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Interspecific hybridization between common and tepary beans: increased hybrid embryo growth, fertility, and efficiency of hybridization through recurrent and congruity backcrossing

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Abstract Cultivated common bean (*Phaseolus vulgaris* L.) and tepary bean (*Phaseolus acutifolius* A. Gray) genotypes possessing desirable agronomic traits were hybridized. The F_1 hybrids were backcrossed twice with the common bean (i.e., recurrent backcrossing). Also, alternate backcrosses with common and tepary beans (i.e., congruity backcrossing) were carried out. Embryo culture was necessary for all initial interspecific crosses, and its requirement was proportionally lower when the common bean was used as the recurrent parent and as the last parent of congruity backcrosses. Modification of the embryo culture technique was necessary to produce congruity hybrids. Effects of both tepary and common bean genotypes on the success rate of hybridization were observed. Tepary accession G 40001 and common bean cultivar ICA Pijao facilitated interspecies hybridization. Growth of hybrid embryos before rescue, recovery of mature hybrid plants, and the vigor and fertility of F_1 hybrids all increased with increased recurrent and congruity backcrosses and intermatings between male-sterile F_1 and selected fertile F_2 plants of the third and fifth congruity backcrosses. Introgression of tepary genes was verified by means of seed protein electrophoretic analysis and morphological markers. The results suggest that congruity backcrossing can help to gradually reduce or overcome *P. vulgaris* × *P. acutifolius* hybridization barriers such as genotype incompatibility, early embryo abortion, hybrid sterility, and lower frequencies of hybridization.

Key words *Phaseolus acutifolius* · *Phaseolus vulgaris* · Common and tepary beans · Interspecific hybridization · Congruity and recurrent backcrosses · In-vitro embryo culture · Gene introgression

Introduction

Of five domesticated *Phaseolus* species, namely, *P. vulgaris* L., *P. acutifolius* A. Gray, *P. coccineus* L., *P. polyanthus* Greenman, and *P. lunatus* L., the common bean (*P. vulgaris* L.) is economically the most important in the world, being involved in more than 90% of all *Phaseolus* bean production (Singh 1992). Certain agronomic traits, such as high levels of resistance to drought, heat, low soil fertility, leafhoppers (*Empoasca kraemeri* Ross and Moore), common bacterial blight caused by *Xanthomonas campestris* pv *phaseoli* (Smith) Dye, been golden mosaic virus (BGMV), and root rots, have not yet been found in cultivated, wild, and weedy forms of the common bean, in spite of the large collections (> 25 000 accessions) available in gene banks around the world. However, one or more of these desirable traits are adequately expressed in other *Phaseolus* species. For example, the tepary bean (*P. acutifolius*) is known to possess relatively high levels of resistance to most of the abiotic and biotic stresses mentioned earlier (Thomas et al. 1983; Parsons and Howe 1984; Zaiter et al. 1989). Because of this, repeated efforts have been made to hybridize the common bean with the tepary (Honma 1956; Smartt 1970; Mok et al. 1978; Alvarez et al. 1981; Prendota et al. 1982; Thomas and Waines 1984). But successful gene transfer for most of the desirable traits, except for resistance to common bacterial blight (Coyne 1964; McElroy 1985; Parker and Michaels 1986), from the tepary to true breeding lines of the common bean has seldom been realized (Pratt 1983). The causes for limited success include: (1) the use of relatively few genotypes from either species for hybridization, (2) the occurrence of F_1 hybrid lethality associated

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with certain genotypes of either species, (3) the use of few flower buds for pollination, (4) the occurrence of post-zygotic embryo abortion, (5) F_1 hybrid sterility, (6) rapid reversion of hybrid progenies to parental types, (7) the limited number of hybrids produced for agronomic evaluation, and (8) inadequate evaluation of interspecific hybrid progenies.

The sterility of interspecies F_1 hybrids could be overcome partially by recurrent backcrossing to the common bean (Thomas and Waines 1984; Pratt et al. 1985; Parker and Michaels 1986; Haghighi and Ascher 1988). But this reduces the possibility of retaining desirable genes from the tepary (Haghighi and Ascher 1988; Waines et al. 1988). Consequently, Haghighi and Ascher (1988) proposed using congruity backcrossing (i.e., backcrossing alternately with the tepary and the common bean) to facilitate genetic recombination between the two species and also increase hybrid fertility.

Hybridization between common and tepary beans was initiated at the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, in 1989. The objective of this work is to transfer multiple desirable traits from selected tepary genotypes into elite lines of the common bean. A modified embryo culture technique was essential to obtain congruity backcross hybrids. Data on the relative efficiencies of recurrent and congruity backcrossing with respect to increased hybrid embryo growth both in situ and in vitro, as well as on hybrid fertility and the introgression of tepary seed protein alleles, are presented.

Materials and methods

Parental genotypes and crossing

The tepary and common bean genotypes used for interspecific hybridization were evaluated and selected for agronomic traits (Table 1). This was essential because striking differences are found among genotypes of both species and our objective was to combine the best level of expression of complementary desirable traits from both of them. All tepary and common bean genotypes used in this study were cultigens. For the initial crosses and subsequent recurrent and congruity backcrosses, the common bean genotypes and resulting hybrids were used as females. In addition, in order to increase the introgression of tepary traits into the common bean, highly-fertile F_2 plants from the third (CBC_3) and fifth (CBC_5) congruity backcrosses were used as males for intermating with their respective male-sterile F_1s. All crosses were made in the greenhouse by hand emasculation and pollination (Buishand 1956; Bliss 1980) to prevent selfing or contamination from foreign pollen.

The following data were obtained from the initial cross and several of the backcross generations: the number and percentage of immature embryos rescued for culture; embryo growth in situ defined as the number of days from pollination to embryo rescue; the number and percentage of mature hybrid plants obtained; the number of mature plants transplanted to soil; the percent fertility (defined as the percentage of F_1 plants that were able to produce at least one selfed (F_2) seed) and the introgression of tepary seed protein alleles into the third and fifth congruity backcrosses.

Embryo rescue and in-vitro culture

Young pods resulting from interspecific crosses were harvested as soon as the first symptoms of embryo abortion were apparent. Abortion symptoms included loss of turgor in pod tissues and interruption of embryo development. Harvested pods were sterilized by submerging for 1 min in 70% ethanol and then for 6 min in a 2.5% solution of sodium hypochlorite with one drop of detergent (Tween 20). This was followed by washing three times with deionized sterile water. Using aseptic techniques, immature embryos, including the embryonic axis and cotyledons, were extracted from pods and testa, using a binocular dissecting scope, and transferred to the surface of the culture medium. The culture medium used was modified after the media reported by Honma (1955), Mok et al. (1978), Prendota et al. (1982), and Pratt et al. (1985). For initial F_1 hybrids and the first recurrent backcross (RBC_1) embryos, a single medium, named SE (for small embryos), was used. The SE medium contained the Murashige and Skoog (1962) mineral salts modified as follows (MS-M salts): a reduction of NH_4NO_3 and $CaCl_2 . 2H_2O$ to half of the original concentraion, i.e., 825 and 220 mg/l, respectively. The SE medium also included vitamins as in Gamborg's B5 medium (Gamborg et al. 1968), 6% sucrose, 200 mg/l of glutamine, and 200 mg/l of casein hydrolysate. The pH of the medium was adjusted to 5.7 and the medium was made semi-solid with 0.6% agarose (Sigma medium EEO) before autoclaving.

Probably because of their very small size and genetic constitution, it was not possible in a first attempt to obtain mature plants from the second congruity backcross (CBC_2) embryos after a single culture on SE medium. In view of this, a two-phase embryo culture protocol was developed. All embryos of this cross and the small hybrid embryos (< 4 mm length, including the cotyledons) of the following congruity backcrosses were cultured first on SE medium in the dark for 1–3 weeks until the plantlets reached over 2 cm in size. These were then transferred to a second medium, named LE (for larger embryos), and cultured in the light (1000 lux and 16:8 h light:dark photoperiod). The components of the LE medium were the same as in SE, but sucrose was reduced to 2%, and the concentration of the MS-M salts

Table 1 Common (*P. vulgaris*) and tepary (*P. acutifolius*) bean genotypes used in interspecific hybridization

Identification	Agronomic characteristics[a]
Phaseolus vulgaris[b]	
ICA Pijao (G 5773), Sacramento (G 17635), A 429, A 752, A 769, A 775, A 779, A 797, A 798, A 800, MAM 38, PEF 14, ARA 9, MAR 1, PVA 1111, CTC 1, IPA 6, Carioca (G 4017), and San Cristóbal 83 (G 17227)	Commercial seed quality, facilitator of hybridization, erect plant type, high yield, earliness, and resistance to bean golden mosaic virus, anthracnose, angular leaf spot, low soil fertility, and drought
P. acutifolius	
G 40001, G 40020, G 40023, G 440063, G 40066, and G 40138	Resistance to common bacterial blight, leafhoppers, drought, heat, and bruchids

[a] More information about specific parents can be obtained from the authors

[b] All *P. vulgaris* entries are resistant (*I* gene) to bean common mosaic virus

was reduced to one half. Embryos with well-developed shoot and root tips were dissected and cultured in vitro. Any embryo larger than 4 mm was cultured directly on LE medium under the light conditions mentioned earlier. The culture medium was poured into 16 × 125 mm (SE) and 18 × 150 mm (LE) test tubes with plastic caps. The cultures were sealed with plastic film and incubated at a temperature of 24–26 °C. A week prior to transfer to soil in the greenhouse, the plastic seal was removed from the test tubes in order to allow loss of humidity. Once the plantlets reached 6–10 cm in size, they were transferred to pots containing equal amounts of soil, sand, and perlite. Pots with the plantlets were placed for the first few days under shade in a fresh and humid area of the greenhouse.

Verification of initial F_1 hybrids and tepary gene introgression

The hybrid origin of F_1 plants of the initial cross was verified using morphological markers, such as growth habit, size and shape of trifoliolate leaves and flower bracteoles (Thomas and Waines 1984), level of self-sterility, and with polyacrylamide-gel electrophoresis (PAGE) of diaphorase isoenzymes in young trifoliolate leaves (Hussain et al. 1986). Furthermore, to assess introgression of tepary alleles, seed protein of selfed progenies of the CBC_3 and CBC_5 hybrids was used for separation by a modified SDS-PAGE (J. Vargas, personal communication 1992). Embryo axes were separated from cotyledons prior to electrophoretic analysis, and transferred to the LE medium for germination. This nondestructive technique made it possible to grow plants from the seeds used for electrophoretic analysis.

Vegetative propagation of hybrid plants

Hybrid plants with superior performance were propagated vegetatively by means of stem cuttings in order to carry out more backcrosses or produce more selfed (F_2) seed. Propagation was done by using a modified rooting solution after Pythoud and Buchala (1989). Young branches, without flowers and pods, were cut and submerged in a 10 mg/l solution of indole butyric acid and 10 mg/l of vitamin D_3 (both were dissolved first with dimethyl sulfoxide at a 1000 × concentration) for 2 days. Subsequently, the cuttings were kept in water for 2 weeks; water was oxygenated with an aquarium air pump and changed every day to avoid stem and root rots.

Results

Embryo culture

All embryos from the initial interspecific cross, the first recurrent backcross, the second congruity backcross, as well as most of the embryos from the third, fourth, and fifth congruity backcrosses, aborted between 8 and 27 days from pollination. Immature embryos had to be aseptically rescued from ovules and cultured in vitro as soon as abortion symptoms were detected.

Establishment of initial interspecific F_1 and first recurrent backcross mature hybrid plants in soil was carried out following immature embryo culture in SE medium. But attempts to establish second congruity backcross (CBC_2) plants in soil failed when the embryos were cultured in SE medium. CBC_2 embryos were, on average, the smallest of the entire backcross program; after culturing in SE medium, they gave rise to weak plantlets that did not withstand transplanting into soil. Only after subculturing such embryos in a second medium (LE) with reduced sucrose and reduced mineral salt concentrations did the resulting plantlets develop further and survive transplanting into soil. A two-phase embryo culture protocol was thus adopted for the CBC_2 and subsequent backcross generations (Table 2). When the medium for the smallest embryos contained low sucrose (i.e., 2%), the embryos either germinated prematurely, giving rise to weak plantlets, or formed only calli without morphogenesis. Substituting agarose for agar prevented oxidation and necrosis of small, young embryos possibly caused by impurities in the agar. Glutamine (Mok et al. 1978) and casein hydrolysate (Bhojwani and Razdan 1983) were considered highly beneficial for the in-vitro growth of immature embryos of the common bean and other species.

Table 2 Effect of embryo culture involving one- and two-phase protocols on the production of mature hybrid plants in two *P. vulgaris* × *P. acutifolius* second congruity backcrosses (CBC_2)

Item	Second congruity backcross[a]			
	CBC_1 × G 40023		CBC_1 × G 40138	
	One-phase culture[b]	Two-phase culture	One-phase culture	Two-phase culture
No. of embryos cultured	29	202	2	11
Embryo growth in situ[c]	15	12	16	10
No. of mature plants obtained	0	37	0	4
% CBC_2 plants obtained	0	18	0	36

[a] G 40023 and G 40138 are the *P. acutifolius* genotypes used in backcrossing as male parents

[b] One-phase culture medium used for small embryos (SE) only; two-phase culture medium used SE first, followed by the medium for large embryos (LE)

[c] Number of days from pollination to the day embryos were rescued for culture

Culturing the smallest embryos in the dark prevented excessive premature growth of foliar tissue at the expense of the embryo axis and radicle. Embryo germination occurred after 1–3 weeks of culture under first-phase conditions. Elongation took place rapidly after transfer of the germinated embryos to LE medium. Removal of the plastic seal from test tubes 1 week before transferring plantlets to soil in the glasshouse allowed a gradual reduction of humidity surrounding the plantlets. This, in turn, prevented or diminished the shock due to transplanting from in vitro to soil conditions. The two-phase embryo culture methodology developed in this study made it possible to recover CBC_2 plants from embryos as young as 9 days of growth, with efficiency rates of 18% and 36% (Table 2).

Effect of common and tepary bean genotypes

Throughout the interspecies crossing program, the genotype of common and tepary parents determined to a large extent the ability to obtain viable F_1s as well as

Table 3 Efficiency of *P. vulgaris* and *P. acutifolius* parental genotypes of the initial interspecific hybridization aided by embryo rescue and in-vitro culture

Parental genotypes		Number of embryos cultured	Number of mature F_1 plants	
P. vulgaris	*P. acutifolius*		Total	% Of embryos cultured
ICA Pijao	× G 40001	63	27	43
	G 40066	154	35	23
	G 40063	19	1	5
Sacramento	× G 40001	21	0	0
	G 40066	34	0	0
	G 40063	14	1	7

the efficiency of using F_1s as females in subsequent backcrossing. For example, using the common bean cultivar ICA Pijao in the initial cross with the tepary bean was more successful than using Sacramento light red kidney (Table 3). No mature hybrids were obtained from the crosses of the common bean cultivar Sacramento with tepary accessions G 40001 and G 40066. Furthermore, tepary bean accession G 40001 gave higher hybrid plant recovery than G 40066 and G 40063 when crossed to ICA Pijao. Similarly, the efficiency in obtaining mature hybrids was higher when backcrossing *P. vulgaris* genotypes onto (ICA Pijao × G 40001) than onto (ICA Pijao × G 40066) F_1s (data not shown). In CBC_2, the most efficient tepary genotype in generating mature hybrid plants was again G 40001.

A high proportion of lethal hybrids was obtained in CBC_1 and/or CBC_3 with crosses involving *P. vulgaris* genotypes MAM 38, A 797, and PEF 14, causing a low efficiency of hybrid plant recovery. Substituting with ICA Pijao and other common bean genotypes as male parents enhanced the success of hybridization. ICA Pijao was not only the best common bean genotype for generating interspecific hybrids, but hybrids involving ICA Pijao were the most efficient for continuing the subsequent congruity backcrossing with *P. acutifolius*. Interestingly, however, although G 40001 and ICA Pijao were the tepary and common bean genotypes, respectively, with which the highest number of embryos were rescued from the CBC_4 and CBC_5 generations, other tepary (e.g., G 40020) and common bean (e.g., ARA 9) genotypes were more efficient in the production of mature hybrid plants (data not shown). Mean differences between the use of tepary and common bean genotypes in hybrid production efficiency were not as large in the fourth and fifth congruity backcrosses as in the first, second, and third congruity backcrosses (Table 4).

Growth of hybrid embryos in situ and recovery efficiency of mature plants

All F_1s of the initial cross were self-sterile. Rescuable embryos were made available only after backcrossing

Table 4 Overall results of the *P. vulgaris* (common) × *P. acutifolius* (tepary) recurrent and congruity backcross program at CIAT, Cali, Colombia

Type of cross	Resultant hybrid[a]	Genetic contribution common: tepary	Requirement for embryo rescue[b]	No. of embryos cultured	Embryo growth in situ[c]	No. of mature plants	% Hybrid plants recovered[d]	% Fertility[e]
P. vulgaris × *P. acutifolius*	F_1	50:50	100.0	312	17.8	64	21	0
F_1 × *P. vulgaris*	$RBC_1 = CBC_1$	75:25	99.1	221	27.6	24	11	46
RBC_1 × *P. vulgaris*	RBC_2	87.5:12.5	2.7	360	32.9	332	92	88
CBC_1 × *P. acutifolius*	CBC_2	37.5:62.5	100.0	250	11.9	56	22	0
CBC_2 × *P. vulgaris*	CBC_3	68.75:31.25	77.8	358	18.7	61	17	48
CBC_3-F_1 × CBC_3-F^f_2	–	–	–	–	–	183	–	–
CBC_3 × *P. acutifolius*	CBC_4	34.38:65.63	95.4	523	16.7	130	25	0
CBC_4 × *P. vulgaris*	CBC_5	67.19:32.81	74.9	865	21.2	202	23	55
CBC_5-F_1 × CBC_5-F^f_2	–	–	–	–	–	600	–	–

[a] After Haghighi and Ascher (1988)

[b] % embryos that required in-vitro culture (i.e., ovules did not form seed in situ)

[c] Number of days from pollination to embryo rescue and culture

[d] % of cultured embryos producing mature hybrid plants

[e] % mature F_1 plants that produced at least one fertile F_2 plant

[f] No embryo rescue was carried out and most hybrid plants were fertile

the F_1 with *P. vulgaris* (RBC_1). The RBC_1 embryos grew more than the F_1 embryos, but produced mature hybrids with lower efficiency (Table 4). Backcrossing of the initial F_1 with tepary did not yield any rescuable embryos (data not shown). In RBC_2, embryo growth increased and reached the most advanced developmental stage in the pods, to the extent that most of the RBC_2 produced mature seeds with more than 30 days of growth in situ (Table 4). By contrast, the second congruity backcross (i.e., RBC_1 × *P. acutifolius*) gave rise to embryos which were the smallest, the most underdeveloped, and the most difficult to culture of the entire backcross program, with a mean of 11.9 days of growth. Growth of embryos from congruity backcrosses increased or decreased depending upon whether the last male parent was *P. vulgaris* or *P. acutifolius*, respectively. The greater the genetic contribution of *P. acutifolius*, with respect to *P. vulgaris*, the less developed were the hybrid embryos. However, at the fourth congruity backcross (CBC_4), it is noteworthy that, although the contribution of tepary increased with regard to the CBC_2, there was an increase in growth achieved by these embryos in situ before being rescued (Table 4). A similar tendency occurred for the CBC_5 embryos with respect to the CBC_3 generation. An increase in the average growth of embryos in situ was accompanied by an increase in the percentage of seed that formed within the pod. Whereas not a single seed was obtained from the second congruity backcross, 4.6% of the embryos in the fourth congruity backcross reached the seed stage. Nonetheless, to increase the probability of germination, mature seeds, without coats, were cultured on LE medium. Similarly, in CBC_5, there was a slight increase in the proportion of ovules that formed seed in situ with respect to CBC_3, and a much higher increase occurred in CBC_3 with respect to the first recurrent or congruity backcross (Table 4).

The rate of mature hybrid plant recovery was influenced by the embryo culture protocol used (Table 2), the genotype of the parents involved in each cross (Table 3), and the type of cross (Table 4). Plant recovery efficiency through embryo rescue was, on average, lowest (11%) in the first recurrent backcross in spite of the fact that embryo growth in situ was higher than in the initial cross (Table 4). In the second recurrent backcross, plant recovery rate increased dramatically and reached 92%. Thus, most of the crosses did not require embryo rescue. Although embryo growth in situ of congruity hybrids involving *P. vulgaris* as the last male parent was higher than growth with hybrids involving *P. acutifolius*, their mature plant recovery rate was slightly lower. But the proportion of mature plants recovered increased with the successive congruity backcrosses having either the common (i.e., $CBC_5 > CBC_3 > CBC_1$) or tepary bean (i.e., $CBC_4 > CBC_2$) as the last parent. From our *P.vulgaris* × *P. acutifolius* hybridization program comprising the initial interspecific crosses, the first and second recurrent backcrosses, and the first through fifth congruity backcrosses, a total of 1 652 mature plants have been produced (Table 4).

Fertility of hybrids

Fertility, measured as the percentage of mature F_1 plants that produced at least one fertile or partially-fertile F_2 plant, was recovered in RBC_1 and increased greatly in RBC_2. In congruity backcrossing, fertility was lost cyclically when *P. acutifolius* was the last male parent (i.e., in CBC_2 and CBC_4), and was recovered partially in CBC_3 and CBC_5 when *P. vulgaris* was the last male parent (Table 4). But fertility recovery efficiency tended to increase as the congruity backcross generations advanced (i.e., $CBC_5 > CBC_3 > CBC_1$). Likewise, production of selfed-seed on partially-fertile hybrid plants increased as recurrent and congruity backcrossing advanced. Thus, a single hybrid plant of the fifth congruity backcross yielded more than 100 F_2 seeds after 5 months in the greenhouse. This is well above the total seeds obtained from the 11 first fertile recurrent backcross hybrids after 8 months (data not shown).

Morphology of interspecific hybrid plants

All F_1 plants of the initial cross had the plant height and growth habit of the tepary parents. These plants also had a pink flower color and leaf and bracteole size intermediate between common and tepary beans. Plant eight and overall morphology varied among the F_1 plants within a given parental combination. As expected, the influence of the tepary bean in the hybrid phenotype decreased in RBC_1 and RBC_2.

Congruity hybrids that had tepary as the last male parent displayed more tepary than common bean characteristics, even more than the initial F_1 hybrids. These characteristics included overall plant morphology and growth habit, small bracteoles and small leaves, and flowers with variable pink and occasionally with a white color of the standards and wings. Similarly, congruity backcross hybrids with *P. vulgaris* as the last male parent showed more common bean characteristics. In advanced generations of congruity backcrossing, the vigor of hybrid plants was higher than in earlier generations. Thus, hybrid plantlets were readily transplanted to soil, and mature hybrids showed vigorous vegetative growth and profuse rooting of cuttings. This made it possible to maintain most CBC_4 and CBC_5 hybrid plants, for backcrossing or seed production, for extended periods in the greenhouse.

Hybrids that included tepary accession G 40001 as the male parent generally displayed growth habit IV – high branching and more vigorous and faster vegetative propagation than the hybrids generated with accessions G 40023, G 40138, and G 40066. The latter displayed a determinate growth habit. Traits not seen in the parental genotypes were expressed in some hybrid

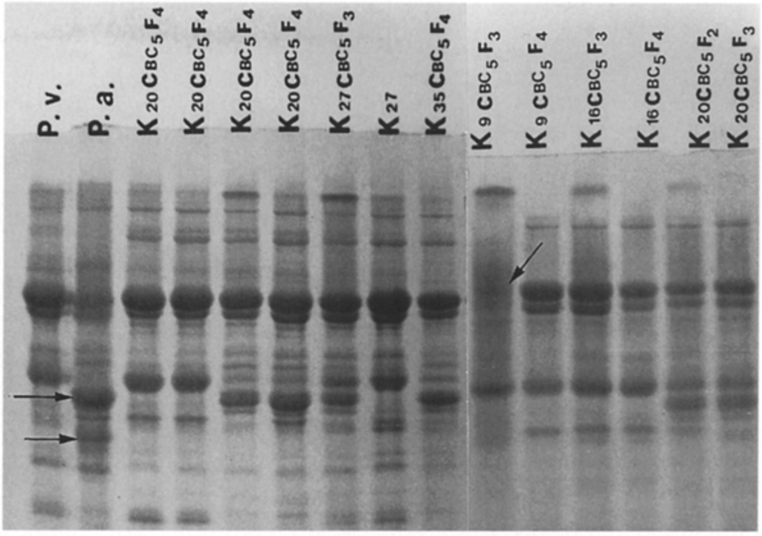

Fig. 1 Seed protein electrophoresis showing evidence for introgression of tepary alleles. From left to right: (*P.v.*) *P. vulgaris* parent (ICA Pijao); (*P.a.*) *P. acutifolius* (G 40001); all other lanes (*K9, K16, K20, K27, K35*) are CBC_5-derived F_3 and F_4 progenies. *Arrows* show tepary alleles whose introgression is monitored in the congruity hybrids. Note that the phaseolin pattern of CBC_5 shown in the first lane is different from the typical ICA Pijao pattern

progenies. These included inflorescences bearing multiple pods, a trait mostly of common beans of Middle American origin (Singh et al. 1991), also observed by Haghighi and Ascher (1988), light brown seed like that of some wild common bean genotypes, new seed phaseolin electrophoretic patterns (Fig. 1), and leathery leaves.

Verification of initial hybrids and monitoring of gene introgression from the tepary bean in subsequent generations

The presence of a low-molecular-weight band of isozyme diaphorase from the male parent *P. acutifolius*, in addition to morphological features and complete self-sterility, confirmed the hybrid origin of F_1 plants of the initial cross. Because the RBC_1, CBC_3, and CBC_5 hybrids were generated from backcrosses involving self-sterile F_1, CBC_2, and CBC_4 plants, respectively, their hybrid nature could be inferred with certainty. The small size of the bracteoles in CBC_2 and CBC_4 hybrids served as a dominant marker of backcrosses with *P. acutifolius*. In these hybrids, bracteoles were similar in size to those of the male parent *P. acutifolius*, and smaller than in the female parents, the respective RBC_1 and CBC_3 hybrids. These observations were correlated with other changes such as decrease in leaf area and loss of fertility (Table 4). Also, monitoring of introgression of genes into progenies of the CBC_3 and CBC_5 hybrids was carried out by analysis of the seed protein electrophoretic bands of the tepary bean in the lectin region; these bands are not present in the common bean (Fig. 1).

Hybrid progeny generated for agronomic evaluation

F_2 seeds were produced from the RBC_1, RBC_2, CBC_3, and CBC_5 hybrids. Intercrossing between the partially fertile F_2 (as males) and self-sterile F_1s of CBC_3 and CBC_5 (as females) also produced large amounts of mature seed without embryo culture (Table 4). Progenies of RBC_1, RBC_2, CBC_3, and CBC_5, and those obtained from intermating within CBC_3 and CBC_5, are being advanced for the development of inbred lines and to provide seed increase for an evaluation for agronomic traits.

Discussion

As in previous hybridizations between *P. vulgaris* and *P. acutifolius* (Mok et al. 1978; Thomas and Waines 1984; Parker and Michaels 1986) our interspecific crossing would not have succeeded without in-vitro embryo culture. Small germinated embryos of CBC_2 failed to reach sufficient development on a high osmolarity medium to survive transplanting to soil. Successful transplanting of rescued embryo-derived plantlets to soil was possible after subculturing of small germinated embryos in a second medium (LE), under decreased sucrose and salt concentration, especially to allow more vigorous growth of the root system.

Haghighi and Ascher (1988) reported congruity backcrossing without the aid of embryo rescue. Other differences include our severe difficulties in backcrossing the initial interspecific F_1 hybrids with tepary genotypes and the recurrence of sterility of congruity hybrids when tepary was used as the last male parent. The actual causes for these differences are not known. But the above authors used wild as well as cultivated tepary genotypes and intraspecies hybrids of common bean, whereas we used only cultivated tepary and common bean. A wild genotype of common bean has been reported to allow interspecific hybridization with less difficulty (Stockinger et al. 1986).

As previously reported for recurrent backcrossing (Thomas and Waines 1984; Pratt et al. 1985; Parker and

Michaels 1986; Scott and Michaels 1990), striking effects on the success of recurrent and congruent hybridization were found in this study due to the genotype of both the tepary and the common bean. The use of common bean cultivar ICA Pijao in the initial cross greatly facilitated the production of vigorous F_1s, which could be backcrossed efficiently with other common bean cultivars to yield a partially fertile RBC_1. Parker and Michaels (1986) identified ICA Pijao as a noncarrier of an incompatibility allele that interacts with a factor in the *P. acutifolius* genome causing lethal F_1 hybrids, and proposed the use of ICA Pijao as a bridging-parent in interspecific crosses with *P. acutifolius*. When difficulties in congruity backcrossing with other common bean genotypes were encountered in our work, the use of ICA Pijao helped to overcome such difficulties. Thus, ICA Pijao not only facilitated the initial cross but also the later congruity backcrosses. ICA Pijao, a common bean of Middle American genepools, was previously identified as a noncarrier of the complementary dominant dosage-dependent lethal (*Dl1* and *Dl2*) genes, and thus served as a bridging-parent between Andean and Middle American incompatible intraspecific crosses (Singh and Gutiérrez 1984).

The tepary bean genotype was also critical for the success of recurrent and congruity backcrosses. G 40001 was the most efficient tepary genotype for the initial cross with ICA Pijao (Table 3) and for the CBC_2. It was also the only tepary giving rise to hybrids that could be effectively used in subsequent congruity backcrossing. But why it could not be successfully hybridized with common bean cultivar Sacramento is not understood. Other initially less efficient tepary genotypes gave rise to partially-fertile progenies when used as parents of advanced congruity backcrosses, apparently after genotypes such as ICA Pijao had helped to overcome or reduce the interspecific incompatibility barrier. Hybrids generally inherited an indeterminate climbing growth habit IV from tepary bean G 40001, which conferred the capacity to elongate in vitro and after transplanting to soil. Hybrids with growth habit IV were also easier to propagate vegetatively and to re-sprout after pruning, allowing protracted greenhouse maintenance of plants.

All common bean genotypes used in the fifth congruity backcross gave rise to some mature, partially-fertile hybrids. Because some common and/or tepary bean genotypes are likely to be carriers of interspecific incompatibility genes (Parker and Michaels 1986; Scott and Michaels 1990), it is probable that the common (e.g., Sacramento) and tepary (e.g., G 40001 and G 40066) bean genotypes that could not be hybridized together successfully in the beginning were carriers of such genes. The generation of viable hybrids using these incompatible genotypes in later backcross generations was possible due to the segregation of viable (from compatible parents, e.g., ICA Pijao) and lethal alleles (Parker and Michaels 1986). Analysis of electrophoretic patterns of seed proteins in segregating advanced congruity backcross populations (CBC_3 and CBC_5) has allowed us to provide evidence of high introgression frequencies of *P. acutifolius* alleles in the lectin region. The increased introgression probably results from the accumulation of favorable gene recombinations between the two species with successive congruity backcrossing.

Growth of hybrid embryos in situ, recovery efficiency of mature plants, and fertility of hybrids, varied cyclically throughout the backcrossing program, depending upon the last male parent. As Haghighi and Ascher (1988) reported, we have also observed a gradual increase in fertility as congruity backcrossing increased. But, in addition to fertility, our data show increased hybrid embryo growth in situ, increased vigor, and the production of mature hybrid plants. For example, the requirement of embryo rescue diminished as congruity backcrosses advanced (Table 4). These effects could be attributed to different causes: (1) an increase in structural congruity of the homologous chromosome segments of the two species, resulting from frequent exchange as backcrossing advances; and (2) an accumulation of complementary favorable gene combinations of the two species, which confer higher fertility and vigor to the hybrids. Moreover, a high proportion of genetic combinations that give rise to lethal, weak, sterile, and low-fertility plants is selected against and automatically eliminated in successive backcross generations. Gene combinations present in fertile and vigorous plants are then transferred to congruity hybrids in the subsequent backcross.

Intercrossing among congruity hybrids, using the highly-fertile F_2 plants as males and self-sterile F_1 as females, has allowed us to obtain a large amount of seed (Table 4). This intermating between hybrid genotypes was proposed by Waines et al. (1988) as a mechanism to combine different donor alleles, and, as a result, achieve higher genetic recombination. Several cycles of congruity backcrossing could then overcome *P. vulgaris* × *P. acutifolius* hybridization barriers such as genotype incompatibility, early embryo abortion, hybrid sterility, and lower frequencies of hybridization. Therefore, hybrid progenies of advanced cycles of congruity backcrossing can serve as a bridge for the transfer of genes between incompatible tepary and common beans. Thus, the use of congruity backcrossing proposed by Haghighi and Ascher (1988) deserves more attention in the future.

In this work, we have generated the largest number (1 652 plants) of *P. vulgaris* × *P. acutifolius* hybrid plants so far obtained. We have also demonstrated high frequencies of tepary gene introgression. Knowledge about facilitator genotypes such as ICA Pijao (Parker and Michaels 1986) and G 40001 (this study), the adoption of the congruity backcross strategy (Haghighi and Ascher 1988), and modification of the embryo culture technique to allow adult hybrid plant recovery from small congruity backcross embryos (this study), have been major factors contributing to the success of this work. We have also developed a pool of congruity hybrids that can be used as a bridge to introgress germplasm from other incompatible tepary to common bean genotypes and vice versa.

Currently, advanced generation lines from the first and second recurrent backcrosses and from the first phase of congruity backcrosses are being selfed to produce inbred lines. This will be followed by seed multiplication and evaluation to identify lines with desirable traits from both species. As shown in this study, fertility, embryo growth in situ, and the recovery efficiency of mature plants from congruity hybrids have apparently not yet reached the desired level. Because of this, our congruity backcrossing program will continue.

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References

Alvarez MN, Ascher PD, Davis DW (1981) Interspecific hybridization in *Euphaseolus* through embryo rescue. Hort Sci 16:541–543

Bhojwani SS, Razdan MK (1983) Plant tissue culture: theory and practice Elsevier Science Publishers, Amsterdam, Netherlands

Bliss FA (1980) Common bean. In: Fehr WR, Hadley HH (eds), Hybridization of crop plants. Am Soc Agron, Madison, Wisconsin, pp 273–284

Buishand TJ (1956) The crossing of bean (*Phaseolus* spp.). Euphytica 5:41–50

Coyne DP (1964) Species hybridization in *Phaseolus*. J Hered 55:5–6

Gamborg OL, Miller RA, Ojina K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50:151–158

Haghighi KR, Ascher PD (1988) Fertile, intermediate hybrids between *Phaseolus vulgaris* and *P. acutifolius* from congruity backcrossing. Sex Plant Reprod 1:51–58

Honma S (1955) A technique for artificial culturing of bean embryos. Proc Am Soc Hort Sci 72:405–408

Honma S (1956) A bean interspecific hybrid. J Hered 47:217–220

Hussain A, Ramírez H, Bushuk W, Roca W (1986) Field bean (*Phaseolus vulgaris* L.) cultivar identification by electrophoregrams of cotyledon storage proteins. Euphytica 35:729–732

McElroy JB (1985) Breeding dry beans, *Phaseolus vulgaris* L., for common bacterial blight resistance derived from *Phaseolus acutifolius* A. Gray. PhD dissertation, Cornell University, Ithaca, New York

Mok DWS, Mok MC, Rabakoarihanta A (1978) Interspecific hybridization of *Phaseolus vulgaris* with *P. lunatus* and *P. acutifolius*. Theor Appl Genet 52:209–215

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 15:473–497

Parker JP, Michaels TE (1986) Simple genetic control of hybrid plant development in interspecific crosses between *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray. Plant Breed 97:315–323

Parsons LR, Howe TK (1984) Effects of water stress on the water relations of *Phaseolus vulgaris* and the drought resistant *Phaseolus acutifolius*. Physiol Plant 60:197–202

Pratt RC (1983) Gene transfer between tepary and common beans. Desert Plants 5:57–63

Pratt RC, Bressan RA, Hasegawa PM (1985) Genotypic diversity enhances recovery of hybrids and fertile backcrosses of *Phaseolus vulgaris* L. and *P. acutifolius*. A. Gray. Euphytica 34:329–344

Prendota K, Baudoin JP, Marechal R (1982) Fertile allopolyploids from the cross *Phaseolus acutifolius* × *Phaseolus vulgaris*. Bull Rech Agron Gembloux 17:177–190

Pythoud F, Buchala AJ (1989) The fate of vitamin D_3 and indolylbutyric acid applied to cuttings of *Populus tremula* L. during adventitious root formation. Plant Cell Environ 125: 489–494

Scott ME, Michaels TE (1990) Genetic control of abnormal seedling development in *Phaseolus vulgaris* L. and *P. vulgaris* × *P. acutifolius* A. Gray hybrids. Plant Breed 104:102–107

Singh SP (1992) Common bean improvement in the tropics. Plant Breed Rev 10:199–269

Singh SP, Gutiérrez JA (1984) Geographical distribution of *Dl1* and *Dl2* genes causing hybrid dwarfism in *Phaseolus vulgaris* L., their association with seed size, and their significance to breeding. Euphytica 33:337–345

Singh SP, Gepts, P, Debouck DG (1991) Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ Bot 45:379–396

Smartt J (1970) Interspecific hybridization between cultivated American species of the genus *Phaseolus*. Euphytica 19:480–489

Stockinger EJ, Waines JG, Italuya FM (1986) Interspecific hybridization between Kenyan common beans and tepary beans. Annu Rep Bean Improv Coop 29:93–94

Thomas CV, Waines JG (1984) Fertile backcross and allotetraploid plants from crosses between tepary beans and common beans. J Hered 75:93–98

Thomas CV, Manshardt RM, Waines JG (1983) Teparies as a source of useful traits for improving common beans. Desert Plants 5:43–48

Waines JG, Manshardt RM, Wells WC (1988) Interspecific hybridization between *Phaseolus vulgaris* and *P. acutifolius*. In: Gepts P (ed) Genetic resources of *Phaseolus* beans. Kluwer Academic Publishers, Dordrecht, Netherlands, pp 485–502

Zaiter HZ, Coyne DP, Vidaver AK, Steadman JR (1989) Differential reaction of tepary bean lines to *Xanthomonas campestris* pv *phaseoli*. Hort Sci 24:134–137