Contribution for improved *in vitro* culture of globular and early heart-shaped embryos in *Phaseolus*

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In the interspecific crosses between *Phaseolus polyanthus* (or *P. coccineus*) as female parent and *P. vulgaris*, the hybrid embryos abort few days after pollination at the globular or early heart-shaped stages. It is therefore essential to develop an *in vitro* culture technique that enables the rescue of very immature embryos. In our laboratory, several trials are conducted since 1990 to test different mineral salt compositions, sugar rates, amino acid contents and physical conditions of culture (Mergeai & Baudoin, 1990; Schmit et *al.*, 1991 and Mergeai et *al.*, 1995). On the basis of our first results, we have developed a technique made of three major steps :

- first, extraction and transfer of the embryos to the culture medium in a high osmolarity liquid environment (containing 120 g/l sucrose and 1.75 g/l agar);
- second, culture of embryos under darkness until germination on a "maturation-germination" medium with the following composition : Gamborg et *al.* (1968) mineral salts, 5 mM/l NH_4NO_3, 1 g/l L-glutamin, 1 g/l casein hydrolysat, 1 mg/l thiamin HCl, 0.5 mg/l pyridoxin, 5 mg/l nicotinic acid, 0.028 mg/l BA, 8 g/l Difco agar and 30 g/l saccharose;
- third, transfer of germinated embryos under light in a second "rooting" medium made of the same mineral salt composition but without NH_4NO_3 and growth regulator and a lower L-glutamin and casein hydrolysat content (100 mg/l).

Application of this technique have yielded good results in the embryoculture of various *P. vulgaris*, *P. coccineus* and *P. polyanthus* genotypes, using 5-6 days old early heart-shaped embryos.

New investigations have been carried on to improve media and techniques for *in vitro* culture of 4-5 days old embryos not only from pure *Phaseolus* genotypes but also from interspecific *Phaseolus* hybrids. Priorities have been given to extraction and "maturation-germination" media, two critical stages of the process.

Table 1 indicates factors which have provided in our experimental conditions significantly increased rates of success in plant regeneration from globular and early heart-shaped embryos.

Concerning the early heart-shaped embryos, the addition of 50 mg/l ascorbic acid in the extraction liquid and of 100 ml/l pod extracts and 0.03 mg/l gibberellic acid in the "maturation-germination" medium have contributed to increase significantly the percent of germinated embryos. Compared to the previous techniques, these modifications have given a 40 % plant regeneration rate (instead of 30 %) after transferring the germinated embryos to the classical "rooting" medium and thereafter the explants to a growth chamber (with high relative humidity) for the hardening phase (Mergeai et *al.*, 1995). The new technique has been applied successfully on early heart-shaped hybrid embryos from the two interspecific combinations : *P. vulgaris x P. coccineus* and *P. vulgaris x P. polyanthus*.

Concerning the globular embryos, a higher germination rate has required the removal of nicotinic acid from the first *in vitro* medium, the addition of 200 ml/l pod extracts, 10 g/l maltose and 3 mg/l gibberellic acid, a cytokinin/auxin ratio of 3, 20 g/l saccharose content and the maintainance of an osmotic pressure ranging from 0.1 et 0.2 osmoles during the maturation process of the young immature embryos. The application of the whole set of modifications has allowed us to obtain for the first time in *P. vulgaris* the regeneration of adult plants from 4 days old globular embryos.

Our results have also evidenced a very significant change in the physiological requirements of the embryos during their maturation and subsequent development (data not shown). This is particularly obvious for the requirements related with growth regulators, vitamins and osmotic pressure between the globular and heart-shaped stages. In order to improve considerably the success rate of *in vitro* globular embryo culture, current investigations are

directed to the definition of a medium with "variable composition" : the latter would provide gradual changes in osmotic pressure, vitamins, growth regulators and mineral salts in relation with the different physiological stages of the embryos.

Table 1. Factors increasing rates of success in plant regeneration from immature embryos (compared to previous technique and media)

Embryos stages	
globular	early heart-shaped
Extraction Solution	
addition 50 mg/l ascorbic acid	addition 50 mg/l ascorbic acid
Maturation-Germination Medium	
reduction in nicotinic acid content	no reduction in nicotinic acid
addition of 200 ml/l pod extracts	addition of 100 ml/l pod extracts
addition of 3 mg/l GA3	addition of 0.03 mg/l GA3
cytokinin/auxin ratio of 3	cytokinin/auxin ratio of 3
20 g/l saccharose	30 g/l saccharose
10 g/l maltose	--------
0.1 to 0.2 osmoles (osmotic pressure adjusted by mannitol addition)	0.3 osmoles (osmotic pressure)

References

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