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 hromonal composition: suppressing N\textsubscript{o}-benzylaminopurine (0.028 mg.l\textsuperscript{-1}) and adding 0.06 mg.l\textsuperscript{-1} gibberelic acid (GA\textsubscript{3}), 25 μg.l\textsuperscript{-1} abscisic acid (ABA) and 200 mg.l\textsuperscript{-1} calcium (CaCl\textsubscript{2} x 2H\textsubscript{2}O) as proposed by Lecomte (1997) and completing Gamborg salts with 500 g.l\textsuperscript{-1} L-glutamine, 100 mg.l\textsuperscript{-1} serine, 100 mg.l\textsuperscript{-1} asparagine, 250 mg.l\textsuperscript{-1} casein hydrolysate, 1 μg.l\textsuperscript{-1} N\textsubscript{o}-benzylaminopurine, 0.1 μg.l\textsuperscript{-1} naphthalen acetic acid (NAA) and 0.01 mg.l\textsuperscript{-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

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.

![Germination rates for different genotypes and media](image)

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.

![Germination rates for different media](image)

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 Ca²⁺, ABA and GA₃, and suppression of N₆-benzylaminopurine).

References:


