SCREENING *PHASEOLUS VULGARIS* L. EMS MUTANTS TO ISOLATE PLANTS FAILING IN SEED DEVELOPMENT AND TO STUDY GENETICS OF EMBRYOGENESIS

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Introduction

In the genus *Phaseolus*, interspecific hybridizations between *P. vulgaris* and the two donor species, *P. coccineus* and *P. polyanthus*, are carried out to introgress desired traits into the recurrent species *P. vulgaris*. Those crosses lead to abortion of immature embryos, mainly at the globular stage, particularly when the donor parents are used as female (Baudoin *et al.*, 2004). These abortions can be caused by the disruption of major genes involved in embryogenesis process, as it is shown in studies using model plants embryogenesis (Devic, 1995; Elster *et al.*, 2000). In order to study genes implied in *P. vulgaris* embryogenesis, an ethyl methane sulphonate (EMS)-induced mutant collection of common bean (Pankhurst & Broughton, 2004) was screened to isolate plants which failed in seed development.

Materials and Methods

Forty grammes of BAT93 seeds were treated with 200 ml of 30mM EMS overnight (approximately 16 hours) at the room temperature with slow shaking. Seeds were rinsed with sterilized water and sown. M1 (first generation) and M2 (second generation) mutants were screened for changes in seed development. Crosses were attempted between interested mutants (i.e. defective plants in seed development) and the original variety BAT93, in order to estimate the genetic determinism of these mutations in progenies.

Results and discussion

M2 mutant seeds were harvested from M1 plants divided into two groups: one which produced mainly empty pods and a second group which produced mature pods containing a high number of degenerated seeds. In total, 343 M2 mutants were tested from 62 lines. Seeds of four plants belonging to a first line (490) failed to develop normally while seeds of two mutants from a second line (522) aborted within 15 to 25 days after anthesis. Embryos of this line failed to grow normally and showed abnormalities at the two heart and cotyledon developmental stages (Figure 1). Abnormalities appeared in the suspensor and the cotyledons. The suspensor plays a role in pushing the embryo into the endosperm to facilitate its nutrition. Therefore defects in the suspensor can disrupt embryo nutrition and induce their abortion (Lecomte, 1997).

Crosses between these two types of mutants and the original variety were carried out to estimate the genetic transmission of the selected mutants in F1 and F2 progenies. F1 seeds were obtained when the original variety BAT93 was used as female parent in the crosses with the two

mutants. In reciprocal crosses, F1 mature seeds were obtained with the mutant line M2490, while crosses with line M2522 as female parent lead to the abortion of immature embryos within 15 to 25 days after pollination. All supposed F1 hybrid mature seeds were sown for phenotypal screening among F2 plants.

Simultaneously with this investigation, we study through semi-quantitative RT-PCR the expression of some major genes (i.e. Lipid Transfer Protein, Heat Shock Protein, KNOX, Leucine Zipper genes, etc.) involved in model plants embryogenesis, during the BAT93 wild-type seed development . This study will be extended to other genes (MONOPTEROS, GNOM, KNOLLE, TWN1, GURKE, etc.) known to be involved in plant embryogenesis. The expression of such genes in the control material (BAT93) will be compared with that observed during the

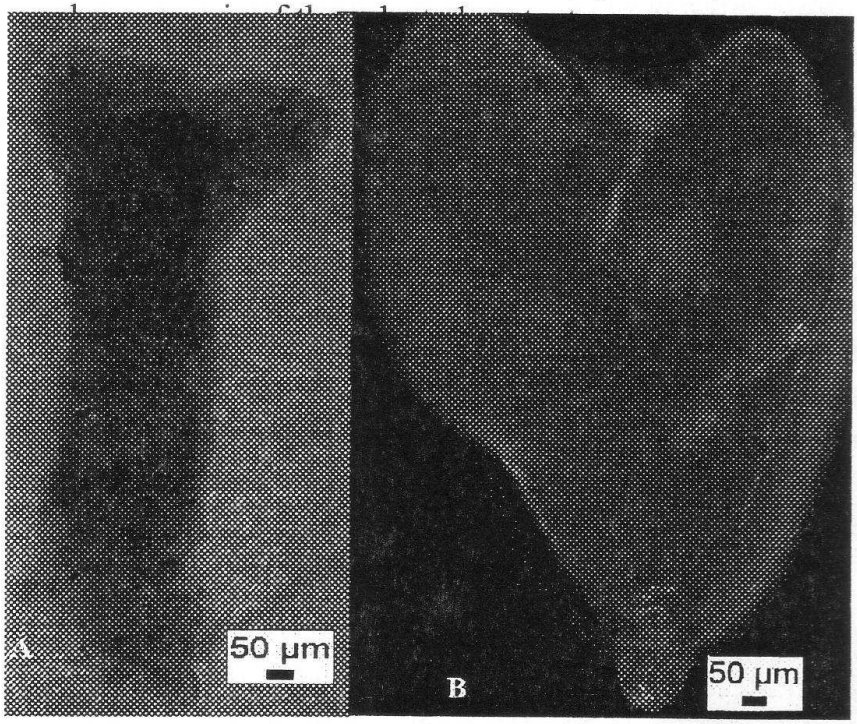

Figure 1. *Phaseolus vulgaris* embryos extracted from ovules 20 days after anthesis.
A: Defective embryo from mutant line 522 with abnormal suspensor (➡) and three cotyledons (+) instead of two. **B**: Wild-type embryo with normal cotyledons.

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