Interspecific Hybridization of *Phaseolus vulgaris, P. acutifolius* and *P. lunatus* Using *in vitro* Technique[1]

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ABSTRACT

Hybrid embryos from the interspecific crosses between *Phaseolus vulgaris* x *P. acutifolius* and *P. vulgaris* x *P. lunatus* were rescued using a culture medium developed at our laboratory. From 44 hybrid embryos, eight differentiated into normal intact plants. Use of White's nutrient before pollination did not increase fertility in the interspecific crosses. The segregant material line F_2, from *P. lunatus*, obtained in the intraspecific cross between cultivars Fava Branca x Fava Pintada, led to a hybrid plant between *P. vulgaris* x *P. lunatus*. Morphological characteristics, such as leaf type and root system, peculiar to the *P. acutifolius* species, were observed in the hybrid from the cross *P. vulgaris* x *P. acutifolius*.

RESUMO

Embriões híbridos provenientes de cruzamentos interespecíficos entre *Phaseolus vulgaris* x *Phaseolus acutifolius* e *Phaseolus vulgaris* x *Phaseolus lunatus* foram inoculados em meio de cultura estabelecido em nossos laboratórios. De 44 embriões híbridos inoculados, 8 se diferenciaram para gerar plântulas intactas ou malformadas. Observou-se que o emprego da solução nutritiva de White antes das polinizações, aplicada diretamente à superfície do estigma da flor utilizada como fêmea, não aumentou a fertilidade nos cruzamentos interespecíficos. O emprego de material segregante (linha de geração F_2, obtida do cruzamento intra-específico entre as cultivares Fava Branca x Fava Pintada) da espécie *P. lunatus*, possibilitou a obtenção de uma planta híbrida entre *P. vulgaris* x *P. lunatus*. Características morfológicas como o tipo de folha e do sistema radicular, peculiar a espécie *P. acutifolius*, foram observadas no híbrido proveniente do cruzamento entre *P. vulgaris* x *P. acutifolius*.

INTRODUCTION

Mendel (7) was the first to obtain successful interspecific hybridization in the bean *Phaseolus*. He described hybrids between *P. vulgaris* x *P. coccineus* and the segregation in the F_2 generation. Recently, achievements in hybridization within the genus *Phaseolus* have been reviewed (8).

P. vulgaris has great importance as a staple food in several regions of the world, but some regions, such as semi-arid northeastern Brazil, are not suitable for growing this crop due to adverse climatic conditions. Other species, such as *P. lunatus* and *P. acutifolius*, can be grown in those regions since they tolerate high temperatures and water stress, but their commercial value is much lower than that of *P. vulgaris*. *P. lunatus* and *P. acutifolius* are important species to be used in interspecific crosses with *P. vulgaris* in order to obtain stress-tolerant germplasm. Embryo-culture techniques have been used to obtain fertile allopolyploids from the cross *P. acutifolius* x *P. vulgaris* (14). The same approach has been used to obtain fertile interspecific hybrids from the same cross (17). More recently, *in vitro* embryo culture has also been used to obtain interspecific hybrids in the cross *P. vulgaris* x *P. angustissimus* (4).

The present study reports that interspecific hybrids between *P. vulgaris* x *P. acutifolius* and *P. vulgaris* x *P. lunatus* have been obtained using the embryo rescue approach.

MATERIALS AND METHODS

Three bean species were used: *P. vulgaris*, *P. acutifolius* and *P. lunatus*. The following cultivars of each species were used: *P. vulgaris*, cvs. Gordo, Goiano Precoce, Canario 101, Oax-62 and California "small white," and the Carioca Precoce 1070 line (mutant, obtained through gamma radiation, selected at M_4 generation); *P. acutifolius*: cvs. G-40018, G-40035, G-40064, G-40002, Tepari Branco, Tepari Roxo; *P. lunatus*: cvs. IPA-1, G-25143 and "Line F_2" generation (from the cross Fava Branca x Fava Pintada).

Pollination, conducted in the greenhouse, followed techniques described by Buishand (3) and Honma (5)

1 Received for publication 3 May 1988.
The authors acknowledge the Commission of the European Economic Community for the grant through Contract no. 33 007 TSD 222 and FINEP/Rio/Brasil through Contract no. 42.87.0411.00.

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with some modifications. The cultivars of *P. vulgaris* were used as female (♀) and the cultivars of *P. acutifolius* and *P. lunatus* as male (♂).

A group of 127 flowers received White's nutrient solution (9) on the surface of the emasculated flower. Another group of 50 flowers was pollinated without White's nutrient solution.

Intact immature embryos excised from hybrid seeds at several stages of development inside the pods were inoculated in the following culture medium: a) mineral salts of Murashige and Skoog (13); sucrose, 30 g/l; enzyme hydrolysed casein, 2 g/l; mio-inositol, 100 mg/l; thiamine HCl, 8 mg/l; nicotinic acid, 5 mg/l; pyridoxine HCl, 0.6 mg/l; niacinamide, 1.25 g/l; glutamine, 25 mg/l; pH 5.6.

Pods were disinfected by immersion in 95% ethanol for 1min., followed by immersion in a 20% hypochlorite commercial solution, for 5 min., then rinsed twice in sterilized de-ionised water. They were then aseptically dissected under the stereomicroscope and intact embryos without maternal teguments were inoculated in the culture medium. The embryo culture was conducted in a growth cabinet under 25°/21°C (day/night), light intensity 54.00 $w\,m^{-2}$, with a 16h/8h photoperiod.

Plantlets were transferred either to sterile solid substrate made of organic matter, vermiculite, and quartz sand sphagnum (3: 1/2: 2/3: 2/3), or to Hoagland and Arnon (2) nutrient solution diluted to 50%.

RESULTS AND DISCUSSION

The most favorable results, due either to the number of interespecific embryo hybrids obtained without White's solution or to the number of hybrid intact plants, were obtained with *P. vulgaris* cvs. Gordo, Goiano Precoce and the mutant line Carioca Precoce 1070. The best cultivars of *P. acutifolius* and *P. lunatus* were G-40035, G-40002 and Line F_2. The latter, from *P. lunatus*, was the best as far as interspecific hybrid plant production is concerned when crossed with cv. Goiano Precoce of *P. vulgaris*. Genetic differences between *P. vulgaris* and *P. lunatus* species, which is a handicap in obtaining interspecific hybrids, have been reported (10, 15). The hybrid plant obtained in the present work was probably due to the fact that a segregating material was used (line F_2 generation) as male, introducing a wider genetic variability than a pure cultivar. In the same type of cross, *P. vulgaris* x *P. lunatus*, the best results were obtained when segregant lines were used (7).

The use of White's nutrient solution did not provide the same results as those obtained in the *P. coccineus* x *P. vulgaris* cross (9). When White's solution was used, the number of pods was higher and the number of embryos smaller. Fig. 1 shows that crosses between California x G-40035, Carioca Precoce x G-40035 and Goiano Precoce x Line F_2 resulted in seven, 10 and eight embryos in four, three and two pods, respectively. Out of 127 flowers which received White's nutrient solution, only 58 pods and six embryos were obtained. On the other hand, out of 50 flowers which did not receive White's solution, 29 pods and 50 hybrid embryos were obtained.

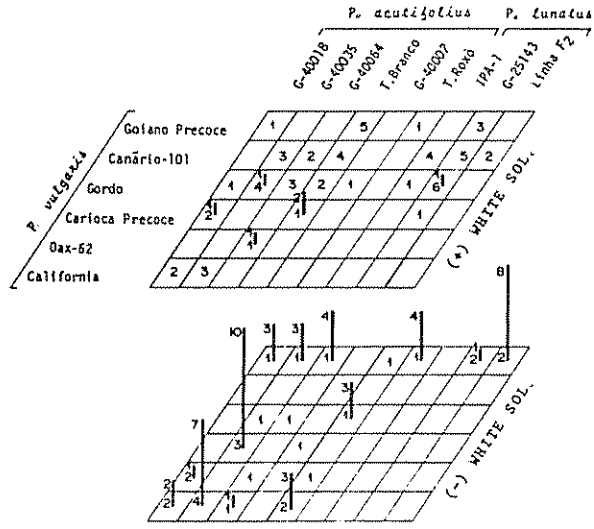

Fig. 1 Effect of White's nutrient solution in obtaining interspecific hybrids between *P. vulgaris* x *P. acutifolius* and *P. vulgaris* x *P. lunatus*. number at bottom bar = pod number; number at top bar = number of hybrid embryos; absence of bar = absence of hybrid embryos; number without bar = pod number.

Embryos from *P. vulgaris* x *P. lunatus* crosses were less developed, abnormal and at the pre-heart-shape stage, as described by other authors (12). However, some of the embryos from *P. vulgaris* x *P. acutifolius* presented two asymetric cotyledons and reached cotyledonary development. Nevertheless, the seed did not mature.

As reported in the literature (17), a great variation was noted in the number of cotyledons in the embryos from *P. vulgaris* x *P. acutifolius* crosses: presence or absence of three abnormal cotyledons. The most vigorous embryos came from Goiano Precoce x T. Roxo, Goiano Precoce x G-40035, Goiano Precoce x G-40018, California x G-40035, Canario 101 x G-40002 crosses, and some other combinations. The immature embryos excised and inoculated 18 days after pollination (18 DAP) did not differentiate in culture. However, embryos of the same age (18-20 DAP), but at the first stage of cotyledonary development (globular), presented a higher rate of differen-

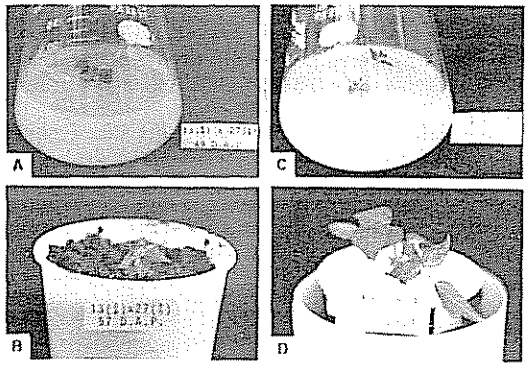

Fig 2 Hybrid plantlet between *P. vulgaris* x *P. lunatus* originated from the cross cv. Goiano Precoce x Line F_2 (2 A, B), with 49 DAP (2A) and 57 DAP (2B); hybrid plantlet between *P. vulgaris* x *P. acutifolius* originated from the cross cv. Gordo x cv. G-40002 (2 C, D), with 70 DAP (2C) and 89 DAP (2D)

tiation, regenerating intact hybrid plants. A possible explanation is that the embryo axis of more developed (torpedo stage) immature embryos utilize the nutritional reserves already existing in the attached cotyledons for their differentiation. A greater contact surface between the embryo axis and the cotyledons, rather than the culture medium, is therefore established. Biochemical processes leading to toxic products would interfere in the relationship embryo axis/cotyledons/culture medium, thereby turning off differentiation and development of hybrid plants. This hypothesis is supported by the findings of other authors (1).

Out of 44 hybrid embryos, from pollination without White's solution, eight differentiated into intact plants. These embryos came from crosses between Carioca Precoce x G-40035, California x G-40002, California x G-40035, Goiano Precoce x Line F_2 and Gordo x G-40002. Fig. 2 shows the hybrid plants obtained in the latter two crosses.

The plantlet shown in Fig. 2A originated from a *P. vulgaris* x *P. lunatus* cross and was transplanted to solid substrate (Fig. 2B). Fig. 2 (C, D) shows the hybrid plantlet from a *P. vulgaris* x *P. acutifolius* cross at different stages of development in different media. This plantlet was transplanted directly from the culture medium to a Hoagland and Arnon (2) nutrient solution diluted to 50%. Thirteen days after transference, the plantlet showed vigorous growth and new leaves were formed, developing morphological characteristics of *P. acutifolius*, such as leaf shape, tendency to bend and petiole type, as has been observed previously (6) in the F_1 generation of hybrids from *P. vulgaris* x *P. acutifolius*, mainly in the leaf shape of young plants. Predominance of *P. acutifolius* in the F_1 generation of such crosses has already been reported (16, 17). The leaves of the hybrid had a rough surface and virus symptoms, also observed by other authors in interspecific crosses between *Phaseolus* species (1, 14). During its development, the plantlet (Fig. 2D) showed characteristics of both parents in the shoot, such as foliar morphology, which had also been observed in previous reports (6). This plant was sterile and did not flower. Indeed the problem of sterility among hybrids in the F_1 generation is a limiting factor in the transference of germplasm among these species (16). This could be overcome either through backcrossing or by the production of allotetraploids through treatment with colchicine (14). Doubling of the chromosome stock in embryo hybrids from *P. vulgaris* x *P. angustissimus* by applying colchicine on embryos 26 hours after planting has recently been reported (14).

Fig. 3 shows the root system of the hybrid plant shown in Fig. 2D. It is a long system, characteristic of *P. acutifolius*, and is not observed in the cultivar Gordo (*P. vulgaris*) used as a pollen source in the cross. These results emphasize the possibility of transferring the genetic character of a long root system from *P. acutifolius* to *P. vulgaris*. The latter would then have higher water absorption efficiency and would therefore be less vulnerable to climatic adversities.

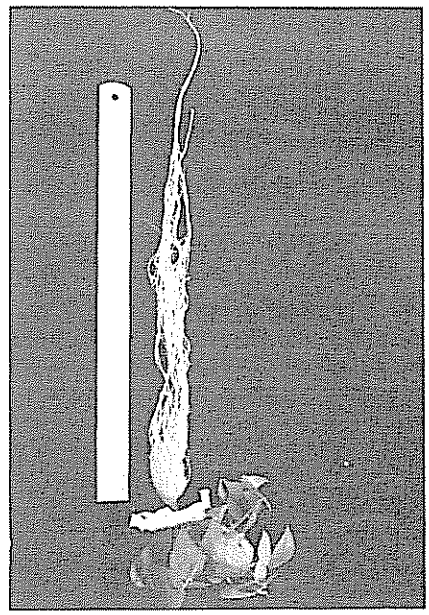

Fig 3 Hybrid intact plant between *P. vulgaris* x *P. acutifolius* (see Fig. 2 C, D) originated from the cross cv. Gordo x cv. G-40002 with, 115 DAP. The long root system, peculiar to the *P. acutifolius* species which has been transferred to the *P. vulgaris*, can be noted

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