

CHARACTERIZATION OF *PHASEOLUS VULGARIS* L. EMS MUTANT FAILING IN SEED DEVELOPMENT

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INTRODUCTION

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 the abortion of immature embryos, particularly when the donor parents are used as female (Baudoin *et al.*, 2004). In order to isolate genes which can cause *Phaseolus* embryo abortion, plants from an ethyl methane sulphonate (EMS) mutagenized seeds of common bean were screened to isolate plants which failed in seed development. The suppressive subtractive hybridization technique was then used to identify transcripts that are differentially expressed in the mutant embryos.

MATERIAL & METHODS

Plants from an EMS mutagenized seeds of *P. vulgaris*, line BAT93 were screened to isolate plants deficient in seed development (Pankhurst *et al.*, 2004; Silue *et al.*, 2006). Seeds (40g) were treated with 200ml of 30mM EMS. The suppressive subtractive hybridization adapted from Diatchenko *et al.* (1996) was performed using degenerated seeds from the selected plants as tester and the normal seeds from the wild type as driver. Seeds were harvested at different stages of development. Fragments revealed after 2nd round PCR were sequenced and submitted to BLAST sequence homology analyses. Reverse transcription PCR was applied in order to study the relative expression of 3 transcripts during seed development 7 and 12 days after anthesis in mutant and wild-type samples.

RESULTS AND DISCUSSION

Among M2, M3 and M4 generations, 416 plants derived from sixty families were screened. Seven plants from family 522 (M 522) showed desired traits on a total of twenty-nine plants observed in this family. All the seeds produced from these plants aborted and embryos inside degenerated seeds failed to grow at different stages of development and showed abnormalities mainly in suspensors and cotyledons.

The suppressive subtractive hybridization allowed us to isolate eight cDNAs fragments. These cDNAs were cloned and sequenced. BLAST sequence homology analyses led to ten groups of proteins encoded by the cDNAs isolated: cytochrome P450 protein, cell wall-associated hydrolase, putative senescence-associated protein, myo-inositol 1-phosphate synthase (MIPS), Sucrose synthase (SUS), voltage-dependent anion channel (VDAC), peroxidase (PEROX), leucine rich protein, IMP dehydrogenase/GMP reductase and serine rich protein. Figure 1 shows the alignment of peroxidase

nucleotide sequence obtained in this study (SSH_PvE6, EF660341) with mRNA sequences in database sharing high identities.

On the basis of their score goal and their homology to gene sequences, five clones which correspond to cytochrome P450, MIPS, PEROX, VDAC and SUS have the best homology results. All of these five genes are expressed in plant seeds and are important for cell survival.

Reverse transcription PCR was applied in order to study the relative expression of VDAC, PEROX and MIPS transcripts during embryo development 7 and 12 days after anthesis in mutant and wild-type samples, with 18S rRNA as internal control (Figure 2). The expression levels of the transcripts differ between mutant and wild-type samples for all the transcripts, with a highest signal for the 7 days old wild-type sample.

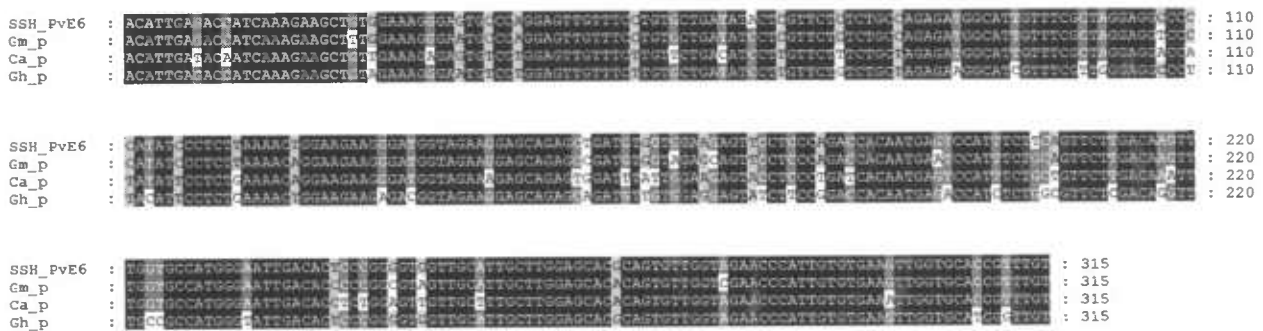


Figure 1. Clustal alignment of SSH_PvE6 (EF660341) nucleotide sequence with *Glycine max*, *Cicer arietinum* and *Gossypium hirsutum* peroxidase mRNA sequences. The sequences identities are 94% (Gm_p, AF039027) for *G. m.*, 87% (Ca_p, AJ271660) for *C. a.*, 82% (Gh_p, L08199) for *G. h.*

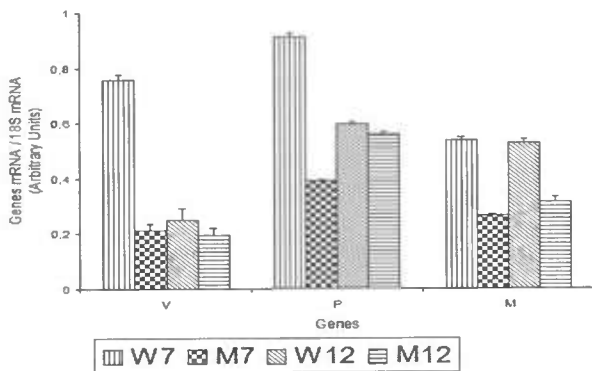


Figure 2. VDAC (V), PEROX (P) and MIPS (M) expression determined by RT-PCR in mutant (M) and wild-type (W) developing seeds at 7 and 12 days after anthesis. Expression of the genes corresponds to the ratio of the quantity of each gene divided by the quantity of the constitutive control 18S rRNA.

PROSPECTS

Crosses between the mutant described here and the wild type were carried out to estimate the genetic transmission of the mutation by analyzing the mutant plants ratio in F2 progenies. This study will be completed by histological comparison between the wild type and the mutant embryos during seed development.

REFERENCES

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