

CRISPR/CAS9-MEDIATED MUTAGENESIS IN TOMATO (*SOLANUM LYCOPERSICUM*) TO IMPROVE THE RESPONSE TO MULTIPLE STRESS

AMOROSO C.G., CHIAIESE P., ANDOLFO G., FORMISANO G., DI MATTEO A.,
ERCOLANO M.R.

Department of Agricultural Sciences, University of Naples, Via Università 100, 80055 Portici
(Italy)

genetics, tomato, stress, target genes, transformation

Tomato (*Solanum lycopersicum*) is an important food crop that can be affected by several biotic and abiotic stresses. The aim of our project is to use a genome engineering approach to improve the tomato resistance against different environmental stresses. A wide literature research was carried out to identify genes having a role in susceptibility. In addition, selected genes were assessed in various stresses transcriptomic experiments to evaluate their activation/repression during tomato response to a given stress (pathogens, drought and salinity) . Taken together the results of these reviews led to the identification of a target gene involved in multiple stress pathways: WATI (Walls are thin 1) previously characterized in Arabidopsis and cotton. To knock-out the gene, we designed two guide-RNA, that have been then introduced singularly or combined, in three *E. coli* vector using the Golden Braid 3.0 system. Transformations experiments were carried out with *Agrobacterium tumefaciens* (strain LBA4404) in Red Setter and San Marzano 2 genotypes. Several putatively transformed shoots containing “nptII” resistance gene have been regenerated on selection media containing kanamycin. Three months after the explants-*Agrobacterium* co-culture, seven putatively transformed plants were obtained. A DNA extraction and a subsequently PCR analysis showed the presence of a transformed plant for double guideRNA-WATI gene. Further analysis will be performed to assess the occurrence of desired mutation and the absence of off-target genes.