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INSERTIONAL MUTAGENESIS AND CRISPR-CAS9 EDITING TO SILENCE *APOSTART* MEMBERS IN ARABIDOPSIS

AIELLO D.*, MARCONI G.*, PALANIVELU R.**, ALBERTINI E.*

*) Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno 74, 06121 Perugia (Italy)
**) School of Plant Sciences, University of Arizona, Tucson, 85721 Arizona (USA)

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Sexual reproduction in plants requires a series of developmental steps that culminate in seed formation. However, this mode of reproduction is not the only way to produce seeds. Apomixis is a naturally occurring mode of asexual reproduction in flowering plants that leads to clonal offspring by conservation of the maternal genotype from the absence of meiosis and fertilization. Apomixis and sexual reproduction are considered to be developmentally and evolutionarily closely related, including sharing many regulatory components. Once apomictic genes initiate embryo development and the initial cell forms and divides, the genes controlling embryo cell formation and patterning are most likely the same as those required for sexual embryo development. Therefore, two different strategies to study apomixis were proposed: the identification of genes exclusively expressed in apomictic plants or the activation of genes encoding proteins with a novel initiating function or an altered function, causing some components of apomixis in sexual plants. Following the first strategy we used the cDNA-AFLP technique to isolate in *Poa pratensis* a gene named *APOSTART* (*PpAPO*) that showed a putative function in programmed cell death, predicted to be involved in the non-functional megaspore and nucellar cell degeneration events that permit enlargement of maturing embryo sacs. APOSTART protein shares high similarity with two *A. thaliana* genes APOSTART1 (At5g45560) and EDR2 (renamed APOSTART2, At4g19040). To investigate the biological role of these two proteins, we analyzed two independent T-DNA insertional mutant lines, *Atapo1* and *Atapo2*. Subtle phenotypical differences in germination rates, plant growing stages and seed production were found comparing these lines with a control line (Col-0). In order to verify if *apo1* and *apo2* have additive or redundant roles we generated and analyzed the *apo1*/*apo2* double homozygous mutants. Double mutants germinated slower and plants appeared smaller than Col-0. Moreover, high variability was found between and within mutant lines. In this context a bisulfite sequencing analyses was performed for checking methylation level on the promoter regions of the two genes.

Furthermore, CRISPR-Cas9 was used to generate double mutant lines to avoid possible influences from not fully silenced single gene mutations. A new multiplex tool was used to streamline and facilitate rapid and simultaneous editing of these genes. In summary, characterization of T-DNA mutant lines, including methylation analysis and genome-editing of the *AtAPOSTART* genes, will be reported and discussed.