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Refinement of an *in vitro* culture technique to rescue immature embryos of *Phaseolus* by microcutting

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Introduction. Crosses between *Phaseolus coccineus* L. or *P. polyanthus* Greenm. (♀) and *P. vulgaris* L. (♂) usually lead to the abortion of hybrid embryos 4 to 8 days after pollinations (DAP) (Lecomte, 1997). *In vitro* culture is the only way to rescue these embryos. Several refinements were made to the embryo rescue technique developed previously in our laboratory. Using the pod culture technique, Geerts (2001) was the first to carry out the regeneration of *P. vulgaris* plantlets from 2 days old embryos. However the plantlets expressed physiological disorders after 14 days (d) of culture with a very low regeneration rate (2.8%). The objective of this study was to find an efficient method for the regeneration of *Phaseolus* sp. from immature embryos by using microcutting of apical or cotyledonary node of *in vitro* regenerants.

Materials and methods. Pods of *P. vulgaris* (NI-637, 2 DAP) and *P. coccineus* (NI-16, 6 DAP) were used as plants material and 2 protocols (P1 and P2) were compared: P1= Pod culture technique as described by Geerts (2001) = control and P2 = P1+ microcutting technique. The different steps of this protocol are shown in fig.1. P1 and P2 were repeated twice with 140 embryos using P1 and 130 embryos using P2. The percentage of intact *in vitro* regenerants (plantlets with a stem, leaves and with or without roots) and adult plants were evaluated on the basis of the number of embryos that has been cultured. Equality test of two proportions per normal approximation was used to analyze our results ($P<0.05$).

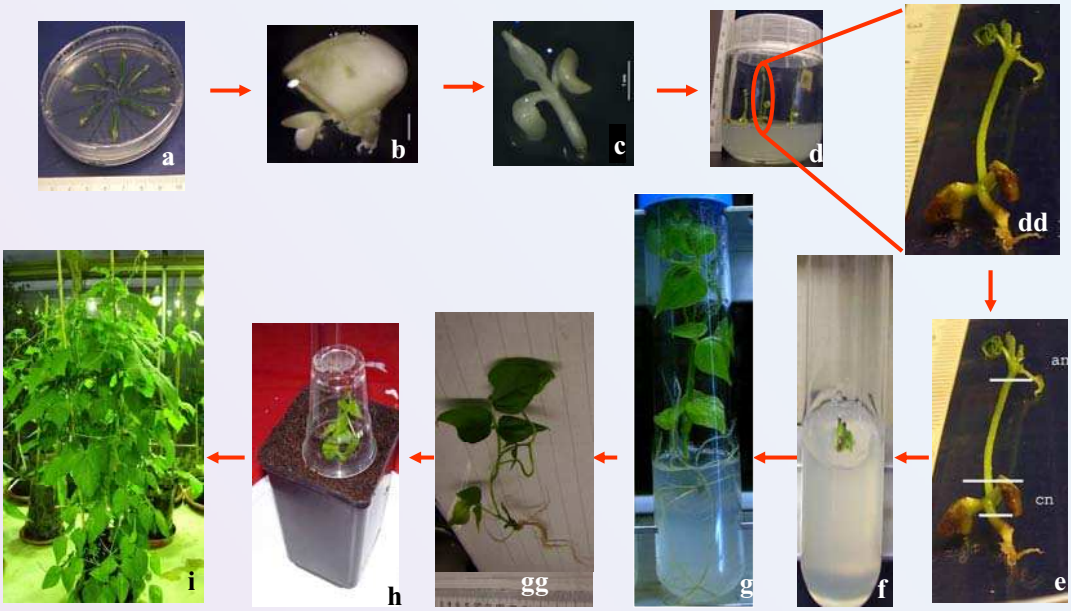

Figure 1: Defferent step of protocol 1 (P1) and protocol 2 (P2) P1 = step a, b, c and d : embryo culture via pod culture (Geerts, 2001) and P2 = P1 steps + step f, e and g.

The pods of *P.coccineus* NI-16, 6 DAP, were cultured in Petri dishes (a). After one week (w), embryos were extracted (b) (bar= 200µM) and they were successively cultured on maturation, dehydration (c), pre germination and germination media (d). Microcuttings containing apical node (an) and cotyledonary node (cn) were excised from 14 days-old *in vitro* regenerants and were subcultured on modified MS medium (f); 4 to 6 w later, plantlets were regenerated (g) and were acclimatised (h) during 2 w and adults plants were developed (i) 12 w later (Pictures: Barikissou E.).

Results and discussion . The regeneration rates obtained are shown in figure. 2. With NI-637, the rate of *in vitro* regenerants obtained reached 26.86% by using P1. In addition, *in vitro* regenrants stopped growing two weeks later, and one of them expressed a gradual necrosis of apical meristem. A similar result was reported by Geerts (2001). On the other hand, no plantlet was obtained by using P2 and all explants from *in vitro* regenerants of *P. vulgaris* became hyperhydric. With NI-16, no significant difference was reported between *in vitro* regenerants rate by using P1 and P2 (61.54% and 53.84%). However, like NI-637 all *in vitro* regenerants stopped growing (fig. 1dd). On the order side, the rate of developed adult plants reached 46.15% by using P2, while no plant was obtained with P1. The success obtained using P2 can be explained by the higher rate of plantlets obtained by microcutting (85.71% evaluated per microcutting subcultured) and the well development of plantlets regenerated (fig 1gg). Similar high rate of *in vitro* regenerants and developed adult plants was obtained from microcuttings excised from plantlets derived from mature seeds germinated *in vitro* in a study aiming to achieve micropropagation in *Phaseolus* sp. (Allavena and Rossetti (1986), Vaquero *et al*. (1993), Ebida (1996), Marta Santalla *et al*. (1998) etc.) .

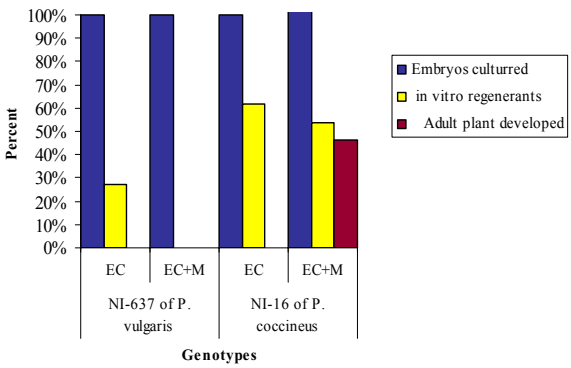

Figure 2 . Regeneration rates of *P. cocineus* and *P. vulgaris* using protocol 1 (P1) and protocol 2 (P2) P1=EC: embryo culture; P2= EC + M (microcutting)

Conclusion and prospects. Its possible to regenerate *in vitro* plantlets from the immature embryos without microcutting . However, plantlets stopped growing after 2 w. The microcutting of these plantlets improved the rates of regenerated plantlets and developed adult plants with only *P. coccineus* (NI-16). For *P. vulgaris* our work currently in progress aim to optimize the microcutting culture medium to support development of pre-existing buds excised from *in vitro* plantlets derived from immature embryos. The results of these works would enable the rescue of interspecific hybrids which abort around 6 DAP.

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