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# Attempts at Interspecific Hybridization Between *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray Using Embryo Rescue

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*With one figure and 3 tables*

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## Abstract

Attempts at interspecific hybridization were made between *Phaseolus vulgaris* (common bean) and *P. acutifolius* (tepary bean) using a new pollination method and embryo rescue. The success of interspecific hybridization depended upon the pollination technique, the species and individual genotypes used as female and male parents, and the growth conditions. A high hybridization efficiency was achieved when *P. vulgaris* was used as female parent. When pods were left on the maternal plants hybrid seeds were obtained which developed into abnormal seedlings. These died at an early stage of growth. Hybrid plants were grown to maturity following embryo rescue, but all showed different developmental abnormalities. The growth of these was determinate like the female parent, *P. vulgaris*, but leaf morphology was closer to the male parent, *P. acutifolius*. The utilization of *P. acutifolius* germplasm for the improvement of common bean remains limited, and further studies are needed to develop promising embryo rescue protocols in such wide crossing programmes.

**Key words:** *Phaseolus vulgaris* — *Phaseolus acutifolius* — common bean — tepary bean — interspecific hybridization — embryo rescue — *in vitro* embryo culture

Successful hybridization techniques which manipulate reproductive systems are important for the utilization of the genetic diversity of *Phaseolus* gene pools. In the past many attempts have been made to incorporate the four most important *Phaseolus* bean species, namely *P. vulgaris* (common bean), *P. coccineus* (runner bean), *P. acutifolius* (tepary bean), and *P. lunatus* (lima bean) into a common gene pool using conventional techniques (HONMA 1956, HONMA and HEECKT 1958, 1959, KEDAR and BEMIS 1960, COYNE 1964, THOMAS 1964, AL-YASIRI and COYNE 1966, SMARTT 1970, HAQ and SMARTT 1978). It has been suggested that this would enable plant breeders to improve these crops for high yield alongside pest and disease resistance and drought and/or heat resistance amongst other characteristics. However, distinct barriers to intra- and interspecific hybridization exist between these *Phaseolus* species (THOMAS 1964, AL-YASIRI and COYNE 1966, SMARTT and HAQ 1972, MOK et al. 1978, SMARTT 1979, HUCL and SCOLES 1985).

The grouping of these four most important *Phaseolus* bean species according to the gene pool concept of HARLAN and DE WET (1971) is convenient for the study and understanding of barriers to hybridization between these species. SMARTT (1981, 1984, 1985 and 1986) has defined the primary, secondary and tertiary gene pools for *P. vulgaris*. The primary gene pool consists of domesticated (GP1A) and wild populations (GP1B); the secondary gene pool of *P. vulgaris* consists mainly of *P. coccineus* (GP2) and the tertiary gene pool consists of *P. acutifolius* (GP3A) and *P. lunatus* (GP3B).

The success to intra- and interspecific hybridization depends on the relationship between several factors such as species and/or genotypes used as male and female parents in

the reciprocal crosses, environmental conditions and pollination techniques. Reciprocal differences have been found in successful interspecific crosses, *P. vulgaris* being in general more successful when used as female parent (HONMA 1956, AL-YASIRI and COYNE 1966, SMARTT 1970, MOK et al. 1978). Nevertheless, THOMAS and WAINES (1984) reported no reciprocal differences between *P. vulgaris* and *P. acutifolius*.

Apparently, no pre-fertilization barriers seem to exist for the interspecific reciprocal crosses between *P. vulgaris* and *P. acutifolius* (MOK et al. 1978, RABAKOARIHANTA et al. 1979). However, post-fertilization barriers to embryo development have been suggested to be the major difficulty in obtaining interspecific hybrids between these two species where normal fertilization can be achieved. Embryos commonly abort between three and 24 days after pollination (HONMA 1956, THOMAS 1964, SMARTT 1970, MOK et al. 1978, PRENDOTA et al. 1982, THOMAS and WAINES 1984). A delay in endosperm and embryo divisions (RABAKOARIHANTA et al. 1979) and further failure of endosperm development appear to be the cause of embryo abortion and subsequent collapse of the pods (AL-YASIRI and COYNE 1964, 1966). Pods appeared initially to develop normally but 16 days after pollination they began to collapse and by 22 days all pods had collapsed.

Embryo rescue and culture techniques are a promising field of research to overcome post-fertilization barriers for the successful growth of interspecific progeny between *Phaseolus* bean species. These would enable plant breeders to transfer useful desirable traits from one species into another (PRATT 1983). The first reported interspecific hybrids between *P. vulgaris* and *P. acutifolius* were obtained using artificial culture medium (HONMA 1955, 1956). From several hundred embryos 14 to 20 days after pollination only four putative hybrid plants were grown to maturity. The morphology of these plants was similar to that of the female parent, *P. vulgaris*, leading SMARTT (1970, 1980) to question their hybrid status, based on results of crosses which he carried out between these species, as well as the high fertility of the plants reported by HONMA. No success was obtained in the reciprocal crosses using *P. acutifolius* as female.

Embryo rescue has recently been used to obtain some hybrids between these *Phaseolus* species, but they were self-sterile or only partially fertile (PRENDOTA et al. 1982, THOMAS and WAINES 1984, PRATT et al. 1985, PARKER and MICHAELS 1986). Some level of fertility has been gradually recovered only by backcrossing to either parent, but mainly to *P. vulgaris*.

In this paper the results of attempts are reported to produce hybrids between *P. vulgaris* and *P. acutifolius* using a new pollination method and embryo rescue.

## Materials and Methods

**Genetic material:** Sixteen accessions of *P. vulgaris* and 20 of *P. acutifolius* from Centro Internacional de Agricultura Tropical (CIAT) were used in this study (Table 1). Plants were grown under varying conditions of temperature and daylength in an attempt to obtain synchronization in flowering between the two species.

**Hybridization techniques:** Initially several hybridization techniques were tried, but with little success. A new method, namely "The Insertion Method" (ANDRADE-AGUILAR and JACKSON 1988) was developed and used with success for most crosses. Essentially this pollination method consists in placing the keel containing the style-stigma and stamens from the male parent over the style-stigma of the female parent after emasculation. The advantage with this method of pollination is that it keeps the anthers and the pollen bearing style-stigma of the male flower in close contact with the stigma of the female flower over a period long enough to ensure fertilization, and reduces the problem of desiccation of the female and male reproductive organs. Hybridization efficiency was scored as the number of successful pollinations/total number of pollinations × 100. A successful pollination was considered as one in which pod growth was normal on the maternal plant just before removal for embryo rescue (2—3 weeks after pollination), or left on the maternal plant for further development.

**Embryo culture:** Two artificial culture media were used, namely B5 (GAMBORG et al. 1968) and MS (MURASHIGE and SKOOG 1962). Both media were supplemented with sucrose 30 g $l^{-1}$ (3 %) and agar 7 g $l^{-1}$ (0.7 %).

Pods of different ages and/or sizes from apparent interspecific crosses were removed from the maternal plants and placed in water. The pods were surface sterilized in 70 % ethanol for 3—5 min, immersed in 10 % sodium hypochlorite (plus one or two drops of

Tween) for 10—15 min and rinsed three times in sterile distilled water. The pods were opened carefully to expose the ovules. Excised embryos were placed either onto B5 or MS medium, or both when enough embryos could be rescued from the same cross. They were cultured in the dark at 25 °C for the first 10 days to induce root formation, after which they were given a 16/8 h day/night photoperiod at 25 °C.

Once roots had developed and the primary unifoliate leaves had turned green, plantlets were transferred individually into 30 ml culture vials containing 10 ml of the corresponding medium and maintained under the same photoperiod and temperature conditions as above. Subcultures were carried out every 2—3 weeks. In the last subculture the roots of some of the plantlets were treated with rooting powder and then placed onto MS medium. Prolific root growth was observed in these plantlets but shoot development was rather poor.

Plantlets of different phenological stages (one or more trifoliate leaves) with a good root system were transplanted into 9 cm plastic pots containing a mixture of peat : vermiculite (1 : 1). The plantlets were covered with transparent plastic hoods and placed in a glasshouse with a minimum controlled temperature of 16 °C. After further growth, the surviving plantlets were transferred into 13 cm pots containing a mixture of peat : loam : sand : grit (3 : 1 : 1 : 1) in the glasshouse where the temperature varied from 16 to 29 °C. The daylength was extended when necessary with 6 h artificial light using mercury vapour lamps MB/U 400W.

## Results

### Hybridization success

All *P. vulgaris* genotypes flowered but only six *P. acutifolius* genotypes did so, although two sowing dates were used in an attempt to obtain synchronous flowering (Table 1). As a consequence *P. acutifolius* was mainly used as male parent in crosses with *P. vulgaris*, but some reciprocal crosses were attempted.

In general, *P. vulgaris* performed better as female (67 % efficiency) than as male parent (28 %) while the opposite was found for

Table 1. Germplasm of *Phaseolus vulgaris* and *P. acutifolius* used in this study, obtained from CIAT (G numbers), and days to flowering

| Species | Days to flower from 10/04/87 | 18/06/87 | Species | Days to flower from 10/04/87 |
|---|---|---|---|---|
| *P. vulgaris* | | | *P. acutifolius* | |
| G-0197 | 41 | 35 | G-40011 | — |
| G-1373 | 45 | 36 | G-40033 | — |
| G-1594 | 43 | 36 | G-40040 | 65 |
| G-4906 | 58 | — | G-40066A | 61 |
| G-4979 | 75 | — | G-40084 | — |
| G-5169 | 69 | — | G-40091 | — |
| G-5307 | 42 | 35 | G-40092 | — |
| G-5343 | 49 | 52 | G-40093 | — |
| G-5677 | 58 | 41 | G-40094 | — |
| G-5974 | 49 | 41 | G-40097 | — |
| G-7339 | 65 | 41 | G-40098 | — |
| G-7418 | 64 | 51 | G-40099 | — |
| G-7537 | 46 | 37 | G-40100 | — |
| G-7624 | 42 | 38 | G-40101 | — |
| G-7715 | 43 | 39 | G-40103 | — |
| G-8115 | 46 | 50 | G-40110 | 65 |
| | | | G-40112 | — |
| | | | G-40120 | 61 |
| | | | G-40122 | 64 |
| | | | G-40123 | 57 |

Table 2. Efficiency of *Phaseolus vulgaris* and *P. acutifolius* as female and male parents in interspecific reciprocal crosses

| Acc. No. | As female | | As male | |
|---|---|---|---|---|
| | Pollinations | Efficiency %* | Pollinations | Efficiency % |
| *P. vulgaris* | | | | |
| G-0197 | 36 | 89 | 6 | 33 |
| G-1373 | 10 | 80 | 10 | 0 |
| G-1594 | 24 | 58 | 15 | 40 |
| G-4906 | 4 | 25 | 19 | 5 |
| G-4979 | 2 | 100 | 15 | 47 |
| G-5169 | 19 | 79 | 21 | 14 |
| G-5307 | 33 | 58 | 6 | 33 |
| G-5343 | 8 | 100 | 4 | 0 |
| G-5677 | 10 | 50 | — | — |
| G-5974 | 15 | 80 | 14 | 36 |
| G-7339 | 11 | 27 | 10 | 10 |
| G-7418 | 8 | 38 | 12 | 25 |
| G-7537 | 22 | 59 | 5 | 40 |
| G-7624 | 30 | 100 | 11 | 64 |
| G-7715 | 3 | 0 | — | — |
| G-8115 | 2 | 100 | — | — |
| 'Red Kidney' | 18 | 28 | 10 | 50 |
| **Total or Mean** | 255 | 67 | 158 | 28 |
| *P. acutifolius* | | | | |
| G-40040 | 24 | 13 | 46 | 63 |
| G-40066A | 8 | 13 | 20 | 75 |
| G-40110 | 8 | 50 | 5 | 20 |
| G-40120 | 64 | 42 | 113 | 62 |
| G-40122 | 23 | 9 | 30 | 77 |
| G-40123 | 31 | 23 | 51 | 80 |
| **Total or Mean** | 158 | 28 | 265 | 68 |

* Efficiency is the ratio of successful pollinations to total pollinations made. A successful pollination was considered as one in which pod growth was normal on the maternal plant just before removal for embryo rescue (2—3 weeks after pollination), or left on the plant for further development

*P. acutifolius* (Table 2). Some accessions of *P. vulgaris* showed 100 % efficiency when pollinated with any of the *P. acutifolius* accessions, particularly G-5343 and G-7624. It was observed that all but four accessions and 'Red Kidney' of *P. vulgaris* achieved at least 50 % efficiency as female parents. Alternatively, all the accessions, except G-7624 and 'Red Kidney', failed to achieve at least 50 % efficiency when used as male parents.

An interesting result is that *P. vulgaris* accession G-7624 showed 100 % efficiency when used as female parent with any of the *P. acutifolius* accessions. In addition, it was the best male parent (64 % efficiency). Although most pods seemed to grow normally in size and shape they had few or no embryos which could be rescued. Accession G-40120 of *P. acutifolius* can be considered as the best female (42 % efficiency) even though G-40110 showed 50 % efficiency. As this latter result was based on only eight pollinations, it cannot be regarded as definitive. However, G-40120 was surpassed by G-40123, G-40122 and G-40066A when used as male parent (Table 2). Nevertheless, it was the most prolific flower-

Table 3. The mean age and size of pods, and the number and mean size of embryos rescued following crosses between *P. vulgaris* × *P. acutifolius* and reciprocal. Sizes of pods and embryos based on dimensions of length and breadth

| Acc. No. | Pods | | | Embryos | | | |
|---|---|---|---|---|---|---|---|
| | Mean age (days) | Size (mm) L | B | Number cultured | Size (mm) L | B | Culture medium |
| *P. vulgaris* | | | | | | | |
| G-0197 | 20 | 82 | 12 | 12 | 1.5 | 0.9 | MS, B5 |
| G-1594 | 23 | 93 | 9 | 12 | 5.8 | 2.7 | MS, B5 |
| G-4906 | 21 | 85 | 10 | 2 | 1.6 | 0.9 | MS |
| G-4979 | 18 | 105 | 11 | 4 | 1.6 | 1.0 | MS |
| G-5169 | 22 | 102 | 11 | 20 | 2.6 | 1.7 | MS |
| G-5307 | 22 | 104 | 9 | 26 | 3.6 | 2.0 | MS, B5 |
| G-5343 | 22 | 135 | 14 | 12 | 3.9 | 2.4 | MS |
| G-5974 | 22 | 117 | 11 | 14 | 2.4 | 1.5 | MS |
| G-7339 | 21 | 82 | 7 | 3 | 2.6 | 1.6 | MS |
| G-7418 | 22 | 110 | 11 | 3 | 3.9 | 2.3 | MS |
| G-7537 | 22 | 112 | 10 | 13 | 2.7 | 1.6 | MS, B5 |
| G-7624 | 20 | 96 | 8 | 19 | 5.5 | 2.8 | MS |
| 'Red Kidney' | 21 | 107 | 11 | 8 | 1.6 | 0.9 | MS |
| *P. acutifolius* | | | | | | | |
| G-40040 | 20 | 51 | 11 | 4 | 0.8 | 0.6 | MS |
| G-40066A | 20 | 55 | 9 | 1 | 1.5 | 1.0 | MS |
| G-40110 | 18 | 47 | 8 | 2 | 0.5 | 0.5 | MS |
| G-40120 | 20 | 68 | 9 | 22 | 2.1 | 1.3 | MS, B5 |
| G-40123 | 21 | 52 | 10 | 4 | 0.9 | 0.8 | MS |

ing, and consequently was the most widely used either as female or male parent. These results indicate that it is potentially useful germplasm for interspecific hybridization programmes.

## Interspecific hybrid seed

From the 122 pods left on the maternal plants only 12 produced seed; the others were empty. Five of these pods produced 13 seeds on *P. vulgaris* as female parent and seven pods produced also 13 seeds on *P. acutifolius* as female parent. Most seeds were abnormal, and they were usually small, shrunken, shrivelled and had a broken and incomplete testa. These seeds were sown but only five seedlings emerged. The seedlings showed developmental abnormalities such as absence of one or both of the primary leaves, variegated leaves and slow growth. Four of these plants died at an early vegetative stage of development, although attempts were made to propagate them by cuttings. The only plant which grew to maturity resembled the maternal parent in its vegetative and floral morphology. Furthermore, pollen stainability was rather high (79.5 %) and it produced seed which resembled that of the female parent in size, shape and colour. These findings strongly suggest that it was derived from a self pollination.

## Embryo rescue

During summer 1987, 89 pods which developed after interspecific crosses, were removed from the maternal plants from which 148 embryos of different sizes were rescued (Table 3). In some developing ovules and apparent hybrid embryos signs of abortion were clearly noted. Ovules were shrunken and cessation of growth was evident. Cessation of growth of the embryos was also evident and an uneven development of the cotyledons was apparent. One cotyledon stopped its development while the other continued for further

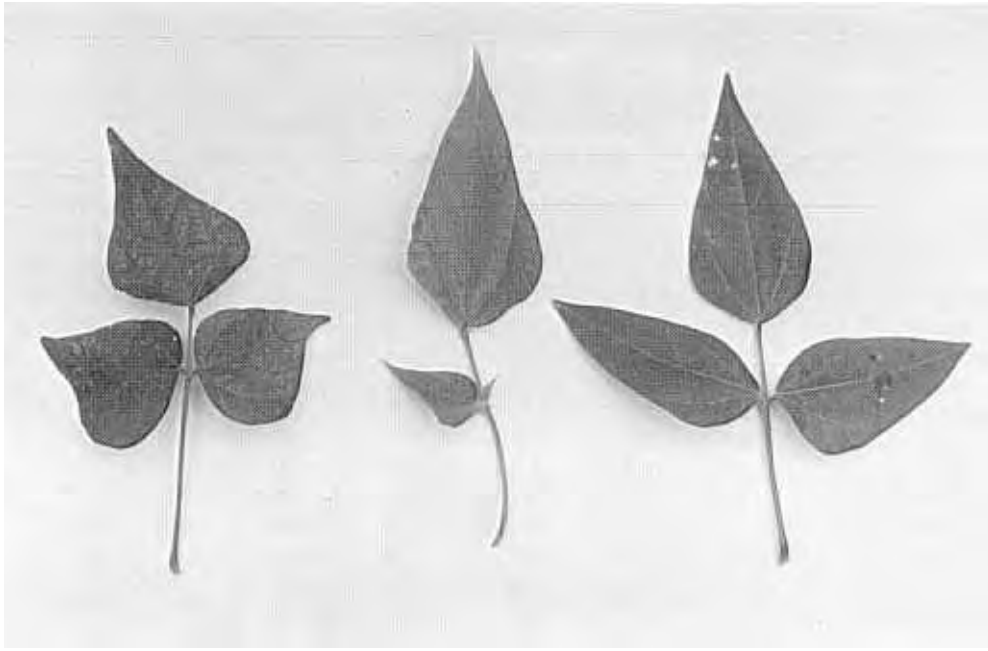

Fig. 1. Leaf morphology of *Phaseolus vulgaris* (left) and *P. acutifolius* (male, right), and the hybrid between them (middle)

growth. Seventy-three embryos died at different stages of development and the rest did not survive in the petri dish to be transferred into individual tubes. Only 63 embryos were transplanted to soil in the glasshouse which developed into plantlets. From these, only two survived to maturity, and they showed different developmental abnormalities, such as variegated and crinkled leaves, and uneven development of the three leaflets. Some leaves had the terminal leaflet longer or shorter than the lateral leaflets whereas the converse was true for others. The growth habit was determinate as in the female parent, *P. vulgaris*, but leaf morphology was similar to that of *P. acutifolius*, clearly suggesting their hybrid status (Fig. 1). Flower development was limited, even after 120 days. Small flower buds often dropped off the plants before pollen fertility could be ascertained or floral morphology determined.

## Discussion

The success of interspecific hybridization between *P. vulgaris* and *P. acutifolius* appeared to be highly influenced by the pollination technique and by the genotype of each species used as female and male parents. It was observed that during the emasculation and pollination of *P. acutifolius* some damage was caused, because flowers are smaller and more delicate and difficult to manage than those of *P. vulgaris*. BUISHAND (1956) obtained 30 and 70 % success in crossing using the rubbing and hooking methods, respectively. In the breeding programme at CIAT 50 % efficiency has been reported using a combination of the rubbing and hooking methods under controlled conditions (18 to 27 °C) (CIAT 1982). In this work, the "Insertion Method" gave an average of 68 % efficiency when using *P. vulgaris* as the female parent. Two other factors affecting hybridization success were: 1. the conditions of growth, and 2. the physiological, phenological and morphological stage of the plants used as female parents. The successful crosses under constant conditions of 25 °C produced pods which were apparently normal and which grew faster compared with those produced under other conditions, but which were nevertheless empty when left on the maternal plant. Also, many pollinations were performed onto both

species as female parents using old plants since synchronization in flowering between accessions of both species was difficult. It is known that considerable flower abscission occurs towards the end of the flowering period as a natural phenomenon (CIAT 1982). Also the growth habit seemed to affect this fact, since it is correlated to the flowering period as well as to the branching pattern. Pollinated flowers on the old lateral branches of *P. acutifolius* generally failed to develop into pods with rescuable embryos.

No true interspecific hybrid plants could be raised from the F1 seeds. From the 26 seeds produced (13 seeds on each species) only five germinated, and the single plant which survived appeared to be a selfed plant as indicated by its morphological characteristics and pollen stainability, even though it showed abnormal development. Furthermore, it set selfed seed which resembled the female parent. One exception is that reported by SMARTT (1970) who obtained hybrid seeds which produced three viable plants from the cross *P. vulgaris* (female) × *P. acutifolius* (male) and six from the reciprocal cross using one particular genotype of each species. However, no further progeny were obtained from these plants. A second exception is that reported by PRENDOTA et al. (1982). From over 3500 pollinations, they obtained 13 seeds from the cross *P. vulgaris* (female) × *P. acutifolius* (male) but only three plants grew to flower, and from almost 1400 pollinations, 23 seeds were obtained from the reciprocal cross of which 21 plants were raised to flower. However they do not report further progeny. They concluded that *P. acutifolius* was more successful as a female parent in producing the hybrid seeds.

Failure of endosperm development appears to be the cause of embryo abortion and subsequent collapse of the pods (AL-YASIRI and COYNE 1964, 1966). MOK et al. (1978) found that a distinct characteristic of the hybrid embryos was the uneven development of the two cotyledons. The rate of growth and final size of these hybrid embryos seemed to be influenced by the genotypes of both parents. This fact may be due to the gradual failure of endosperm development as suggested by RABAKOARIHANTA et al. (1979). Moreover, in the present work, a relationship was observed between the uneven development of the cotyledons and the uneven development of the primary unifoliate leaves. This relationship may be a useful marker to distinguish and verify hybrid embryos at an early stage of development, but it was noted only in particular crosses and not as a general pattern of hybrid embryos.

In many respects the present work has confirmed the findings of earlier workers. It is clear that hybridization between *P. vulgaris* and *P. acutifolius* remains problematical, even with the help of embryo rescue. In terms of germplasm utilization, the level of success is extremely low, and will continue to present problems for the plant breeder wishing to utilize *P. acutifolius* for its drought tolerant properties, and pest and disease resistance, unless protocols can be developed which promise a high level of success.

## Zusammenfassung

**Herstellung von Artbastarden zwischen *Phaseolus vulgaris* L. und *P. acutifolius* A. Gray mittels Embryokultur**

Mit Hilfe einer neuen Bestäubungstechnik und anschließender Embryokultur wurde versucht, *Phaseolus vulgaris* (gewöhnliche Gartenbohne) und *P. acutifolius* (Texas-Bohne) miteinander zu kreuzen. Der Bastardierungserfolg war von der Bestäubungstechnik, von den als Mutter oder Vater verwendeten Arten und Genotypen sowie von den Wachstumsbedingungen abhängig. Die Kreuzungen gelangen am besten, wenn *P. vulgaris* als Mutter verwendet wurde. Beließ man die Hülsen an den Mutterpflanzen, erhielt man Bastardsamen, aus denen sich anomale Keimpflanzen entwickelten, die bereits im frühen Stadium ihrer Entwicklung abstarben. Über Embryokultur hingegen gelang es, Pflanzen bis zur Reife heranzuziehen, die allerdings auch verschiedenartige Anomalien aufwiesen. Das Wachstum dieser Pflanzen war, wie bei dem mütterlichen Elter *P. vulgaris*, terminiert; aber die Morphologie der Blätter war der des Vaters, *P. acutifolius*, sehr ähnlich. Eine Nutzung von Genen aus *P. acutifolius* zur Verbesserung der Gartenbohne bleibt begrenzt. Es sind daher weitere Untersuchungen nötig, geeignete Techniken der Embryokultur zu entwickeln, um diese in solch weiten Kreuzungen einsetzen zu können.

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## References


AL-YASIRI, S. A., and D. P. COYNE, 1964: Effect of growth regulators in delaying pod abscission and embryo abortion in the interspecific cross *Phaseolus vulgaris* × *P. acutifolius*. Crop Sci. **4**, 433—435.

— —, and — —, 1966: Interspecific hybridization in the genus *Phaseolus*. Crop Sci. **6**, 59—60.

ANDRADE-AGUILAR, J. A., and M. T. JACKSON, 1988: The insertion method: a new and efficient technique for intra- and interspecific hybridization in *Phaseolus* beans. Bean Improv. Coop. Ann. Rept. (Submitted for publication).

BUISHAND, T. J., 1956: The crossing of beans (*Phaseolus* spp.). Euphytica **5**, 41—50.

CIAT, 1982: Cruzamiento del frijol. Study guide. Centro Internacional de Agricultura Tropical. Cali, Colombia.

COYNE, D. P., 1964: Species hybridization in *Phaseolus*. J. Hered. **55**, 5—6.

GAMBORG, O. L., R. A. MILLER, and K. OJIMA, 1968: Nutrient requirements of suspension cultures of soybean root cells. Expl. Cell Res. **50**, 151—158.

HAQ, N., and J. SMARTT, 1978: Interspecific hybridization in *Phaseolus* and the production of inbred lines. Bean Improv. Coop. Ann. Rept. **21**, 62—63.

HARLAN, J. R., and J. M. J. DE WET, 1971: Toward a rational classification of cultivated plants. Taxon **20**, 509—517.

HONMA, S., 1955: A technique for artificial culturing of bean embryos. Proc. Amer. Soc. Hort. Sci. **65**, 405—408.

— —, 1956: A bean interspecific hybrid. J. Hered. **47**, 217—220.

— —, and O. HEECKT, 1958: Bean interspecific hybrid involving *Phaseolus coccineus* × *P. lunatus*. Proc. Amer. Soc. Hort. Sci. **72**, 360—364.

— —, and — —, 1959: Interspecific hybrid between *Phaseolus vulgaris* and *P. lunatus*. J. Hered. **50**, 233—237.

HUCL, P., and G. J. SCOLES, 1985: Interspecific hybridization in the common bean: a review. Hort. Science **20**, 352—357.

KEDAR, N., and W. P. BEMIS, 1960: Hybridization between two species of *Phaseolus* separated by physiological and morphological blocks. Proc. Amer. Soc. Hort. Sci. **76**, 397—402.

MOK, D. W. S., M. C. MOK, and A. RABAKOARIHANTA, 1978: Interspecific hybridization of *Phaseolus vulgaris* with *P. lunatus* and *P. acutifolius*. Theor. Appl. Genet. **52**, 209—215.

MURASHIGE, T., and F. SKOOG, 1962: A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. **15**, 473—497.

PARKER, J. P., and T. E. MICHAELS, 1986: Simple genetic control of hybrid plant development in interspecific crosses between *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray. Plant Breeding **97**, 315—323.

PRATT, R. C., 1983: Gene transfer between tepary and common beans. Desert Plants **5**, 57—63.

— —, R. A. BRESSAN, and P. M. HASEGAWA, 1985: Genotypic diversity enhances recovery of hybrids and fertile backcrosses of *Phaseolus vulgaris* L. × *P. acutifolius* A. Gray. Euphytica **34**, 329—344.

PRENDOTA, K., J. P. BAUDOIN, and R. MARECHAL, 1982: Fertile allopolyploids from the cross *Phaseolus acutifolius* × *Phaseolus vulgaris*. Bull. Rech. Agron. Gembloux **17**, 177—190.

RABAKOARIHANTA, A., D. W. S. MOK, and M. C. MOK, 1979: Fertilization and early embryo development in reciprocal interspecific crosses of *Phaseolus*. Theor. Appl. Genet. **54**, 55—59.

SMARTT, J., 1970: Interspecific hybridization between cultivated American species of the genus *Phaseolus*. Euphytica **19**, 480—489.

— —, 1979: Interspecific hybridization in the grain legumes — a review. Econ. Bot. **33**, 329—337.

— —, 1980: Evolution and evolutionary problems in food legumes. Econ. Bot. **34**, 219—235.

— —, 1981: Gene pools in *Phaseolus* and *Vigna* cultigens. Euphytica **30**, 445—449.

— —, 1984: Gene pools in grain legumes. Econ. Bot. **38**, 24—35.

— —, 1985: Evolution of grain legumes. IV. Pulses in the genus *Phaseolus*. Expl. Agric. **21**, 193—207.

— —, 1986: Evolution of grain legumes. VI. The future — the exploitation of evolutionary knowledge. Expl. Agri. **22**, 39—58.

— —, and N. HAQ, 1972: Fertility and segregation of the amphidiploid *Phaseolus vulgaris* L. × *P. coccineus* L. and its behaviour in backcrosses. Euphytica **21**, 496—501.

THOMAS, H., 1964: Investigations into the interrelationships of *Phaseolus vulgaris* L. and *P. coccineus* Lam. Genetica **35**, 59—74.

THOMAS, C. V., and J. G. WAINES, 1984: Fertile backcross and allotetraploid plants from crosses between tepary beans and common beans. J. Hered. **75**, 93—98.


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