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DATOS DE CONTACTO

NOMBRE Y APELLIDOS: BEGOÑA OROZCO NAVARRETE

CENTRO: IHSM-UMA-CSIC

e-mail: borozco@uma.es

TELÉFONO: 952134265

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Studying the role of the strawberry Fra protein family in the flavonoid metabolism during fruit ripening

Begoña Orozco-Navarrete, Araceli G. Castillo, Ana Casañal, Victoriano Valpuesta, and Catharina Merchante

Instituto de Hortofruticultura Subtropical y Mediterránea-Universidad de Málaga-CSIC, Málaga, Spain.

Strawberry fruits are highly appreciated worldwide due to their pleasant flavor and aroma and to the health benefits associated to their consumption. An important part of these properties is due to their content in secondary metabolites, especially phenolic compounds, of which flavonoids are the most abundant in the strawberry fruit. Although the flavonoid biosynthesis pathway is uncovered, little is known about its regulation.

The strawberry *Fra a* (*Fra*) genes constitute a large family of homologs of the major birch pollen allergen Bet v 1 and for which no equivalents exist in Arabidopsis. Our group has shown that Fra proteins are involved in the formation of colored compounds in strawberries (Muñoz et al., 2010), which mainly depends on the production of certain flavonoids; that they are structurally homologs to the PYR/PYL/RCAR Arabidopsis ABA receptor, and that they are able to bind flavonoids (Casañal et al., 2013). With these previous results, our working hypothesis is that the Fra proteins are involved in the regulation of the flavonoids pathway. They would mechanistically act as the ABA receptor, binding a protein interactor and a ligand to regulate a signaling cascade and/or act as molecular carriers.

The main objective of this research is to characterize the Fra family in strawberry and gain insight into their role in the flavonoid metabolism.

By RNAseq expression analysis in ripening fruits we have identified transcripts for 10 members of the Fra family. Although expressed in all tissues analyzed, each family member presents a unique pattern of expression, which suggests functional specialization for each Fra protein.

Then, our next approach was to identify the proteins that interact with Fras and their ligands to gain knowledge on the role that these proteins play in the flavonoids pathway.

To identify the interacting partners of Fras we have performed a yeast two hybrid (Y2H) screening against cDNA libraries of strawberry fruits at the green and red stages. A protein that shares a 95% homology to the Heat stress transcription factor A-4-C like of *Fragaria vesca* (HSA4C) interacts specifically with Fra1 and not with other family members, which suggests functional diversification of Fra proteins in specific signaling pathways. The Y2H screening is not yet saturated, so characterization of other interacting proteins with other members of the Fra family will shed light on the functional diversity within this gene family.

This research will contribute to gain knowledge on how the flavonoid pathway, and hence, the fruit ripening, is regulated in strawberry; an economically important crop but for which basic research is still very limited.

References:

Muñoz, C, et al. (2010). The Strawberry Fruit Fra a Allergen Functions in Flavonoid Biosynthesis. *Molecular Plant*, 3(1): 113–124.

Casañal, A, et al (2013). The Strawberry Pathogenesis-related 10 (PR-10) Fra a Proteins Control Flavonoid Biosynthesis by Binding Metabolic Intermediates. *Journal of Biological Chemistry*, 288(49): 35322–35332.