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

Poster Communication Abstract – 7.24

SETTING UP OF VIRUS-INDUCED GENOME EDITING (VIGE) PROTOCOL IN EGGPLANT

MAIOLI A.*, NAGALAKSHMI U.**, DINESH-KUMAR S.**, MOGLIA A.*

*) DISAFA, Plant Genetics, University of Torino, Grugliasco (TO), Italy
**) Department of Plant Biology, College of Biological Sciences, University of California, Davis, California, USA

VIGE, TRV, genome editing, eggplant, CRISPR/Cas9

Amongst genome editing tools CRISPR/*Cas9* is the most used and advanced. The development of new genome editing technologies in plant breeding has fostered a growing interest for *in vitro* culture and regeneration protocols, which represent a major bottleneck in the application of these techniques in many crop species.

Up to date, in eggplant only two CRISPR/*Cas9* editing events are reported both relying on *A. tumefaciens* transformation followed by *in vitro* regeneration. Unfortunately, available protocols are mostly inefficient, highly dependent on the genotype and time-consuming, and thus new cutting-edge solutions need to be established.

Over the past several years, there has been a growing interest in using plant viruses as vectors to deliver gene-editing reagents. A possible approach is Virus-Induced Genome Editing (VIGE) based on delivering gene editing elements to plant's tissue using viral constructs. VIGE in *Solanaceae* makes use of Tobacco Rattle Virus (TRV) based construct and protocols are already available for *Nicotiana benthamiana*, *Nicotiana attenuata* and *Arabidopsis thaliana*.

TRV is affected by two main flaws: its reduced cargo capacity and its limited mobility to the meristems. To overcome the first limitation, transgenic plants expressing *Cas9* should be used in combination with RNA viruses used to deliver sgRNAs to these plants through infection. To increase the mobility into the meristems, the sgRNAs may be fused with RNA mobile elements such as Flowering locus T (FT) and tRNA-like structures.

In our work we propose an *in vitro*-free protocol for VIGE in eggplant based on TRV construct. To maximize viral expression and persistence inside the plant tissue, we optimized VIGS approach previously developed in eggplant by targeting a reporter gene (*SmChl*). We compared two infection methods (agro-infection and *Nicotiana benthamiana* sap inoculum) in two plant tissues (leaves and cotyledons). Our results highlighted that sap inoculum on cotyledons is the best suiting approach (90% infected plant showing expected phenotype and average gene silencing of 60%) to be used for VIGE.

Three VIGE constructs have been developed, with sgRNA targeting eggplant *Chl* fused to: (a) isoleucine tRNA (b) FT and (c) truncated version of FT. 40% and 30% of the plants infected with the first two constructs showed the expected phenotype, no changes were observed upon the infection with the third construct. Infected plantlets were checked for mutations in the target region and sequencing analysis highlighted editing efficiency spanning from 22 to 54%.

While editing on somatic tissue was successful, its spread to meristems and therefore seeds must be still verified.