Development of an in vitro culture method for heart-shaped embryo in Phaseolus vulgaris

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Introduction

Since in Phaseolus the frequent failure of interspecific embryo growth in situ is due to incompatibility between the embryo and the endosperm and since this abortion occurs precociously in hybrids between distantly related species, it is mostly important to define a technique and a medium suitable for the very young hybrid embryos. We focus our research on two main objectives :

- The improvement of the techniques of extraction and transfer to the culture medium of very small embryos (5 to 6 days old after pod setting with a size varying between 0,25 and 0,65 mm).
- The elaboration of a culture medium especially well-adapted for in-vitro culture of young immature embryos in the genus Phaseolus.

In a first step all trials on in-vitro culture have been made with embryos of the Phaseolus vulgaris genotype NI 637, a Brazilian variety commonly known as Bico de Ouro.

Results :

After the cultivation of more than 750 embryos under various conditions of in-vitro culture, we developed a method allowing a success rate of more than 35 % from heart-shaped embryo to hardened plant. This method consists of the following stages.

a) The pods are removed from the plants 5 to 6 days after fruit setting. Collected pods are surface-sterilized in 70 % ethanol for 60 seconds, and then immersed in 5 % calcium hypochlorite for 5 minutes and rinsed in sterile disionized water. Under a laminar-flow transfer hood, ovules are extracted from the pod and placed on a sterile petri dishes in a drop of sterile distillated water solution containing 120 g/l sucrose and 1,75 g/l agar.

The embryos are excised under a stereo dissecting microscope. The transfer of the excised embryos to the culture medium is made in the same liquid environment with the aid of a "pasteur pipette" whose top had been fitted to the size of the embryo. After being aspirated, the embryo is blown out from the pasteur pipette with a drop of transfer solution on the surface of a petri dishes containing the culture medium. This system allows the maintenance of the embryo in a high osmolarity environment similar to the high osmolarity conditions that prevail in the endosperm of the ovule. Both the extraction and the transfer techniques of the embryos in a liquid environment allow a survival rate superior to 90 % .

b)the young immature embryos are laid down in a first culture medium which is aimed at favoring the germination. Its composition is as follows :

- Salts of Murashige and Skoog (1962)
- Vitamins : 1 mg/l Thiamin HCL, 5 mg/l nicotinic acid, 0,5 mg/l pyridoxin HCL, 100 mg/l Myoinositol
- Amino acids : 1000 mg/l L-glutamine, 1000 mg/l caséine hydrolysate
- Growth regulators : 0,028 mg/l de N6 Benzyladenin
- Sugar : 30 g/l of sucrose

For the germination of the embryos, the culture medium is dispensed into sterile polyethylene petri dishes. Up to the germination stage (usually one week after transfer), the embryos are placed in a growth chamber with no light and at 26 degree Celsius. Around 60 % of the embryos placed on this culture medium germinate normally.

c) After the phase of germination, the embryos are transferred into 20 x 150 mm borosilicate-glass culture tubes containing 20 ml of a second culture medium. The latter is designed to favor the rooting of the embryos and is composed of the following elements:

- One half of the normal Gamborg et al (1968) salts composition
- Vitamins : 1 mg/l Thiamin HCL, 5 mg/l nicotinic acid, 0,5 mg/l pyridoxin HCL
- Amino acids : 100 mg/l L-glutamine, 100 mg/l caséine hydrolysate
- Sugar : 30 g/l of sucrose

The tubes are placed in a growth chamber at 26 degree Celsius with 12 hours/day light (200 micro einstein /m2.s).

Almost all the germinated embryos placed on the rooting medium develop roots. After formation of the first true leaves, the plantlets with a good rooting system are hardened in jiffy pots containing 1 coarse vermiculite - 1 sand - 1 mould. The pots are placed into a closed glass box and watered with a solution containing one quarter of the normal Murashige and Skoog (1962) salts composition. During all the hardening period, the plants stay in a growth chamber with a day and night temperature of 24 degree Celsius and receive 12 hours continuous illumination a day. After three days, the cover of the glass box is progressively opened. With this method, more than 50 % of the germinated embryos give hardened plants.

Prospects

Investigations are being carried on to improve the rate of successful regeneration. NH_4^+ concentration plays probably a major role in promoting embryo development and germination. A better balance between this element and the other mineral components of the germination medium may allow more embryos to germinate normally.

References

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