

ROLE OF CXE15 IN STRIGOLACTONE DEGRADATION UNDER DROUGHT STRESS IN TOMATO

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Strigolactones, carboxylesterase, drought stress, gene editing, tomato

Strigolactones (SLs) are plant hormones produced in very low quantities mainly in the roots and in part released into the soil. They are involved in various plant functions such as development, root architecture and relationships with other organisms (hyphal growth in arbuscular mycorrhizae, seed germination in parasitic plants and nodulation in legumes). In addition, they contribute to tolerance to abiotic stresses (related to acclimation following drought and N and P deprivation), making them an interesting object of study for different markets. However, their extraction from the producing tissues is challenging, and chemical synthesis of their analogues (e.g. GR24) is expensive and limiting. SLs produced naturally by the plant provide a wider range of bioactive molecules with diversified effects than synthetic analogues or mimics. The use of new technologies as CRISPR/Cas9 to increase the level of SLs in the plant, coupled with an optimised protocol for their extraction, could be an effective way to overcome these obstacles. Recently, specific carboxylesterases such as AtCXE15 have been shown to play a key role in the catabolism of SLs in *A. thaliana*. The aim of the work is to characterise and edit *CXE15* genes in order to assess their role under drought stress in tomato.

In this study, bioinformatic analysis allowed the selection of two putative orthologs of *AtCXE15* in *Solanum lycopersicum* (*SLCXE15_p1* and *SLCXE15_p2*). Their transcripts were preliminarily quantified by qRT-PCR on different tissues and conditions (GR24 foliar application, P and N starvation and osmotic stress). *SLCXE15_p2* seems to be modulated by exogenous GR24 and stress, whereas the other appears to be stable and less affected by treatments.

To further investigate the transcript profiles of both genes, a water stress experiment was performed on cv. M82 wt and the SL-defective line 6936 (silenced in the SL biosynthetic gene *CCD7*). Physiological measurements such as stomatal conductance (gs), net photosynthesis (An) and stem water potential were collected; plants were sampled at different water potentials. RNA was extracted from the leaf samples and qRT-PCR analysis of the candidate genes is underway.

Since their deletion has the potential to produce high-SL plants, two guide RNAs were designed for each gene after off-targets analysis. The guides were inserted into pDGB3 vectors and used to transfect tomato leaf protoplasts for both single and double knockout, using GFP as a positive control. The experiment was repeated three times with comparable levels of GFP expression and protoplasts are going to be regenerated following the protocol published by Liu and colleagues. Edited plants are going to be evaluated for SLs content and performance under drought stress.