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Grafting induces hormonal and metabolic responses enhancing citrus scion tolerance to combined abiotic stress

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INTRODUCTION

Carrizo citrange genotype presents a higher tolerance to drought and heat stress combination than Cleopatra mandarin and this is, in part, due to a higher antioxidant capacity, a better stomatal regulation that allows leaf cooling, and the accumulation of protective proteins. A previous study in Carrizo and Cleopatra plants under drought and heat stress combination demonstrated that the induction of a metabolic reprogramming in the plant (specially of the secondary metabolism), relied on plant ability to cope with oxidative stress (Zandalinas et al., 2017). Furthermore, when used as a rootstock, Carrizo increases scion tolerance to drought and heat stress combination, whereas other rootstocks such as Cleopatra have the opposite effect. This is due, at least in part, to the ability of Carrizo to activate the antioxidant system of the scion, enhancing protection against oxidative stress and reducing cell damage (Balfagón et al., 2021).

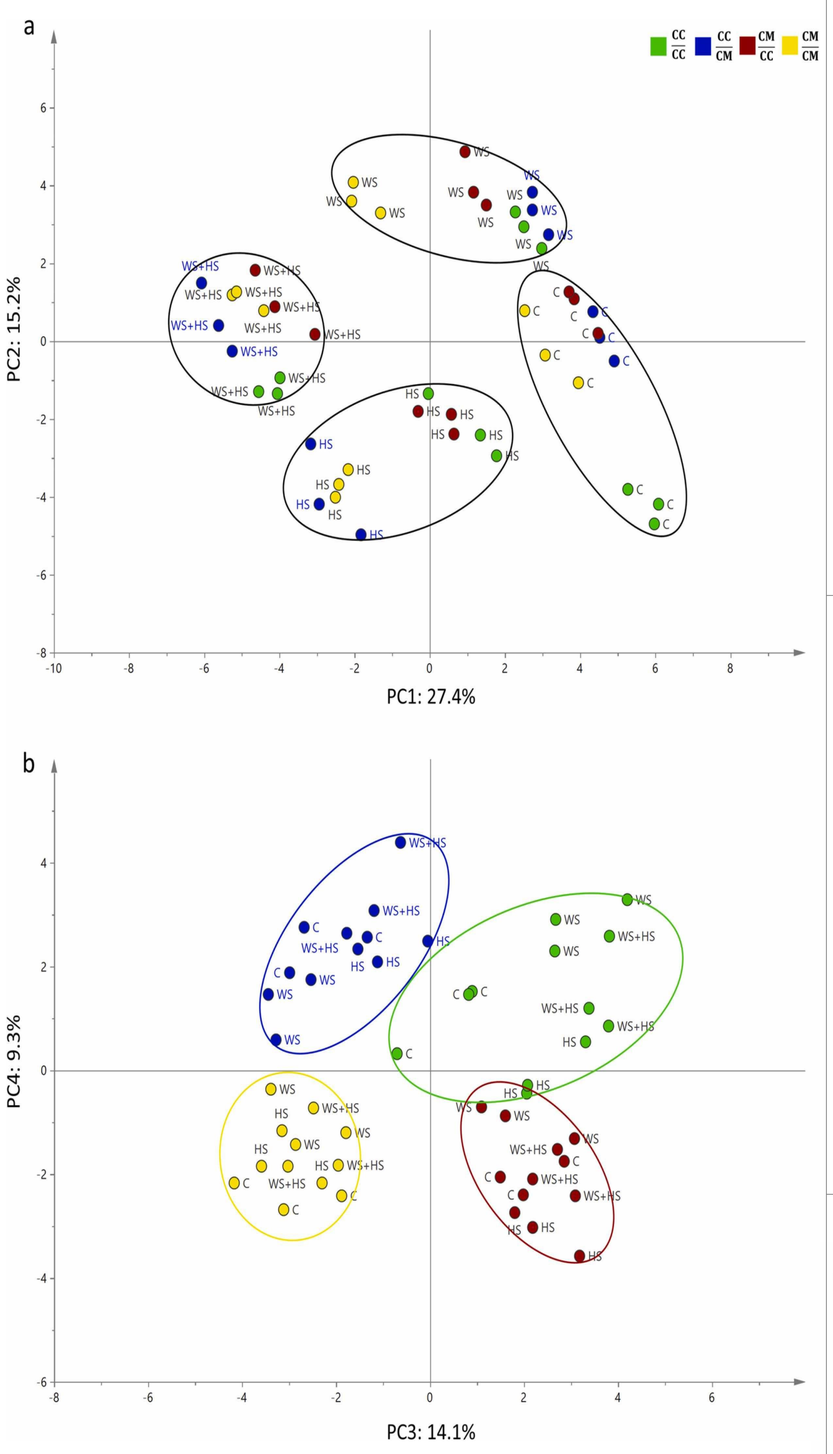
The aim of this work is to assess the importance of the rootstock on scion metabolic and hormonal responses to drought and heat stress combination by using reciprocal grafting between Carrizo and Cleopatra genotypes.

MATERIALS AND METHODS

Reciprocal and self-grafted plants of Carrizo citrange (*Poncirus trifoliata* × *Citrus sinensis*, CC) and Cleopatra mandarin (*Citrus reshni*, CM): $\frac{CC}{CC}, \frac{CC}{CM}, \frac{CM}{CC}$ *and* $\frac{CM}{CM}$ were acquired from an authorized commercial nursery. Four different groups of plants were established: well-watered plants at 25 °C (CT) or 40 °C (HS) and plants subjected to water stress at 25 °C (WS) or 40 °C (WS+HS). The relative levels of polar metabolites and the hormonal content in the leaves were determined by GC-MS and LC-MS respectively.

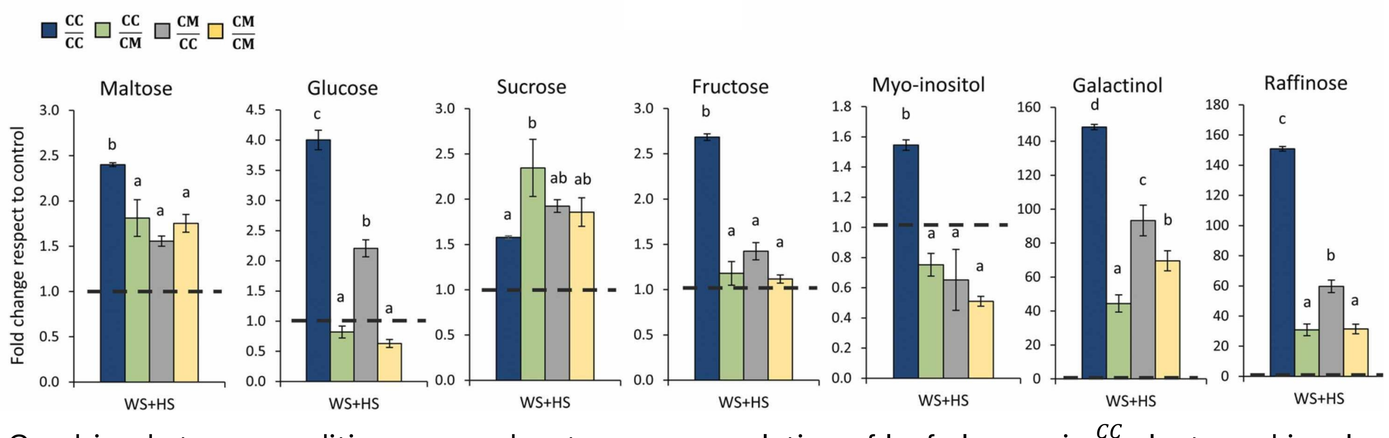
RESULTS

Principal Component Analysis (PCA) score plot of leaf metabolite and hormonal profiles



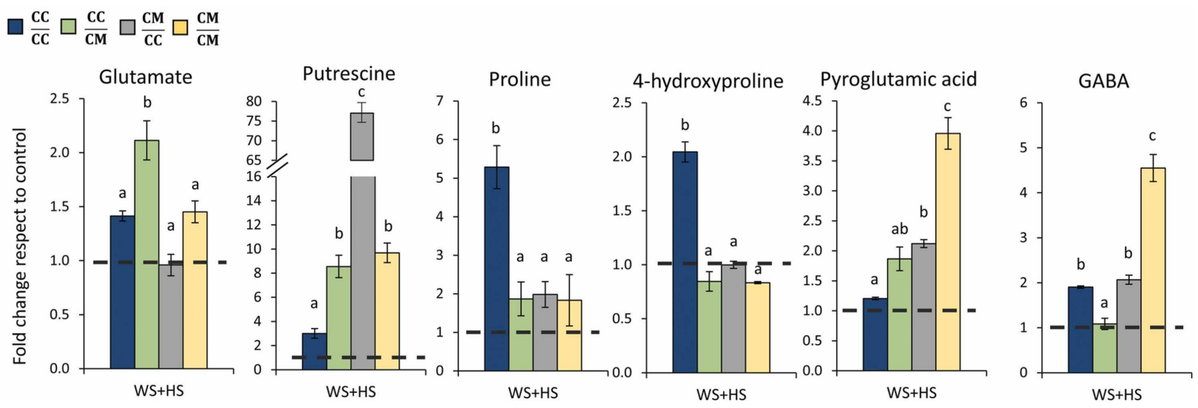
Stress treatments were the main source of variability in leaf metabolites, grouping the samples independently of their genotype (PC1 and PC2). PC3 separated samples based on their rootstock; while PC4 separated samples based on the scion genotype.

Rootstock affects scion sugar metabolism and accumulation of protective galactinol and raffinose under drought and heat stress conditions



Combined stress conditions caused a strong accumulation of leaf glucose in $\frac{CC}{CC}$ plants and in a lower extent in $\frac{CM}{CC}$ plants, whereas in plants that carried Cleopatra as a rootstock ($\frac{CC}{CM}$ and $\frac{CM}{CM}$), levels remained similar to those observed in plants subjected to control conditions or even decreased. Contents of raffinose and galactinol were higher in leaves of plants with Carrizo as a rootstock.

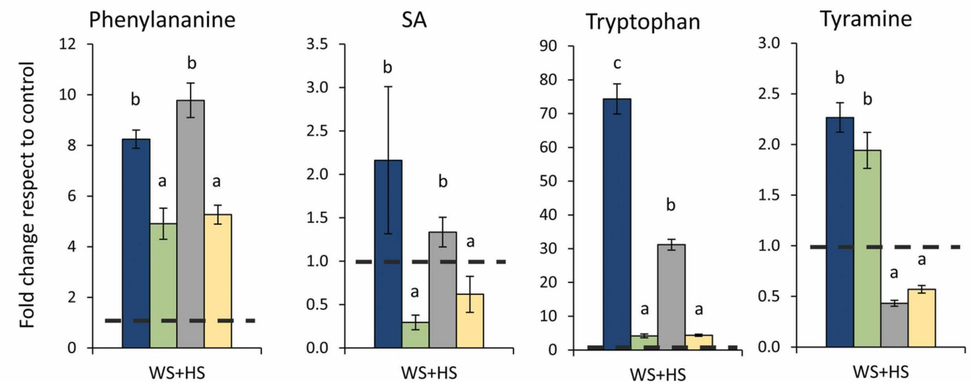
Differential accumulation of glutamate-derived metabolites was driven by the grafting combination



$\frac{CC}{CC}$ plants strongly accumulated proline and 4-hydroxyproline in their leaves, whereas this response was not observed in $\frac{CC}{CM}$, $\frac{CM}{CC}$ and $\frac{CM}{CM}$ plants

Leaf levels of putrescine, pyroglutamic acid and GABA were lower in Carrizo self-grafted plants than in Cleopatra ones, showing that glutamate metabolism is directed to these metabolites in Cleopatra while it seems to be directed to proline in Carrizo.

Shikimate derived metabolism in the scion is highly influenced by the rootstock



Under stress conditions, the accumulation of phenylalanine and salicylic acid (SA) increased in the leaves of citrus plants with Carrizo as a rootstock, while the increase in phenylalanine was less pronounced and SA content decreased in plants with Cleopatra as a rootstock. The accumulation of tryptophan in scion leaves under stress combinations was found to be dependent on the rootstock, while tyramine content was found to be uniquely dependent on scion metabolism.

CONCLUSIONS

Metabolic reconfiguration, including changes in carbohydrate and amino acid fluxes, revealed as key responses for plant acclimation to stress conditions. Moreover, the importance of the rootstock on scion metabolic and hormonal responses to drought and heat stress combination has been addressed by using reciprocal grafting between Carrizo and Cleopatra genotypes. Thus, Carrizo as a rootstock improves the metabolic and hormonal response of Cleopatra scions to the stress combination by inducing the accumulation of protective compounds such as raffinose, galactinol and salicylic acid. In turn, Cleopatra as a rootstock reduces the levels of raffinose, galactinol, proline, phenylalanine and tryptophan in Carrizo scions, which impairs plant tolerance to this stress combination. Our findings show the effect of the rootstock on scion metabolic response to stress combination and remark the importance of the rootstock in citrus plants exposed to harsh environmental conditions.

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