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Study of genes involved in *Phaseolus* embryogenesis

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Our research is aimed at understanding mechanisms of *Phaseolus* embryogenesis. Indeed, interspecific hybridizations between the recurrent species, *P.vulgaris*, and the two donor species, *P.coccineus* and *P.polyanthus,* reveal incompatibility barriers, particularly when the donor parents are used as female, a condition favouring the introgression of desired genes in the breeding process. Crosses between *P.vulgaris,* as paternal genotype, and the two other species lead to abortion of immature embryos, usually at the globular or early heart-shaped developmental stages, with most embryos aborting 3-6 days after pollination. Causes of early embryo abortion in several reciprocal interspecific crosses were studied through histological examinations. When the donor parent (*P.coccineus* or *P.polyanthus*) is used as female, early nutritional barriers are related to a deficient endosperm or suspensor development, while in reciprocal crosses, endothelium proliferation and, in some extent, hypertrophy of the vascular elements might be the cause of early embryo abortion. (Baudoin *et al,* 1992; Lecomte, 1997; Geerts, 2001).

Our specific objective is to identify genes involved in embryogenesis and whose disruption can cause the degeneration of interspecific embryos. The investigations are carried out according to two approaches : the molecular analysis of model plant genes involved in embryogenesis with a view to identify *Phaseolus* homologous genes, and the isolation of specific genes implicated in *Phaseolus* embryogenesis. For the latter, self-pollinations and reciprocal interspecific hybridizations are made using a wide array of parental *Phaseolus* genotypes (both wild and cultivated forms) and with the aim at obtaining several selfed and hybrid embryos (*P.vulgaris* x *P.coccineus*, *P.vulgaris* x *P.polyanthus*) and comparing embryo development in these distinct genotypes at different stages up to abortion or maturity. Histological studies and *in situ* hybridization with the genes analysed will be also carried out at different evolution stages of the ovule resulting from self pollinations or interspecific combinations.

Significant genes in the embryogenesis process of model plants such as *Arabidopsis thaliana, Zea mays, Oryza sativa,* etc., have been studied. Such genes have already been sequenced; the effects of proteins resulting from their expression on the embryonic development as well as the consequency of possible disruption on the embryo survival are known. Among the genes studied in model plants, MONOPTEROS, GURKE, FACKEL, GNOM/EMB30 and BODENLOS are implied in apical-basal axis formation; KNOLLE and KEULE are implied in radial axis formation; TWN1, SUSP and RASPBERRY are involved in suspensor formation. Other genes, such as homeobox genes (knotted-like, GLABRA2, etc.), EMP2 (Empty Pericarp 2), LTP (Lipid Transfer Protein) genes play also a significant role in the normal development of the embryo (Meinke *et al,* 1998; Wetering *et al,* 2001; Barnes, 2002; Hamann *et al,* 2002).

Phaseolus homologous genes are isolated by PCR and RT-PCR with genes specific primers and degenerate primers designed on the basis of conserved domains in the genes above mentioned. All nucleic and proteinic sequences from the model species used in

multiple alignment are available via EMBL and GENEBANK sites. DNA extracted from leaves are used for PCR amplification, and mRNA extracted from young leaves and from ovules at different developmental stages are used for RT-PCR amplification. The amplified DNA and cDNA will be cloned and sequenced. Nucleic probes derived on the basis of the obtained sequences will be used in histological studies (*in situ* hybridization) to determine the spatial pattern of these genes inside the ovule.

"Differential display" technique (Liang and Pardee, 1992) will be applied to identify specific genes involved in *Phaseolus* embryogenesis. This technique reveals the differential expression of target genes after a PCR amplification with arbitrary primers of small size.
For this purpose, mRNA will be extracted from the self-pollinated ovules of various parental genotypes and from the degenerated ovules from interspecific hybridization. The fragments so revealed will be isolated, cloned, sequenced and compared with other already isolated genes. Further investigations will be developed with the genes having the most significant contribution in *Phaseolus* embryogenesis.
At this stage of the study, several interspecific hybridizations were attempted and classified according to the level of incompatibility barrier severity. Two crosses involving *P.vulgaris* as male parent were succeeded and their hybrid nature confirmed through morphological and molecular marker (SSR) characterisation, i.e. NI 889 (wild *P. coccineus* as female) x G 21245 (wild *P. vulgaris*) and G 35348 (*P. polyanthus* as female) x G 21245. Abnormalities at the cotyledon level (one surnemary cotyledon) and the growth level were observed in the cross between a cultivated form of *P. coccineus* (NI16) and *P. vulgaris* (NI637). The importance of the abnormalities observed during embryo development were also influenced by the compatibility between the two parental genotypes. Two gene families (knotted-like and Lipid Transfer Protein) involved in model plant embryogenesis, were identified in this genus. Lipid Transfer Protein gene was revealed by RT-PCR amplification with following primers 5'-GAG-TTG-TTT-CCA-TGG-CCA-CC-3' and 5'-GAG-TAG-TTT-TCA-GTG-CCT-TC-3'. The presence of Knotted-like genes was revealed by PCR amplification with degenerate primers (PELDQFM: 5'-CCN-GAR-YTN-GAY-CAR-TTY-ATG-3' and QINNWFI: 5'-CAA-TGA-CGC-TTA-CGT-TGG-TT-3'). These primers correspond to the conserved amino acids region containing the homeodomain of knotted-like genes.

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