Workshop SOI-SIGA
30/09/2021

CRISPR/Cas9 editing for improving the nutritional quality of tomato fruits

Luigia Principio[1]*, Amalia Barone[1], Caterina D'Ambrosio[2], Adriana Lucia Stigliani[2], Giovanni Giorio[2], Maria Manuela Rigano[1]

[1]Department of Agricultural Sciences, University of Naples "Federico II", Portici (Na), Italy

[2]Metapontum Agrobios Research Center, Agenzia Lucana di Sviluppo e di Innovazione in Agricoltura (ALSIA), Metaponto (MT), Italy

BACKGROUND

Tomato (*Solanum lycopersicum* L.) consumption has been associated with a reduced risk of cancer, inflammation and cardiovascular diseases. These effects are attributed to the presence of antioxidant molecules including ascorbic acid (AsA). This study aims at obtaining new genotypes with improved nutritional traits by exploiting the great potential of the introgression lines (ILs) obtained by crossing *S. lycopersicum* cv. M82 x *S. pennellii*, which carry a small portion of wild *pennellii* genome in the cultivated background.

MATERIALS AND METHODS

Plant material consisted of the cultivated tomato line M82 (accession LA3475) and the IL R182. Fruits of M82 and R182 were collected at the mature red stage to evaluate yield *per* plant and nine qualitative traits. CRISPR / Cas9 gene editing method was applied to generate mutation within the coding sequence of two genes potentially involved in the production of AsA: Nucleobase Ascorbate Transporter (NAT) and Major Facilitator Superfamily Protein (MFSP) genes. A DNA target region inside each gene was chosen by Crispr-P web tool and the sequences were cloned into the binary vector pKSE401. Cotyledons of R182 and M82 tomato lines were infected with *Agrobacterium tumefaciens* LBA4404 carrying the binary vector. Regenerated plants were characterized by PCR analysis and DNA sequencing to detect the transgene insertion and the mutations.

RESULTS

Statistical analysis on R182 and on parental line M82 demonstrated that R182 showed better performance in terms of yield and fruit qualitative traits most considered for tomato processing (**Table 1 and Figure 1**). A higher amount of AsA in fruit from R182 was also demonstrated. The sgRNA/Cas9 cassette targeted to NAT gene was detected into 12 R182 and 3 M82 regenerated plants; while 14 R182 and 9 M82 plants were shown to carry the transgene for the editing of MFSP gene. A *posteriori* analyses of the genomic editing experiments will be performed by characterizing and quantifying the insertion, deletion and homologous recombination events (**Figure 2**).

Table 1: Yield *per* plant and fruit quality traits of two tomato genotypes. The values are means ±SD (n = 9). The differences between the two genotypes (M82 *vs.* R182) were evaluated by the Student's t-test (*P < 0.05; **P < 0.01; ***P < 0.001).

Genotype	Yield (Kg/pt)	Soluble solids (°Brix)	Titratable acidity (g/100 g FW)	Firmness (Kg/cm²)	Total carotenoids (mg/100g FW)	Lycopene (mg/100g FW)	β-carotene (mg/100g FW)
M82	0.37 ± 0.06	5.60 ± 0.39	0.33 ± 0.01	5.73 ± 0.68	16.44 ± 1.83	1.01 ± 0.13	0.14 ± 0.01
R182	0.48 ± 0.02 *	7.54 ± 0.38 ***	0.59 ± 0.06 ***	6.15 ± 0.59	21.32 ± 1.94 ***	1.32 ± 0.13 ***	0.18 ± 0.01 ***

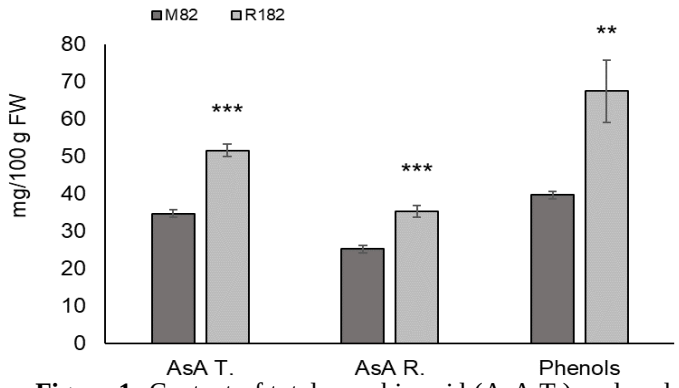

Figure 1: Content of total ascorbic acid (AsA T.), reduced ascorbic acid (AsA R.) and total phenols in M82 and R182. Values are means ± SD (n = 9). Asterisks indicate statistically differences of R182 compared to M82 by Student's t-test (*P < 0.005; **P < 0.01; ***P < 0.001).

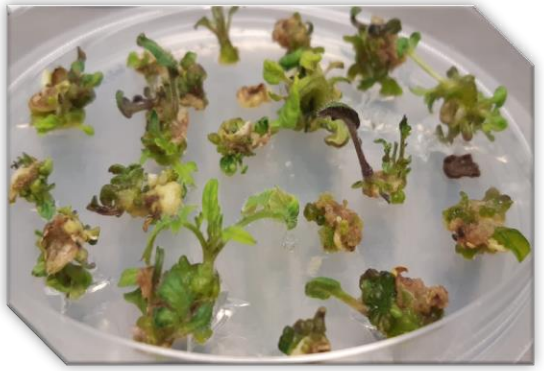

Figure 2: Regeneration of putative transgenic shoots from cotyledon explants of tomato seedlings after infection with *Agrobacterium tumefaciens* and selection on Kanamycin containing substrate.

SUMMARY AND CONCLUSIONS

These analyses confirmed that the line R182 has better performance than the cultivated line M82, probably due to the presence of wild alleles deriving from *S.pennellii* in genes controlling fruit quality and nutritional traits. Using genome modification techniques (CRISPR / Cas9) the function and mechanisms of action of genes identified in the introgression line are being confirmed.

Luigia Principio
luigia.principio@unina.it