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

Oral Communication Abstract – 5.05

CRISPR/CAS9-MEDIATED MUTAGENESIS OF A POLYAMINE OXIDASE GENE INCREASES TOMATO PLANT TOLERANCE TO DROUGHT STRESS

D'INCÀ R.*, MATTIOLI R.*, TOMASELLA M.**, TAVAZZA M.***, MACONE A.****, TAVAZZA R.***, MARTIGNAGO D.*****, INCOCCIATI A.****, FRAUDENTALI I.*, CONA A.*, ANGELINI R.*, NARDINI A.**, TAVLADORAKI P.*

*) Department of Science, University 'Roma Tre', Rome, Italy.
**) Dipartimento di Scienze della Vita, Università di Trieste, Trieste, Italy.
***) Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), UTAGRI-INN C.R. Casaccia, Rome, Italy.
****) Department of Biochemical Sciences 'A. Rossi Fanelli', 'Sapienza' University of Rome, Rome, Italy.
*****) Department of Biosciences, University of Milano, Milano, Italy.

polyamine metabolism, stress tolerance, plant development, CRISPR/Cas9-mediated mutagenesis, tomato plants

In plants, the polyamines putrescine, spermidine, spermine (Spm) and thermospermine (T-Spm) are involved in developmental and defense processes. In particular, T-Spm is implicated in the control of plant growth and xylem differentiation interfering with auxin and cytokinin signaling. Polyamine levels are finely tuned through biosynthesis and catabolism. In Arabidopsis, five FAD-dependent polyamine oxidases (AtPAO1 to AtPAO5) are involved in polyamine catabolism exhibiting distinct expression patterns, substrate specificity and subcellular localization. Notably, the cytosolic AtPAO5, which oxidizes Spm and T-Spm, contributes to the control of plant development, xylem differentiation and abiotic stress tolerance. In tomato (*Solanum lycopersicum*), three *AtPAO5* homologs were identified (*SlPAO2*, *SlPAO3* and *SlPAO4*), and CRISPR/Cas9 mediated loss-of-function *slpao3* mutants were obtained. Transgenic tomato plants ectopically expressing *AtPAO5* (*AtPAO5over*) were also obtained. Phenotypical, molecular, and physiological analyses evidenced that *slpao3* mutants and *AtPAO5over* tomato plants exhibit altered T-Spm levels, growth parameters, number and size of xylem elements, and expression levels of genes (*SlPIN1* and *SlPIN6*) related to auxin signaling

pathways, with respect to the wild-type plants. Furthermore, as determined by observation of wilting symptoms and measurement of water-loss rates, *slpao3* mutants are characterized by increased tolerance to drought stress compared to wild-type plants, whereas on the contrary the *AtPAO5over* tomato plants appear hypersensitive to this stress. In addition, preliminary data from hydraulic conductance measurements evidence that *slpao3* mutants present lower water transport capacity and vulnerability to xylem embolism than the wild-type plants. Collectively, our data suggest that the *slpao3* plants are characterized by improved water-use efficiency. This study highlights that polyamine metabolism can be exploited to transfer stress tolerance traits into crop plants.