plants of Tainan #1 x NI208 and M865 x NI208 to vigorous like the plants of M865 x T-9.

The hybrid plants resembled the female parent for certain characters; the male for other characters; or else was intermediate in expression (Table 4). The morphological characteristics similar to male parent or intermediate between two parental species were the most important traits to use as markers to verify hybridity. In the cross of V. radiata x V. mungo, the characters of ovoid seeds, seed L/W ratio and concave hilum cushion liked the male parent; whereas the seed color and seed weight were intermediate.

The $F_1$ plants were partially fertile and flowered profusely and continuously, and had a low pod-set. The infrequent mature pods harvested from self-pollinated flowers usually contained one or two fully developed seeds. Many pods aborted prematurely with only empty or ruptured seeds. Pollen stainability ranged from 17 to 47% depending upon the combination of parental cultivars (Table 3). The hybrid plants of M865 x T-9 had the highest percentage of stainable pollen grains with an average of 47% compared to over 90% for the two parental species (Table 4). The stained pollen grains were

*Table 4. Comparison of the morphological characteristics of V. radiata (M865), V. mungo (T-9) and their interspecific hybrid.*

Table 4. Comparison of the morphological characteristics of *V. radiata* (M865), *V. mungo* (T-9) and their interspecific hybrid.

| Character | V. radiata (female) | Hybrid | V. mungo (male) |
|---|---|---|---|
| Margin of leaflet | lobed | lobed | entire |
| Terminal leaflet (cm) | | | |
| L/W (ratio) | 16.1/11.7 (1.38) | 15.6/11.6 (1.34) | 13.2/7.6 (1.74) |
| Lateral leaflet (cm) | | | |
| L/W (ratio) | 15.6/10.9 (1.43) | 14.9/10.5 (1.42) | 11.5/6.7 (1.72) |
| Days to first flowering (dap)[1] | 52 | 47 | 37 |
| Pollen stainability (%) | 93.4 | 46.7 | 96.9 |
| Pod pubescence | long | long | short |
| Pod at maturity | radiated or drooping | radiated | upright |
| Pod length (cm) | 8.6 | 3.2 | 4.1 |
| No. seeds per pod | 10.8 | 1.7 | 5.5 |
| Shape of seed | globose | ovoid | ovoid |
| Color of seed | green | brown | black mottle |
| Hilum cushion | not concave | concave | concave |
| Seed size (mm) | | | |
| L/W (ratio) | 4.8/3.8 (1.26) | 5.8/3.7 (1.57) | 5.6/3.6 (1.56) |
| Seed weight (mg) | 49.7 | 51.7 | 59.3 |

[1]Planted on February 12; dap = days after planting.

relatively plump and uniform, whereas the unstained pollen grains were smaller and variable in size.

Crossability of V. radiata and V. umbellata. The cross between *V. radiata* and *V. umbellata* was only successful when *V. radiata* was used as the seed parent. The pod-set was high (85% average) and few viable hybrid seeds were obtained (Table 5). When *V. umbellata* was used as the female, pod-set was only 21% and no viable seed was produced. The reciprocal cross differences for both pod-set and number of viable seeds were highly significant.

No differences in pod-set between the parental cultivars was found when *V. radiata* was used as the female parent. However, there were significant differences in numbers of viable seeds obtained for this interspecific cross (Table 5). Pods developed normally to maturity. The mature pods contained seeds which appeared from very shrivelled to partially filled. Upon germination only 1 to 11% of the total seeds harvested were viable. The percentages of viable seeds depended upon the hybrid combinations. The combination of M865 x HK produced the most viable seeds per 100 attempts, 48, while Tainan #1 x S-91 produced only 8 viable seeds. Though no differences in viable seed numbers were found between the interspecific crosses

Table 5. Crossability for the interspecific hybridization of *V. radiata* (M) and *V. umbellata* (R).

| Parents[1] Female | Male | No. flowers crossed | Pod-set (%) | Total seeds obtained | Viable seeds (%) | Viable seeds No./100 attempts |
|---|---|---|---|---|---|---|
| M1 | R1 | 154 | 89.6 | 878 | 6.7 | 38.3 |
| M1 | R2 | 160 | 90.0 | 996 | 1.3 | 8.1 |
| Mean | | 157 | 89.8 | 937 | 3.8 | 22.9 |
| M2 | R1 | 142 | 74.5 | 699 | 9.7 | 47.9 |
| M2 | R2 | 155 | 85.2 | 538 | 7.8 | 27.1 |
| Mean | | 149 | 79.9 | 619 | 8.9 | 37.5 |
| (M1xM2) | (R2xR1) | 210 | 92.9 | 1483 | 11.1 | 78.6 |

| Comparison | % pod set | Viable seeds per 100 attempts | Comparison | % pod-set |
|---|---|---|---|---|
| Within M x R | ns[2] | ** | Within R x M | ** |
| between M | ns | ns | between R | ** |
| between R | ns | ** | between M | ns |
| cultivars vs $F_1$s | ns | ** | cultivars vs $F_1$s | ns |
| M x R vs R x M | | | | |
| cultivar x cultivar | ** | ** | | |
| $F_1$ x $F_1$ | ** | ** | | |

[1]M1 = Tainan #1, M2 = M865, R1 = HK, R2 = S-91.

[2](**) significant at 1%; ns = not significant by chi-square test.

for the two V. radiata cultivars, the difference between the two V. umbellata cultivars as the pollen parents was highly significant. The combination of intraspecific hybrids of the two species significantly increased the number of viable seeds, 79 per 100 attempts as compared to 30 for the parental cultivars.

In the cross of V. umbellata x V. radiata, pod-set differences were highly significant among the four combinations of parental cultivars. No effect of two V. radiata pollinators on pod-set was observed, but the differences in pod-set were highly significant between two V. umbellata cultivars. The pod-set on HK (32%) was higher than that on S-91 (11%). Pods on V. umbellata usually abscised within a week of pollination. The use of heterozygous parents did not improve the low pod-set and embryo abortion.

The reciprocal cross differences in crossability suggest that cytoplasmic factors play an important role in the unidirectional success of this interspecific cross.

Growth and morphology of V. radiata x V. umbellata hybrids. The germination habit of hybrid seedlings was intermediate between the parental species which were epigeal and hypogeal for V. radiata and V. umbellata, respectively (Table 6, Fig. 1-e). The cotyledon

Table 6. Comparison of the morphological characteristics of *V. radiata* (Tainan #1), *V. umbellata* (HK) and their interspecific hybrid.

| Character | *V. radiata* (female) | Hybrid | *V. umbellata* (male) |
|---|---|---|---|
| Germination | epigeal | intermediate | hypogeal |
| Color of upper epicotyl | green | light purple | purple |
| Hypocotyl length (mm)[1] | 64.7 ± 3.5 | 24 ± 4.5 | 0 |
| Petiole of primary leaves | sessile | short | long |
| Primary leaf (mm) | | | |
| L/W (ratio) | 61.5/18.5 (3.32) | 31.6/11.1 (2.85) | 60.4/19.3 (3.13) |
| Growth habit | determinate | indeterminate | indeterminate |
| Terminal leaflet (cm) | | | |
| L/W (ratio) | 15.4/11.9 (1.29) | 21.9/15.8 (1.39) | 16.6/9.6 (1.73) |
| Lateral leaflet (cm) | | | |
| L/W (ratio) | 16.5/11.3 (1.46) | 20.6/13.8 (1.49) | 14.6/8.5 (1.72) |
| Days to first flowering (dap)[2] | 45 | 54 | 50 |
| Top of keel color | greyish | light greyish | bright yellow |
| Raceme | often compound | simple | simple |
| Pollen stainability (%) | 97.8 | 1.8 | 94.5 |
| Pubescence of young pods | hairy | hairy | glabrous |

[1] Mean and standard deviation of 40 seedlings.

[2] Planted on February 12; dap = days after planting.

positions of the hybrid seedlings were at the soil surface, and the average length of hypocotyl was 24 mm compared to 65 mm for the *V. radiata* parent.

The hybrid seedlings showed poor vigor and the initial growth was slow. Abnormal seedlings with three or four primary leaves were noted. Growth of the hybrid plants was vigorous once the seedling stage was past (Fig. 1-f). Hybrid plant growth usually exceeded the parental species and was characterized by a thicker stem, larger leaves and more branches (Table 6).

The hybrid plants were also intermediate for expression of petiole length of the primary leaves and flower color (Table 6). The *V. umbellata* characters of indeterminate growth and simple raceme were dominant in the $F_1$ plants. The indeterminate growth habit of the hybrid plants produced continuously juvenile shoots and behaved as a perennial with no signs of senescence.

The $F_1$ plants flowered profusely and continuously within the same racemes for a prolonged period and set few pods during cool temperature period, but abscised shortly thereafter. Stainable pollen grains were from 1.2 to 2.0% depending upon the hybrid combination of parental cultivars (Table 7). The unstained pollen grains were variable in size, while the stained pollen

Table 7. Effects of parental cultivar cross on growth and fertility of the interspecific hybrid of *V. radiata* x *V. umbellata*.

| Interspecific hybrid pedigree | Seedling[1] height (cm) | Leaf size (LxW, cm) Terminal leaflet | Leaf size (LxW, cm) Lateral leaflet | Pollen stain-ability (%) |
|---|---|---|---|---|
| Tainan #1 x HK | 9.3 | 21.9 x 15.8 | 20.6 x 13.8 | 1.8 |
| Tainan #1 x S-91 | 10.8 | 22.4 x 15.9 | 20.8 x 14.4 | 2.0 |
| M865 x HK | 10.4 | 18.0 x 12.2 | 16.2 x 11.3 | 1.5 |
| M865 x S-91 | 7.9 | 19.1 x 14.5 | 18.2 x 13.4 | 1.2 |
| (Tainan #1 x M865) x (S-91 x HK) | 10.2 | 17.7 x 13.5 | 16.8 x 12.0 | 1.3 |

[1]Planted on June 10; seedling height was measured at 3 weeks after planting.

grains were somewhat larger than the parental pollen. This was also observed by other workers (Ahn & Hartmann, 1977; Machado et al., unpublished). The sterility of the hybrid plants might be caused by the lack of chromosomal homology and resultant meiotic irregularities (Dana, 1967; Ahn & Hartmann, 1977; Machado et al., unpublished).

Crossability of *V. radiata* and *V. angularis*. Neither the parental cultivars of *V. radiata* nor *V. angularis* affected pod-set when *V. radiata* was used as the female parent (Table 8). The percentages of pod-set noted on the fourth day after pollination were high; viz., 77% for the four combinations of parental cultivars and 85% for the interspecific cross between the two intraspecific hybrids. However, the pods only grew to about 3-5 cm in length and finally abscised some 1 to 3 weeks after cross-pollination. A few pods were retained longer on the plants but embryo abortion eventually occurred. The empty pods contained very small, shrivelled, nonviable seeds. No differences in pod-set and seed development were observed between the use of parental cultivar and intraspecific hybrid as the parents. The 15-day-old hybrid embryos appeared small, averaging 1.2 mm in length.

There were significant differences for pod-set between the parental cultivars of seed parent in the crosses of *V. angularis* x *V. radiata*. However, pod-set

Table 8. Crossability for the interspecific hybridization of *V. radiata* (M) and *V. angularis* (A).

| Parents[1] Female | Male | No. flowers crossed | Pod-set (%) | Pod abscision[2] (days) |
|---|---|---|---|---|
| M1 | A1 | 130 | 80.8 | 8.6 |
| M1 | A2 | 206 | 79.1 | 13.4 |
| Mean | | 168 | 79.8 | 11.0 |
| M2 | A1 | 133 | 72.2 | 5.2 |
| M2 | A2 | 115 | 76.5 | 8.2 |
| Mean | | 124 | 74.2 | 6.7 |
| (M1xM2) | (A2xA1) | 214 | 84.6 | 9.8 |
| A1 | M1 | 106 | 62.3 | 11.8 |
| A1 | M2 | 118 | 68.6 | 15.6 |
| Mean | | 112 | 66.1 | 13.7 |
| A2 | M1 | 125 | 24.0 | 11.0 |
| A2 | M2 | 131 | 35.1 | 10.2 |
| Mean | | 128 | 29.7 | 10.7 |
| (A2xA1) | (M1xM2) | 147 | 57.8 | 10.8 |

| Comparison | % pod-set | Comparison | % pod-set |
|---|---|---|---|
| Within M x A | ns[3] | Within A x M | ** |
| between M | ns | between A | ** |
| between A | ns | between M | ns |
| cultivars vs $F_1$s | ns | cultivars vs $F_1$s | ns |
| M x A vs A x M | | M x A vs A x M | |
| cultivar x cultivars | ** | $F_1$ x $F_1$ | * |

[1]M1 = Tainan #1, M2 = M865, A1 = Chien Shien, A2 = KS#210.

[2]Mean of days to pod abscision after pollination.

[3]Significant at 5% (*) or 1% (**); ns = not significant by chi-square test.

was equally affected by the two pollen parent *V. radiata* cultivars. Pod-set on the two parental cultivars was not significantly different from the intraspecific hybrids. The pods stopped growing about 10-15 days after cross-pollination, then either abscised or the embryos aborted leaving empty pods on the plants.

Significant reciprocal differences in pod-set were found within parental cultivars and intraspecific hybrids used as parents in this interspecific cross. These results were not similar to findings of Ahn and Hartmann (1978a), perhaps due to cultivar differences. In this study, pod-set on *V. radiata* was higher than that on *V. angularis*. Pod-set on *V. radiata* and *V. angularis* averaged 77 and 47% respectively, where cultivars were used as parents, whereas, the use of intraspecific hybrids as parents increased pod-set to 85 and 58%, respectively (Table 8).

Growth and morphology of V. radiata x V. angularis hybrids. As mentioned previously, the reciprocal crosses of *V. radiata* x *V. angularis* did not produce viable seed. The hybrid embryos of *V. radiata* x *V. angularis* were very small, but appeared normal. Embryo culture of 20 embryos from 14-18 day old pods produced 15 seedlings of which only 3 plants reached flowering. The other 12 plants died during early seedling stages. Two of the hybrid plants were cultured from the cross of Tainan #1 x KS#210, the other one from the cross of (Tainan #1 x M865) x (KS#210 x Chien Shien).

The plants derived by embryo culture were definitely hybrid (Table 9, Fig. 2-a,b,c). They were intermediate between their two parents for petiole length and shape of primary leaves. However, the hybrids resembled the *V. angularis* pollen parent for color of epicotyl, lobing of leaflet margins, and simple compact racemes.

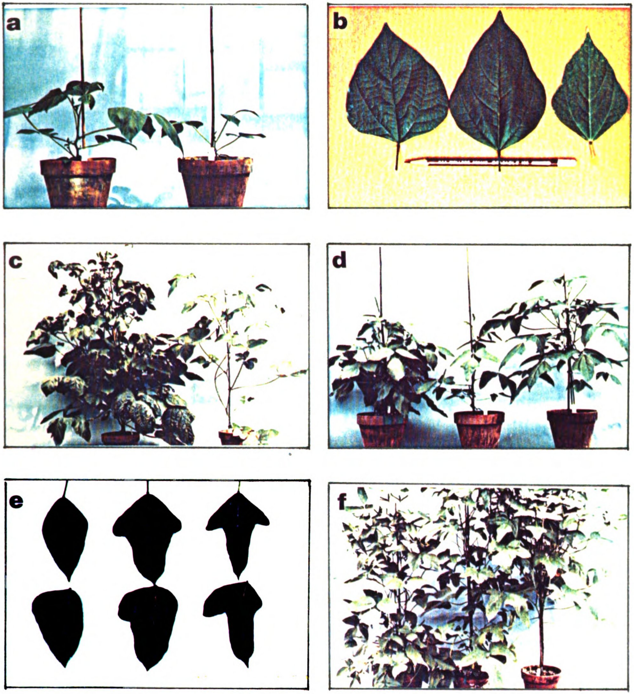
The Tainan #1 x KS#210 hybrid plants grew vigorously with thicker stems, larger leaves and many branches, compared to the parental cultivars. However, abnormal growth occurred after the plants reached the 8 to 10 trifoliate leaf stage. The plants formed crinkled leaves and gradually ceased vegetative growth. The hybrids rarely flowered as flower buds aborted prior to anthesis. Slightly distorted plant growth and development was noted for the hybrid plant derived from the cross of (Tainan #1 x M865) x (KS#210 x Chien Shien). This difference might be accounted for by the parental heterozygosity of this interspecific hybrid pedigree.

No pod-set was observed on any of these hybrid plants. The flowers contained small numbers of pollen grains with 0.6 - 9.8% of stainable pollen grains as

Table 9. Comparison of the morphological characteristics of *V. radiata* (Tainan #1), *V. angularis* (KS#210) and their interspecific hybrid.

| Character | *V. radiata* (female) | Hybrid | *V. angularis* (male) |
|---|---|---|---|
| Epicotyl color | purple | green | green |
| Petiole of primary leaves | sessile | short | long |
| Shape of primary leaves | lanceolate | ovate | cordate |
| Margin of leaflets | entire | lobed | lobed (often) |
| Terminal leaflet (cm) L/W (ratio) | 17.8/15.4 (1.16) | 25.3/20.3 (1.25) | 16.9/11.3 (1.50) |
| Lateral leaflet (cm) L/W (ratio) | 19.0/14.2 (1.34) | 22.6/16.9 (1.34) | 13.9/11.1 (1.25) |
| Top of keel color | greyish | light greyish | light yellow |
| Pollen stainability (%) | 97.8 | 0.6 - 9.8 | 92.0 |
| Raceme | elongated (often compound) | compact (simple) | compact (simple) |

Figure 2. Interspecific hybrids of _V. radiata_ x _V. angularis_ and _V. mungo_ x _V. angularis_. 2-a: Hybrid plants of _V. radiata_ (Tainan #1) x _V. angularis_ (KS#210). 2-b: Terminal leaflets of _V. radiata_ (Tainan #1, left), _V. angularis_ (KS#210, right) and their interspecific hybrid (middle). 2-c: Interspecific hybrid plants of _V. radiata_ x _V. angularis_: Tainan #1 x KS#210 (left) and (Tainan #1 x M865) x (KS#210 x Chien Shien). 2-d: Plants of _V. mungo_ (NI208 x T-9, left), _V. angularis_ (KS#210 x Chien Shien, right) and their interspecific hybrid (middle). 2-e: Terminal and lateral leaflets of _V. mungo_ (NI208 x T-9, left), _V. angularis_ (KS#210 x Chien Shien, right) and their interspecific hybrid (middle). 2-f: Mature plants of _V. mungo_ (NI208 x T-9, left), _V. angularis_ (KS#210 x Chien Shien, right) and their interspecific hybrid (middle).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compared to over 90% for the parental species (Table 9). The sizes of unstained pollen grains were highly variable. Chromosomal irregularity and a low frequency of pairing for this species cross have been reported (Ahn & Hartmann, 1978a).

Crossability of V. mungo and V. umbellata. When *V. mungo* was used as the female, significant differences in pod-set among the different combinations of parental cultivars were determined (Table 10). Regardless of the pollen parent cultivar, T-9 set significantly more pods than NI208. The averages for pod-set on T-9 and NI208 were 82% and 53%, respectively. There were no differences between the *V. umbellata* cultivars when used as the pollen parent. Pod-set was not affected by the use of $F_1$ parental species for the cross. The hybrid pods developed normally and reached maturity. About 43 to 71% of the pods were harvested depending upon the combinations of parental species used for the cross. The mature pods contained two kinds of seeds; viz., small, undeveloped and normal full-sized seeds which were slightly shrivelled. The slightly shrivelled seeds appeared normal when imbibed with water, but failed to germinate with highly distorted cotyledons and poorly developed embryo axes.

In reciprocal crosses where *V. umbellata* was used

Table 10. Crossability for the interspecific hybridization of V. mungo (B) and V. umbellata (R).

| Parents[1] Female | Parents[1] Male | No. flowers crossed | Pod-set (%) | Pod abscision[2] (days) |
|---|---|---|---|---|
| B1 | R1 | 202 | 79.7 | 8.0 |
| B1 | R2 | 165 | 83.6 | 8.4 |
| Mean | | 184 | 81.5 | 8.2 |
| B2 | R1 | 166 | 49.4 | 7.2 |
| B2 | R2 | 181 | 56.4 | 6.6 |
| Mean | | 174 | 52.9 | 6.9 |
| (B2xB1) | (R2xR1) | 231 | 87.9 | 6.3 |
| R1 | B1 | 152 | 48.7 | 5.5 |
| R1 | B2 | 132 | 36.4 | 4.3 |
| Mean | | 142 | 43.0 | 4.9 |
| R2 | B1 | 118 | 33.1 | 4.0 |
| R2 | B2 | 124 | 58.9 | 4.4 |
| Mean | | 121 | 46.3 | 4.2 |
| (R2xR1) | (B2xB1) | 177 | 45.8 | 4.9 |

| Comparison | % Pod-set | Comparison | % Pod-set |
|---|---|---|---|
| Within B x R | **[3] | Within R x B | * |
| between B | * | between R | ns |
| between R | ns | between B | ns |
| cultivars vs $F_1$s | ns | cultivars vs $F_1$s | ns |
| B x R vs R x B | | B x R vs R x B | |
| cultivar x cultivar | * | $F_1$ x $F_1$ | ** |

[1]B1 = T-9, B2 = NI208, R1 = HK, R2 = S-91.

[2]Mean of days to pod abscision after pollination.

[3]Significant at 5% (*) or 1% (**); ns = not significant by chi-square test.

as the seed parent, the pod-set was significantly affected by the hybrid combination of parental cultivars (Table 10). However, there were no significant differences for pod-set between V. umbellata cultivars, V. mungo cultivars and intraspecific hybrids as the parents. Reduced pod growth was followed by abscision within one week of pollination. Reciprocal differences for both pod-set and subsequent pod growth was observed as pod-set was higher and pod retention longer when V. mungo was used as the seed parent than V. umbellata.

Embryo culture of hybrid embryos from the cross of V. mungo x V. umbellata was attempted. Thirty-eight embryos taken from 15 to 19 day-old pods of (NI208 x T-9) x (S-91 x HK) were cultured. Normal seedlings were obtained from 6 embryos, 22 formed callus and/or etiolated shoots with deformed leaves, and 10 did not grow. The hybrid embryos appeared normal in shape and size (4 mm in length). However, all 6 plantlets died during early seedling stages. Hybrids obtained through embryo culture have been reported (Ahn & Hartmann, 1977; Biswas & Dana, 1975). In one case, the plants flowered but were completely sterile (Biswas & Dana, 1975).

Other attempts to make this interspecific cross have been reported (Chowdhury & Chowdhury, 1977). When V. umbellata was used as the female, the pollen tubes did not penetrate the stigma, while in the reciprocal cross the embryos aborted due to early degeneration of the endosperm.

Crossability of V. mungo and V. angularis. There was a significant difference in pod-set for the cross of *V. mungo* x *V. angularis* depending on the cultivar used as the pollen parent. Regardless of the *V. mungo* cultivar, pod-set on Chien Shien and KS#210 was 49 and 73%, respectively (Table 11). The difference in pod-set between the two *V. mungo* cultivars used as seed parent was not significant. The use of intraspecific hybrids as parents significantly increased the number of pods that reached maturity without a significant increase in pod-set. The mature pods were generally empty as only 28 partially filled and shrivelled seeds were obtained. Of these seeds, 7 germinated but 6 seedlings died soon after germination. They were observed to have distorted cotyledons and poor root development. Only one seedling survived and flowered. This was the first successful interspecific hybrid plant obtained from seed. The use of heterozygous parental species did successfully enhance the crossability of this species cross. On the other hand, cultures of 8 embryos (2.5 mm in length) from 14-18 day-old pods produced 3 seedlings which died at the early seedling stages. The root systems were poorly

Table 11. Crossability for the interspecific hybridization of *V. mungo* (B) and *V. angularis* (A).

| Parents[1] Female | Male | No. flowers crossed | Pod-set (%) | Pods harvested (%) | Pod abscision[2] (days) |
|---|---|---|---|---|---|
| B1 | A1 | 118 | 52.5 | 35.6 | 8.8 |
| B1 | A2 | 186 | 78.5 | 53.8 | 9.1 |
| Mean | | 152 | 64.4 | 46.7 | 9.0 |
| B2 | A1 | 135 | 45.9 | 33.3 | 7.9 |
| B2 | A2 | 123 | 64.4 | 42.3 | 8.2 |
| Mean | | 129 | 54.3 | 37.6 | 8.1 |
| (B2xB1) | (A2xA1) | 164 | 84.2 | 63.4 | 9.3 |
| A1 | B1 | 112 | 36.6 | 0 | 7.7 |
| A1 | B2 | 108 | 21.3 | 0 | 6.8 |
| Mean | | 110 | 29.1 | 0 | 7.3 |
| A2 | B1 | 124 | 12.9 | 0 | 4.8 |
| A2 | B2 | 120 | 8.3 | 0 | 5.0 |
| Mean | | 122 | 10.7 | 0 | 4.9 |
| (A2xA1) | (B2xB1) | 138 | 36.2 | 0 | 5.8 |

| Comparison | % Pod-set | % Pod harvested | Comparison | % Pod-set |
|---|---|---|---|---|
| Within B x A | *[3] | ns | Within A x B | ** |
| between B | ns | ns | between A | ** |
| between A | * | ns | between B | ns |
| cultivars vs $F_1$s | ns | * | cultivars vs $F_1$s | * |
| B x A vs A x B | | | | |
| cultivar x cultivar | ** | ** | | |
| $F_1$ x $F_1$ | ** | ** | | |

[1] B1 = T-9, B2 = NI208, A1 = Chien Shien, A2 = KS#210.

[2] Mean of days to pod abscision after pollination.

[3] Significant at 5% (*) or 1% (**); ns = not significant by chi-square test.

developed as was previously reported to be a primary cause of seedling mortality (Ahn & Hartmann, 1977). Successful hybrid plants might possibly be attained by either modifying the culture medium, particularly the sucrose content, as reported by Honma (1955) to encourage better growth of the root system or by grafting onto parental rootstocks as described by McLean (1946).

When V. angularis was used as the female, there was a highly significant difference in pod-set between the two cultivars of V. angularis. Pod-set on Chien Shien and KS#210 were 29 and 11%, respectively (Table 11). The differences in pod-set between the two V. mungo cultivars used as male parents was not significant. Moreover, a significant increase in pod-set was obtained by using intraspecific hybrids as parents; viz., 36% compared to 19% for parental cultivars. Unfortunately, all pods abscised within 10 days after pollination.

Reciprocal differences in crossability were clearly shown in crosses between these two species. When cultivars were used as parents for hybridization, pod-set on V. mungo and V. angularis were 62 and 19%, respectively; whereas pod-set increased to 84 and 36%, respectively, when intraspecific hybrids were used as parents (Table 11). Pod abortion was delayed by using V. mungo as the female parent.

_Growth and morphology of V. mungo x V. angularis hybrid_. The single plant obtained from the cross of (NI208 x T-9) x (KS#210 x Chien Shien) grew slowly during the early seedling stage, but became increasingly vigorous as it became more established (Fig. 2-d,f).

An examination of morphological characteristics strongly suggested that the plant was a species hybrid (Fig. 2-e). It was intermediate between the two species parents for germination habit, petiole length and shape of primary leaves, and growth habit (Table 12). The hybrid resembled the _V. angularis_ parent in color of epicotyl, lobing of leaflet margins, and simple compact racemes.

_Crossability of V. umbellata and V. angularis_. In the cross of _V. umbellata_ x _V. angularis_, no significant difference for pod-set was found between the two cultivars of _V. umbellata_ or between the use of cultivars and intraspecific hybrids as female parents (Table 13). Pod-set was significantly influenced by the cultivar of _V. angularis_ used as pollen parent. The pollen parent KS#210 produced a higher pod-set (29%) than Chien Shien (45%). The pods developed slowly and abscised 1 to 2 weeks after pollination.

The reciprocal cross using _V. angularis_ as the female showed no differences in pod-set between the

Table 12. Comparison of morphological characteristics of *V. mungo* (NI208 x T-9), *V. angularis* (KS#210 x Chien Shien) and their interspecific hybrid.

| Character | *V. mungo* (female) | Hybrid | *V. angularis* (male) |
|---|---|---|---|
| Germination | epigeal | intermediate | hypogeal |
| Epicotyl color | purple | green | green |
| Petiole of primary leaves | sessile | short | long |
| Shape of primary leaves | lanceolate | ovate | cordate |
| Margin of leaflet | entire | lobed | lobed |
| Terminal leaflet (cm) L/W (ratio) | 16.9/10.3 (1.64) | 20.7/12.6 (1.64) | 18.0/11.6 (1.55) |
| Lateral leaflet (cm) L/W (ratio) | 15.7/10.1 (1.55) | 18.6/11.6 (1.60) | 15.0/10.9 (1.38) |
| Grow habit | determinate | indeterminate | indeterminate |

Table 13. Crossability for the interspecific hybridization of *V. umbellata* (R) and *V. angularis* (A).

| Parents[1] Female | Male | No. flowers crossed | Pod-set (%) | Pod abscision[2] (days) | Shrivelled seeds (no./attempt) |
|---|---|---|---|---|---|
| R1 | A1 | 126 | 27.8 | 5.6 | 0 |
| R1 | A2 | 118 | 36.4 | 9.3 | 0 |
| Mean | | 122 | 32.0 | 7.5 | 0 |
| R2 | A1 | 148 | 31.1 | 5.0 | 0 |
| R2 | A2 | 194 | 53.6 | 6.6 | 0 |
| Mean | | 171 | 43.9 | 5.8 | 0 |
| (R2xR1) | (A2xA1) | 188 | 52.1 | 10.5 | 0 |
| A1 | R1 | 138 | 65.2 | 24.3 | 1.6 |
| A1 | R2 | 116 | 74.1 | 21.7 | 1.8 |
| Mean | | 127 | 69.3 | 23.0 | 1.7 |
| A2 | R1 | 166 | 63.9 | 22.1 | 2.6 |
| A2 | R2 | 104 | 75.0 | 21.4 | 3.1 |
| Mean | | 135 | 68.2 | 21.8 | 2.8 |
| (A2xA1) | (R2xR1) | 146 | 84.9 | 23.5 | 3.3 |

| Comparison | % Pod-set | Comparison | % Pod-set |
|---|---|---|---|
| Within R x A | *[3] | Within A x R | ns |
| between R | ns | between A | ns |
| between A | * | between R | ns |
| cultivars vs $F_1$s | ns | cultivars vs $F_1$s | ns |
| R x A vs A x R | | R x A vs A x R | |
| cultivar x cultivar | ** | $F_1$ x $F_1$ | ** |

[1]R1 = HK, R2 = S-91, A1 = Chien Shien, A2 = KS#210.
[2]Mean of days to pod abscision after pollination.
[3]Significant at 5% (*) or 1% (**), ns = not significant by chi-square test.

parental cultivars of both species, nor differences between cultivars compared to heterozygous hybrids as parents (Table 13). Pods on V. angularis developed normally up to 3 weeks, then aborted.

Pod-sets were significantly higher for V. angularis x V. umbellata than the reciprocal. Crosses using parental cultivars of V. umbellata and V. angularis resulted in 39 and 69% pod-set, respectively; whereas, 52 and 85%, respectively, were achieved by using intraspecific hybrids as parents. No viable seeds were obtained for this cross. From the culture of 20 embryos (1.3 mm in length), 3 hybrid plants were obtained. Two of these hybrid plants were from the cross of V. umbellata x V. angularis and the other from its reciprocal cross. Thus, interspecific hybrids were successfully secured for the reciprocal crosses of V. umbellata x V. angularis (Fig. 3-f).

Growth and morphology of V. umbellata x V. angularis hybrid. The two plants derived by embryo culture from the cross of HK x Chien Shien grew normally (Fig. 3-a). Epicotyl color, shape of primary leaves, seed size and seed weight of the hybrid plants were intermediate between the two parental species (Table 14). The hybrid plants resembled the female parent, V. umbellata (HK), for indeterminate growth habit, elongated racemes and concave

Figure 3. Interspecific hybrids of V. umbellata x V. angularis and the reciprocal hybrid. 3-a: Plants of V. umbellata (HK, left), V. angularis (Chien Shien, right), and their interspecific hybrid (middle). 3-b: Leaves of V. umbellata (HK, left), V. angularis (Chien Shien, right) and their interspecific hybrid (middle). 3-c: Hybrid plants of V. umbellata x V. angularis, showing fruiting (left) and continuous vegetative growth (right). 3-d: $F_2$ seedlings of V. umbellata x V. angularis, showing the variation of primary leaf shapes and their parents, V. umbellata (left) and V. angularis (right). 3-e: Terminal leaflets of V. umbellata x V. angularis $F_2$ plants, showing the variation of leaf shapes with their parents, V. umbellata (top left two) and V. angularis (top right two). 3-f: Reciprocal interspecific hybrid plant, V. angularis x V. umbellata.

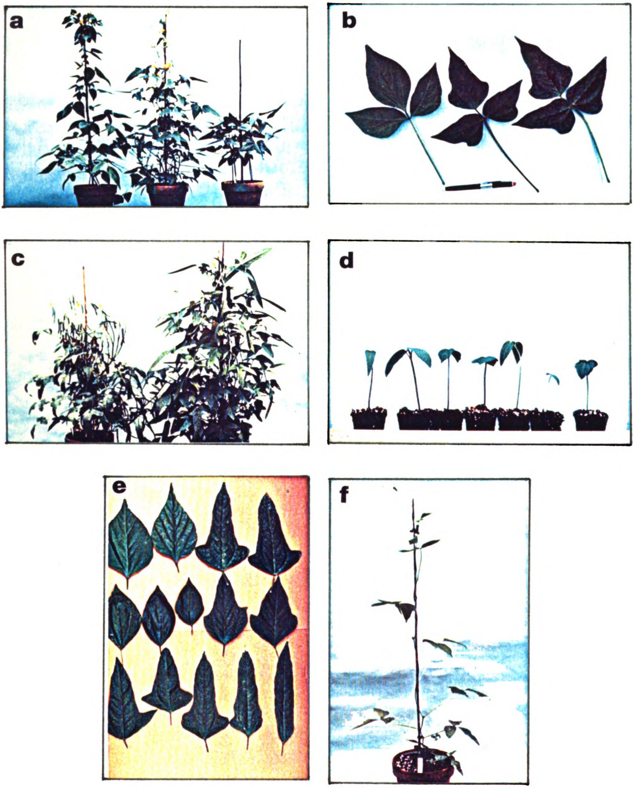
a
b
c
d
e
f

Table 14. Comparison of the morphological characteristics of _V. umbellata_ (HK), _V. angularis_ (Chien Shien) and their interspecific hybrid.

| Character | _V. umbellata_ (female) | Hybrid | _V. angularis_ (male) |
|---|---|---|---|
| Epicotyl color | purple | light purple | green |
| Shape of primary leaves | lanceolate | ovate | cordate |
| Margin of leaflet | entire | lobed | lobed |
| Terminal leaflet (cm) L/W (ratio) | 16.6/9.6 (1.73) | 19.0/11.0 (1.73) | 16.4/9.6 (1.71) |
| Lateral leaflet (cm) L/W (ratio) | 14.6/8.5 (1.72) | 15.5/10.1 (1.53) | 14.0/9.5 (1.47) |
| Growth habit | indeterminate | indeterminate | determinate |
| Raceme | elongated | elongated | compact |
| Flower color | bright yellow | bright yellow | light yellow |
| Pollen stainability (%) | 94.5 | 77.4 | 92.9 |
| Pod length (cm) | 9.3 | 7.3 | 8.2 |
| No. of seeds per pod | 6.0 | 2.8 | 5.3 |
| Seed size (mm) L/W (ratio) | 7.8/3.3 (2.36) | 7.6/4.1 (1.85) | 7.9/5.5 (1.44) |
| Seed weight (mg) | 74.2 | 85.5 | 167.5 |
| Hilum cushion | concave | concave | not concave |

hilum cushion. The pollen parent, V. angularis (Chien Shien), character of lobed leaflet was dominant in the hybrid to the entire leaflet margin of the female, V. umbellata (HK). The presence of lobed leaflets in the hybrid plants clearly indicated a hybrid (Fig. 3-b).

The hybrid plants expressed indeterminate growth by producing juvenile shoots even after flowering (Fig. 3-c). They flowered profusely and produced viable seeds, though the fertility was less than the parental species (Fig. 3-c). The pollen stainability of the hybrids was 77% compared to 95 and 93% for HK and Chien Shien, respectively (Table 14). The length of pod and number of seeds per pod for the $F_1$ plants was less than the parental species due to the low fertility.

Ahn and Hartmann (1978) reported that the hybrid of V. umbellata x V. angularis had regular meiosis with 11 bivalents and a high degree of chromosome homology. In the current study, $F_2$ seeds from $F_1$ plants germinated normally. From 60 $F_2$ plants, a continuous variation was observed for epicotyl color and shape of primary leaves (Fig. 3-d,e). Combination of parental types was observed; e.g., the purple epicotyl of V. umbellata with the cordate primary leaves of V. angularis and vice versa were found in the population (Fig. 3-d).

The reciprocal crossability of this hybrid and the relatively high fertility of the $F_1$ and $F_2$ generations indicated that the only isolating barrier to this species cross was embryo abortion which can be overcome by embryo culture. Accordingly, *V. umbellata* and *V. angularis* are probably closely related and genetic exchange between them may be feasible.

# GENERAL DISCUSSION AND CONCLUSIONS

Stebbins (1958) reviewed several mechanisms responsible for the isolation of species including hybrid inviability, lack of vigor and sterility. These general mechanisms might operate between the four species of *Vigna* in this study. Five interspecific hybrids were successfully made from the six possible crosses of four species. In general, pod-set occurred in all of the interspecific crosses. Embryo abortion took place in the crosses of *V. radiata* x *V. angularis*, *V. mungo* x *V. umbellata* and *V. umbellata* x *V. angularis*. The remaining interspecific crosses produced viable hybrid seeds with varying degrees of success.

The degree in crossability based on the use of two cultivars per species, and for cultivars versus intraspecific hybrid parents, suggested that parental genotypes and heterozygous parental genotypes might be important factors in successful interspecific crosses. Growth and development of hybrid seedlings was also influenced by the parental genotypes of both parents. As a result, the lethality and vigor of hybrid seedlings could be improved and recovered by judicious selection of parental genotypes. In this study, the interspecific hybrid of *V. mungo* x *V. angularis* and the reciprocal hybrid, *V. umbellata* x *V. angularis*, were produced through the use of proper parental genotypes.

Reciprocal difference in crossability for pod-set, embryo abortion and viable seed were found for all six interspecific crosses. Except for the cross between *V. umbellata* and *V. angularis*, no other reciprocal interspecific crosses were successful. The unilateral success of most crosses suggested that cytoplasmic factors may be involved in the isolating mechanisms between these species (Ahn & Hartmann, 1977).

In the present study, viable hybrid seeds were only obtained for the interspecific crosses of *V. radiata* x *V. mungo*, *V. radiata* x *V. umbellata* and *V. mungo* x *V. angularis*. Hybrid seedlings of these species crosses can also be obtained through embryo culture. Hybrid seedlings obtained through embryo culture grew normally and reached flowering for the following $F_1$ crosses: *V. radiata* x *V. angularis*, *V. umbellata* x *V. angularis* and *V. angularis* x *V. umbellata*.

The hybrid plants of *V. radiata* x *V. mungo* and *V. umbellata* x *V. angularis* were partially fertile, while the hybrids of *V. radiata* x *V. umbellata*, *V. radiata* x *V. angularis* and *V. mungo* x *V. angularis* were sterile. Cytological studies have shown poor chromosome pairing probably due to lack of homology (Sawa, 1973; Biswas & Dana, 1975; Ahn & Hartmann, 1977; Machado et al., unpublished).

Ahn and Hartmann (1977) postulated the evolutionary relationships of these four species based on crossability and divided them into two subgroups; viz., *V. radiata* with *V. mungo* and *V. umbellata* with *V. angularis*. Electrophoretic survey of isozyme patterns of these four *Vigna* species made no suggested genetic relationship between these four species due to the large number of variant bands (Bassiri & Adams, 1978). Based on the cultivars used in this study, the degree of crossability for these four *Vigna* species might be divided into four groups. The cross of *V. radiata* x *V. mungo* yielded most $F_1$ seeds which suggested these two species are closely related. Few $F_1$ seeds of *V. radiata* x *V. umbellata* and *V. mungo* x *V. angularis* were obtained which suggested these two pair of species are slightly related. Interspecific $F_1$ plants of *V. radiata* x *V. angularis* and *V. umbellata* x *V. angularis* could only be obtained by embryo culture indicating these two pairs of species are remotely related. Neither seeds nor embryos of *V. mungo* x *V. umbellata* were obtained as these two species are probably more distantly related.

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# SECTION II

## EFFECT OF E-AMINO-N-CAPROIC ACID ON INTERSPECIFIC HYBRIDIZATION IN THE GENUS *VIGNA*

ABSTRACT

The possibility of using chemicals to circumvent crossability barriers in interspecific hybridization was studied. Three chemicals, E-amino-n-caproic acid (EACA), gentisic acid and L-lysine were tested for their effect on interspecific crosses in the genus *Vigna*. The EACA as a foliar spray was the most effective chemical studied. Daily foliar applications of EACA to the plants of the maternal parent, *Vigna radiata* (mungbean) for 14 days before cross-pollination with *V. umbellata* (rice bean) significantly increased the numbers of viable hybrid seeds. Concentrations of EACA less than 100 mg/l appeared ineffective, whereas concentrations over 2500 mg/l were phytotoxic. Concentrations of 100 to 1000 mg/l were the most effective. The overall numbers of viable seeds obtained were increased from 2- to 9-fold by EACA treatment depending upon the experiment number. Different cultivars of maternal *Vigna* species responded differently to EACA concentrations. Additionally, 1000 mg/l EACA applied as a post-pollination treatment increased numbers of viable seed similar to pre-pollination treatments. Treatment by either EACA or gentisic acid significantly enhanced the development of normal hybrid seeds produced for the *V. radiata* x *V. umbellata* cross. Applications of equal concentration EACA and L-lysine were as effective

as EACA applied alone for producing $F_1$ seeds of this interspecific cross. Appropriate chemical treatments increased the frequencies of normally appeared hybrid embryos by 15 to 22% relative to the control. The success with which the embryos could be cultured *in vitro* was improved accordingly. The proposed use of EACA for interspecific hybridization involving selected cultivars of *V. radiata* and *V. umbellata* reduced crossability barriers and greatly facilitated the movement of germplasm between these two *Vigna* species.

## INTRODUCTION

Interspecific hybridization has played an important role in the evolution of flowering plants in nature (5). In plant breeding, interspecific hybridization has been used when a desired character can not be identified in the gene pool of a given species, but is present in a second species. The Leguminosae species are endowed with a richness of genetic diversity unparalleled by other economic plants; e.g., N-fixation, protein quality and quantity, oil content. The relatively narrow gene base (17, 19, 20) of some individual species, however, has limited varietal improvement. The use of interspecific hybridization significantly enlarges the genetic base of individual food legume species.

Interspecific hybridization of *Vigna* food legumes was recently studied at the Asian Vegetable Research and Development Center (AVRDC). The *V. umbellata* (rice bean) is highly resistant to bean fly (*Melanagromyze phaseoli*), *Cercospora* leaf spot and other diseases, but of little agronomic value as a cultivated species (2, 3). The nature of reproductive barriers between the major Asian food legumes in the genus *Vigna* has been studied (1, 14, 15, 16). Interspecific crosses were made with difficulty. The use of large numbers of pollinations have yielded extremely low numbers of interspecific hybrid plants. These occasional interspecific hybrid plants are nearly always sterile (1, 3, 13).

Bates and Deyoe (6) theorized that crossability barriers evolved during speciation are mediated through a specific inhibition reaction (SIR) similar to immune responses in animals. The SIR may effect fertilization and development of hybrid embryos in wide crosses. Animal-effective immunosuppressants were applied to various cereal species to test the hypothetical model of interspecific and intergeneric crossability barriers (6, 7). An immunosuppressant, E-amino-n-caproic acid (EACA), did overcome crossability barriers in interspecific and intergeneric crosses of cereals (8, 9, 10, 21). Application of EACA by either injecting the solution into the leaf sheath or by foliar sprays increased embryo formation and seed set in several intergeneric crosses of cereals. In food legumes, injection of EACA was less effective, while foliar sprays with aqueous solutions of EACA to the maternal parent significantly increased viable hybrid seed set of the interspecific cross of *V. radiata* x *V. umbellata* (4, 13).

The present study was undertaken to investigate the effects of EACA on the crossability of *Vigna* species to create new genetic variation for plant breeding programs for food legume improvement.

## MATERIALS AND METHODS

Plant material. A series of experiments was conducted using *Vigna radiata* (mungbean) as the maternal parent and *V. umbellata* (rice bean) as the pollen parent. Two cultivars of mungbean, Tainan #1 and PHLV #18, and one cultivar of rice bean, HK, were used. These were obtained from the Asian Vegetable Research and Development Center (AVRDC) as AVRDC accession nos. 2013, 2184 and 4006, respectively. Two other interspecific crosses, *V. mungo* (black gram) x *V. radiata* and *V. umbellata* x *V. angularis* (adzuki bean) were attempted to test their response to EACA treatments. The cultivars used were black gram, "T-9", and adzuki bean, "Chien Shien", which were AVRDC accession nos. 3115 and 5124, respectively.

Cultural conditions. All plants were grown in the greenhouse. During winter, temperatures were adjusted to 26°C (day) and 18°C (night) with a photoperiod of 14 h light supplied with high intensity metal-halide lamps (10-12 klx). Plants used as maternal parents were grown in 20 cm pots containing either a mixture of soil, sand and peat (3:1:1) or a "Peat Lite Mix". Plants were fertilized with 3 g/pot of "Peter's" fertilizer (20N-20P-20K) biweekly. Two plants were maintained in each pot.

Chemical treatments. The chemicals, E-amino-n-caproic acid (EACA), gentisic acid and L-lysine HCl were tested and compared for their success on the interspecific crosses of *Vigna*. The chemical treatments were made as aqueous solutions applied to the maternal plant as foliar sprays to run-off. Spray treatments were initiated prior to the meiotic stage of floral development. In *V. radiata*, meiosis occurs when the floral envelope of the bud is 2-3 mm in size. Daily treatments of EACA were initiated at this pre-meiotic stage and continued over a 14-day period before cross-pollination. The surfactant, "Tween 20", was used in all treatments. For post-pollination treatments, EACA was applied daily for 14 consecutive days after cross-pollination. A "cotton wrap" method was also tested and compared to foliar spray as a post-pollination treatment. In this method, the pedicels of cross-pollinated (hybrid) pods were wrapped with cotton into which an EACA solution was slowly dripped once a day continuously for 14 days.

*Crossing techniques*. Flowers for hybridization were emasculated the day before anthesis and immediately pollinated. The hybridization technique described by Buishand (12) and Boling et al. (11) was used. Plants were pollinated for five to seven consecutive days after termination of chemical treatments. Approximately equal numbers of pollination attempts were made for each treatment. After cross-pollinating for 5-7 days, all subsequent flowers were removed to eliminate competition between hybrid and naturally self-pollinated pods.

Experimental design and data analysis. Randomized complete block and split-plot designs were used. The pods were harvested individually and the number of shrivelled and "normal" seeds noted. Seeds were then germinated to confirm their hybridity.

Two experiments were conducted to evaluate the chemical effects on the development of the hybrid embryos. Fertilized ovules were collected 12 or 14 days after pollination and the excised embryos were examined using a dissecting microscope. The development of embryos was classified as either normal or abnormal based upon visual examination of their gross morphological appearance and apparent degree of differentiation.

Analyses of variance were performed on all data after using either square root ($\sqrt{x + \frac{1}{2}}$) or arcsin transformations. Duncan's multiple range test was used to compare treatment means.

## RESULTS AND DISCUSSION

Effect of EACA on V. radiata-V. umbellata hybrid cross. Foliar sprays with a $\log_{10}$ concentration series of EACA from 0 to 10000 mg/l were tested. A large number of viable hybrid seeds was obtained (Table 1). In Experiment 1, the treatment of 100 mg/l EACA was superior to all other treatments including the control except for 1000 mg/l. The efficiency of viable hybrid seeds produced was 50.1 seeds per 100 attempts compared to 17.7 seeds for the control. Treatment with the highest concentration of EACA (10000 mg/l) was toxic to the plants.

In Experiment 2, three concentrations of EACA (100, 500 and 1000 mg/l) were tested. The treatment of 1000 mg/l EACA significantly increased the number of viable seed as compared to the control (Table 1). The numbers of viable seed per 100 attempts for the treatment and control were 13.6 and 2.3, respectively. Differences among EACA treatments, however, were not significant. The number of viable seed obtained coincided with the percent pods that produced viable seed; i.e., the higher the percentage of pods with viable seeds the greater the number of viable seeds observed in both experiments. Differences between treatments for percent pods with viable seeds were not significant in the second experiment.

In subsequent experiments, attempts to identify the optimum concentration were made with no one consistent

Table 1. Effect of EACA treatments on the success of the interspecific cross of *V. radiata* x *V. umbellata*.

| EACA treatment (mg/l) | No. flowers crossed | Total viable seeds obtained | No. of viable seeds per 100 attempts | Pods with viable seeds (%) |
|---|---|---|---|---|
| Experiment 1[z] | | | | |
| 0 | 137 | 25 | 17.7b[y] | 11.5ab |
| 10 | 159 | 19 | 12.0b | 8.9bc |
| 100 | 142 | 69 | 50.1a | 24.6a |
| 1,000 | 124 | 24 | 22.3ab | 15.7ab |
| 10,000 | 76 | 0 | 0 b | 0 c |
| Experiment 2[x] | | | | |
| 0 | 88 | 2 | 2.3b | 2.3[ns] |
| 100 | 86 | 6 | 6.9ab | 6.9 |
| 500 | 93 | 8 | 8.5ab | 7.4 |
| 1,000 | 96 | 13 | 13.6a | 10.3 |

[z]Data represent the total number and the mean of 6 replications.

[y]Mean separation in columns within experiment by Duncan's multiple range test, 5% level.

[x]Data represent the total number and the mean of 4 replications.

ns= Not significant.

concentration as the "best" EACA treatment. Concentrations of EACA less than 100 mg/l seemed ineffective, whereas concentrations over 2500 mg/l appeared phytotoxic. Concentrations in the range of 100 to 1000 mg/l seemed most effective (Table 1). In general, high concentrations of EACA (over 2500 mg/l) caused chlorosis and abnormal floral morphogenesis on the treated plants. Morphogenetic anomalies induced by EACA treatments have been reported for *Dolichos lab-lab* (18).

The total number of viable seeds obtained and viable seeds per 100 attempts are higher in Experiment 1 than Experiment 2 for all corresponding treatments including controls (Table 1). The relative difference in degree of response between the two experiments and subsequent experiments was probably due to differences in environmental conditions. The application of EACA to the maternal parent significantly improved the development of embryos from wheat-rye crosses, but was not independent of the temperature regime (22) suggesting that in the present study, depending upon environmental conditions, the magnitude of response could vary from one experiment to another.

To determine the effect of plant growth stage on chemical treatment, an experiment was designed using various durations of pre- and post-pollination EACA treatments. Plants of the maternal parent, *V. radiata*, were subjected to daily treatments of 100, 500 and 1000 mg/l EACA at various growth stages. No significant differences were observed for the number of viable seed produced by EACA treatments made at different stages of plant development.

Post-pollination EACA effects on V. radiata-V. umbellata hybrid cross. The possibility of post-pollination treatments with EACA to reduce hybrid embryo abortion or breakdown was explored. Plants of the maternal parent, *V. radiata*, were sprayed daily with 100 and 1000 mg/l EACA for 14 days after hybridization. Treatments with EACA increased 2- to 4 times the number of viable hybrid seeds as compared to the control (Table 2). A concentration of 1000 mg/l EACA was optimal for post-pollination treatment. At this concentration, numbers of viable seeds were significantly higher than that from untreated plants. As mentioned previously, foliar application of EACA in concentration greater than 2500 mg/l were phytotoxic. An experiment to test cotton wraps for post-pollination EACA treatments was conducted to possibly reduce phytotoxic effects. Two concentrations of EACA (2500 and 5000 mg/l) were used to compare with the control and the "optimal" 1000 mg/l EACA treatment. An increase in numbers of viable hybrids seed were obtained using the "cotton wrap" method (Table 2). No phytotoxic effect was observed from these treatments with relatively high concentration of EACA. Although EACA treatments significantly increased the number of viable

Table 2.

| EAC treatm (mg/ |
| --- |
| Foliar sp |
| 0 |
| 100 |
| 1,000 |
| Cotton wr |
| 0 |
| 1,000 |
| 2,500 |
| 5,000 |

[z]Data rep attempts

[y]Mean sep range te

[x]Data rep attempts

ns=Not s

Table 2. Effect of EACA applied as post-pollination treatments on the success of the interspecific cross of *V. radiata* x *V. umbellata*.

| EACA treatment (mg/l) | Total viable seeds obtained | No. of viable seeds per 100 attempts | Pods with viable seeds (%) |
|---|---|---|---|
| Foliar spray experiment[z] | | | |
| 0 | 21 | 32.8b[y] | 23.5b |
| 100 | 48 | 75.0b | 31.3b |
| 1,000 | 99 | 154.7a | 42.2a |
| Cotton wrap experiment[x] | | | |
| 0 | 24 | 30.8c | 22.7[ns] |
| 1,000 | 70 | 87.8a | 32.2 |
| 2,500 | 43 | 53.5abc | 35.9 |
| 5,000 | 67 | 83.3ab | 38.7 |

[z]Data represent the mean of 4 replications. A total of 64 pollination attempts was made in each treatment.

[y]Mean separation in columns within experiment by Duncan's multiple range test, 5% level.

[x]Data represent the mean of 4 replications. A total of 80 pollination attempts was made in each treatment.

ns=Not significant.

seeds obtained, differences among treatments were not significant suggesting that the treatment with 1000 mg/l was equal or superior to other treatments.

To define more clearly the optimum duration for EACA application, an experiment was conducted using 1000 mg/l EACA applied daily as a foliar spray for 7 and 14 consecutive days as a pre-pollination or post-pollination treatment. The EACA applied daily either for 14 days before cross-pollination or for 7 days after cross-pollination significantly increased the number of viable seeds relative to the control (Table 3). There were no significant differences in effects among EACA treatments. The relatively low number of viable seeds obtained in all treatments, including the control, was probably due to the high temperatures encountered during this experiment.

Cultivar response to EACA treatment. Two cultivars of *V. radiata* were used to determine their crossability with *V. umbellata* and their responses to EACA treatments. Plants of the two cultivars, Tainan #1 and PHLV #18, were treated with a series of EACA concentrations (0 to 4000 mg/l) for 14 days before anthesis, and then crossed with the same cultivar (HK) of *V. umbellata*. Crossability was higher for Tainan #1 which yielded more viable hybrid seeds than PHLV #18 regardless of the chemical treatment (Table 4). These data suggest that cultivar may play an important role in the success of interspecific hybridization.

Table 3. Effect of duration of treatment with 1,000 mg/l EACA on viable seed set of the interspecific cross of *V. radiata* x *V. umbellata*.[z]

| EACA treatment | Total viable seeds obtained | No. of viable seeds per 100 attempts | Pods with viable seeds (%) |
|---|---|---|---|
| Pre-pollination | | | |
| 7 days | 5 | 6.9ab[y] | 5.6 |
| 14 days | 9 | 12.5a | 9.7 |
| Post-pollination | | | |
| 7 days | 7 | 9.7a | 9.7 |
| 14 days | 5 | 6.9ab | 6.9 |
| Control | 1 | 1.4b | 1.4 |

[z]Data represent the mean of 3 replications. A total of 72 pollination attempts was made in each treatment.

[y]Mean separation in column by Duncan's multiple range test, 5% level.

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Table 4. Response of two varieties of V. radiata to EACA treatments by interspecific hybridization with V. umbellata.[z]

| EACA treatment (mg/l) | Variety: Tainan #1 Total viable seeds | Tainan #1 Viable seeds per 100 attempts | PHLV #18 Total viable seeds | PHLV #18 Viable seeds per 100 attempts | Mean viable seeds/100 attempts |
|---|---|---|---|---|---|
| 0 | 34 | 40.5c[y] | 22 | 26.2c | 33.4d |
| 100 | 133 | 158.3a | 43 | 51.2b | 104.7a |
| 500 | 46 | 54.8b | 63 | 75.0a | 64.9b |
| 1,000 | 52 | 61.9b | 46 | 51.2b | 56.5c |
| 4,000 | 43 | 51.2bc | 19 | 22.6c | 36.9d |
| Mean | 62 | 73.3 | 39 | 45.2 | |

[z]Data represent the mean of 6 replications. A total of 84 pollination attempts was made in each treatment.

[y]Mean separation in columns by Duncan's multiple range test, 5% level.

Treatments with EACA were effective on both mungbean cultivars as the number of viable seeds was increased (Table 4). However, the cultivars responded differently to the EACA concentrations. The interaction between cultivar and EACA treatment was highly significant ($\alpha = .01$). The highest number of viable seed was exhibited by the treatment of 100 mg/l EACA in "Tainan #1", while the highest number of viable seed in "PHLV #18" was produced from the treatment with 500 mg/l EACA. The effective concentrations of EACA for the interspecific cross of _V. radiata_ x _V. umbellata_ ranged from 100 mg/l to 1000 mg/l.

In an intergeneric cross of cereals, involving 10 cultivars, EACA generally enhanced total seed set of _Triticum turgidum_ var. _durum_ x _Secale cereale_ by 45%, although the crossability of the 10 cultivars differed while certain cultivars were unaffected by EACA treatment (10). Such interactions between maternal cultivars and EACA treatments confound the development of a specific methodology for using EACA in interspecific and intergeneric hybridization.

_Effects of EACA on other interspecific crosses_. Interspecific hybridization of _V. mungo_ x _V. radiata_ has not been reported in the literature. The cross of _V. umbellata_ x _V. angularis_ was accomplished by culturing immature embryos _in vitro_ (1). The possibility of successfully making these two interspecific crosses using EACA treatments was explored.

Plants of the maternal parent, V. mungo, were treated daily with 100, 1000 and 2000 mg/l EACA for 14 days; then cross-pollinated with V. radiata for 5 days. No viable hybrid seeds were produced by any of the treatments including the untreated plants. Treatments with 1000 and 2000 mg/l EACA induced phytotoxicity and premature pod abortion. For the interspecific cross of V. umbellata x V. angularis, maternal plants were subjected to daily treatments with the same concentrations of EACA described in the cross of V. mungo x V. radiata with the same results, viz., no viable hybrid seeds were obtained.

Effects of other chemicals on V. radiata-V. umbellata hybrid cross. Several chemicals including EACA, acriflavin, gentisic acid, salicylic acid and chloramphenicol have been used to enhance the crossability of interspecific and intergeneric hybridization in cereals with varying degrees of success (7, 8). Gentisic acid was tested to compare its efficiency to EACA. An experiment was designed using three concentrations (10, 100 and 1000 mg/l) of each chemical. Maternal plants, V. radiata, were sprayed daily with appropriate concentration of either EACA or gentisic acid for 14 days; then cross-pollinated with V. umbellata.

Both chemicals were effective in increasing the numbers of viable seeds obtained for this interspecific hybrid cross (Table 5). The EACA was a more effective promoter of

Table 5. Relative effect of EACA and gentisic acid on the success of the interspecific cross of _V. radiata_ x _V. umbellata_.[z]

| Chemical | Treatment (mg/1) | Total viable seeds obtained | No. of viable seeds per 100 attempts | Pods with viable seed (%) |
|---|---|---|---|---|
| EACA | 10 | 13 | 20.3bc[y] | 9.4c |
| | 100 | 14 | 21.9bc | 14.1abc |
| | 1,000 | 36 | 56.3a | 25.0a |
| | Mean | 21 | 32.8 | 16.2 |
| Gentisic acid | 10 | 11 | 17.2bc | 7.8c |
| | 100 | 8 | 12.6bc | 11.0bc |
| | 1,000 | 20 | 31.3b | 23.5ab |
| | Mean | 13 | 20.4 | 14.1 |
| Control | | 4 | 6.3c | 6.3c |

[z]Data represent the mean of 4 replications. A total of 64 pollination attempts was made in each treatment.

[y]Mean separation in columns by Duncan's multiple range test, 5% level.

hybrid seed production than gentisic acid. Treatments with 1000 mg/l of either EACA or gentisic acid produced totals of 36 and 20 viable seeds or 56 and 31 viable seeds per 100 attempts, respectively. The untreated plants, however, yielded only 4 viable seeds or 6 viable seeds per 100 attempts. Obviously, the use of these chemicals to accomplish the interspecific cross of *V. radiata* x *V. umbellata* is advantageous as indicated by 5- to 9-fold increases in seed numbers compared to the control (Table 5).

An analogue of EACA, L-lysine, was also compared to EACA for overcoming crossability barriers in the *V. radiata* x *V. umbellata* cross. Two concentrations (100 and 1000 mg/l) of each chemical and two combinations in equal concentration of these two chemicals were compared with the control. Chemical treatments were applied daily as pre-pollination treatments described previously. Treatments with 100 and 1000 mg/l of either EACA or L-lysine resulted in increased numbers of viable hybrid seeds (Table 6). However, the differences between L-lysine treatments and the control were not significant. Treatment of the maternal plants by a combination of equal concentration of the two chemicals (50 mg/l + 50 mg/l) was superior to other treatments in terms of number of viable seeds produced, but it was not significantly different from that obtained with either chemical applied separately.

*Chemical effects on V. radiata-V. umbellata hybrid embryo development*. In the previous studies, the frequency

Table 6. Relative effect of EACA and L-lysine on the success of the interspecific cross of *V. radiata* x *V. umbellata*.[z]

| Treatment (mg/l) | Total viable seeds obtained | No. of viable seeds per 100 attempts | Pods with viable seeds (%) |
|---|---|---|---|
| Control | 2 | 2.3c[y] | 2.3c |
| EACA | | | |
| 100 | 8 | 9.1abc | 9.1ab |
| 1,000 | 11 | 12.5ab | 11.4ab |
| L-lysine | | | |
| 100 | 5 | 5.7bc | 5.7bc |
| 1,000 | 9 | 10.3abc | 9.1ab |
| EACA + L-lysine | | | |
| 50 + 50 | 14 | 15.9a | 14.8a |
| 500 + 500 | 4 | 4.7bc | 4.6bc |

[z]Data represent the mean of 4 replications. A total of 88 pollination attempts was made in each treatment.

[y]Mean separation in columns by Duncan's multiple range test, 5% level.

of fertilization in *V. radiata*, using *V. umbellata* as the pollen parent, was relatively high as indicated by a high percentage of pod-set. However, most embryos aborted within 10-14 days resulting in empty pods at maturity. Extremely low numbers of embryos developed to mature seeds (14). An experiment was conducted using EACA and L-lysine to determine their effects on interspecific hybrid embryo development *in vivo*. Plants of the maternal parent were subjected to treatments with either various concentrations of single chemicals or combinations of two chemicals for 14 days prior to hybridization and post-pollination treatments for 7 consecutive days following cross-pollination. Hybrid ovules were sampled at 14 days after pollination. Excised embryos were examined and classified as either normal or abnormal based on their size and morphological appearances.

The development of young hybrid pods and seeds measured at 14 days after pollination were not affected by the chemical treatments (Table 7). Treatments with 100 and 1000 mg/l EACA, 100 mg/l lysine, and a combination of 500 mg/l EACA plus 500 mg/l lysine significantly increased the frequencies of normal embryos ($\alpha$=.05) relative to non-treatments. Generally, embryo abortion occurred during all stages of embryogenesis; however, the critical stage of embryo abortion or breakdown was observed to occur 10 to 14 days after pollination. The low frequencies

Table 7. Effects of EACA and L-lysine on development of 14-day-old pods, seeds and embryos produced from the cross of V. radiata x V. umbellata.[z]

| Chemical treatment (mg/l) | Pod size | | Length of seed (mm) | Fertilized ovules examined (no.) | Normal embryos (%) |
|---|---|---|---|---|---|
| | Length (mm) | Width (mm) | | | |
| Control | 65.0 | 5.5bc[y] | 4.0 | 147 | 0.7d |
| EACA | | | | | |
| 100 | 65.0 | 5.7a | 4.0 | 131 | 9.3ab |
| 1,000 | 58.1 | 5.6ab | 4.1 | 115 | 7.0bc |
| L-lysine | | | | | |
| 100 | 64.5 | 5.4bc | 4.3 | 134 | 8.4ab |
| 1,000 | 63.9 | 5.3c | 4.2 | 144 | 2.9d |
| EACA + L-lysine | | | | | |
| 50 + 50 | 68.7 | 5.4bc | 4.2 | 155 | 3.3cd |
| 500 + 500 | 66.2 | 5.4bc | 4.2 | 144 | 13.1a |

[z]Data represent the mean of 15 samples.

[y]Mean separation in columns by Duncan's multiple range test, 5% level.

of normal-appeared embryos observed in all treatments were probably attributed to a delay in collecting the hybrid ovules. Therefore, the experiment was repeated and expanded by adding the treatments with gentisic acid. Hybrid ovules were sampled at 12 days instead of 14 days after pollination. The frequencies of normal-appeared embryos in all treatments including control were correspondingly higher in this latter experiment relative to those shown in Table 7 (Table 8). The EACA concentrations of both 100 mg/l and 1000 mg/l resulted in significantly increased frequencies of normal-appeared embryos ($\alpha$=.05). Differences between treatments of lysine and control, however, were not significant. The application of EACA (500 mg/l) in combination with an equal concentration of lysine (500 mg/l) was as effective as EACA applied alone. The frequency of "normal" embryos produced as a result of EACA + lysine treatments was significantly increased as compared with that obtained from untreated, but did not differ significantly from that of EACA applied alone. On the other hand, treatment with 1000 mg/l gentisic acid was found to be effective and similar to EACA treatments. It increased the frequency of "normal" embryos by 13.8% (Table 8). Differences between treatments with gentisic acid and EACA, however, were not significant. The combination of gentisic acid with lysine had no effect on normal embryo development relative to the control.

Table 8. Effects of EACA, gentisic acid and L-lysine on development of 12-day-old *V. radiata* x *V. umbellata* embryos.[z]

| Chemical treatment | Concn (mg/1) | No. fertilized ovules examined | Normal embryos (%) |
|---|---|---|---|
| Control | 0 | 217 | 13.2d[y] |
| EACA | 100 | 216 | 35.2a |
| | 1,000 | 198 | 32.8ab |
| Gentisic acid | 100 | 221 | 19.6cd |
| | 1,000 | 208 | 27.0abc |
| L-lysine | 100 | 223 | 20.1cd |
| | 1,000 | 217 | 21.5bcd |
| EACA + L-lysine | 50 + 50 | 214 | 20.9cd |
| | 500 + 500 | 204 | 28.6abc |
| Gentisic acid + L-lysine | 50 + 50 | 193 | 22.2bcd |
| . | 500 + 500 | 223 | 13.9d |

[z]Mean of 4 replications.

[y]Mean separation in column by Duncan's multiple range test, 5% level.

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In general, EACA either applied alone or combined with lysine enhanced normal embryo development by 15 to 22% (Table 8). Both chemicals consist of six-carbon-chain skeletons carrying an amino group at the epsilon position. Therefore, it was presumed that both chemicals might behave similarly in promoting embryo development. Both chemicals were reported to improve significantly the formation of embryos in a wheat-rye cross (21) which agrees with the present study. Such chemical treatments are promising for plant breeding programs particularly for the interspecific cross of *V. radiata* x *V. umbellata*.

*Suggested methodology*. The chemical EACA, was the most effective for increasing both the total numbers of viable seeds and "normal" embryos formed among the chemicals tested. The use of EACA provides a valuable technique to circumvent certain crossability barriers to facilitate the movement of germplasm between distantly related species.

The use of EACA for interspecific hybridization of *V. radiata* and *V. umbellata* is suggested as follows: (a) Initiate daily treatments of maternal parent at premeiotic (2 mm bud size) or desired stage of bud development, approximately 14 days before anthesis; use 100 to 1000 mg/l EACA as an aqueous foliar spray to run-off. (b) Emasculate flowers the day before anthesis and cross-pollinate either immediately or the subsequent day; daily pollination of additional flowers should be continued for 3 or more days. (c) Continue daily spray for 7 days after cross-pollination. (d) Remove all self-pollinated flowers to prevent competition. (e) Harvest pods either at immature stage (10 to 12 days after pollination) for embryo culture or at maturity (20 to 22 days). (f) Verify hybridity of hybrid seedlings by genetic markers and/or cytogenetic analysis.

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# SECTION III

# TECHNIQUES IN CIRCUMVENTING CROSSABILITY BARRIERS IN THE INTERSPECIFIC CROSS, *VIGNA RADIATA* x *V. UMBELLATA*

# SUMMARY

Application of techniques such as partial detachment of pods, defoliation, pollen mixture, interspecies grafting, species bridge and amphiploidy were studied to facilitate the crossing of *Vigna radiata* x *V. umbellata*. Number of viable $F_1$ seeds was increased in pods with partially broken pedicels and in pods excised from the plant 10-14 days after hybridization and cultured *in vitro*. Partial or complete removal of leaves from the maternal plants 4 days after pollination also increased the number of viable $F_1$ seeds. The "side-grafting" of two species, and the use of a third species bridge were effective in reducing embryo abortion and increasing the number of viable $F_1$ seeds.

Hybrid sterility was circumvented by doubling the chromosome number. Five triploid plants were derived from cultured embryos of the backcross of the amphidiploid to *V. radiata*. The triploid plants grew vigorously and flowered profusely with partial fertility.

# INTRODUCTION

*Vigna radiata* (L.) Wilzcek (mungbean) is an ancient and important pulse crop in Asia and parts of Africa. It is an excellent source of protein, but its yield potential is low and it is susceptible to many insect and disease pests (AVRDC, 1974). The *V. umbellata* (Thunb.) Ohwi & Ohashi (rice bean), a closely related species of *V. radiata*, was reported to be highly resistant to bean fly (*Melanagromyze phaseoli*) but of little agronomic value as a cultivated species. *V. umbellata* is also highly resistant to *Cercospora* leaf spot, powdery mildew and root diseases (AVRDC, 1975). The present study was to incorporate this resistance from *V. umbellata* to *V. radiata*.

Interspecific hybridization between *V. radiata* and *V. umbellata* has been reported with limited success (Dana, 1967; AVRDC, 1974, 1975; Ahn & Hartmann, 1977; Chen et al., 1977). The interspecific cross was only successfully made when *V. radiata* was used as the female parent, with difficulty due to low numbers of hybrid plants obtained. Most of the embryos were observed to abort prematurely (AVRDC, 1976; Ahn & Hartmann, 1977; Chen & Baker, in press). The hybrid plants obtained grew vigorously, but were highly sterile. Apparently, there were barriers to crossability between these two species.

Chen and Baker (in press) found that foliar applications of E-amino-n-caproic acid significantly increased the number of viable *V. radiata* x *V. umbellata* $F_1$ seeds.

In the reciprocal cross between Phaseolus vulgaris and P. coccineus, Ibrahim and Coyne (1975) used the techniques of partially breaking the pedicels of pods or pod culture in Ziploc plastic bags to obtain several viable $F_1$ seeds.

Mixed species pollen were used to overcome interspecific incompatibility for a number of plant taxa including Populus (Stettler & Guries, 1976), Sesamum (Sastri & Shiranna, 1976) and Ipomea (Guries, 1978). The incompatible or "foreign" pollen is generally used with a killed compatible, or "mentor" pollen which can ultimately effect interspecific fertilization. The use of third species "bridges" to overcome interspecific incompatibility have been successfully used (Dionne, 1963; Hermsen & Bamanna, 1973; Ronald & Ascher, 1976; Sams et al., 1977).

For the interspecific cross, V. radiata x V. umbellata, hybrid sterility was overcome by doubling the chromosomes to induce amphidiploid (Dana, 1967; Ahn & Hartmann, 1977). The fertility of the amphidiploids was increased up to 80% as indicated by pollen stainability. However, the use of V. radiata x V. umbellata or of the amphidiploids in a practical breeding program has not been reported. This paper reports on the use of several techniques to overcome crossability barriers in the interspecific cross of V. radiata x V. umbellata and their possible implications in plant breeding.

## MATERIALS AND METHODS

Plant materials. The cultivars used in the interspecific hybridization were *V. radiata* cv. Tainan #1 and *V. umbellata* cv. HK. Three other species, *V. mungo*, *V. angularis* and *V. radiata* var. *sublobata*, were used as "species bridges". The cultivars used were *V. mungo*, "T-9", *V. angularis*, "Chien Shien" and *V. radiata* var. *sublobata*, "S1" and "S2". All materials were obtained from the Asian Vegetable Research and Development Center (AVRDC). A spontaneous amphidiploid "MR51" (AVRDC, 1976) of *V. radiata* x *V. umbellata* was used to backcross to *V. radiata* to derive triploid progenies.

Cultural conditions. All plants were grown in a glasshouse. During the winter, temperatures were adjusted to 26$^{o}$C (day) and 18$^{o}$C (night) with a photoperiod of 14 hr light supplied with high intensity mercury lamps (10-12 klx). Plants were grown in 20 cm pots using a "Peat Lite Mix". The fertility program was to apply 3 g/pot of 20N-20P-20K biweekly.

Crossing Method. Flowers for hybridization were emasculated the day before anthesis and immediately pollinated. The hybridization technique used in crosses was described by Boling et al. (1961). After hybridization, all subsequent flowers were removed to eliminate competition.

Partial detached and detached pods. The techniques of partial pod attachment and excised pods were described by Ibrahim and Coyne (1975). In this study, pedicels of cross-pollinated pods were partially cut with a sterilized razor 10-12 days after hybridization. For excised pods, pods were removed from the plant at 10-12 days after pollination and surface sterilized with 10% chlorox followed by four rinses in sterilized water and were placed in sterile petri dishes and sealed with "parafilm". The dishes were cultured in the laboratory at $22^{o} \pm 2^{o}C$ supplied with 16 hr artificial light. To determine the best time for detaching the pods, hybridization was made to obtain pods that were 10 to 18 days old. The pods were either partially detached or detached.

Defoliation treatments. All leaves were excised from the plant 8-12 days after cross-pollination. To determine the most effective time for defoliation, hybridizations were made at two-day intervals to provide 6 ages, from 2 to 12 days post-pollination. Four defoliation treatments were compared including a control. They were:
(a) excise one leaflet from every leaf (approx. 33%);
(b) excise two leaflets from every leaf (approx. 67%);
(c) excise all leaves from the plant (100%); and
(d) control.

Pollen mixture. The use of pollen mixture to increase the percent of the hybridization between *V. radiata* and *V. umbellata* was through the use of the third species, *V. angularis*. Pollen from the third species was mixed with pollen of *V. umbellata* for cross-pollination with *V. radiata* and compared to the use of *V. umbellata* pollen alone (control). The mixing was accomplished by successively touching stigmata containing pollen of *V.*, *umbellata* and *V. angularis* to the stigma of *V. radiata*.

*Interspecies grafting*. Grafting between two species was made to increase the number of viable $F_1$ seeds in the cross of *V. radiata* x *V. umbellata*. Two types of grafting were used; viz., side-grafting and approach-grafting. In side-grafting, the scions from *V. radiata* were side-grafted onto the plant of *V. umbellata*. In approach-grafting, plants of *V. radiata* and *V. umbellata* were grown side-by-side and the approach-grafting was made at 3 weeks after planting. Grafted plants were placed in a mist chamber for 2 weeks to facilitate survival of the grafted plants without excising the shoots or roots of either plant.

*Species bridges*. Two $F_1$ hybrids, *V. radiata* x *V. mungo* and *V. umbellata* x *V. angularis*, were used to test the "bridging species", *V. mungo* and *V. angularis*. The hybrid of *V. radiata* x *V. mungo* was hybridized with *V. umbellata* while the hybrid of *V. umbellata* x *V. angularis* was crossed with *V. radiata*. Two accessions of *V. radiata* var. *sublobata* were crossed with *V. radiata*. The resultant hybrid plants were hybridized with *V. umbellata* which used *V. radiata* var. *sublobata* as a bridging species for the interspecific cross of *V. radiata* x *V. umbellata*.

*Amphiploidy*. To induce amphiploids, cuttings from sterile hybrid plants were treated with 0.25% (2.5 mg/ml) colchicine for 8 hr; and then, rooted in a mist chamber. The induced amphiploids were backcrossed to *V. radiata* and the backcross embryos cultured *in vitro* on a Linsmaier and Skoog medium (1965). The successful plants derived from cultured embryos were grown to flowering and backcrossed again to *V. radiata*.

*Experimental design and data analysis*. Randomized complete block and split-plot designs were used. Data were taken on the number of flowers crossed, total number of seeds obtained, number of viable seeds and percentage of pods that produced viable seeds. The authenticity of hybrid plants from viable seeds was confirmed by several genetic markers (Chen & Baker, in press). The analysis of variance was performed and L.S.D. or Duncan's multiple range test used to compare the treatment means.

## RESULTS AND DISCUSSION

Growth of pod, seed and embryo. Pods from the cross of *V. radiata* x *V. umbellata* and self-pollinated pods from *V. radiata* were excised at 4-day intervals and the length of pods, seeds and embryos measured. The growth rate of hybrid pods, seeds and embryos was relatively slow as compared to the selfed (Fig. 1). The growth of hybrid embryos increased from 4 days after pollination to a maximum at 12 days; whereupon, growth ceased.

Partial and detached pods. Partially detached and detached pods contained significantly more viable seeds than the control "attached" pods (Table 1). Partially detached pods was more effective than detached. However, more defined techniques for pod sterilization and cultural conditions were needed to further improve the success of *in vitro* pod culture. For example, the relatively poor results obtained from the detached pods in Experiment 1 were mainly due to incomplete sterilization that permitted the pods to rot before attaining maturity. Furthermore, the high humidity conditions in the petri dishes also caused the seeds to germinate (viviparity) before pod maturity. Thus, it is necessary to carefully control both sterilization procedures and relative humidity conditions to assure pod maturation.

The detaching technique used to overcome embryo abortion was based on the assumption that inhibiting

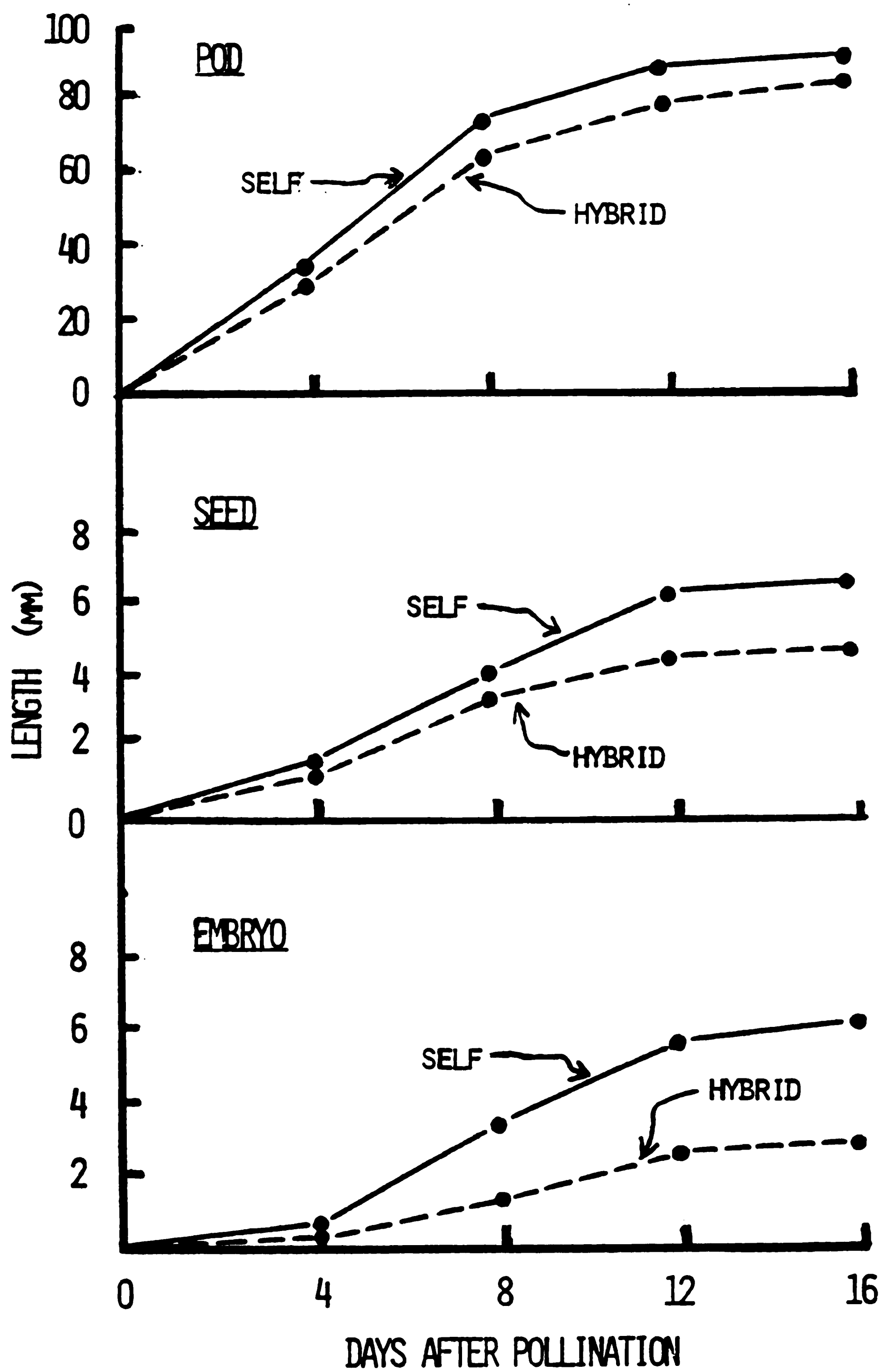



Figure 1. Comparative growth of pod, seed and embryo of natural self of *V. radiata* and the interspecific hybrid, *V. radiata* x *V. umbellata*.

Table 1. Effect of the partial detached and detached pods on the success of the interspecific cross, _V. radiata_ x _V. umbellata_.

| Treatment | Experiment 1[1] | | | Experiment 2[2] | | |
|---|---|---|---|---|---|---|
| | Viable seeds (no.) | Viable seeds per 100 attempts | % pods with viable seeds | Viable seeds (no.) | Viable seeds per 100 attempts | % pods with viable seeds |
| Partial detached pods | 17.3 | 86 | 58.8 | 35.8 | 143 | 55.4 |
| Detached pods | 3.0 | 15 | 13.8 | 16.5 | 66 | 43.0 |
| Control | 2.3 | 11 | 10.0 | 6.3 | 25 | 17.9 |
| L.S.D. 5% | 2.9 | - | 12.9 | 9.1 | - | 18.4 |

[1]Mean of 4 replications; 20 pods per replication.

[2]Mean of 4 replications; 25 pods per replication.

substances are prevented from being translocated to the pods (Ibrahim & Coyne, 1975). Partially detaching the pedicels of pods permitted sufficient water absorption to continue physiological processes, but may have reduced the translocation of inhibitors. In vitro pod culture the inhibiting substances are eliminated and the pods continue necessary physiological and developmental functions under controlled temperature and light conditions. The best time to apply these techniques to reduce $F_1$ embryo abortion was 10 to 12 days after pollination (Table 2). The highest number of viable $F_1$ seeds (12) was obtained from the 10 day-old pods with partial detachment, while the maximum number of viable $F_1$ seeds (7.3) was produced from the 12 day-old pods with in vitro pod culture.

Defoliation treatments. As mentioned above, inhibiting substances may be translocated from the leaves to the pods and cause embryo abortion. Thus, partial or complete defoliation might reduce or eliminate hybrid embryo abortion. Complete removal of leaves from the maternal plants significantly increased the numbers of viable $F_1$ seeds from 5 to 12 times as compared to the controls (Table 3). The defoliation treatments also increased the percentages of pods that produced viable seeds (from 17 to 29%). Generally, the greater defoliation resulted in the highest number of viable seeds (Table 4). Regardless of the time of defoliation, the average

Table 2. Effect of post-pollination time for partial detached and detached pods on the success of the interspecific cross, *V. radiata* x *V. umbellata*.[1]

| | Pod size | | Partial detached pods | | | Detached pods | | |
|---|---|---|---|---|---|---|---|---|
| Days after pollination | Length (mm) | Width (mm) | Viable seeds (no.) | Viable seeds per 100 attempts | % pods with viable seeds | Viable seeds (no.) | Viable seeds per 100 attempts | % pods with viable seeds |
| 10 | 53.9c[2] | 2.8c | 12.0a | 240 | 56a | 2.8bc | 56 | 45abc |
| 12 | 59.4bc | 3.6b | 11.8a | 235 | 67a | 7.3a | 146 | 75a |
| 14 | 64.4ab | 4.6a | 6.0b | 120 | 52a | 4.0ab | 80 | 55ab |
| 16 | 69.1a | 4.8a | 4.5b | 90 | 47a | 1.8bc | 36 | 30bcd |
| 18 | 68.4a | 4.8a | 1.3c | 25 | 25b | 0.8c | 16 | 10d |
| Control | - | - | 1.0c | 20 | 20b | 1.0bc | 20 | 15cd |

[1] Mean of 4 replications; 5 pods per replication.

[2] Mean separation in columns by Duncan's multiple range test, 5% level.

Table 3. Effect of defoliation on the success of the interspecific cross, *V. radiata* x *V. umbellata*.

| Treatment | Viable seeds (no.) | Viable seeds per 100 attempts | % pods with viable seeds |
|---|---|---|---|
| | | Experiment 1[1] | |
| Defoliated | 28.7 | 73.3 | 26.7 |
| Undefoliated | 2.3 | 7.5 | 3.3 |
| L.S.D. 5% | 4.6 | | 4.7 |
| | | Experiment 2[2] | |
| Defoliated | 12.7 | 66.3 | 38.4 |
| Undefoliated | 1.5 | 8.8 | 9.4 |
| L.S.D. 5% | 1.1 | | 12.0 |
| | | Experiment 3[2] | |
| Defoliated | 7.6 | 48.4 | 26.6 |
| Undefoliated | 1.5 | 9.6 | 9.4 |
| L.S.D. 5% | 0.9 | | 8.4 |

[1]Mean of 3 replications.

[2]Mean of 4 replications.

Table 4. Effect of time and degree of defoliation on the success of the interspecific cross, *V. radiata* x *V. umbellata*.[1]

| Time[2] of defolia-tion (days) | Degree of Defoliation | | | | | | | | Overall Mean | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0% | | 33% | | 67% | | 100% | | | |
| | Viable seeds per 100 attempts | % pods with viable seeds | Viable seeds per 100 attempts | % pods with viable seeds | Viable seeds per 100 attempts | % pods with viable seeds | Viable seeds per 100 attempts | % pods with viable seeds | Viable seeds per 100 attempts | % pods with viable seeds |
| 2 | 17a[3] | 17a | 63bc | 25b | 79bc | 50ab | 150bc | 58a | 77bc | 38b |
| 4 | 33a | 25a | 238a | 75a | 300a | 100a | 325a | 71a | 224a | 68a |
| 6 | 13a | 13a | 213ab | 50ab | 63bc | 46ab | 63bc | 50a | 88b | 40b |
| 8 | 13a | 13a | 88abc | 63ab | 175ab | 38b | 113bc | 67a | 97b | 45b |
| 10 | 8a | 8a | 113abc | 42ab | 133ab | 67ab | 200a | 50a | 114b | 42b |
| 12 | 0a | 0a | 25c | 50ab | 17c | 42ab | 17c | 25b | 15c | 29b |
| Overall Mean | 14 | 13 | 123 | 51 | 128 | 57 | 145 | 54 | - | - |

[1]Mean of 4 replications; split plot design.

[2]Days after pollination.

[3]Mean separation in columns by Duncan's multiple range test, 5% level.

numbers of viable seeds per 100 attempts were 123, 128 and 145 for 33, 67 and 100% defoliation, respectively, compared to 14 for the control. Complete defoliation (100%) following pollination caused a large number of pod abscission. The time of defoliation seemed to affect embryo abortion. Defoliation at 4 days after pollination was the most effective for obtaining $F_1$ seed as opposed to leaf removal 12 days after pollination (Table 4).

Adverse effect of defoliation on grain yield has been reported for several legume crops (Stewart et al., 1978; Enyi, 1979). The mechanism by which defoliation facilitated hybrid embryo development in this interspecific cross of *V. radiata* x *V. umbellata* is unknown. Defoliation of the maternal plant may have eliminated or reduced the synthesis of inhibitory substances in the leaves and be transported to the pods and seeds.

*Pollen mixture*. Use of pollen mixture combining a compatible pollen with a foreign species pollen to overcome interspecific incompatibility has been successful in *Populus* (Stettler & Guries, 1976). Application of pollen mixture to the cross of *V. radiata* x *V. umbellata* was made to learn regards its possible effect on embryo abortion.

Pollen of a third species, *V. angularis*, which was partially compatible with *V. radiata* (Ahn & Hartmann, 1978;

Chen & Baker, in press) was mixed with *V. umbellata* and used to pollinate *V. radiata*. The use of pollen mixture increased the number of viable hybrid seeds 3 times that of the control (Table 5). The number of viable seeds and percent pods producing seeds were increased. Possibly, either the pollen of *V. angularis* and/or the hybrid embryos of *V. radiata* x *V. angularis* stimulated the growth and development of the hybrid embryos. The effect of mixed pollen might be similar to that of "double pollination" described by De Vaulx and Pitrat (1977).

*Interspecies grafting*. Grafting between *V. radiata* and *V. umbellata* was successful. The grafted plants grew vigorously and produced seeds in both the scion and stock species. Moreover, a large number of viable hybrid seeds resulted by grafting as compared to control (Table 6). The use of side-grafting in which *V. radiata* was used as the scion on *V. umbellata* was the most effective in overcoming embryo abortion in the interspecific cross of *V. radiata* x *V. umbellata*. The number of viable seeds per 100 attempts was 187 using side-grafting as compared to 7 for the ungrafted control (Table 6). An increase in percentages of both viable seeds and pods that produced viable seeds was realized for the side-grafted plants. Approach-grafting was not as successful as side-graft.

Table 5. Effect of mixed pollen on facilitating interspecific hybridization of *V. radiata* and *V. umbellata*.[1]

| Treatment | Total seeds obtained | Viable seeds No. | Viable seeds % | Viable seeds per 100 attempts | % pods with viable seeds |
|---|---|---|---|---|---|
| Experiment 1 | | | | | |
| Mixed pollen | 232 | 9 | 4.0 | 22.5 | 17.7 |
| Control | 246 | 3 | 1.2 | 7.5 | 7.5 |
| Experiment 2 | | | | | |
| Mixed pollen | 487 | 25 | 5.1 | 31.3 | 18.8 |
| Control | 455 | 7 | 1.5 | 8.8 | 8.8 |

[1]Mixed pollen of *V. umbellata* and *V. angularis* were cross-pollinated onto *V. radiata*. A total of 40 pollination attempts was made in each treatment.

Table 6. Influence of interspecies grafting on overcoming crossability barriers for the interspecific cross of _V. radiata_ x _V. umbellata_.

| Graft[1] treatment | No. flowers crossed | Total seeds obtained | Viable seeds No. | Viable seeds % | Viable seeds per 100 attempts | % pods with viable seed |
|---|---|---|---|---|---|---|
| Side-graft | 38 | 236 | 71 | 30.1 | 186.8 | 76.0 |
| Approach-graft | 45 | 260 | 12 | 4.6 | 26.7 | 15.0 |
| Control | 85 | 586 | 6 | 1.0 | 7.1 | 7.1 |

[1]Side-grafting was made by using _V. radiata_ as the scion on a _V. umbellata_ stock. Plants of the grafted two species were grown without excising respective shoots and roots of the grafts.

Species bridge. Three potential bridging species, *V. mungo*, *V. angularis* and *V. radiata* var. *sublobata*, were tested. Pod-set was observed for the crosses of (*V. radiata* x *V. mungo*) x *V. umbellata* and (*V. umbellata* x *V. angularis*) x *V. radiata*, however, the pods abscised prematurely and no viable seed was produced. One hybrid plant was obtained from the cross, (*V. radiata* x *V. mungo*) x *V. umbellata*. The hybrid plant grew normally but was sterile and succumbed soon after flowering.

A promising potential bridging species was found for this cross. By using *V. radiata* var. *sublobata* as a bridging species, considerably higher numbers of viable seeds were produced from the crosses of (*V. radiata* x *V. radiata* var. *sublobata*) x *V. umbellata* as compared to that of the cross, *V. radiata* x *V. umbellata* (Table 7). The number of viable seeds per 100 attempts were 497 and 296 for where a third species was involved as compared to only 11 for direct hybridization of the two species.

*V. radiata* var. *sublobata* has been considered a wild progenitor of cultivated *V. radiata* and *V. mungo* (Arora et al., 1973). It was originally classified as *Phaseolus sublobatus* Roxb., but it has been separated from *Phaseolus* and given varietal status under *V. radiata* by Verdicourt (1970). The morphological diversity in *V. radiata* var. *sublobata* and its possible value as a source of resistance to yellow mosaic virus have been reported (Arora et al., 1973; Singh & Ahuja, 1977).

Table 7. Effect of *V. radiata* var. *sublobata* as a bridge species to improve the crossability of *V. radiata* and *V. umbellata*.

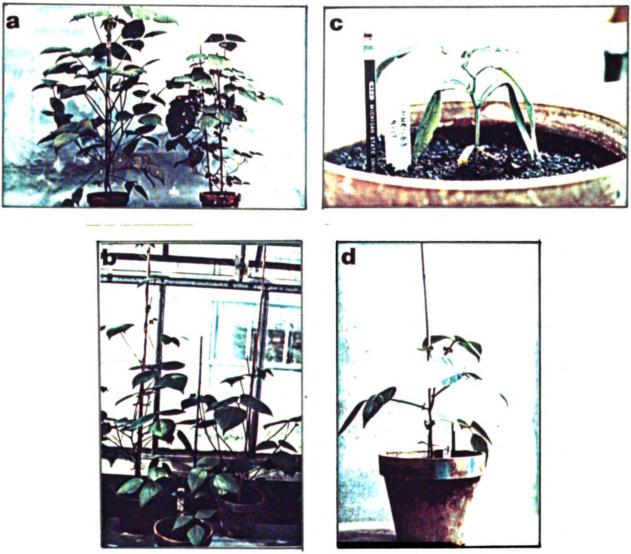
| Cross | No. flowers crossed | Total seeds obtained | Viable seeds No. | Viable seeds % | Viable seeds per 100 attempts | % pods with viable seed |
|---|---|---|---|---|---|---|
| (*radiata* x *sublobata* cv. S1) x *umbellata* | 36 | 278 | 179 | 88.9 | 497 | 64.4 |
| (*radiata* x *sublobata* cv. S2) x *umbellata* | 28 | 116 | 83 | 89.3 | 296 | 71.6 |
| *radiata* x *umbellata* | 64 | 390 | 7 | 1.8 | 11 | 9.4 |

The use of *V. radiata* var. *sublobata* as a bridging species provides another means to circumvent barriers to crossability in the interspecific hybridization of *Vigna*.

*Amphiploidy*. To circumvent hybrid sterility, the ploidy of sterile hybrid plants can be doubled with colchicine to produce amphiploids (Dana, 1967; Ahn & Hartmann, 1977). Amphiploids were induced by treating cuttings of the sterile hybrid with 0.25% colchicine (Fig. 2). The amphidiploid plants were partially fertile with pollen stainability to 81%. However, the vigorous vegetative growth, photoperiod sensitive, and poor pod-set suggest that it will not succeed as a new crop without improvement. So, one method to transferring the desirable genes is by backcrossing the amphidiploid to *V. radiata*.

A spontaneous amphidiploid (AVRDC, 1976) was used to backcross with *V. radiata*. Five triploid plants were obtained with the aid of embryo culture (Fig. 2). The triploid plants grew vigorously and flowered profusely with infrequent pod-set. Cytogenetic studies (Machado, 1978) showed meiosis in the triploid plants (amphidiploid x *V. radiata*) was irregular with 11 bivalents and 11 univalents at metaphase I. The second division was also abnormal with multipolar division and non-congregating and lagging chromosomes. A number of progenies from the triploids were produced by natural self-pollination and by backcrossing to *V. radiata* (Fig. 2). Variation in

Figure 2. Plants of interspecific hybrid *V. radiata* x *V. umbellata*, amphidiploid, triploid and progenies of the triploid. 2-a: Hybrid plants (2X, right) and amphidiploid (4X). 2-b: Triploid plants derived from embryo culture (amphidiploid x *V. radiata*). 2-c: Plant of triploid x *V. radiata*, showing abnormal monoleaflet of leaves. 2-d: Progeny of the triploid derived from natural self-pollination.


a
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d

morphological characteristics and levels of fertility were found among these progenies. Mitotic studies revealed that 2n=22 and 2n+1=23 somatic chromosome numbers occurred in these triploid progenies (Machado, 1978). Improvement and selection of economic traits could be accomplished by backcrossing to *V. radiata* to recover desirable traits.

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