Involvement of *GRAS9/GRAS10* genes in tomato ripening

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Abstract:

The steady increase of the World's population exerts a tremendous amount of pressure on the already inadequate global food supply systems, posing increased challenges regarding food security. Improper storage, handling and transportation, together with the intrinsically fast ripening of climacteric fruits leads to increased post-harvest losses. To try to mitigate this issue, one of the main efforts has been to find ways to increase the fruits' shelf-life without compromising key nutritional and organoleptic properties. In this regard, tomato (*Solanum lycopersicum* L.), apart from being an extremely important crop due to its nutritional benefits, is also considered a model plant for the study of climacteric fruit ripening. It is estimated that around 25-40% of tomato production is lost every year, and as such it is imperative to address this issue. Previous studies have described the GRAS gene family as transcription factors and highlighted their importance in the regulation of a wide array of biological plant processes across the plant kingdom, including the ripening process (Neves et al., 2023). In tomato, this gene family is composed by 53 genes, including the *SlGRAS10* gene whose expression was shown to increase during flower anthesis and fruit ripening, namely during the breaker stage (Huang et al., 2015). This gene and its grapevine (*V. vinifera*) ortholog, *VviPAT6*, were previously suggested to be putatively involved in the ripening of climacteric and non-climacteric fruits, respectively (Grimplet et al., 2016). Additional phylogenetic, sequence and expression analysis showed that the *SlGRAS10* gene possessed a duplicate gene, the *SlGRAS9*, which was also shown to be expressed during ripening, more specifically 10 days after the breaker stage (Grimplet et al., 2016; Huang et al., 2015). Hence, to further our knowledge regarding the ripening process and the possible role played by the *SlGRAS9* and *SlGRAS10* genes, with the main goal being to increase the shelf-life of tomato fruits, different lines of tomato mutant plants (single mutants *SlGRAS10* and double mutants *SlGRAS9/SlGRAS10*) were created using CRISPR/Cas9 technology. The majority of the *SlGRAS10* single mutant lines showed differential expression of genes related with the ripening process compared to the Wt, namely a decreased expression of the *RIN*, *PYL9*, and *PSY* genes in specific lines. These results were in accordance with the results obtained with the total carotenoid quantification, which showed a decrease in the total carotenoid content at a late ripening stage (breaker +10 days). In these mutants, the total carotenoid content 10 days after breaker was comparable to Wt fruits at 5 to 7 days after breaker, translating into a ripening delay of almost 3 to 5 days in the mutant fruits. The double mutants were created to overcome the possible functional redundancy between the *SlGRAS10* gene and its paralog, the *SlGRAS9* gene. Preliminary results show an overall significant reduction of fruit size, weight and seed number across most lines studied. Nevertheless, more in-depth studies will be performed once the lines have been stabilized. In

summary, our findings lay the groundwork for the future development of new tomato varieties with increased shelf-life.

Keywords:

Tomato, Climacteric Fruit, Ripening, CRISPR/Cas9, GRAS

References:

Grimplet, J., Agudelo-Romero, P., Teixeira, R. T., Martinez-Zapater, J. M., & Fortes, A. M. (2016). Structural and functional analysis of the gras gene family in grapevine indicates a role of GRAS proteins in the control of development and stress responses. *Frontiers in Plant Science*, *7*, 353. https://doi.org/10.3389/fpls.2016.00353

Huang, W., Xian, Z., Kang, X., Tang, N., & Li, Z. (2015). Genome-wide identification, phylogeny and expression analysis of GRAS gene family in tomato. *BMC Plant Biology*, *15*(1), 1–18. https://doi.org/10.1186/s12870-015-0590-6

Neves, C., Ribeiro, B., Amaro, R., Expósito, J., Grimplet, J., & Fortes, A. M. (2023). Network of GRAS transcription factors in plant development, fruit ripening and stress responses. *Horticulture Research*, *10*(12). https://doi.org/10.1093/HR/UHAD220