Osmotic environment of *Phaseolus polyanthus* Greenm. and *P. vulgaris* L. embryos during early development.

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Introduction

The interest to improve an embryo rescue technique that enables the regeneration of early hybrid embryos obtained by crossing *P. polyanthus* Greenm. (use as female) with *P. vulgaris* L. has been described by Baudoin *et al.* (1992). According to Ryczkowski (1960), to set up an *in vitro* device that can support very immature embryos it is necessary to use solutions presenting properties similar to those of the natural environment constituted within the seed. The study of the osmotic potential evolution during the early development of *Phaseolus* embryos is essential to define an *in vitro* culture media that takes into account the *in vivo* properties and could be an important step for regenerating plants. This is supported by preliminary results obtained by Mergeai *et al.* (1990) showing the positive role played by a double layer medium on the development of *Phaseolus* globular embryos.
Although data for *Phaseolus polyanthus* are not available, Smith (1971) demonstrated that, in young *Phaseolus vulgaris* embryos, osmolarity decreased from 0.7 in the heart stage to 0.5 osmolar in the late cotyledon stage. These values were confirmed later by Yeung and Brown (1982). No data are given for globular embryos.
In the present study, we compare the stage of embryo development with the evolution of osmolality within developing pods, seeds and embryos of a *Phaseolus polyanthus* and a *P. vulgaris* genotypes. It is suggested that the osmolality evolution could be an important factor in the abortion of hybrid embryos between these two species.

Material and Methods

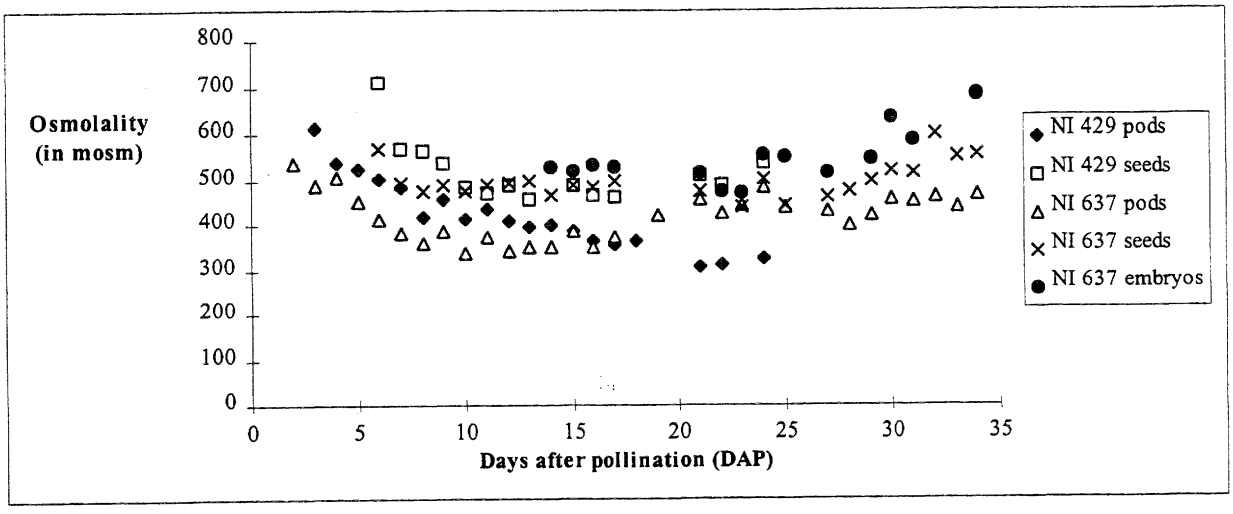
Two cultivated genotypes : NI 637 (*P. vulgaris*) and NI 429 (*P. polyanthus*) were grown in a growth chamber under controlled conditions (day/night temperature of 24/20°C, light intensity of 580 µE/m²sec. and a day length of 11h30). Twelve plants per genotype were grown to produce enough plant material. Pods were harvested early in the morning when plants were at full turgor. To avoid evaporation, they were carried from the growth chamber to the laboratory in a freeze box maintained at 19°C.
A method adapted from several authors (Oparka and Wright 1988, Garcia-Maya and Chapman 1990, Santos-Diaz and Ochoa-Alejo 1994) was applied on pods, seeds and embryos of the two genotypes. About 600 mg of plant material were crushed in a sample tube. Tubes were then centrifuged for 1 hour at 16000 rpm and 19°C. A 150-µl aliquot of the supernatant was taken to determine osmolality (Cryoscopic method, Fiske ONE-TEN osmometer, Massachusetts, USA). This method was applied on pods from 2 to 35 days after pollination (DAP), on seeds from 8 to 35 DAP and on embryos from 14 to 35 DAP.

Results and discussion

Data obtained with NI 637 (Figure I) were in agreement with the results of Yeung and Brown (1982) : the level of osmotic pressure in *P. vulgaris* pods between 8 to 14 DAP is relatively constant while a rapid increase of osmolality is observed after 22 DAP in relation with dehydration and dormancy. However, our data also showed a high variability of these values in pods just after pollination. A rapid decrease from 550 mosm (2 DAP) to 350 mosm (11 DAP) when embryos reached the cotyledon stage was observed for NI 637 pods and could be expected for their seeds and embryos. Indeed, the development of embryo in *P. vulgaris* depends partly of an osmotic gradient between young developing seeds and the pod (Yeung and Brown 1982). In particular, these authors (1982) showed that, at cotyledon stage, osmolalities were the highest in the embryo axis in comparison with cotyledons, seed membrane and pod. These differences were maintained during all the

maturation when potentials became more negative. The existence of a gradient between embryo, seed and pod was confirmed by our data showing that young seeds maintained an osmolality about 50 mosm higher than pods and 20 mosm lower than embryos (Figure I).

Figure I. Evolution of pod, seed and embryo osmolality for a *P. vulgaris* (NI 637) and *P. polyanthus* (NI 429) genotypes during early development (means of three replicates).



The results obtained with *P. polyanthus* pods and seeds showed also a rapid decrease of osmolality the first days after pollination (Figure I). However, this decrease was slower compared with *P. vulgaris* (about 40 %). The level of osmolality in NI 637 pods was similar to the level observed in 4 days older NI 429 pods. This observation can be correlated with the lower rate of development in *P. polyanthus*. For example at 7 DAP, *P. vulgaris* embryos are at heart-shaped stage while the same stage in *P. polyanthus* is reached only at 10 DAP (Lecomte 1997). A similar stage of embryo development of the two studied species corresponded to a same level of osmolality. This level decreases until cotyledons are initiated, i.e. 5 days after pollination for *P. vulgaris* and 9 days after pollination for *P. polyanthus*.

It is thus expected that a correlation exists between the level of osmotic potential and the early stage of development within *Phaseolus*. In the case of hybrid between *P. polyanthus* (as female) and *P. vulgaris*, the differences in osmolality evolution of the parents could be an important factor in abortion processes. Indeed, the rate of evolution of osmotic potential in *P. polyanthus* seeds could be too low for the hybrids *P. polyanthus* (as female) x *P. vulgaris*, and as a result, leads to a discordance between nutrient balance and stage of development.

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