

## IMPROVING THE BENEFICIAL EFFECTS OF TOMATO ON HUMAN HEALTH BY REDUCING THE LEVELS OF ALLERGENIC MOLECULES

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*Solanum lycopersicum* (also known as tomato) is one of the most cultivated fruit crops all over the world. Nowadays, its importance is due to the high content of bioactive compounds beneficial to human health. It is known that fresh tomatoes are rich in vitamins, flavonoids, folates, fibres, minerals, and carotenoids like lycopene, which has an antioxidant function. Previous studies suggest that consuming 100g of raw tomatoes can provide from 50% to 120% recommended daily intake of the vitamin C. Furthermore, tomatoes are considered the second most important source of vitamin C in both Italian and Spanish diets.

However, tomatoes are also known for containing anti-nutritional compounds and allergenic proteins. Anti-nutritional compounds, like glycoalkaloids, are toxic biomolecules for human health. Instead, allergenic proteins such as Sola I 1-7, Thaumatin-like proteins, and polygalacturonase 2a can induce allergic reactions.

Studies show that about 1.5% of the population in Northern Europe and up to 16% of Italians with oral allergy syndrome are affected by tomato allergy.

To this end, it is crucial to reduce the number of allergens in tomatoes to improve their qualitative aspects, as well as their beneficial properties. In previous work, we edited a tomato line knocking out two genes: i) the GAME4 gene, involved in the biosynthetic pathway of the glycoalkaloids, and ii) the Sola l 4, one of the major allergens in tomato.

In this work, we leveraged the previously edited tomato line and the Moneymaker cultivar, and we applied the CRISPR/Cas9 genome editing to knockout two additional allergenic proteins: *thaumatin-like proteins (TLPs)*, belonging to the PR-5 family, with the PR-NP24 protein expressed in the tomato exocarp; and *polygalacturonase 2a (PG2a)*, an enzyme involved in the degradation of pectin. We designed different gRNAs for the knockout of these genes, then we assembled the DNA construct using Goldenbraid and performed the tomato transformations using *Agrobacterium tumefaciens*. A total of 45 shoots carrying the kanamycin selection and the CRISPR Cas 9 editing machinery were first selected and later were able to root in the presence of kanamycin in the media. Editing results will be presented at the meeting.

Eventually, our approach will allow us to obtain tomato lines with low or no allergenic proteins to improve the beneficial effects of tomatoes. In addition, our results will show that it is possible to target and stack different genes using CRISPR/Cas9 genome editing.