PLASMID-TAGGED IN TRANGENIC BEANS IDENTIFIES A HAPLOID-SPECIFIC GENE IN BIOBALISTIC PROCESS

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Genes introduced in plants are normally transferred to the progeny. However, transformants where the transgenes fails to segregate in a Medelian fashion may occur. These altered patterns of segregation could be due to different mechanisms, including insertional mutagenesis of genes that are essential to gametophyte development. Considering the difficulties encountered in isolation of gamethophytes, insertional mutagenesis becomes an important alternative for isolation of haploid-expressed genes. The rationale of this approach is that mutations that affect gametophyte viability and function lead to a defect in the transmission of gametes. Particularly megagametophytic mutated genes reduce the number of seeds per pod. Pollens of transgenic bean plants were analyzed by Alexander and histochemical GUS assay, in order to determine their viability. Phenotypic analysis of female gametophyte mutants is facilitated by the fact that this kind of mutation results in transgenic plants with pods presenting a reduced number of seeds. We identified two lines with a severe reduction of the number of seeds per pod. The plant lines were individually analyzed by PCR for the presence of the transgenes. A ratio of 1:10 (transgenic:non-transgenic) was observed in the R_1 generation. After six progenies from self-pollination all transgenic plants (more than 50) remained generating transgenic and non-transgenic plants indicating that all transgenic plants were heterozygous. These data obtained by self-pollination suggest that the mutagenized gene is essential. In fact, lethal mutations that are fully penetrant in one gender, but are transmitted partially through the other, are recovered as heterozygotes. The reciprocal crosses with non-transformed plants corroborated with this hypothesis. The morphological analysis of the pods suggests that the tagged gene affects the female viability with incomplete penetrance. The number of aborted seeds was reduced about 30% and fully penetrant gamethophytic mutations that disrupt female functions should reduce seed set (50%). The data obtained by self-pollination and reciprocal crosses with non-transformed plants suggest that the tagged gene acts as a recessive lethal gene with complete penetrance in the male gametophyte and incomplete penetrance in female gametophyte. This work reports for the first time the identification of a plasmid-tagged megagamethophytic mutant in a transgenic bean line.

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