Proceedings of the LXV SIGA Annual Congress
Piacenza, 6/9 September, 2022
ISBN: **978-88-944843-3-5**

Poster Communication Abstract – 7.35

BIOTECHNOLOGICAL STRATEGIES FOR IMPROVING IN VITRO REGENERATION AND CRISPR/CAS9 EDITING IN BELL PEPPER (CAPSICUM ANNUUM L.)

POMPILI V.*, MOGLIA A.*, ACQUADRO A.*, LANTERI S.*, COMINO C.*

*) DISAFA, Plant Genetics and Breeding, University of Torino, Grugliasco, Italy

gene editing, CRISPR/Cas9, in vitro regeneration, Capsicum annuum

Since its first application in plants, CRISPR/Cas9 gene editing has been used in a growing number of monocot and dicot species to enhance yield, quality, nutritional value as well as to introduce or enhance tolerance to biotic and abiotic stresses. Considerable progress has been made in optimizing CRISPR/Cas9 systems in plants, particularly for targeted gene mutagenesis, however its application is still challenging in species such as bell pepper (*Capsicum annuum* L.), which is recalcitrant to *in vitro* regeneration and lacks efficient transformation protocols. With the goal to investigate the *in vitro* organogenesis in two bell pepper genotypes ('Cuneo Giallo' and 'California Wonder') and assess the efficiency in delivery the CRISPR/Cas 9 machinery, we set up transformation and regeneration protocols based on the ectopic expression of developmental regulators and the CRISPR/Cas9 machinery delivered in plant tissues *via Agrobacterium tumefaciens*.

In order to improve the regeneration efficiency and simultaneously induce the editing in a targeted genomic site, cotyledons explants of the two bell pepper genotypes were transformed by *in vitro* co-culture with *A. tumefaciens* containing vectors with different combinations of growth regulators (*WUS*, *STM*, *BBM* and *IPT*) as well as the CRISPR/Cas9 system targeting the *PDS* gene. The disabling of the latter disrupts chlorophyll biosynthesis allowing for visual assessment of the knockout efficiency. The same vectors were also used to transform *in vitro* shoot apical meristems and lateral nodes deprived of buds in soil-acclimated plantlets, the latter as a strategy for a *de novo* induction of meristems.

The protocol based on cotyledons and shoot apical meristems transformation

with the vector harboring the combination of *WUS* and *BBM* induced a marked increase in the organogenesis efficiency, up to 80-90% compared to control treatment. The regenerated plants will be phenotyped, PCR validated for the presence of T-DNA and investigated to detect putative CRISPR/Cas9-induced mutations via high-throughput sequencing.

At last, to assess the way of producing T-DNA free edited plants, procedures for protoplasts isolation and their transformation with CRISPR/Cas9 ribonucleoproteins are in progress.