Proceedings of the LXIV SIGA Annual Congress
Online, 14/16 September, 2021
ISBN: **978-88-944843-2-8**

Poster Communication Abstract – 7.17

EVALUATION OF GRNAS AND VALIDATION OF DCAPS FOR THE EDITING OF THE LIN5 GENE IN TOMATO

PALMA D.*, D'ORSO F.**, D'AGOSTINO N.***, MORELLI G.**, CARDI T.*, SESTILI S.*

*) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca Orticoltura e Florovivaismo
**) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Genomica e Bioinformatica
***) Dipartimento di Agraria, Università degli Studi di Napoli Federico II

tomato, invertase, °Brix, gene editing, hairy roots

One of the main challenges of tomato breeding is fruit quality, characterized mainly by the content of total soluble solids (°Brix), also indicative of an increase in aromatic compounds, a better content of vitamins and a longer shelf-life. Solanum pennellii LIN5, encoding for the apoplastic invertase that irreversibly hydrolyzes sucrose into glucose and fructose, is more efficient than the cultivated tomato allele (SILIN5) in increasing °Brix. The SNP2878, responsible for the replacement of aspartic acid (Asp348), plays a predominant role in increasing the invertase activity. The aim of our work is to introduce the SNP2878 in SlLIN5 by a geminivirus-mediated Gene Targeting (GT) approach. This system combines CRISPR/Cas-induced double-strand break (DSB) with the homology-directed repair (HDR) in which DONOR DNA is supplied by the geminivirus replicon. Critical steps in a GT experiment include the selection of efficient gRNAs and the screening of GT positive events. Guide RNA efficiency was assessed by the frequency of random mutations induced in a hairy root (HR) based system using Agrobacterium rhizogenes. A dCAPS-based analytical method was developed to screen for the desired base change in the edited plants. Several gRNAs were selected for the CRISPR/Cas9 (three sgRNAs) and CRISPR/Cpf1 (four crRNAs) systems to be validated by HRs of the CREA-SAAB genotype (Abruzzo Pear). The three sgRNAs (#3, #4 and #5) for CRISPR/Cas9 were designed using three dedicated software (GT-Scan, CRISPR direct and CAS designer) and were positioned downstream and upstream of SNP2878. Sequence analysis revealed the presence of several mutations in the HRs obtained with sgRNA#3 and relatively few mutations in the sequences obtained with sgRNA#4 and sgRNA#5. SgRNA#3 was selected for the final construct with geminivirus hosting the specific donor DNA. The four crRNAs for the CRISPR/cpf1 system were designed by the CRISPOR software and all were positioned relatively close to the SNP. The HRs obtained from three crRNA guides were sequenced and two of them showed high efficiency in inducing mutations close to the SNP (66.7% and 90%) while the other provided only 11,11% of mutagenesis and therefore was not selected. The fourth guide produced a low number of roots and the sequences obtained were unclear. The preparation of vectors for stable transformation by Agrobacterium tumefaciens with the selected guides for CRISPR/Cpf1 system is in progress. Based on these preliminary results, we performed the Agrobacterium tumefaciens-based stable transformation of different Solanum lycopersicum genotypes using the construct with the sgRNA#3. In addition, the developed dCAPS marker, validated using the HRs obtained with the sgRNA#3, allowed early and easy detection of the replacement of the DONOR carrying the SNP2878. It represents a valid tool for the screening of positive GT events. The resulting transformed plants will be

screened by the dCAPS method and finally genotyped by sequencing.