DEVELOPMENT OF AN *IN VITRO* CULTURE METHOD OF VERY IMMATURE EMBRYOS TO ASSIST WIDE INTERSPECIFIC HYBRIDISATION IN *PHASEOLUS*

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Summary

The objective was to develop an in vitro culture technique of early heart-shaped embryos adapted for different Phaseolus genotypes. Best results were obtained on a modified germination medium of Phillips et al. (6) but with a high genotype dependent variability.

Key Words : *embryoculture, Phaseolus*

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*. In the reciprocal crosses between *P. polyanthus* and *P. vulgaris*, the use of *P. polyanthus* cytoplasm avoid a quick reversal to the recurrent parent *P. vulgaris* (1). Unfortunately, the level of incompatibility barriers is very high when using *P. polyanthus* cytoplasm. In trials conducted in greenhouses, Lecomte (3) 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 and only 7% after *in vitro* cultivation could reach the cotyledonar stage before dying.

Materials and methods

Our investigations were based on the technique developed by Mergeai *et al.* (5) for *in vitro* culture of early heart-shaped *Phaseolus* embryos. The following factors were tested: **1.** 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.** mineral composition: salts of Mergeai *et al.* (5) and salts of Phillips *et al.* (6), particularly rich in calcium and in minor salts; **3.** 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 $\mu g.l^{-1}$ naphthalene acetic acid (NAA) and 0.01 $mg.l^{-1}$ adenine as proposed by Bodanese-Zanettini *et al.* (2). Factors **2** and **3** were studied separately on the single *P. vulgaris* 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 (5) and Phillips medium (6) are designated respectively as M and P. Indices 1, 2 and 3 are used to represent respectively the original medium, M1 or P1, the medium modified by Lecomte (3), M2 or P2, and the medium modified by Bodanese-Zanettini *et al.* (2), M3. Results are given in figure 1.

The best embryo development was observed on P2. Germination rate is increased by more than 20 % compared to M1. The M3 medium did not bring significant changes.

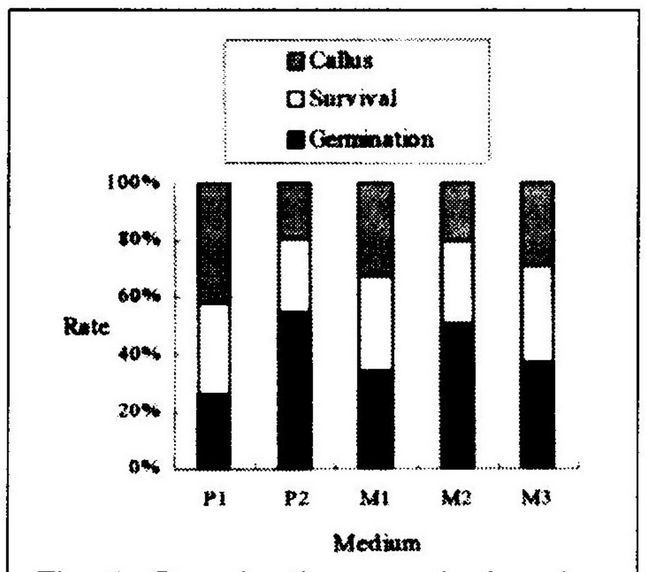

Fig. 1. Germination, survival and callus rates for the different tested media (genotype NI 637).

According to the results of Liu *et al.* (4) better development on P2 could be attributed to a more balanced ratio NO_3^-/NH_4^+ in the salts of Phillips for *Phaseolus vulgaris* embryos. Addition of ABA, that prevents precocious germination (7), could also be an important factor.

A high variation of the embryos response to *in vitro* culture was observed among the four *P. polyanthus* genotypes tested on M1 and P1 media (data not shown). 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 the three other genotypes).

Perspectives

Conduct of factorial experiments should allow us to understand better the relative influence of each of the modifications proposed by Lecomte (3) and their interactions (addition of Ca^{2+}, ABA and GA_3, and supression of N_6-benzylaminopurine). Moreover, more attention should be paid on genotypic response to *in vitro* culture.

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