In vitro CULTURE OF IMMATURE EMBRYOS OF *Phaseolus polyanthus* Greenm. AND *Phaseolus vulgaris* L.

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Introduction

Intraspecific hybrids in the common bean *Phaseolus vulgaris* L. have allowed to develop very high seed yielding cultivars, but without a significant improvement of the resistance to several diseases and pests responsible for half of the losses of yields in the tropics (Singh 1999). Interspecific hybridization between *P.vulgaris* and species belonging to the secondary gene pool, such as *P. polyanthus* Greenm., would allow to introgress genes of resistance to some economically important diseases (Schmit, Baudoin 1992). In order to maintain the desired characteristics, it is essential to use *P. polyanthus* as the female parent. In this case, however, the early abortion of hybrid embryos constitutes a very strong incompatibility barrier (Baudoin *et al.* 1992; Geerts 2001). One of the techniques used for the rescue of embryos is the *in vitro* culture (Sharma *et al.* 1996). Geerts *et al.* (2001) have generated and acclimatized plantlets of *P. vulgaris* from two days old pods cultivated *in vitro*. This study compares the growth and development parameters of immature embryos of *P. vulgaris* and *P. polyanthus* with the aim to create interspecific crosses between the two species.

Material and Methods

The tested genotypes are NI 637 for *P. vulgaris* and NI 1015 and G 35348 for *P. polyanthus*, all cultivated forms. Pod and embryo culture techniques are those described by Geerts *et al.* (2001). These are summarized in Table 1.

Table 1. *In vitro* culture stages of pods and embryos and for each stage technical conditions.

Technical conditions	*Pod culture*	***Embryo maturation***	**Embryo dehydration**	***Germination, growth and rooting***	***Acclimatization***
Media	P_{00}[(1)] (580 mosm) P_{01}[(1)] (450 and 350 mosm)	P_{01}[(1)] at 350 mosm	G6[(2)]	G7[(3)]	garden mould [(4)]
Light	60 µmol m^{-2} s^{-1}	darkness	darkness	60 µmol m^{-2} s^{-1}	580 µmol m^{-2} s^{-1}
Photoperiod	16h	-	-	16h	11h30
Temp. (D/N)	25/25°C	25/25°C	25/25°C	25/25°C	24/20°C
Cult. duration	7 days	14 days	14 days	14 days [(5)]	
Containers	Petri dish	Petri dish	Petri dish	Petri dish	polyethylene pot

[(1)] P_{00} & P_{01} : Phillips modified medium (Geerts 2001); [(2)] G6 : Hu et Zanettini modified medium (Geerts 2001); [(3)] G7 : Mergeai *et al.* modified medium (Geerts 2001); [(4)] Klasmann 4 special n^r 26 (80 %), moss peat (15 %), Rhine sand (5 %) & 100 mg organic fertilizer (2.9 % total N, 2.9 % P_2O_5, 2.0 % K_20); [(5)] duration average according to the vigour of the plantlet before acclimatization.

The osmotic pressure of culture media evolves according to pod age. It varies in continuous way from 580 to 350 mosm when using liquid medium but by steps, successively 580, 450 and 350 mosm when using solid media. The studied parameters concern the influence of the genotype, the maturity level of pods before its *in vitro* culture (from 3 to 5 days after pod setting on the mother plant) and pod culture techniques (comparing liquid and solid media).

Results and Discussion

Pod growth rates differ between the two tested species according to the culture technique : NI 637 shows a 32.3 % increase (with number of embryos : n = 48) in solid medium and 48.2 % increase (n = 24) in liquid medium, while NI 1015 and G 35348 show on an average a lower rate, respectively 22.7 % and 19.8 % increase (n = 86 and n = 77) in solid medium and 30.9 % and 27.3 % increase (n = 86 and n = 89) in liquid medium.

After seven days of *in vitro* pod culture, ovules, and then embryos, are extracted and placed in petri dishes containing *in vitro* solid media. For ovules, one notes a regular growth of half of these in NI 637 (*P. vulgaris*) and only of 21.5% and 23.5%, respectively in NI 1015 and G 35348, the two *P. polyanthus* genotypes. During ovule growth, many ovules died due to several factors : the disinfection technique of the plant material (phytotoxicity, necrosis), the *in vitro* conditions or a poor pollination and/or fertilization of mother plants maintained in growth chamber. The rate of embryo extraction is double in *P. vulgaris* compared to *P. polyanthus*. This may be related with a delay of embryos evolution in *P. polyanthus*. Indeed, in *P. vulgaris*, the extracted embryos had often reached heart-shaped or cotyledonar stage, while in *P. polyanthus*, embryos were hardly developed. On the other hand, no difference in rate of embryo extraction is observed between the two *P. polyanthus* genotypes NI 1015 and G 35348.

Germination of extracted embryos is higher in *P. vulgaris* (68.7%) than in *P. polyanthus* (28.4% in NI 1015 and 20.7% in G 35348). Pod age at the time of *in vitro* culture does not appear to influence the germinating capacity of the embryo. No significant difference has been observed between the three durations pods stay on the mother plant (3,4 and 5 days after pod setting). The ratio between the number of plantlets under acclimatization conditions and the number of germinated embryos is higher in *P. polyanthus* (76.2% for NI 1015 and 73.7% for G 35348) than in *P. vulgaris* (51,1%). Nevertheless, six weeks after the onset of acclimatization, the percent of growing plantlets out of the number of extracted embryos is higher in *P. vulgaris* (> 30%) than in *P. polyanthus* (5%).

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