First results on the development of an *in ovulo* culture technique of globular embryos in *Phaseolus*

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Introduction

In *Phaseolus*, hybrid embryos produced from crosses between remote species generally abort at the globular or early heart-shaped stages. It is therefore essential to develop an *in vitro* culture technique that allows the rescue of very immature embryos. *In ovulo* embryoculture offers interesting prospects to reach this objective. Indeed, this technique has been successfully used in different interspecific breeding programmes involving other major grain legumes such as groundnut (STALKER & EWEDA, 1988) and soybean (NEWELL & HYMOWITZ, 1982). Preliminary investigations have been carried out in Gembloux (DHERTE, 1995) in order to develop an efficient regeneration method adapted to this kind of explants. These researches concerned mainly the assessment of the main nutritive factors affecting the *in ovulo* development of globular zygotic embryos of *Phaseolus vulgaris*.

Material and method

The "maturation-germination" medium developed in Gembloux for the rescue of early heart-shaped embryos (MERGEAI *et al.*, 1995) constitutes the base of our investigations.

This control medium is made of : GAMBORG et *al.* (1968) mineral salts, 5 mM/L NH4NO3, 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 BAP, 8 g/L Difco Agar and 30 g/L sucrose. Growth regulators and vitamins are sterilised by ultrafiltration while the rest of the medium is autoclaved at 120°C for 20 minutes.

The following factors were tested : **1**. Sucrose content (2, 3, 4, 5, 6, 8, 10 %); **2**. Total mineral nitrogen content (25, 35, 45, 60, 75 mM/L with $[NH_4^+]/[NO_3^-] = 4$); **3**. $[NH_4^+]/[NO_3^-]$ ratio (1, 2, 3, 4, 5 with a total mineral nitrogen content = 35 mM/L); **4**. Kinetin content (0, 0.01, 0.1, 0.5, 1 mg/L).

The plant material is made of 4 days old *P. vulgaris* self pollinated ovules (NI 637 : variety Bico de Ouro from Brazil) extracted from pods produced in controlled conditions (day/night temperature : 24°C/20°C, light intensity : 325 µEinstein/m².sec, 60 to 70 % relative humidity).

After 10 days of *in ovulo* culture, the embryos were extracted from the ovules and transferred for germination on the same medium.

Results and discussion

A rather high proportion of immature embryos (30 to 50 %) have developed *in ovulo* and reached the heart-shaped stage (early or late) or even the early cotyledonary stage.

The sucrose content, the total mineral nitrogen concentration and the kinetin content are the major factors affecting the growth and the development of the embryos.

Figure 1 shows the effect of each of these factors on *in ovulo* development of *Phaseolus* globular embryos. The best results on *Phaseolus* embryos are obtained by increasing the content of these components to 60 g/L for sucrose, 60 mM/L for total mineral nitrogen and 1 mg/L for kinetin.

The $[NH_4^+]/[NO_3^-]$ ratio of the control medium (ratio = 4) gives the best results in terms of embryo development.

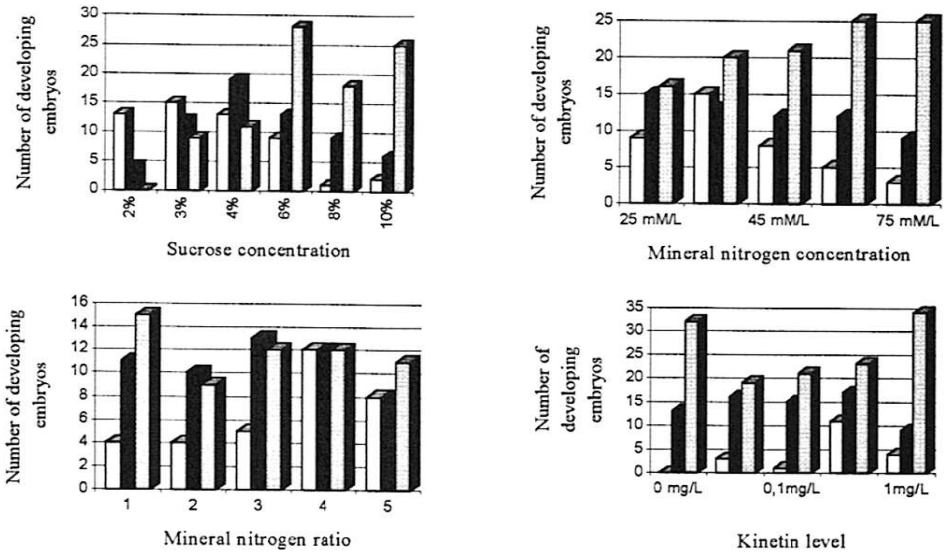

Figure 1: Effect of sucrose, mineral nitrogen, nitrogen ratio and kinetin on the *in ovulo* development of *P.vulgaris* embryos.

Complementary investigations are still needed to improve the development and the growth of the embryos after incubation in the ovule. On an average, about 5 % of the embryos germinated after extraction and only 1 % gave rise to an adult plant.

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