The effect of the light on the control of anthocyanin pigmentation of fruits, flowers, and shoots of Citrus and relatives

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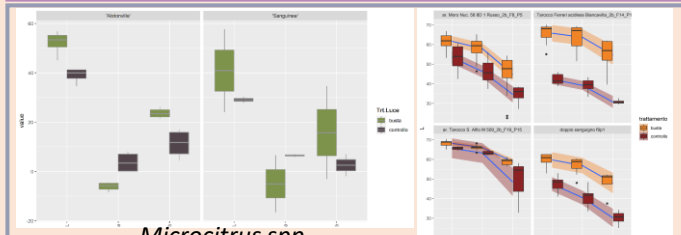
BACKGROUND

In plant anthocyanins are the results of a manifold interaction between biosynthetic pathway, controlled by the Myb-bHLH-WD complex, and other transcription factors (TF) interacting with each other coping with external factors, such as cold and light. In citrus, anthocyanins pigmentation is a trait quite diffused among primitive, wild, and cultivated species. In particular, the purple coloration is under the control of a positive activator (Ruby1)[1], of a competitor of that (Ruby2)[2], and a repressor working in an activator-and-repressor loop (CsMYB3)[3]. The role of HY5 (bZIP, binding to the G-box motif within the promoter of R2R3 Myb-like) and COP1 (in the dark it degrades, by ubiquitination, all the positive regulators) has been also evaluated similarly to *Arabidopsis* and *Malus*[4,5].

RESULTS

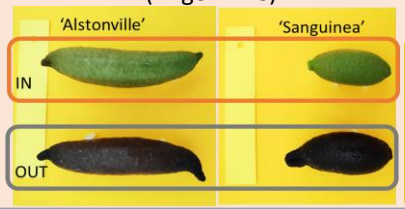
Phenotypically the light affected the pigmentation of all tissues, except fruits of **Chinese box orange** (looking light and cold-independent).

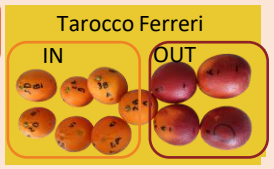
This phenomenon led us to suppose the existence of a genetic source that is different compared to sweet orange, also totally cold-independent.



Microcitrus spp
(finger lime)

Citrus sinensis
(sweet orange)





"L" showed that no-covered fruits of **finger lime** and **sweet orange** were externally much deep-colored for the presence of anthocyanins .

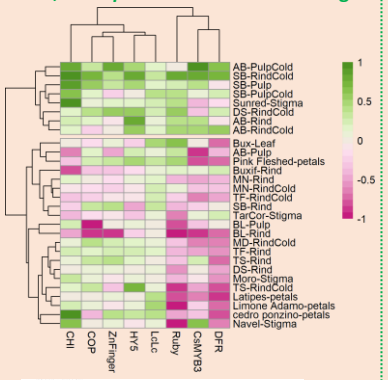
Otherwise, no difference was observed in the flesh pigmentation of sweet oranges.

Moreover, in the finger lime 'Sanguinea' variety (naturally internally and externally pigmented) the enveloping induced the development of overall non-pigmented fruits.

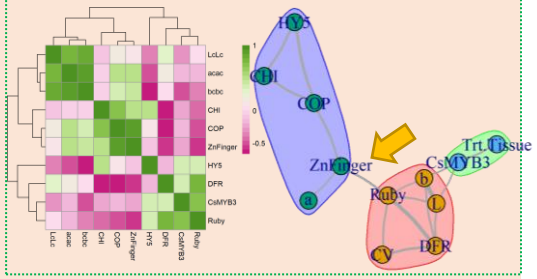
RESULTS

To investigate the effect of the light and the role of a new TF **Zinc Finger-like** in the anthocyanin production, the co-expression analysis (which infers the function of a gene basing on similar expressions) have been carried out on comparing several tissues under dark-light conditions, focusing on already known TFs (Ruby1, Ruby2, CsMYB3, HY5, COP1) and two biosynthetic genes, CHI and DFR.
We selected ……

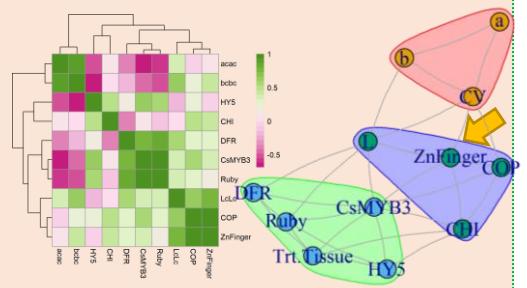
…..several tissues of **sweet oranges, lemon, citron, *C. latipes*** and Chinese box orange…



… accessions of **sweet orange**…



…. and **finger lime** not equally anthocyanins-rich in the flesh and in the rind



MATERIALS AND METHODS

(A) Sweet orange fruits were covered with black envelope bags before the external turning from green to orange; fruits were collected after 3 months. **(B)** Finger lime fruits were covered in the early phase of fruit development, and they were collected after 6 months. **(C)** In addition, stigma of pigmented and non-pigmented sweet orange varieties, petals of lemon (*C. limon*), citron (*C. medica*), and *C. latipes*, fruits and shoots of Chinese box orange (*Atalantia buxifolia,* an ancient *Citrus* species) were covered and sampled after from 3 to 4 weeks. The color was measured using a Konica minolta CR-400 and through image analysis. The genes expression was evaluated through Real time PCR.

SUMMARY AND CONCLUSIONS

(Co)Expression analysis showed the putative involvement of the new **Zinc Finger-like** in the response to **light**, leading to hypothesize that the atavism of anthocyanins accumulation in response to external factors has been recovered in pigmented oranges, similarly to other *Citrus* species.

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