

**TITLE: Changes in xylem metabolite profile during fruit development in peach trees affected by iron deficiency.**

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**ABSTRACT:** Peach (*Prunus persica* (L.) Batsch) trees are among the most Fe-deficiency susceptible fruit crops. An untargeted metabolomic analysis was carried out to assess changes in xylem sap constituents under Fe deficiency. Sixteen-year-old peach trees (cv. 'Miraflores' grafted on 'GF677' rootstock) grown on a flood-irrigated calcareous soil were used. Twelve trees were selected at the end of May and grouped into two classes: six with no Fe chlorosis (+Fe; 240  $\mu\text{mol Chl m}^{-2}$ ) and six with Fe chlorosis (-Fe; 108  $\mu\text{mol Chl m}^{-2}$ ). Six current-year branches were taken from each tree at three dates, each corresponding to a fruit development stage: middle of cell division (Stage I), end of pit hardening (Stage II) and fruit maturity (Stage III). Xylem sap was obtained from shoots using a Schölander chamber. The metabolite profiles of the xylem sap were analyzed by GC-MS, following the Metabolomics Standards Initiative. A total of 159 metabolites were consistently found (in  $\geq 80\%$  of the samples in at least a sampling time), and 77 of them were identified using the database at <http://fiehnlab.ucdavis.edu/Metabolite-Library-2007>. The -Fe and +Fe samples clustered separately using PLS. Metabolite response ratios, defined as the level in the -Fe treatment divided by that in the +Fe treatment, were obtained for each sampling time. Iron deficiency caused changes (mean response ratios above 1.5 or below -1.5) in the levels of 14, 13 and 34 identified metabolites at Stages I, II and III, respectively, and also in those of 9, 10 and 24 unknowns at Stages I, II and III, respectively. Metabolites decreasing with Fe deficiency were 14 out of 23, 12 out of 23 and 40 out of 58 at Stages I, II and III, respectively. Major decreases were found at Stage III for aspartic acid (94%), glutamine (83%), sucrose (75%), oxoproline (75%) and mannose (67%), whereas the largest increases corresponded to tyrosine (5- and 2-fold at Stage I and II, respectively), cyano-L-alanine (4-fold at Stage I), asparagine (2-fold at Stage II) and arabitol (2-fold at Stage III). Changes in xylem sap metabolite profiles with Fe deficiency were found during fruit development, and the effects were largest at the fruit maturation stage.

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