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 deouro 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-benzylaminopurine (0.028 mg.l⁻¹) and adding 0.06 mg.l⁻¹ gibberellic acid (GA₃), 25 mg.l⁻¹ abscisic acid (ABA) and 200 mg.l⁻¹ calcium (CaCl₂ x 2H₂O) 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 mg.l⁻¹ N-benzylaminopurine, 0.1 mg.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 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. According to the results of Liu et al. (4) better development on P2 could be attributed to a more balanced ratio NO₃/NH₄⁺ in the salts of Phillips for Phaseolus vulgaris embryos. Addition of ABA, that prevents precocious germination (7), could also be an important factor. Therefore, 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).

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.

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

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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 Ca³⁺, ABA and GA₃, and suppression of N-benzylaminopurine). Moreover, more attention should be paid on genotypic response to in vitro culture.