Proceedings of the LXII SIGA Annual Congress
Verona, Italy – 25/28 September, 2018
ISBN **978-88-904570-8-1**

Poster Communication Abstract – 1.11

NON-ALLELIC GENE RESHUFFLING INDUCED BY CRISPR/CAS9-MEDIATED DOUBLE STRAND BREAKS IN A PLANT GENOME

FRANCO-OROZCO B., BUNELLO F., FELICE N., ZAINA G., DE PAOLI E.

Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine,
Via delle Scienze 206, 33100 Udine (Italy)

genome editing, homologous recombination, CRISPR/Cas9, Nicotiana benthamiana

Unrepaired DNA double strand breaks (DSBs) are harmful events for the plant cell, leading to chromosome loss, gamete sterility or cell death. Organisms have developed several molecular repair mechanisms to correct these lesions, including Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ). Nevertheless, repair of DSBs by these pathways, while being essential for the maintenance of genome integrity, may also be associated with insertion/deletion mutations, ectopic recombination and gene conversion events that modify the original DNA sequences. In some circumstances, these events may alter the allele segregation ratios and contribute to shaping the genomic diversity within a species.

The features of plant HR-mediated repair have increasingly attracted attention in the context of CRISPR/Cas9-mediated DSBs for the appealing application of HR in site-specific gene knock-in and non-disruptive genome editing. While NHEJ has been established as the most active repair pathway in plant somatic cells, induced DSBs can enhance HR-repair, albeit at very low frequency. Donor templates can be provided by transgenic DNA, endogenous homologous partners and, according to recent studies, also homologous alleles.

Our experimental work in this field is based on the use of a multiplex CRISPR/Cas9 vector system for the co-expression of multiple guide RNAs via *Agrobacterium*-mediated transient transformation and Next Generation Sequencing in *Nicotiana benthamiana*. With this approach we are exploring the gamut of CRISPR/Cas9-mediated repair products, including deletions with pre-defined boundaries, that may promote recombination. Interestingly, beyond the usual effects of targeted mutagenesis by CRISPR/cas9, we also observed significant instances of Non-Allelic Homologous Recombination (NAHR) eliciting the reciprocal exchange of DNA stretches between paralogous genes present in the *N. benthamiana* genome. These observations of NAHR add further examples of gene reshuffling among the possible outcomes of CRISPR-based genome editing in plants. Moreover, they offer new sparks for the application of New Breeding Techniques (NBTs) and the investigation of genome topology through the effects of homologous recombination.