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

Oral Communication Abstract – 2.05

ENGINEERING WATER USE IN TOMATO BY GENERATING SLMYB60 MUTANTS USING A CRISPR-CAS9- BASED APPROACH

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Solanum lycopersicum, abscisic acid, drought tolerance, stomatal closure

The demand for water by agriculture is increasing rapidly due to climate change. Developing crops with higher water use efficiency (WUE) is therefore urgently needed. Tomato (*Solanum lycopersicum*) is a major crop plant cultivated under green house and, importantly, open field conditions. Hence, it would be important to identify genotypes of tomato that maintain high productivity and fruit quality with a reduced water footprint. This objective aligns with a nationwide effort (Cisgenesis and genome editing in tomato – CISGET) to leverage emerging breeding technologies for expediting genetic enhancement in tomato plants. Our study focuses on targeted mutagenesis of Abscisic acid (ABA) signaling, which is key in mediating a plant's acclimation to water deficit by stimulating stomata closure, thus reducing transpiration. We aimed at eliminating the *SlMYB60* gene, encoding a transcription factor and putative positive regulator of stomatal opening. Here we successfully generated deletion mutants in the *SlMYB60* coding sequence with multi-guide Crispr-Cas9 approaches in two genetic backgrounds (Ailsa Craig -AC and Red Setter -RS). Mutant lines display a clear reduction in water loss under optimal and water deficit conditions, and a slight improvement in drought resistance in the AC background. This was consistent with an increased leaf water content compared with the wild type. However, relative water consumption in response to the water deficit was unaffected. These phenotypes possibly derived from (i) a reduction in stomata opening, suggesting a functional conservation of *MYB60* in tomato and, (ii) an increase in permeabilization of the leaf cuticle. We speculate that gains in water loss, derived from reduced stomatal transpiration in

the mutants, might be compromised by unknown effects on cuticle permeability, this is an aspect that warrants deeper investigations. Preliminary observations indicate a slight growth reduction in *SlMYB60* mutants compared with the wild type, although this effect appears to be background specific. Detailed analysis of the expression of *SlMYB60* at the cellular resolution will provide information to separate direct and indirect effects of SlMYB60 on plant growth and leaf development. In summary, our data support the idea that reducing water loss by enhancing stomatal closure can be achieved through impairment of *SlMYB60* function, without detrimental effects on growth, at least under greenhouse conditions. Future field trials will allow us to determine if these newly generated alleles can be used to confer increased water deficit tolerance without impairing productivity traits.