

PRELIMINARY RESULTS OBTAINED IN INTERSPECIFIC CROSSES BETWEEN *PHASEOLUS VULGARIS* AND *PHASEOLUS ACUTIFOLIUS*

Veronica Dincă and Florentina Răducanu

ABSTRACT

Phaseolus vulgaris, as female, was crossed to *Phaseolus acutifolius* as male form, in order to diversify the starting breeding material in dry bean, particularly for drought resistance. Hybrid plants produced pods in different development stages, but although fecundation took place, embryo abortion occurred in early seed formation. For saving embryos before abortion embryo-rescue technique was used. Fifteen days aged embryos were extracted and inoculated under aseptic conditions on Murashige - Skoog (MS) medium without growth regulators. Immature embryo culture was kept at 17-18°C and continuous illumination. After 12 days, the seedlings with normal roots were transferred into a mixture of soil and sand, and maintained under controlled conditions.

Key words: dry bean, *Phaseolus vulgaris*, *Phaseolus acutifolius*, interspecific crosses, embryo culture.

INTRODUCTION

Drought and heat, frequently occurring in southern area of Romania, affect grain yield and quality of dry bean. Higher temperatures than 30°C, combined with a low relative air moisture occurring during flowering and seed setting, decrease to a large extent the fructification, lower number of pods/plant are produced, with small and shriveled seeds (Popa and Dincă, 1985).

Releasing drought tolerant varieties of dry bean is the only way to reduce the losses caused by drought and heat.

Since resistant forms are not known within the species *Phaseolus vulgaris*, obtaining of genetic variability of this trait could be achieved by interspecific crosses of agronomic superior varieties of *P. vulgaris* to *P. acutifolius*, a species adapted to arid areas (Ockendon, 1983). Hybrid plants obtained from such crosses bear usually pods in different development stages. (Dincă and Popa, 1984), some of them having a normal growth till maturity, but do not produce seeds.

Although fertilization takes place, embryo abortion occurs during seed development. Embryo culture is necessary to obtain normal and mature seeds.

MATERIALS AND METHODS

Three varieties and two breeding lines of *P. vulgaris* (Ami, Avans, Fundulea 332, F 88-1632 and F 88-1254) as female parents and *P. acutifolius* (P.I.440803) as male parent were crossed. The parents were grown under controlled biological requirements of bean conditions, in 51 pots, in a mixture of 40% soil, 30% fermented manure and 30% sand. Particular attention was paid at flowering and pollinating time, when temperature was maintained at 20-22°C during the day and 18°C at night and relative air moisture between 75-80%.

Pollination with mature pollen, freshly collected from the flowers of the male parent in the same day, was made for increasing the level of hybridization and to avoid the accidental selfing.

Fifteen days after pollination normally developed pods were harvested, sterilized with mercuric chloride (HgCl 1‰) for 10 minutes and finally washed with distilled water three times. Hybrid embryos were extracted from immature seeds contained by the pods, and cultured on MS medium (Murashige and Skoog, 1962) with 40 g saccharose/1 liter medium without growth regulators. After 7 days of incubation at 17-18°C and under permanent illumination conditions, embryos were transferred on liquid MS medium without NH₄NO₃, for root initiation. The normally developed seedlings were transferred and grown in pots with a 3:1 mixture of soil and sand, under controlled conditions, with 16h of light, at 20-22°C during the day and 16-18°C at night.

RESULTS AND DISCUSSIONS

Pods in different development stages resulted from crossing the five bean genotypes of *P. vulgaris* with *P. acutifolius*.

After 5-6 days from pollination, some pods stopped growing and aborted while others continued to grow and develop normally so that, after 15 days from pollination (Table 1), 38 pods were harvested from a total of 140 pollinations (27.1% hybridization efficiency). A number of 92 immature seeds were collected from 38 pods, and an equal number of embryos were extracted (Table 2).

Table 1. Results obtained with *P. vulgaris* x *P. acutifolius* crosses

Female	No. of pollinations	Results of the cross		% hybrids pods
		No. of 15 days pods	No. of immature seeds	
Ami	30	8	23	26.6
Avans	30	6	9	20.0
Fundulea 332	30	9	20	45.0
F 88-1632	30	5	15	16.7
F 88-1254	30	10	25	33.3
Total	140	38	92	27.1

Table 2. Efficiency of immature embryo culture in *P. vulgaris* x *P. acutifolius* crosses

Female	No. of inoculated embryos	No. of embryos germinated	% from total inoculated embryos	No. of seedlings transferred into soil
Ami	23	19	82.6	11
Avans	9	2	22.0	1
Fundulea 332	20	14	70.0	7
F 88-1632	15	9	60.0	3
F 88-1254	25	21	84.0	15
Total	92	65	70.6	37

From the total of 92 cultured immature embryos a number of 65 germinated (70.6% average efficiency). The largest efficiency (84%) was obtained when the line F. 88-1254 was used as female in crosses, while the lowest embryo germination efficiency was produced by variety Avans (22.2%).

After the transfer on the liquid medium, from 65 germinated embryos, 37 seedlings formed roots, were transferred into soil and grown under controlled conditions. The normal development of the seedlings continued only 5-6 days after their transfer into soil. After that period, the seedlings ceased to grow and develop normally and perished.

CONCLUSIONS

More investigation are needed for finding the causes leading to the perishing of seedlings after their transfer into soil. Further improvement of the procedure would result in an efficient method to be successfully used by bean breeders in *P. vulgaris* x *P. acutifolius* crosses.

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