CANDIDATE GENE FOR BRANCHED-CHAIN AMINO ACID CONTENT IN STRAWBERRY

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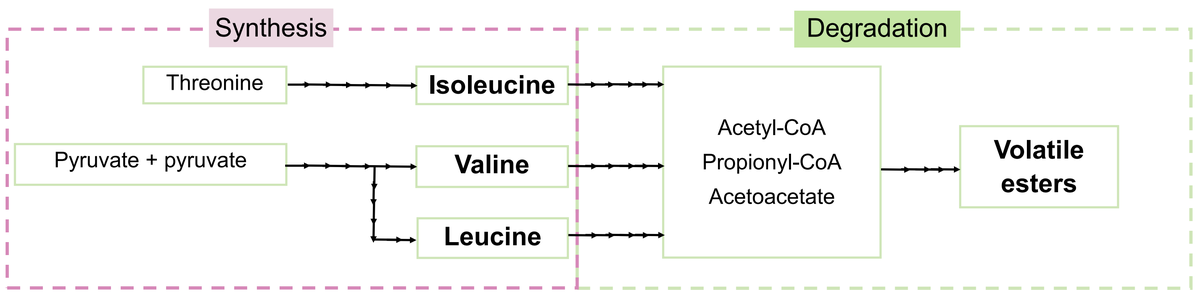
Nowadays, strawberry breeding programs are mainly focused in the improvement of its unique organoleptic characteristics, as production was prioritized over flavour and aroma. In relation to the latter, the main contributors to this characteristic in this fruit are esters, which some of them are produced from the degradation of branched-chain amino acids. We identified a possible candidate gene that could be involved in these amino acids metabolism. In last instance could be related to the production of important volatile compounds in strawberry fruits, and could be a target for future breeding programs.

The objective of this work is to identify and validate a candidate gene that regulates the content of branched-chain amino acids in strawberry fruit.

Scheme of branched-chain amino acid metabolism



Adapted from Binder, Knill & Schuster 2007 and Chen et al., 2010)

Workflow

1. Candidate gene selection based on:
- Differential expression in contrasting strawberry lines for BCAA content
- Annotation

2. Overexpression in *Nicotiana benthamiana*
- Metabolic changes by GC-MS
- Subcellular location by confocal microscopy

Ongoing

3. Stable overexpression in *Fragaria* × *ananassa*
- Metabolic changes by GC-MS

RESULTS

Candidate gene: amino acid transporter (AAAP)

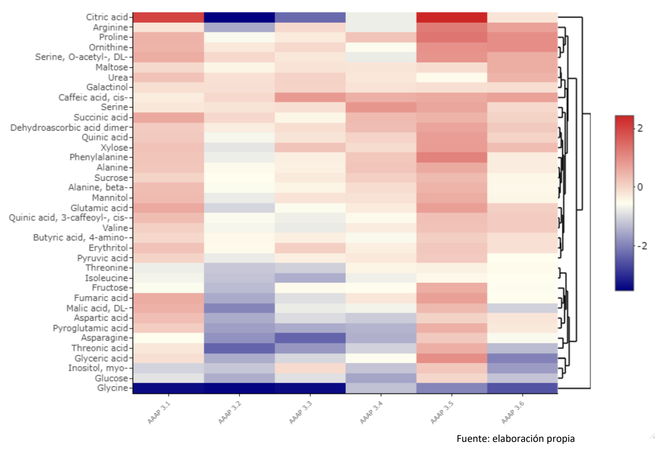
Gene	log_2FC	Annotation
AAAP	2,596	Solute transporter. APC superfamily. AAAP family.

The selected gene as a **candidate**, included in a QTL associated with BCAA content, presented higher expression in lines with higher content of valine and isoleucine.

It belongs to the **AAAP family**, which has been described to transport neutral amino acids.

Overexpression of AAAP leads to a decrease of isoleucine and its precursors in *N. benthamiana*

A) Relative content of the detected metabolites by GC-MS in *N. benthamiana* after the transient overexpression of *AAAP*

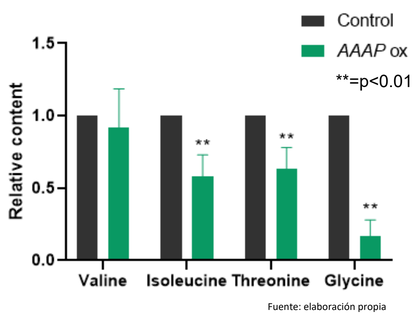


Fuente: elaboración propia

All the biological replicates present a decrease in **isoleucine** in comparation to the control.

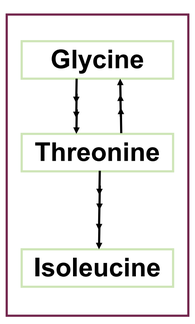
No significant changes are observed regarding **valine** content

B) BCAA relative content and significal metabolites changes in overexpressing *AAAP* lines compared to control plants



Fuente: elaboración propia

Scheme of the relation between the amino acids that present significal changes



Fuente: elaboración propia

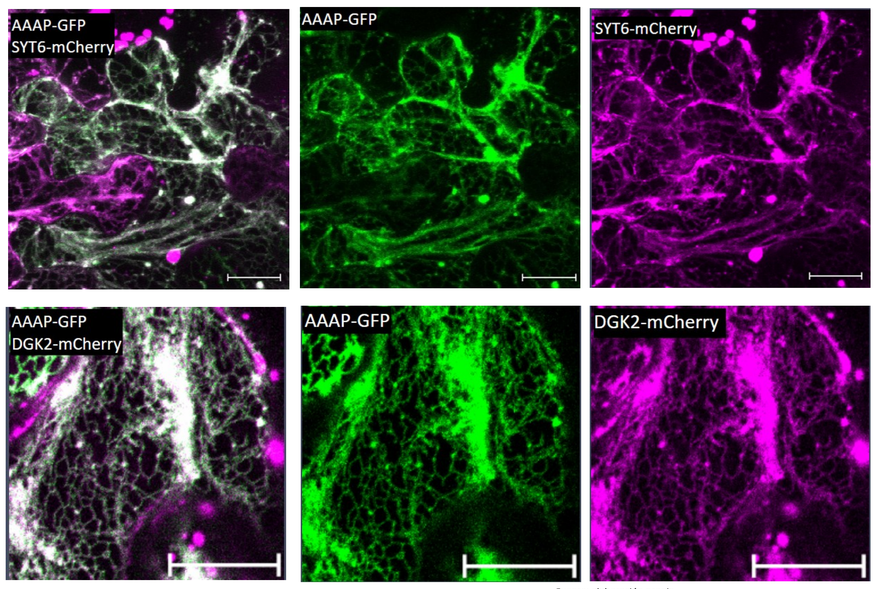
The overexpression of *AAAP* leads to a decrease of isoleucine, which is the opposite effect observed in strawberry. Our hypothesis is that what we are observing is an exclusion of this metabolite from the source to the sink.

Acknowledgements & Funding

This work was supported by grants RTI 2018-099797-B-100 (Ministerio de Ciencia, Innovación y Universidades, Spain) and UMA18-FEDERJA-179 (FEDER-Junta Andalucía). In addition, we acknowledge partial funding by PY20_00408 (PAIDI 2020-Junta de Andalucía). The attendance to this meeting was supported by Plan Propio de Investigación, Transferencia y Divulgación Científica de la Universidad de Málaga.

AAAP-GFP localizes in endoplasmic reticulum

AAAP with the tag GFP in the C-terminus under the control of the promotor 35SCaMV colocalizes with proteins that have been described to express in the reticulum



Fuente: elaboración propia

Scale bar: 20 um

Conclusions

- AAAP modulates **isoleucine** content, as well as it precursors glycine and threonine. The overexpression of this gene in *N. benthamiana* leaves leads to a decrease in the content of these metabolites, which is the **opposite effect** observed in *F.* × *ananassa* fruits.
- Although the QTL was the same for valine and isoleucine, no significant changes were observed in **valine** content.
- The addition of the GFP tag to the C-terminal end of AAAP leads to its expression in the **endoplasmic reticulum**, contrary to what has been observed in other species. This result could be due to a massive expression of the gene.

Future perspectives

- Studying the effect of this gene overexpression in **strawberry** fruits, as using a different biological system could lead to different outcomes.
- Confirm the **subcellular location** using different tags and promotors.
- Determine the transporter **substrate/s and activity**.

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