

Succeeded plant regeneration from very immature embryos of *Phaseolus vulgaris*

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Introduction

In the framework of a research program aiming at developing an *in vitro* culture technique adapted to very immature *Phaseolus* zygotic embryos, our analyses showed that osmolality within young pods evolved *in vivo* from 580 mosm to 350 mosm during the first ten days after pollination (Geerts *et al.* 1999a). Upon the basis of these observations, we assessed the possibility to develop a culture technique of 2 to 3 days old *Phaseolus* pods by comparing the effect of three different osmolality conditions, (i) : a constant osmolality of 350 mosm, (ii) : a constant osmolality of 580 mosm, or (iii) : an osmolality varying progressively from 580 mosm to 350 mosm in 7 days, using a modified Phillips *et al.* (1982) maturation medium.

Material and method

Our investigations were based on the technique developed by Geerts *et al.* (1999b) for *in vitro* culture of early heart-shaped *Phaseolus* embryos. Pods, collected on a single *P. vulgaris* variety (Bico de Ouro or NI 637), were sterilized and directly cultivated under light in Petri dishes on a Phillips *et al.* (1982) medium containing 1 mg.l⁻¹ thiamin HCl, 5 mg.l⁻¹ nicotinic acid, 0.5 mg.l⁻¹ pyridoxine, 1.000 mg.l⁻¹ L-glutamine, 1.000 mg.l⁻¹ casein hydrolysate, 100 mg.l⁻¹ myoinositol, 25 µg.l⁻¹ abscisic acid (ABA), 1.128 µg.l⁻¹ tryptophan, 19 µg.l⁻¹ Naphtalen acetic acid (NAA) and 30 g.l⁻¹ sucrose, described as P1 medium. Three osmolality conditions were compared : (i) culture on a gelified P1 medium (constant osmolality of 350 mosm or LOSM), (ii) culture on a gelified P1 medium completed with 50 g/l sucrose (constant osmolality of 580 mosm or HOSM), or (iii) culture on a liquid P1 medium with osmolality evolving from 580 to 350 mosm (EVOSM). Evolution of osmolality was obtained by dripping a LOSM medium in a HOSM medium in which pods were cultivated. After one week, embryos were extracted and cultivated on a gelified P1 medium in which abscisic acid and tryptophan were replaced by 1.350 µg.l⁻¹ adenin and 225 µg.l⁻¹ N6 bezylaminopurin for rooting.

Results and discussion

Figure 1 indicates the frequencies of pods in relation with the number of ovules reaching at least 2 mm long within the pod after one week culture. Results show the interest to use evolving osmolality conditions (EVOSM) as observed *in vivo* by Geerts *et al.* (1999a). Indeed, under LOSM conditions only 2 to 4 ovules could reach 2 mm while 4 to 6 under HOSM and EVOSM conditions. Moreover, in 18 % of the pods cultivated under EVOSM conditions, 6 ovules reached more than 2 mm while such evolution was observed in only 13 % of the pods when cultivated under HOSM conditions.

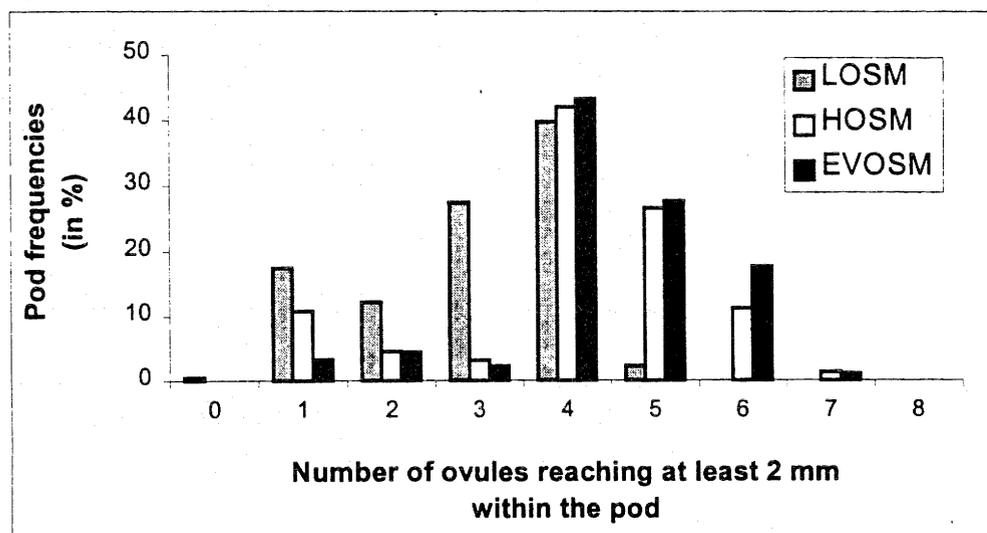


Figure 1. Pod frequencies (in %) in relation with the number of ovules reaching at least 2 mm long within a pod after one culture week. Three culture conditions are compared (LOSM, HOSM, EVOSM). Frequencies are calculated on 181 pods under LOSM conditions, on 313 pods under HOSM conditions and on 100 pods under EVOSM.

Among the embryos extracted from the 2 mm long ovules, 60.4 % reached heart-shaped stage and 0.08 % reached cotyledonar stage. For heart shaped and cotyledonar embryos, germination rates were respectively of 18.5 % and 50 %. From all the germinated embryos, 3 % survived the first 50 days of culture giving rise to well-developed plantlets. These plantlets were rescued after the cultivation of three days old pods under EVOSM conditions during one week and after the cultivation of the extracted embryos on P1 rooting medium.

Conclusion

Our results show the interest to adapt *in vitro* culture conditions to those observed *in vivo*. Particularly, we demonstrated that the use of adapted osmolality conditions are important. Indeed, satisfactory germination rates and, for the first time, plantlets could be rescued from 2 to 3 days old embryos when osmolality of the culture medium evolved as in *in vivo* conditions. More investigations are needed to obtain a higher percentage of plantlet regeneration. Indeed, regeneration rates of cultivated heart-shaped embryos of *P. vulgaris* obtained by Mergeai *et al.* (1997), Lecomte (1997) and Geerts *et al.* (1999b) reached 30 % while only 3 % of the extracted embryos from cultivated pods gave rise to plantlets.

References

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