Proceedings of the LX SIGA Annual Congress
*Catania, Italy – 13/16 September, 2016*
ISBN **978-88-904570-6-7**

**Oral Communication Abstract – 3.05**

# CRISPR/CAS9-MEDIATED HQT GENE EDITING TO STUDY CHLOROGENIC ACID BIOSYNTHESIS IN TOMATO

D'ORSO F.*,**, LAWRENSON T.*, HARWOOD W.*, ZHANG Y.*, LI J.*,
TOMLINSON L.***, MORELLI G.**, MARTIN C.*

*) John Innes Centre, Norwich Research Park, Colney, Norwich (UK)
**) Food and Nutrition Research Centre, Council for Agricultural Research and Economics, Rome (Italy)
***) The Sainsbury Laboratory, Norwich Research Park, Norwich (UK)

*Genome Editing, CRISPR/Cas9, chlorogenic acid, HQT, Solanum lycopersicum*

Caffeoylquinic acids (CQAs), and in particular chlorogenic acid, are the most abundant polyphenols in many plant species such as potato, tomato, eggplant, tobacco, coffee and apple. Consequently, they are important compounds present in human diet with several biological properties. Their biosynthesis could occur by several different proposed pathways, but considerable evidence suggests that the most active pathway is mediated by Hydroxycinnamoyl CoA Quinate Transferase (HQT). HQT converts caffeoyl-CoA and quinic acid to chlorogenic acid (CGA) and in some Solanaceous species its role has been demonstrated; in tomato the silencing of SlHQT resulted in a significant reduction of CGA and the overexpression of SlHQT led to an accumulation of CGA. Moreover HQT is also involved in di- and tri-caffeoylquinic acid biosynthesis. To study the regulation of chlorogenic acid metabolism in tomato further, we edited the *SlHQT* gene using CRISPR/cas9. We induced mutagenesis of the *SlHQT* gene at two different positions directing the cas9 cut in the *SlHQT* genomic sequence, the first in the 5'UTR and the second in the first exon of the HQT-coding sequence. We undertook full genotypic analysis of a large number of T0 transformed tomato plants, and observed a very high mutation frequency but also considerable variability in terms of the number of alleles and kinds of mutations in any one plant. This variability allowed us to investigate the role of HQT in the CGA production; plants with KO mutations produced no chlorogenic acid indicating that HQT is necessary for CGA biosynthesis in tomato and that its pathway is the only one active in this species. In addition, the mutagenesis of specific codons in the coding sequence strongly reduced the activity of HQT, allowing us to assess the importance of the alterated amino acids for protein folding and/or activity. Finally, mutations in the 5'UTR and the promoter were examined to show whether there are specific regulatory motifs controlling *SlHQT* gene expression in these regions.

Our results show that the CRISPR/cas9 system can be used successfully to edit the *SlHQT* gene, generating large number of allelic variants and modifying the production of chlorogenic acid. These edits may also alter the phenylpropanoid pathway with pontential impacts on nutritional value.