

METABOLIC CHANGES IN TRANSGENIC TOMATO PLANTS REVEAL THE ROLE OF ORGANIC ACIDS IN FRUIT QUALITY AND CROP GROWTH

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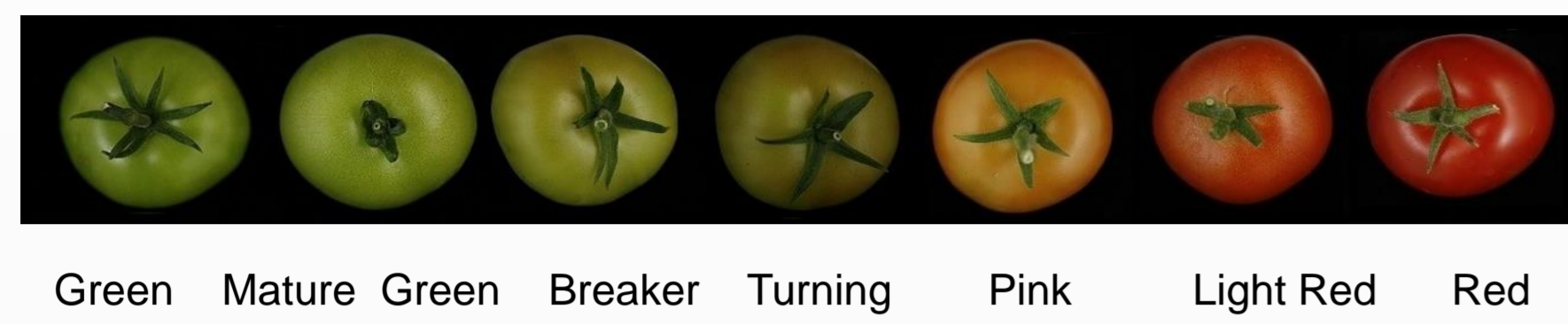
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Introduction

Organic acids are important in all plant species because their roles as **photosynthates** or energy production, furthermore they are important for **taste**.

In **tomato fruit** the levels of this acid are related to the maintenance of the quality of the fruit during postharvest ^{1,2}.

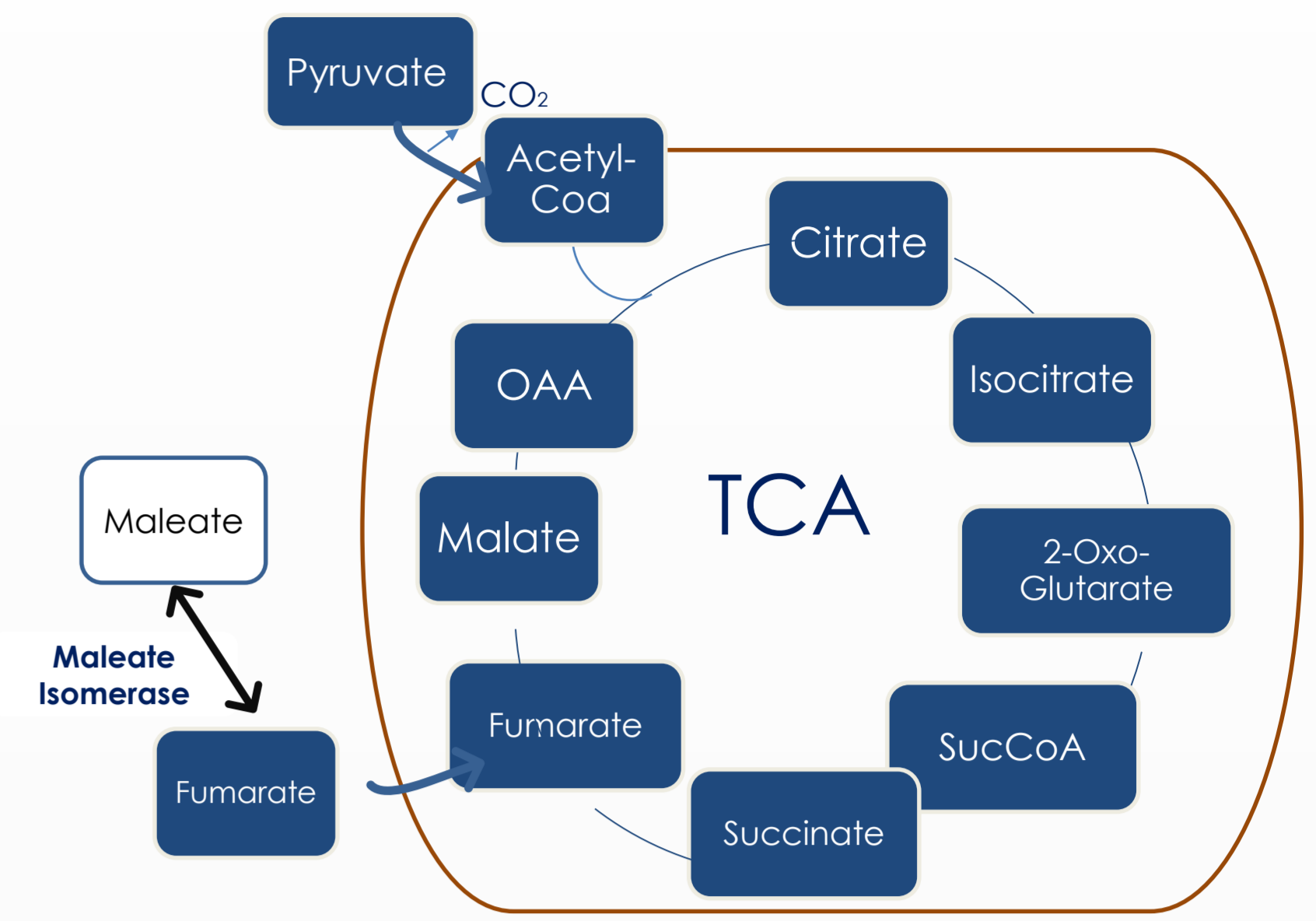
We investigated the **photosynthesis** and **primary metabolome**, of **leaves and fruits** jointly with ripening-related gene expression of fruit from transgenic tomato plants overexpressing a bacterial **maleate isomerase (MI)** gene to better understand the factors that influence the concentration of two important acids, fumarate and malate, in fruit and plant.



Ripening stages of tomato fruit from cultivar MoneyMaker (MM) selected based on color.

Metabolite profiling of the MI lines and wild type from different stages of maturity of tomato fruit and leaves:
-By Gas chromatography-mass spectrometry (GC-TOF-MS)

Identification and semi-quantification of 50 primary metabolites from different classes: amino acids, organic acids, sugars, sugar alcohols and other non-classified groups.



Results

Ripening fruit metabolome

Heatmap of primary metabolite pointing out some relevant metabolites with level changes during ripening.

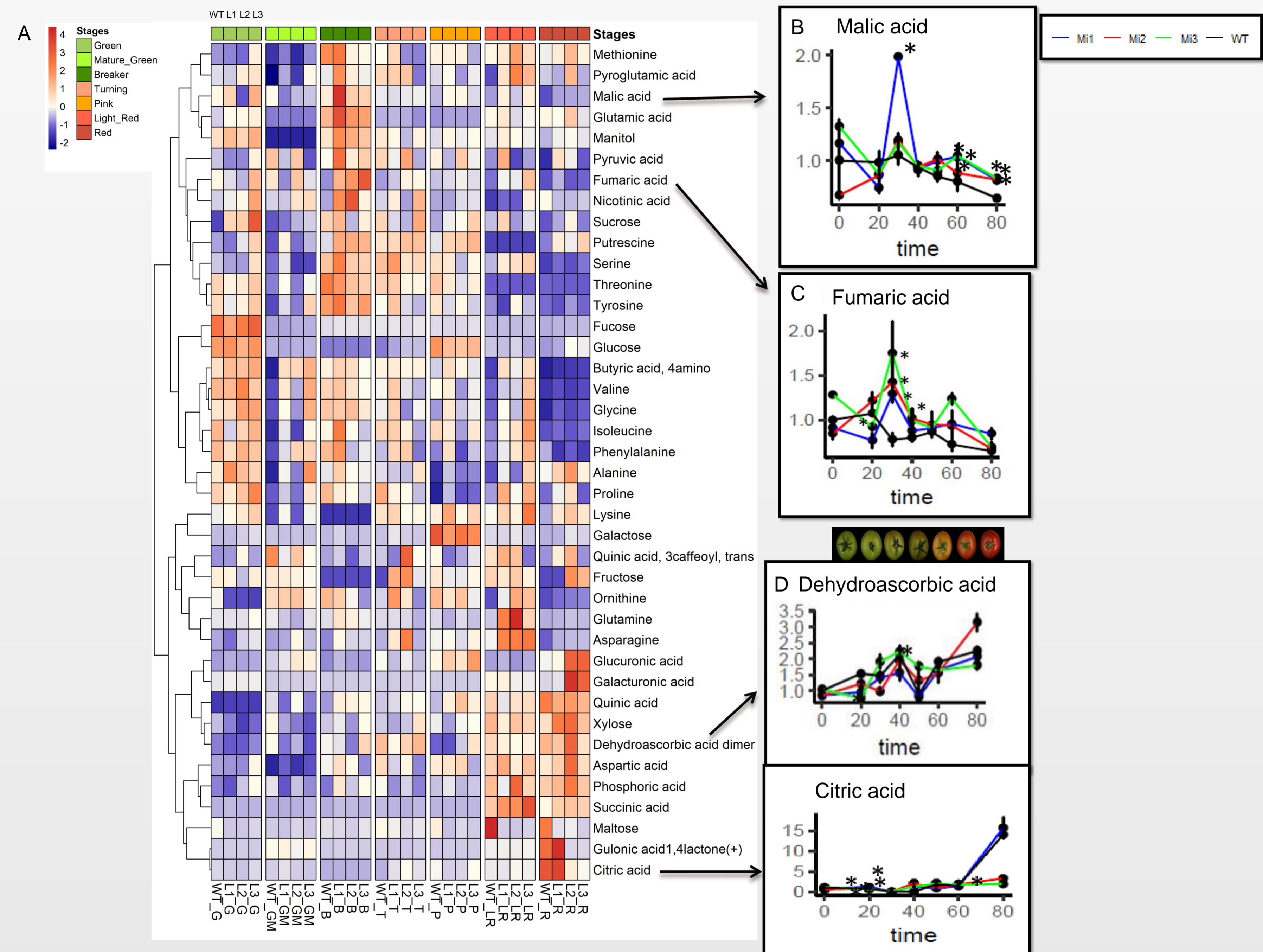


Figure 1: A. Heatmap comparison between WT and three MI-lines during 7 ripening stages of tomato fruits. B. Malic acid, C. Fumaric acid, D. Dehydroascorbic acid and E. Citric acid evolution level during ripening fruit of tomato. Values are presented as mean of 6 to 9 fruit per line, and the error bars indicate ± SE. Asterisks indicate significant differences between wild type and transgenic lines ($P < 0.05$).

Postharvest fruit life

Transpirational water loss is enhanced in detached MI-fruits, leading to a significantly increase of weight loss and a detrimental postharvest life.

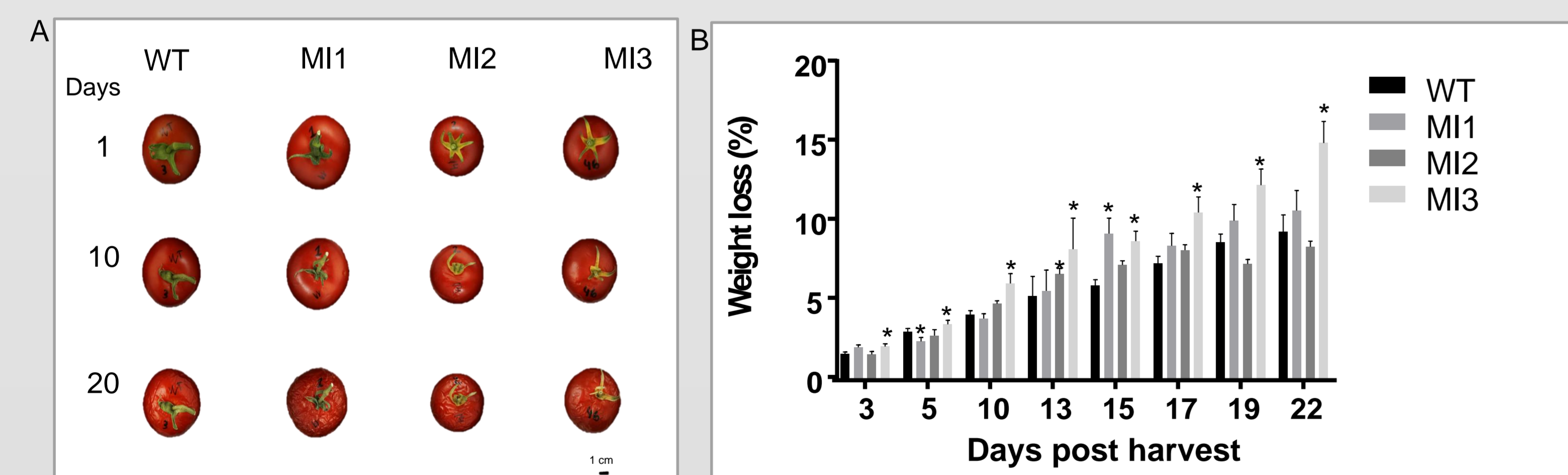


Figure 2: A. The postharvest senescence of the MI-fruit is affected. B. Percentage of weight loss from detached MI-fruits. Values are presented as mean of 6 to 9 fruit per line, and the error bars indicate ± SE. Asterisks indicate significant differences between wild type and transgenic lines ($P < 0.05$).

At red stage the transgenic fruits showed expression changes in ripening-related genes implicated in cell wall degradation.

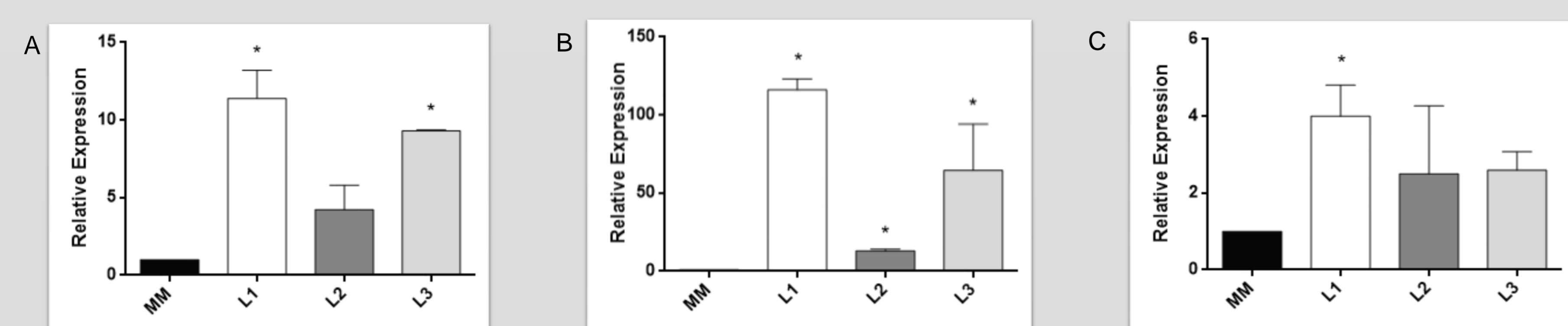


Figure 3: A. Expression level of *L-expansin*, in wild type and MI lines. B. Expression level of *cellulase-2* in wild type and MI lines. C. Expression level of *pectate lyase*, in wild type and MI lines. Values are presented as mean of 3 replicate per line, and the error bars indicate ± SE. Asterisks indicate significant differences between wild type and transgenic lines ($P < 0.05$).

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Leaves metabolome during a day

Log₂ fold change of primary metabolite presents in leaves samples showed changes in the metabolism and multi-line plots pointing out relevant metabolites in leaves during a photoperiod with light.

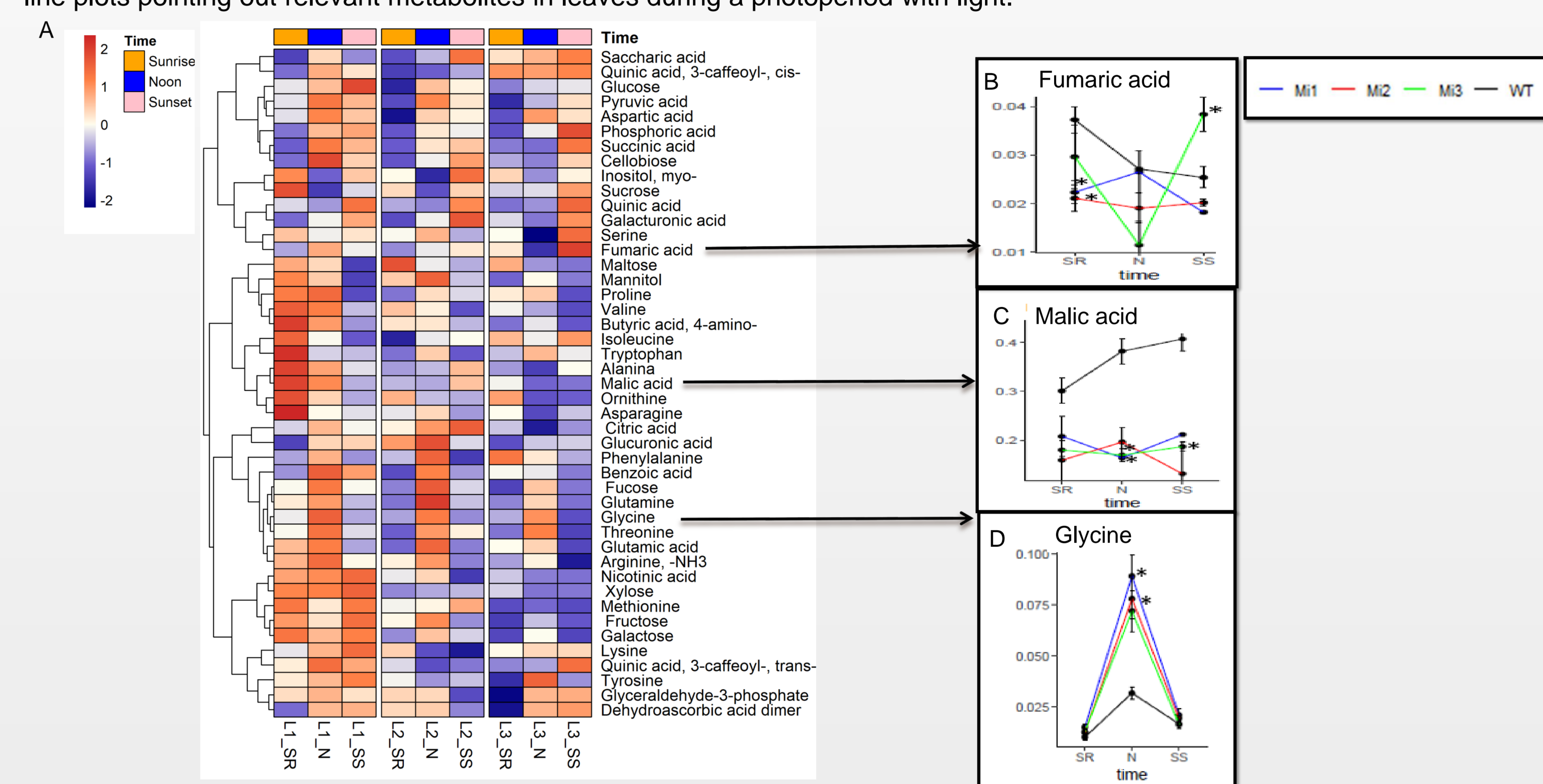


Figure 4: A. log₂ fold change in leaves tissue from WT and three MI-lines during a day taking three point: sunrise (SR), noon (N) and sunset (SS). B. Fumaric acid, C. Malic acid and D. Glycine evolution level during a day in leaves. Values are presented as mean of 3 replicates per point, and the error bars indicate ± SE. Asterisks indicate significant differences between wild type and transgenic lines ($P < 0.05$).

Photosynthesis

Study of efficiency of photosynthesis regarding photosynthesis variables, which are affected. Furthermore, the number of stomata is affected rather the aperture of the pore in the lines.

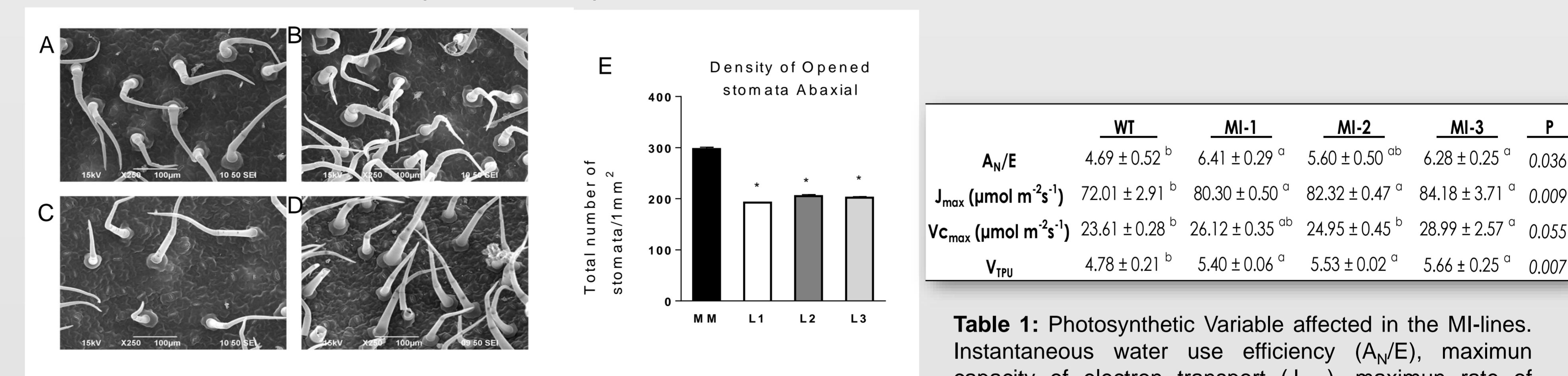


Figure 5: Images taken with a scanning electro microscopy from the surface of tomato leaves, abaxial part. A. L1. B. L2. C. L3. D. Wild type plants. E. Bar graphics showing density of opened stomata in the leaves. Values are presented as mean of 3 replicates per point, and the error bars indicate ± SE. Asterisks indicate significant differences between wild type and transgenic lines ($P < 0.05$).

Growth

Dwarf phenotype of the transgenic lines, pointed out the relevance of changes in metabolite in the control of development.

The transgenic plants showed a dwarf phenotype and a delay in development and flowering. A. Wild type. B. L1. C. L2. D. L3

Figure 7:



Conclusions

- Metabolites affected through the overexpression of *maleate isomerase* were increased in breaker stage indicating an essential role in ripening regulation (Figure 1):
 - **Malic acid:** associated with starch, soluble solid content of Tomato fruit ¹.
- The fruit showed worse **postharvest** (Figure 2) which is a described role of malic acid ¹ and affected to the expression of cell wall degradation genes ³ (Figure 3)
- In leaves the level of **malic acid** and **fumaric acid** (Figure 4) are also affected, impacting also in **photosynthesis** (Table 1) and in the **development** of the plants (Figure 7).

References

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