Development of an *in vitro* culture technique adapted to *Phaseolus polyanthus* and *P. vulgaris* embryos

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Introduction

Embryoculture is required to succeed some *Phaseolus* interspecific hybrids. It allows the rescue of aborting immature embryos and the regeneration of mature plants suitable for breeding work. A critical case study is the introgression of *Ascochyta* blight resistance from *P. polyanthus* into the common bean *P. vulgaris*. The best strategy is to use the donor parent (*P. polyanthus*) as maternal genotype in the interspecific hybridisation (Baudoin *et al.*, 1992). Unfortunately, the level of incompatibility barriers is very high when using *P. polyanthus* cytoplasm. In trials conducted in greenhouses, Lecomte (1997) observed that more than 60 % of the *P. polyanthus* (♀) x *P. vulgaris* embryos usually abort within eight days after pollination at the globular or early heart-shaped stages.

Material and method

Our investigations were based on the technique developed by Mergeai *et al.* (1997) for *in vitro* culture of early heart-shaped *Phaseolus* embryos. The following factors were tested : **1.** the genotype : four *P. polyanthus* cultivars (NI 429, NI 519, NI 553 and NI 1340) and one *P. vulgaris* variety (Bico de Ouro or NI 637); **2.** the mineral composition : salts of Gamborg *et al.* (1968) modified by Mergeai *et al.* (1997) and salts of Phillips *et al.* (1982), particularly rich in calcium and in minor salts; **3.** the organic and hormonal composition : suppressing N_6-benzylaminopurine (0.028 $mg.l^{-1}$) and adding 0.06 $mg.l^{-1}$ gibberellic acid (GA_3), 25 $\mu g.l^{-1}$ abscisic acid (ABA) and 200 $mg.l^{-1}$ calcium ($CaCl_2 \times 2H_2O$) as proposed by Lecomte (1997) and completing Gamborg salts with 500 $g.l^{-1}$ L-glutamine, 100 $mg.l^{-1}$ serine, 100 $mg.l^{-1}$ asparagine, 250 $mg.l^{-1}$ casein hydrolysate, 1 $\mu g.l^{-1}$ N_6-benzylaminopurine, 0.1 $\mu g.l^{-1}$ naphthalen acetic acid (NAA) and 0.01 $mg.l^{-1}$ adenine as proposed by Bodanese-Zanettini *et al.* (1996). Factors **1** and **2** were studied in a first trial and factor **3** was studied separately on single genotype NI 637.
The parameters assessing embryo growth and development after 10 days of *in vitro* culture were as follows : germination rate, survival rate or rate of embryos maintained in a survival state (i.e. presenting a white color but without growth and development), necrosis rate or rate of embryos presenting more than 50 % necrotic tissues and calli.

Results and discussion

Mergeai *et al.* medium (1997) and Phillips medium (1982) are designated respectively as G and P. Indices 1, 2 and 3 are used to represent respectively the original medium, G1 or P1, the medium

modified by Lecomte (1997), G2 or P2, and the medium modified by Bodanese-Zanettini *et al.* (1996), G3.

A high variation of the embryos response to *in vitro* culture was observed among the *P. polyanthus* genotypes (figure I). NI 429 surpasses all the other tested cultivars in terms of growth and development (50 % of germination for NI 429 compared to a mean germination rate of 20 % for all the other genotypes). Moreover, all *P. polyanthus* genotypes showed a better germination rate than the *P. vulgaris* variety NI 637. Genotypic response to *in vitro* culture is therefore a key factor to develop interspecific hybrids in which embryo rescue techniques have to be applied.

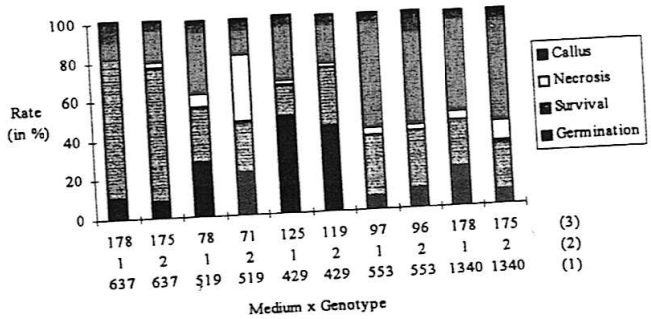

Figure I. Germination, survival, necrosis and callus rates for 10 studied objects. Objects are described as follows : (1) tested genotypes (NI); (2) the two tested media (1 for P1 and 2 for G1); (3) number of cultivated embryos.

The best embryo development was observed on P2 (figure II). Germination rate is increased by more than 20 % compared to G1. The G3 medium did not bring significant changes.

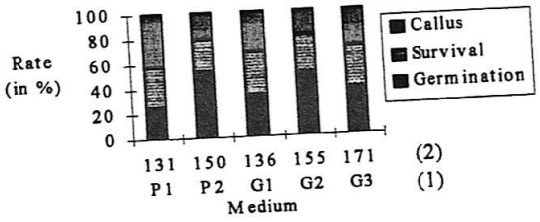

Figure II. Germination, survival and callus rates for the different tested media. (1) the tested media; (2) the number of cultivated embryos.

Conduct of factorial experiments should allow us to understand better the relative influence of each of the modifications proposed by Lecomte (1997) and their interactions (addition of Ca^{2+}, ABA and GA_3, and supression of N_6benzylaminopurine).

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