Proceedings of the LXVI SIGA Annual Congress
*Bari, 5/8 September, 2023*
ISBN: **978-88-944843-4-2**

**Poster Communication Abstract – 7.17**

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# IMPLEMENTATION OF PRECISION BREEDING STRATEGIES FOR MALE STERILITY INDUCTION IN SOLANACEAE MODEL SPECIES

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*male sterility, Solanaceae, genome editing, CRISPR/Cas9, RNP complex*

Male sterility (MS) trait in plants is defined like the failure to produce functional anthers, pollen, or male gametes. Incorporating MS into plant breeding programs is a critical task to reduce the cost of hybrid seed production and ensure high varietal purity for the production of F1 hybrids in many horticultural crops. On the contrary, in ornamental plants, this aspect has not been deeply investigated to date, even if the production of male-sterile ornamental plants could be of great interest for many purposes, in addition to hybrid seed production, as eliminating pollen allergens (i.e., gene escape), reduce the need for deadheading to extend the flowering period, and increase flower longevity and self-life. The recent developments in Genome Editing (GE) technology have opened a new era to study gene function and develop new plant cultivars suitable for any condition. The latest version of the GE technology, based on CRISPR/Cas9 methodology, provides a potential method for producing MS lines in several major species. Starting from these assumptions, in this work we describe the setting of preliminary stages for developing of a CRISPR/Cas9-based breeding strategy for the implementation of MS trait in two model systems of *Solanaceae*, *Solanum lycopersicum* var. microtom and *Petunia hybrida*, referent respectively in crop and ornamental research. The final goal is generating CRISPR/Cas-edited DNA-free plant material mediating transient protoplast transfection system, by direct delivery of a ribonucleoprotein (RNP) preassembled complex consisting of purified Cas9 protein and in vitro synthetized single guide RNA molecules (sgRNA). In particular, the targeting of *SolycMYB80* and *PethMYB80* candidate genes, orthologue to *AtMYB80* , as well as *OsMYB80*, whose central role in the development of pollen and

tapetum was well described, are now under investigation in both our biological systems. Here we report the results obtained, and work in progress, especially regarding tomato system. The amplification and isolation of genomic CDS and basal promoter regions of target loci were performed confirming the identity degree between amplified regions and reference sequences in databases. Several sgRNAs were then identified and selected by CRISPOR web application, based on their potential ability to target the investigated sequence at CDS level and predicted TSS region. The target specificity was determined by the following *in vitro* sgRNA synthesis/transcription to evaluate their later potential use *in vivo* condition. Moreover, an expression kinetics analysis of target gene in tomato system was performed on vegetative tissues and flower buds at different phenological stages, confirming the gene expression in stamens at specific stages, with a significant decrease after the degradation of the tapetum, and absent in the rest of the plant tissues, speculating on its importance in the development and formation of pollen and relative anther tissues.