Refinement of the media composition for the in vitro culture of early heart-shaped embryos in *Phaseolus vulgaris* .

V. SCHMIT , J.P. BAUDOIN and G. MERGEAI
Faculté des Sciences Agronomiques, Gembloux, BELGIUM

In *Phaseolus* , very few experiments have been carried out in the in vitro culture of the very young immature embryos. Breakthrough in this field will open the way to obtain hybrids between very distantly related species or between species showing unilateral or total incompatibility. In our laboratory, research works are undertaken to define a technique and media suitable for the 5-6 days old, pre-heart shaped embryos of various *Phaseolus* genotypes. First trials yielded promising results and indicated several trends (MERGEAI and BAUDOIN, 1990) :

- extraction and transfer of the young embryos to the culture medium in a high osmolarity environments (drop of water with 120 g/l sucrose and 1.75 g/l agar) allow a survival rate superior to 90 %,
- nitrogen level and form in the culture medium is critical, NH_4 being essential during the first developmental stage of the embryo,
- among the various tested media, Gamborg's medium appears to have a beneficial effect not to favour embryo growth initiation but to favour embryo growth up to a stage where the germination can be expressed; this can be due to its low ionic and NH_4 concentration, compared to the other media.

In our investigation, embryos are transferred to two distinct media before the hardening stage of the plantlets in soil substrate :

- a first medium aimed at promoting embryo growth, up to the development of apical buds and leaf primordia,
- a second medium aimed at stimulating both shoot and root development.

Progress were made to refine the two media with embryos of 3 *Phaseolus vulgaris* genotypes : NI 637 (a Brazilian variety used as a check), G 12582 (a Colombian variety) and Anc 182 (a Peruvian variety). Donor plants are cultivated in growth cells with 26°C temperature, 12 h day light and 60 % relative humidity.

Refinement in the first medium (growth initiation)

We studied the influence of 4 mineral salts compositions : Murashige and Skoog, Gamborg, L_6 and Yeung, supplemented with various concentrations of amino acids (from 100 to 1000 mg/l L-glutamine and casein hydrolysate) and NH_4NO_3 (from 0 to 7.5 mM/l). Each medium contained vitamins (1 mg/l thiamin HCl, 7.5 mg/l nicotinic acid, 0.5 mg/l pyridoxin HCl, 100 mg/l myoinositol), growth regulators (0.028 mg/l N_6 benzyladenin) and sugar (30 g/l sucrose). Culture media were dispensed into petri dishes placed in a growth chamber with no light and under 26°C temperature. Table 1 gives the results of one of the most significant trials: it was implemented to compare the effect of the 4 media, each supplemented with 1000 mg/l a.a. and 5 mM/l NH_4NO_3, on the embryos growth of NI 637.

Table 1. Influence of the 4 media on the embryos growth of NI 637.

Media	Nb of tested embryos	Nb of cankered embryos (%)	Nb of embryos showing growth initiation
Murashige & Skoog	117	1 (0.9)	44 (37.6)
Gamborg	108	3 (2.8)	65 (60.2)
L_6	116	7 (6.1)	65 (56.5)
Yeung	119	4 (3.4)	45 (37.8)

We observed from 37 to 61 % of embryos which remained green but did not show apical buds, leaf primordia or both of them; no plantlets could be regenerated from these embryos. The results indicated the best performance of Gamborg's medium and this was confirmed in other trials. In order to assess any genotypic response, we examined the effect of this medium on the

embryos growth of three *P. vulgaris* genotypes : out of 200 excised embryos, we observed 64.6 %, 57.5 % and 70.3 % growing embryos respectively for G 12582, Anc 182 and the check NI 637. Gamborg's medium supplemented with 1000 mg/l amino acids and 5 mM/l NH_4NO_3 appears to be well suited for other genotypes of the common bean.

Refinement in the second medium (shoot and root development)

For this second stage of in vitro culture, the embryos were transferred into media contained in glass tubes and the latter were placed in a growth chamber at 26°C temperature and with a photoperiod of 16 h (day)/8 h (night). In a first set of trials, we did not find any influence of the composition of the 4 media (growth initiation) on the subsequent behaviour of the growing embryos. The Gamborg's mineral salts composition yielded the best results to promote shoot and root development, provided it was supplied with a moderate amino acids content. We report in table 2 the results obtained from one trial; the embryos belong to the variety NI 637 and two major treatments are compared :

- one half of the normal Gamborg's medium, with 100 mg/l amino acids (L-glutamine and casein hydrolysate), with 0.028 mg/l N_6 benzyladenin, without myoinositol and with 30 g/l sucrose.
- normal Gamborg's medium, with 100 mg/l L-glutamine, with 100 mg/l myoinositol, without both casein hydrolysate and growth regulators and with 30 g/l sucrose.

Table 2. Influence of 2 media on the shoot and root development of embryos NI 637.

Media	Nb of embryos transferred in glass tubes	Nb of regenareted plantlets (%)
1/2 Gamborg's salts	138	13 (9.4)
normal Gamborg's salts	145	49 (33.8)

The normal salts composition of Gamborg allowed to regenerate the highest number of plantlets : the latter showed well developed leaves and a good rooting system after the in vitro culture in the second medium. The seedlings were hardened in jiffy pots containing 1 coarse vermiculite - 1 sand - 1 mould and watered regularly with one quarter of the Murashige and Skoog solution. The plants were covered by a small plastic glass and placed in a growth chamber, with 24°C temperature and 12 h day light. This step in the embryoculture process remains however the most critical : we did not always obtain the same percentage of regenerated plantlets in other experiments repeated during the year; success depended also very much upon the genotypes used in the trials : in one experiment, G 12582 and Anc 182 performed better than the control variety NI 637.

Prospects

We are investigating the response of other *Phaseolus* materials, such as *P. lunatus* , *P. coccineus* and *P. polyanthus* to the in vitro culture of pre-heart shaped embryos. It is also intended to test various combinations of amino acids, NH_4NO_3, growth regulators and myoinositol on both the initiation of embryons growth and the shoot and root development. Major focus will be given to the refinement of the second medium.

References

MERGEAI G. and BAUDOIN J.P. (1990). Bean Improvement Cooperative. Annual Report : 115-116.