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Interspecific Hybridization of Phaseolus vulgaris with *P. lunatus* and *P. acutifolius**

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Summary. The influence of genotypic combinations on the growth of hybrid embryos between *Phaseolus vulgaris* and *P. lunatus*, and between *P. vulgaris* and *P. acutifolius* was examined. All embryos obtained from *P. vulgaris* x *P. lunatus* crosses developed only to a stage which appears to be comparable to the pre-heart-shape stage of selfed embryos. Reciprocal crosses were attempted, but pods abscised at a very early stage. Embryos derived from *P. vulgaris* x *P. acutifolius* and reciprocal crosses attained the cotyledon stage although no mature seeds were formed. A distinct characteristic of these 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.

Immature embryos were cultured on defined medium and the effects of glutamine and gibberellin (GA_3) were examined. Glutamine was effective in increasing the survival rate; gibberellin had no apparent effect. Plants derived from cultured embryos of *P. vulgaris* x *P. lunatus*, *P. vulgaris* x *P. acutifolius* and *P. acutifolius* x *P. vulgaris* were obtained.

Key Words: *Phaseolus* – Interspecific hybridization – Embryo culture

Introduction

Phaseolus lunatus (lima bean) and *Phaseolus acutifolius* (tepary bean) contain genetic resistance to *Fusarium solani* f. sp. *phaseoli* (root rot) and *Xanthomonas phaseoli* (common bacterial blight) which is not apparent in *Phaseolus vulgaris* (Baggett et al. 1965, Coyne and Schuster 1973, Coyne et al. 1963, Wallace and Wilkinson 1965).

The transfer of genetic resistance to *P. vulgaris* through interspecific hybridization has been difficult due to the premature abortion of hybrid embryos (Smartt 1970). However, Honma and Heeckt (1959) described high seed set in an attempt to cross *P. vulgaris* (female) with *P. lunatus* (male). All fertile progeny resembled the seed parent (*P. vulgaris*); the remaining two plants were sterile and did not resemble the maternal parent. It was assumed that all these plants were interspecific hybrids. Extensive efforts to repeat the same cross were not successful (Smartt 1970) and it was suggested that the fertile progeny obtained earlier (Honma and Heeckt 1959) might have resulted from selfing (Smartt 1970).

Fertilization occurred in matings of *P. vulgaris* x *P. acutifolius* (Honma 1956, Smartt 1970), but the hybrid embryos collapsed 17-21 days after pollination. The immature embryos were excised and cultured on artificial medium, which resulted in viable plantlets (Honma 1955). The frequency of surviving plants was low (four out of several hundred). However, an exception was the formation of mature seeds in reciprocal crosses between two particular genetic lines (Smartt 1970).

It appears that much information is still needed on the basis of reciprocal cross differences between these species, the early hybrid embryo development, the genotypic effects on embryo growth and the effects of culture medium on hybrid survival. Added knowledge in these areas would permit the design of methods to optimize interspecific hybrid recovery which is necessary to provide sufficient materials for the studies of the genetic control of hybrid fertility. Adequate fertility of interspecific hybrids will no doubt be the prerequisite for efficient utilization of the germplasm contained in *P. lunatus* and *P. acutifolius*, and is also expected to be a formidable

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challenge. This paper describes the effects of genotypes on later embryo growth and influence of culture medium on embryo survival. Results of studies on the fertilization process and early embryo development in reciprocal crosses will be reported elsewhere.

Materials and Methods

Plant materials

Two genotypes each of *Phaseolus vulgaris* (cvs. 'Great Northern' (GN), 'Gallatin 50' (G50)), *P. lunatus* (cvs. 'Kingston' (K), 'Burpee Hybrid' (B)) and *P. acutifolius* (P.I. 310800 (AC 1), P.I. 321637 (AC 2)) were used. Each *P. vulgaris* genotype was used as the female parent and mated to the two genotypes of *P. lunatus* and *P. acutifolius*. Reciprocal crosses were also attempted. Flowers were emasculated and pollinated one day before opening. Developing pods were collected every three days; the sizes of pods, seeds and embryos were measured.

Embryo culture

Developing pods were surface sterilized with 95% ethanol for one minute, immersed in 1% sodium hypochlorite for five minutes, and rinsed in sterile water. Seeds and embryos were excised with the aid of a stereo-microscope in a laminar flow transfer hood. Hybrid embryos were identified by their distinct morphology which differed from that of selfed embryos (see results). The embryos were planted on culture medium.

The medium consisted of mineral nutrients as described by Murashige and Skoog (1962) with the following organic substances added: sucrose (30 g/1), myo-inositol (100 mg/1), thiamine.HCl (1 mg/1), nicotinic acid (5 mg/1) and pyridoxine.HCl (0.5 mg/1).Glutamine (10 or 100 mg/1) was supplied when indicated. The pH of the medium was adjusted to 5.7 and Difco bacto-agar (8 g/1) was added. The medium was dispensed into sample bottles (25 ml/bottle) and autoclaved at 120°C for 15 minutes. To test the effect of gibberellin (GA_3), the appropriate amount of GA_3 was dissolved in dimethyl sulfoxide, and 0.0125 ml of the solution was added to each bottle after autoclaving to give a final concentration of 0.1 μM.

Embryo cultures were maintained at 28°C under constant light (8000 lux) for the initial four weeks. Embryos that survived were transferred to fresh medium in mason jars (100 ml/jar) and then maintained at a photoperiod of 18 hours at 28°C. The resulting plants were transferred to Jiffi Mix Plus and placed in a misting chamber in the greenhouse.

Results

The sizes of the pods, seeds and embryos as measured at three day intervals are presented in Tables 1, 2 and 3, for the crosses *P. vulgaris* x *P. lunatus*, *P. vulgaris* x *P. acutifolius* and *P. acutifolius* x *P. vulgaris*, respectively. Crosses between *P. lunatus* (female) and *P. vulgaris* (male) were also attempted and resulted in fertilization (unpublished) but the majority of pods abscised in less than three days after pollination and those remaining had no developing seeds. The data on matings between *P. acutifolius* (female)

Table 1. The average sizes and standard deviations of pods, seeds and embryos obtained from *P. vulgaris* X *P. lunatus* crosses as measured at three day intervals after pollination

	Cross	'GN' X 'K'		'G50' X 'K'		
Day	Pod (cm)	Seed (mm)	Embryo (mm)	Pod (cm)	Seed (mm)	Embryo (mm)
3	3.5 ± 0.7 (14)[1]	0.6 ± 0.3 (54)	0.02 ± 0.01 (53)	5.5 ± 0.5 (9)	1.5 ± 0.2 (27)	0.05 ± 0.02 (27)
6	4.5 ± 0.4 (15)	0.9 ± 0.3 (51)	0.02 ± 0.01 (51)	7.2 ± 0.5 (8)	1.7 ± 0.2 (44)	0.10 ± 0.02 (44)
9	4.9 ± 0.8 (14)	1.7 ± 0.3 (51)	0.05 ± 0.02 (50)	9.3 ± 0.4 (10)	2.8 ± 0.7 (10)	0.23 ± 0.09 (20)
12	6.9 ± 1.2 (21)	2.0 ± 0.6 (61)	0.14 ± 0.03 (58)	8.7 ± 1.0 (13)	3.4 ± 0.5 (33)	0.24 ± 0.04 (30)
15	6.2 ± 2.1 (27)	2.3 ± 0.5 (81)	0.30 ± 0.16 (79)	8.8 ± 0.6 (11)	3.0 ± 0.5 (30)	0.35 ± 0.02 (28)
18	6.3 ± 0.5 (21)	2.3 ± 0.3 (67)	0.29 ± 0.07 (66)	9.0 ± 0.3 (8)	3.2 ± 0.2 (24)	0.40 ± 0.03 (21)
21	6.0 ± 0.8 (12)	2.8 ± 0.4 (38)	0.34 ± 0.08 (35)	10.0 ± 0.5 (6)	3.5 ± 0.3 (24)	0.40 ± 0.02 (20)
24	6.7 ± 1.2 (5)	3.0 ± 0.3 (15)	0.32 ± 0.06 (15)	10.5 ± 0.5 (9)	4.3 ± 0.4 (7)	0.42 ± 0.05 (21)
	Cross	'GN' X 'B'		'G50' X 'B'		
Day	Pod (cm)	Seed (mm)	Embryo (mm)	Pod (cm)	Seed (mm)	Embryo (mm)
3	3.5 ± 0.8 (11)	1.0 ± 0.3 (10)	0.02 ± 0.01 (10)	5.8 ± 0.3 (10)	1.5 ± 0.4 (21)	0.05 ± 0.02 (19)
6	4.5 ± 0.4 (11)	1.2 ± 0.3 (10)	0.02 ± 0.02 (10)	7.5 ± 0.4 (10)	2.0 ± 0.4 (20)	0.05 ± 0.03 (18)
9	5.6 ± 0.4 (7)	1.6 ± 0.3 (10)	0.05 ± 0.03 (9)	8.3 ± 1.1 (10)	2.4 ± 0.8 (31)	0.10 ± 0.04 (30)
12	5.4 ± 0.4 (18)	1.5 ± 0.3 (53)	0.12 ± 0.02 (37)	8.0 ± 0.5 (8)	2.5 ± 0.5 (24)	0.15 ± 0.03 (24)
15	6.6 ± 0.6 (9)	1.6 ± 0.4 (22)	0.14 ± 0.04 (22)	8.5 ± 0.7 (8)	3.0 ± 0.4 (19)	0.26 ± 0.02 (19)
18	6.5 ± 0.7 (11)	1.6 ± 0.3 (13)	0.13 ± 0.03 (10)	10.0 ± 0.8 (7)	4.0 ± 0.3 (14)	0.25 ± 0.05 (14)
21	6.6 ± 0.5 (8)	1.8 ± 0.4 (8)	0.15 ± 0.03 (8)	11.0 ± 0.7 (8)	4.5 ± 0.3 (16)	0.27 ± 0.04 (15)

[1] Number of individuals examined

and *P. vulgaris* (male) are limited to the genotype AC 2, since AC 1 flowered poorly and only a small number of crosses could be made during the period of the experiment.

In the four *P. vulgaris* x *P. lunatus* crosses (Table 1), very few hybrid embryos did exceed 0.5 mm in size after 24 days. The rate of embryo growth appeared to be influenced to a large extent by the maternal parent, especially within 15 days following pollination. Hybrid embryos developing on 'G50' exhibited faster growth than those set on 'GN' with identical pollen parent. Also the final size of the embryos in 'G50' x 'K' and 'G50' x 'B' crosses was larger than those derived from 'GN' x 'K' and 'GN' x 'B' crosses, respectively. The development of the hybrid embryos as determined by the rate of growth and final size, seemed to be affected by the genotype of the pollen parent as well. When 'B' was used as the male parent resulting embryos grew at a much slower rate and the final size at 21 days was smaller.

The size of the embryos was measured in each cross when spontaneous pod abscission occurred. Although the maximum number of days of pod retention after pollina-

Table 2. The average sizes and standard deviations of pods, seeds and embryos obtained from *P. vulgaris* X *P. acutifolius* crosses as measured at three day intervals

	Cross	'GN' X 'AC 1'			'G50' X 'AC 1'	
Day	Pod (cm)	Seed (mm)	Embryo (mm)	Pod (cm)	Seed (mm)	Embryo (mm)
3	4.5 ± 0.3 (10)[1]	2.8 ± 0.3 (31)	0.2 ± 0.0 (31)	5.7 ± 0.3 (6)	2.1 ± 0.3 (11)	0.2 ± 0.0 (11)
6	5.5 ± 0.4 (11)	3.5 ± 0.5 (28)	0.3 ± 0.1 (27)	6.6 ± 0.5 (6)	2.5 ± 0.5 (10)	0.4 ± 0.0 (10)
9	6.6 ± 0.6 (10)	4.3 ± 0.6 (31)	0.3 ± 0.1 (31)	7.0 ± 0.5 (6)	3.0 ± 0.5 (11)	1.5 ± 0.1 (11)
12	8.5 ± 0.7 (10)	3.8 ± 0.1 (34)	1.1 ± 0.1 (34)	8.2 ± 0.7 (7)	3.5 ± 0.4 (15)	1.8 ± 0.2 (14)
15	8.0 ± 0.8 (10)	5.0 ± 0.2 (25)	2.0 ± 0.2 (25)	8.5 ± 0.6 (10)	4.3 ± 0.8 (20)	2.4 ± 0.1 (18)
18	9.0 ± 0.5 (10)	5.4 ± 0.5 (33)	2.8 ± 0.2 (30)	8.8 ± 0.9 (8)	5.2 ± 0.4 (13)	3.2 ± 0.2 (13)
21	9.2 ± 1.0 (10)	5.3 ± 0.4 (30)	3.2 ± 0.3 (28)	9.2 ± 0.7 (5)	5.5 ± 0.7 (8)	3.6 ± 0.3 (8)
24	9.5 ± 0.8 (9)	5.5 ± 0.4 (24)	4.0 ± 0.4 (24)	10.5 ± 0.5 (6)	5.5 ± 0.6 (9)	3.9 ± 0.4 (9)
	Cross	'GN' X 'AC 2'			'G50' X 'AC 2'	
Day	Pod (cm)	Seed (mm)	Embryo (mm)	Pod (cm)	Seed (mm)	Embryo (mm)
3	4.5 ± 0.4 (10)	2.3 ± o.4 (32)	0.2 ± 0.0 (30)	5.6 ± 0.3 (10)	2.0 ± 0.3 (12)	0.2 ± 0.0 (10)
6	5.7 ± 0.8 (12)	2.8 ± 0.3 (40)	0.3 ± 0.0 (39)	6.5 ± 0.4 (10)	2.5 ± 0.4 (10)	0.4 ± 0.0 (14)
9	7.5 ± 1.2 (11)	3.6 ± 0.7 (42)	0.4 ± 0.1 (41)	7.5 ± 0.8 (10)	3.3 ± 0.5 (12)	0.6 ± 0.1 (12)
12	8.1 ± 1.2 (17)	3.3 ± 0.8 (60)	1.3 ± 0.4 (60)	8.0 ± 0.9 (8)	3.8 ± 0.4 (16)	1.5 ± 0.2 (15)
15	7.5 ± 1.0 (7)	3.8 ± 0.5 (19)	1.4 ± 0.6 (19)	8.8 ± 0.7 (8)	4.5 ± 0.4 (22)	2.1 ± 0.1 (22)
18	9.3 ± 0.8 (10)	4.0 ± 0.8 (41)	2.6 ± 0.6 (40)	8.5 ± 0.4 (10)	5.0 ± 0.7 (20)	2.4 ± 0.2 (20)
21	9.2 ± 0.8 (11)	4.5 ± 0.5 (30)	3.0 ± 0.4 (28)	9.8 ± 0.8 (7)	5.3 ± 0.4 (15)	2.8 ± 0.3 (15)
24	9.5 ± 0.7 (10)	5.0 ± 0.4 (28)	3.5 ± 0.2 (24)	11.0 ± 0.5 (8)	6.0 ± 0.4 (24)	3.5 ± 0.3 (24)

[1] Number of individuals examined

Table 3. The average sizes and standard deviations of pods, seeds and embryos obtained from *P. acutifolius* X *P. vulgaris* crosses as measured at three day intervals

	Cross	'AC 2' X 'GN'			'AC 2' X 'G50'	
Day	Pod (cm)	Seed (mm)	Embryo (mm)	Pod (cm)	Seed (mm)	Embryo (mm)
3	3.5 ± 0.4 (5)[1]	2.1 ± 0.3 (8)	0.2 ± 0.03 (8)	3.6 ± 0.4 (4)	2.3 ± 0.3 (5)	0.3 ± 0.0 (5)
6	4.2 ± 0.3 (4)	2.5 ± 0.4 (6)	0.4 ± 0.04 (6)	4.8 ± 0.2 (4)	2.4 ± 0.3 (6)	0.4 ± 0.0 (6)
9	5.2 ± 0.2 (5)	2.8 ± 0.3 (5)	0.8 ± 0.11 (5)	5.7 ± 0.4 (5)	2.9 ± 0.2 (5)	1.0 ± 0.1 (5)
12	6.0 ± 0.5 (4)	3.7 ± 0.2 (3)	1.5 ± 0.15 (3)	6.1 ± 0.4 (5)	4.0 ± 0.3 (5)	1.2 ± 0.1 (5)
15	6.8 ± 0.5 (3)	4.0 ± 0.3 (4)	1.7 ± 0.10 (4)	6.2 ± 0.6 (6)	4.3 ± 0.4 (7)	1.9 ± 0.2 (7)
18	7.0 ± 0.5 (4)	4.5 ± 0.2 (5)	1.6 ± 0.12 (5)	7.5 ± 0.6 (4)	5.0 ± 0.4 (6)	2.0 ± 0.2 (6)
21	7.5 ± 0.4 (5)	4.5 ± 0.3 (6)	2.0 ± 0.13 (6)	8.2 ± 0.5 (4)	5.1 ± 0.5 (4)	2.5 ± 0.4 (4)
24	7.2 ± 0.3 (6)	5.0 ± 0.5 (8)	2.3 ± 0.40 (8)	9.0 ± 1.5 (3)	5.5 ± 0.5 (5)	3.0 ± 0.2 (5)

[1] Number of individuals examined

tion varied within and between crosses (from 22 to 33 days), the sizes of embryos contained in abscised pods were not significantly different from the final embryo sizes presented in Table 1.

The development of all *P. vulgaris* x *P. lunatus* embryos was atypical of that observed in selfed embryos of these species. The hybrid embryos had an oval-rod configuration (Fig. 1a), very similar to the pre-heart-shape embryos in intraspecific crosses. However, subsequent enlargement was not accompanied by morphological changes. Advancement to the heart-shape or cotyledon stage was not observed in any of the hybrid embryos, while selfed embryos were all at heart-shape stage six days after fertilization. The maximum length of the hybrid embryo was approximately 0.45 to 0.50 mm with no substantial change in the morphology indicating differentiation of embryonic plant parts. No exception of atypical development of hybrid embryos was found. (For the development of intraspecific embryos, see Von Wettstein (1926)).

In *P. vulgaris* x *P. acutifolius* matings (Table 2), the growth of hybrid embryos was also affected by the maternal parent. However, the effect was apparent only within 15 days after pollination. The growth rate of hybrid embryos was higher when 'G50' was the female parent. After 24 days, however, embryos developing on 'G50' and 'GN' attained the same size. The male parents did not seem to differ in their influence on embryo growth.

Crosses between *P. acutifolius* (female) and *P. vulgaris* (male) were successful (Table 3). The genotype of the pollen appeared to affect the later development of the hybrid embryos; the final size of hybrid embryos attained with 'G50' as the male parent was larger. However, the early growth rates (within 15 days after pollination) of embryos obtained from the two crosses were not significantly different.

Embryos derived from crosses between *P. vulgaris* and *P. acutifolius* appeared to develop normally until late heart-shape or early cotyledon stage. The distinct morphological feature detected was the uneven growth of the two sides or of the young cotyledons (Fig. 1b). This characteristic was accentuated during further growth and at 12 days after pollination, one cotyledon expanded to approximately twice the size of its counterpart. Furthermore, the cotyledons were open rather than closely adjoining each other. These characteristics were observed on all six crosses between *P. vulgaris* and *P. acutifolius* and served as markers in distinguishing hybrid embryos from selfed embryos (Fig. 1c).

The effects of glutamine and gibberellin in supporting the growth of immature embryos in culture were tested. The embryos were classified into two groups according to size: larger or smaller than 0.3 mm for *P. vulgaris* x *P. lunatus* embryos and larger or smaller than 1 mm for *P. vulgaris* x *P. acutifolius* (and reciprocal) embryos. The survival rates of embryos after a culture period of four weeks are presented in Tables 4, 5 and 6. The addition of glutamine to the culture medium had a beneficial effect on hybrid embryo survival. This effect was most pronounced on the very small (<0.3 mm) embryos of 'GN' x 'K' and 'G50' x 'K', since none of the hybrid embryos survived on medium devoid of glutamine. However, the survival rate of the very small (<0.3 mm) 'GN' x 'B' and 'G50' x 'B' embryos was extremely low regardless of the addition of glutamine. Enhancement of the survival rate was also observed on the larger (>0.3 mm) embryos of 'GN' x 'K' and 'G50' x 'K' as well as on the smaller (<1 mm) *P. vulgaris* x *P. acutifolius* embryos. The larger >1 mm) embryos of the latter cross survived at an equally high rate on medium with or without glutamine.

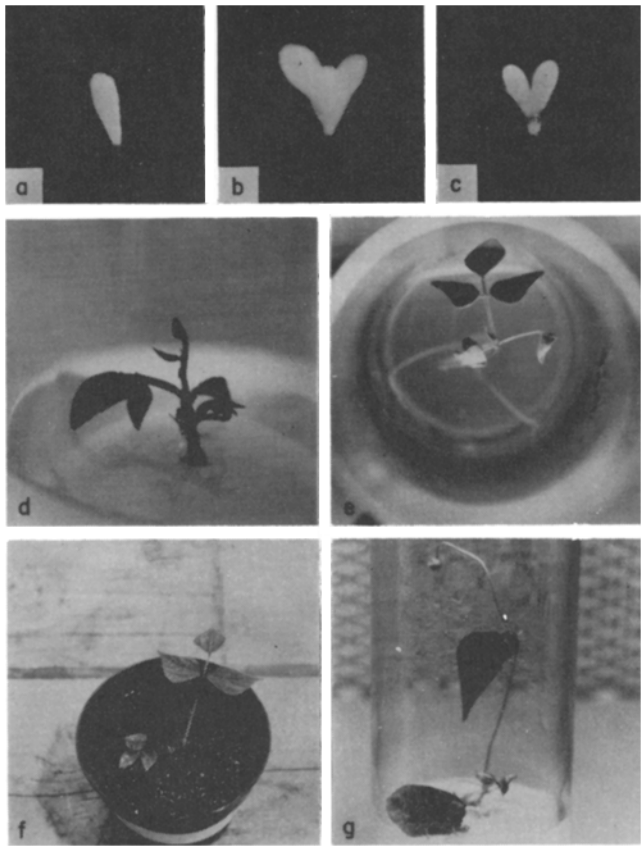

Fig. 1. Embryos of inter- and intra-specific hybridization and plantlets obtained from cultured hybrid embryos. a) Hybrid embryo of 'G50' X 'K', 15 days after pollination (80 X). b) Hybrid embryo of 'GN' X 'AC 1', 12 days after pollination (80 X). c) Selfed embryo of 'G50', 12 days after pollination (40X). d) Plantlet derived from cultured embryo of 'G50' X 'K' (45 days). e) Plantlet derived from cultured embryo of 'GN' X 'AC 1' (42 days). f) Plantlet derived from cultured embryo of 'G50' X 'K' transplanted after 72 days. g) Plantlet derived from cultured embryo of 'AC 2' X 'G50' (60 days)

Table 4. The effects of glutamine and gibberellin on the survival rate of hybrid embryos obtained from *P. vulgaris* × *P. lunatus* crosses

Cross	Embryo size (mm)	Survival Rate				
		Control	Glutamine	(mg/l)	Gibberellin + Glutamine	(mg/l)
			10	100	10	100
'GN' × 'K'	< 0.3	0% (33)[1]	17% (60)	21% (70)	16% (67)	21% (68)
	0.3–0.5	5% (21)	30% (23)	35% (31)	32% (31)	35% (31)
'GN' × 'B'	< 0.3	0% (21)	0% (27)	4% (25)	0% (25)	4% (26)
'G50' × 'K'	< 0.3	0% (14)	16% (32)	26% (31)	14% (35)	24% (38)
	0.3–0.5	11% (18)	29% (17)	50% (18)	35% (17)	56% (18)
'G50' × 'B'	< 0.3	0% (12)	3% (34)	5% (37)	3% (34)	5% (37)

[1] Number of embryos cultured

Table 5. The effects of glutamine and gibberellin on the survival rate of hybrid embryos obtained from *P. vulgaris* × *P. acutifolius* crosses

Cross	Embryo size (mm)	Survival Rate				
		Control	Glutamine	(mg/l)	Gibberellin + Glutamine	(mg/l)
			10	100	10	100
'GN' × 'AC 1'	< 1	14% (22)	30% (23)	33% (21)	30% (23)	28% (25)
	1–4	45% (49)	50% (38)	50% (34)	56% (36)	59% (29)
'GN' × 'AC 2'	< 1	13% (24)	24% (25)	29% (21)	28% (25)	24% (21)
	1–4	47% (15)	43% (51)	50% (40)	44% (52)	58% (33)
'G50' × 'AC 1'	< 1	7% (14)	27% (11)	27% (11)	25% (12)	25% (12)
	1–4	50% (10)	64% (11)	58% (12)	75% (12)	69% (13)
'G50' × 'AC 2'	< 1	13% (8)	31% (16)	14% (14)	21% (14)	13% (16)
	1–4	50% (14)	50% (18)	52% (21)	60% (15)	63% (19)

Table 6. The effects of glutamine and gibberellin on the survival rate of hybrid embryos obtained from *P. acutifolius* × *P. vulgaris* crosses

Cross	Embryo size (mm)	Survival Rate				
		Control	Glutamine	(mg/l)	Gibberellin + Glutamine	(mg/l)
			10	100	10	100
'AC 2' × 'GN'	< 1	0% (6)[1]	40% (15)	20% (5)	20% (5)	25% (4)
	1–4	50% (8)	43% (7)	50% (6)	33% (6)	43% (7)
'AC 2' × 'G50'	< 1	14% (17)	25% (4)	25% (4)	25% (4)	25% (4)
	1–4	44% (9)	37% (8)	57% (7)	50% (8)	43% (7)

[1] Number of embryos cultured

The addition of gibberellin did not enhance the survival of hybrid embryos, however gibberellin seemed to hasten the greening of surviving embryos and the seedlings subsequently obtained elongated at a faster rate.

Plantlets obtained from embryo cultures (Figs. 1d, 1e and 1g) were transplanted into Jiffi Mix Plus when they were approximately 10 cm in height. At present, six plantlets of *P. vulgaris* x *P. lunatus*, four of *P. acutifolius* x *P. vulgaris* and one of *P. acutifolius* x *P. vulgaris* have been obtained. They grew vigorously for two weeks but subsequent growth was extremely slow and no new development of vegetative parts was observed in 60 days.

Discussion

The abnormal development of hybrid embryos obtained from *P. vulgaris* x *P. lunatus*, *P. vulgaris* x *P. acutifolius* and *P. acutifolius* x *P. vulgaris* crosses precluded the recovery of mature hybrid seeds and the culturing of immature embryos on artificial medium appeared to be necessary. The formation of mature seeds in crosses between *P. vulgaris* and *P. lunatus* (Honma and Heeckt 1959) may be an exception. Since the parents involved in those crosses were intraspecific hybrids, specific genetic combinations could have resulted in the formation of interspecific hybrids that were capable of normal development.

The reciprocal crosses (*P. lunatus* x *P. vulgaris*) resulted in very early abortion of embryos which appears to be in agreement with previous reports (Smartt, 1970). However, the development of *P. vulgaris* x *P. acutifolius* and *P. acutifolius* x *P. vulgaris* embryos were not found to be different, although the final size of the embryos of 'GN x AC 2' was slightly larger than of those obtained from the reciprocal cross.

The atypical morphologies of hybrid embryos facilitated the initial identification of hybrids during excision. Since most genetic markers in *Phaseolus* can only be observed on mature plants or seedlings, the availability of morphological traits which can be recognized at early stages is essential. It is not certain at this time whether the embryonic morphologies are intrinsic characteristics of hybrid embryos obtained from all *P. vulgaris* x *P. lunatus* and/or *P. vulgaris* x *P. acutifolius* crosses, or are limited to certain genotypic combinations.

The addition of glutamine to the medium was clearly beneficial for the survival of *P. vulgaris* x *P. lunatus* embryos, however its effect on larger *P. vulgaris* x *P. acutifolius* embryos was less apparent. It appears that a more advanced stage of development has been attained by these larger embryos.

It was reported that *P. coccineus* embryos before or at the heart-shape stage require the association of the suspensor for normal development in culture, while more mature embryos did not have such requirements (Cionini et al. 1976). The addition of gibberellin to the culture medium could replace the need of the young embryos for the suspensor. However, the addition of gibberellin to the medium in the present study did not enhance the survival rate of immature embryos. It is of particular interest in this aspect to note that hybrid embryos obtained from *P. vulgaris* x *P. lunatus* crosses did not have a suspensor or suspensor-like structure and yet gibberellin was not essential for further growth.

For the purpose of generating interspecific hybrids, the genotypes of the parents appear to be essential. The much higher growth rate of embryos developing on 'G50' also suggests the relative importance of the maternal parent. As the effectiveness of the present medium varied with genotypic combinations, nutrient requirements of immature embryos may be genotype specific. It is possible that other particular combinations of genotypes are capable of supporting even further development of hybrid embryos than those reported here.

The optimal environment for the survival of plantlets in non-sterile conditions has not been extensively evaluated. However, a limited number of plantlets derived from selfed embryos of 'GN' and 'K' have been used for initial tests. Under misting conditions, nine of 12 and 10 of 14 of the selfed plantlets of 'GN' and 'K', respectively, survived to maturity. It appears that favorable environmental conditions can be designed to optimize the survival of hybrid plantlets as well. The retarded growth of the 11 hybrid plants obtained thus far suggests that terminal development could result from particular genotypic combinations. However, since hybrid embryo development appears to be greatly affected by the parental genotypes, it is conceivable that selection of particular crosses can result in interspecific hybrids which are capable of normal development.

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