Overexpression of endogenous *lipoyl synthase* (*SlLIP1*) in *Solanum lycopersicum* fruits to increase their lipoic acid content

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I. ABSTRACT

Oxidative stress is generated by an increase in reactive oxygen species (ROS) which can cause cell damage. As a response, antioxidants are produced due to their capacity to neutralise ROS. Of these, lipoic acid is extremely powerful, as well as being amphipathic, regenerating other antioxidants and functioning in both oxidised and reduced forms. Also, lipoic acid is a cofactor that is associated with several enzymes, including the pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (kGDH) complexes. The lipoylation process occurs through two routes, de novo synthesis and the salvage of free lipoate. Lipoyl synthase (LIP1) is common in both pathways. In order to obtain a tomato fruit with a higher content of lipoic acid, lipoyl synthase gene (*SlLIP1*) from *Solanum lycopersicum* was expressed under the control of a fruit-specific promoter (Polygalacturonase, PG). The tomato cv "Micro-Tom" was successfully transformed and 3 lines overexpress *SlLIP1* transcripts in fruits. These lines do not show altered vegetative growth and their fruits are similar in size to those of wild type plants, but a parthenocarpic phenotype was detected. The degree of lipoylation is being determined and we hope to obtain a correlation between the levels of lipoic acid and the antioxidant capacity in transgenic tomato fruits. In addition, the PG promoter is induced under high salt conditions, so the progeny of transgenic lines are being molecularly verified to study their response to saline stress conditions and the effect on their development.

II. METHODS

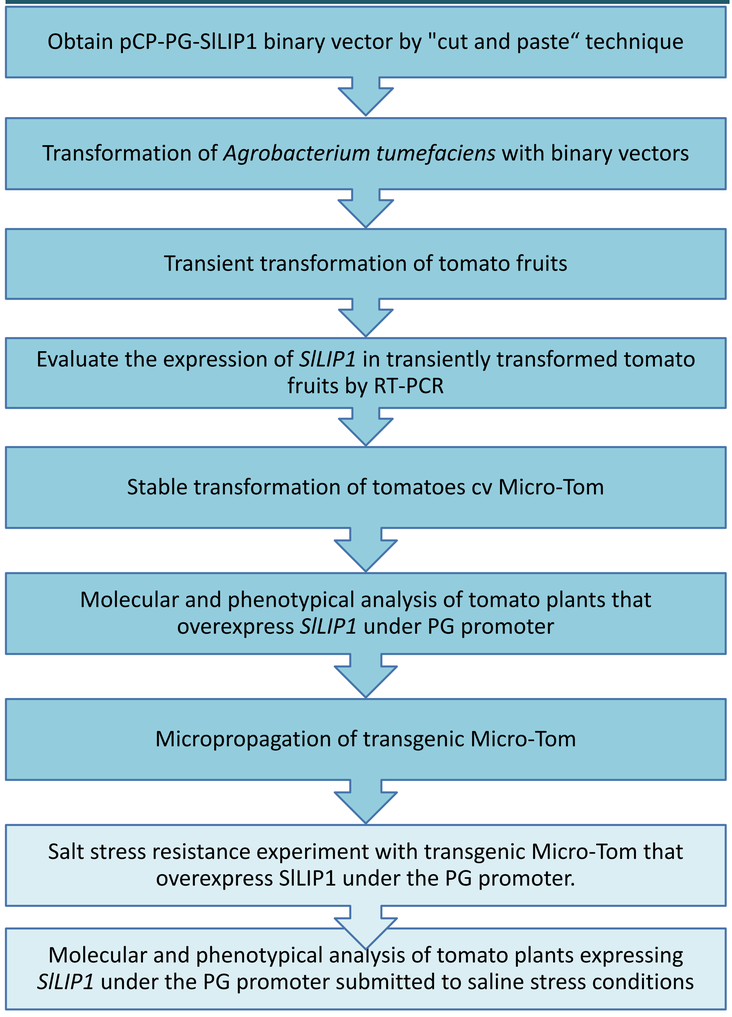

Figure 1: Work flow of research

III. RESULTS

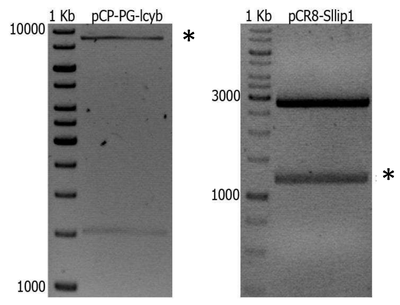

Figure 2: Obtaining pCP-PG and SlLIP1. Both initial vectors were digested with AscI and visualised in an agarose gel (asterisks show the bands for pCP-PG, left, and SlLIP1, right). Both fragments were then ligated, thus obtaining the pCP-PG-SlLIP1 vector

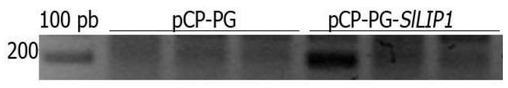

Figure 3. Expression of *SlLIP1* after transient transformation of tomatoes. Three commercial tomatoes were transiently-transformed with *Agrobacterium* harbouring pCP-PG-SlLIP1, using pCP-PG as a negative control. RNA was extracted from fruits and subjected to RT-PCR to detect a fragment of *SlLIP1*. The more intense band in pCP-PG-SlLIP1 fruits shows the overexpression of this gene, and thus the functionality of the vector.

Table 1. Number of plants obtained after stable transformation of Micro-Tom explants by *A. tumefaciens* harbouring pCP-PG and pCP-PG-SlLIP1. These plants were Basta-resistant, tolerance to which is provided by the vector pCP.

Construct	N° of explants used	N° of transformed plants (T0)	Efficiency of regeneration
pCP-PG	142	7	**4.9%**
PG-SlLIP1	161	42	**26%**
Total	303	49	**16%**

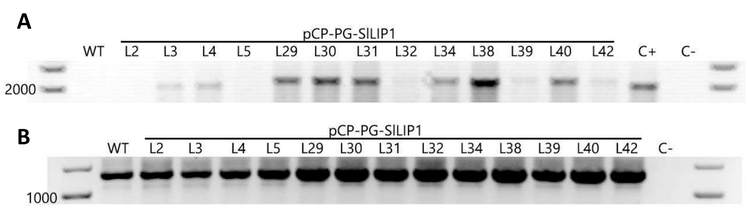

Figure 4: Genotypification of Basta-resistant PG-SlLIP1 tomato plants. A. PCR to detect a fragment spanning the PG promoter (5' end) to the His tag (3' end). in order to identify Basta-resistant transgenic plants. **B.** PCR to a fragment of *EF1*, as a control of DNA extraction. In total 13 lines were analysed and 11 were PCR positive, indicating 85% transgenic efficiency. As a positive control (C+) a miniprep was used. As a negative control (C-) water was used as template.

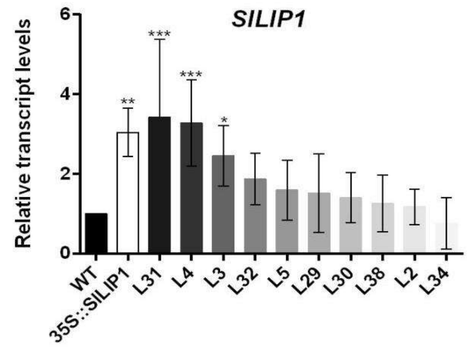

Figure 5: Analysis of *SlLIP1* expression in transgenic tomato fruits. *SlLIP1* expression was quantified by qRT-PCR, and calibrated to levels in WT fruits (1). Ten lines were analysed, using 3 fruits of each as biological replicates and 2 technical replicates. The means (±SD) of 6 data points were statistically analysed using one way ANOVA Dunnett's multiple comparison test (*p* value < 0.05).

Figure 6: Vegetative phenotype of transgenic Micro-Tom plants overexpressing *SlLIP1* in fruits. Lines 31, 3 and 4 overexpress *SlLIP1* compared to WT. The transgenic lines were obtained by somatic regeneration and numbers correspond to days (d) in the greenhouse. On the other hand, the WT plant was sown directly in soil in the greenhouse, hence the number reported corresponds to the sowing date.

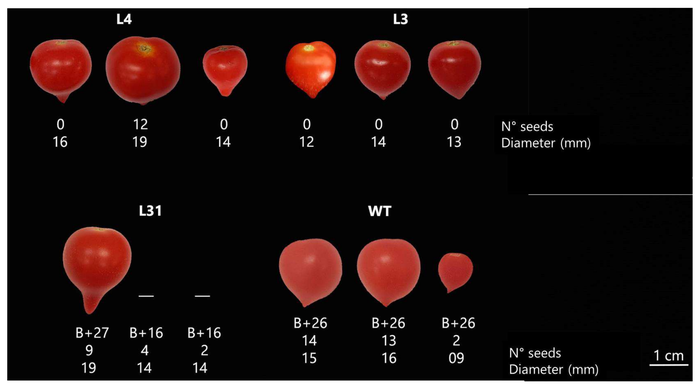

Figure 7: Reproductive phenotype of transgenic Micro-Tom fruits overexpressing *SlLIP1*. Three fruits from lines 3, 4 and 31 and WT are shown indicating the number of seeds and the equatorial diameter of each fruit. Also, for lines 31 and WT, days after 'Breaker' are indicated (B+).

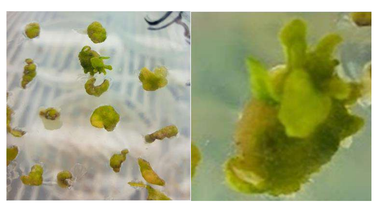

Figure 8. *In vitro* micropropagation of second generation transgenic lines in callus and shoot induction medium. For the salinity test, clones will be obtained from the transgenic lines obtained. To do so, leaf explants are being cultured in Callus and Shoot Induction Medium (MS 1X, 3% sucrose) supplemented with Trans-zeatin 2 µg/mL and Indole Butyric Acid (IBA) 0.1 µg/mL.

IV. CONCLUSIONS

1. The pCP-PG-SlLIP1 vector was able to trigger *SlLIP1* expression in commercial tomatoes after transient transformation.
2. Regeneration efficiency (Basta-resistant plants) was greater after transformation with PG-SlLIP1 than with pCP-PG.
3. 42 Basta-resistant PG-SlLIP1 plants were obtained, of which 17 were analysed after acclimatation in the greenhouse.
4. 13 lines were transgenic, of which 3 overexpress *SlLIP1* in fruits.
5. Overexpressing lines show no visible phenotypical differences with WT, in vegetative growth or in fruit morphology.
6. L3 has a parthenocarpic phenotype, L4 a partial parthenocarpic phenotype and L31 did not shown a reduced number of seeds (similar to WT).
7. Micropropagation of transgenic lines is underway for the use of clones in the salt stress tests.

V. ACKNOWLEDGMENTS

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