Glutathione and ascorbic acid levels in iron deficient plants

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Background
Reduced glutathione (GSH) and ascorbic acid (ASA) play an important role in maintaining the intracellular redox status in plant cells. Both metabolites act in the so-called ascorbate-glutathione cycle, helping to prevent and/or minimize damages caused by reactive oxygen species (ROS). Therefore, it is important to be able to measure both forms without the less influence of the iron deficiency paradox [4]. We have developed an HPLC-MS(TOF) method [5] to measure both forms of glutathione (reduced and oxidized) in plant tissues. The method has been used to evaluate the influence of iron deficiency in the levels of glutathione (reduced and oxidized) in different plant species under different iron regimes and with different levels of iron chlorosis.

Experimental
Three plant species were used: tomato, sugar beet and pear. Tomato and sugar beet were grown hydroponically in a chamber in a half strength Hoagland nutrient solution, and kept under iron deficiency or with 45 μM Fe-EDHA for 10 to 14 days. Leaf discs were sampled and immediately frozen in liquid nitrogen and stored at -80°C until analysis. Pear trees were located in an orchard in the Ebro river basin, Zaragoza. Leaf discs were collected in the field and immediately frozen in liquid nitrogen.

Frozen samples were extracted at 4 °C under green safelight with metaphosphoric acid 5%. Samples were analyzed by HPLC-MS(TOF) as described elsewhere [5]. Labelled ASA (ASA*) and GSH (GSH*) were used as internal standards. The method is capable of analyze both GSH and GSSG as well as ASA and other thiols like homoglutathione and nitrosoglutathione. Fig 1.

Table 1. Concentration of the analytes measured in green and chlorotic leaves of different species

<table>
<thead>
<tr>
<th>Sample</th>
<th>ASA</th>
<th>GSH</th>
<th>GSGG</th>
<th>ASA/GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorotic pear leaves</td>
<td>4.136</td>
<td>1251</td>
<td>42.3</td>
<td>ND</td>
</tr>
<tr>
<td>Green pear leaves</td>
<td>5.130</td>
<td>352</td>
<td>205</td>
<td>8.6</td>
</tr>
<tr>
<td>Chlorotic tomato leaves</td>
<td>1720</td>
<td>125</td>
<td>877</td>
<td>15.4</td>
</tr>
<tr>
<td>Green tomato leaves</td>
<td>2160</td>
<td>151</td>
<td>774</td>
<td>4.8</td>
</tr>
<tr>
<td>Chlorotic sugar beet leaves</td>
<td>753</td>
<td>97</td>
<td>118</td>
<td>4.0</td>
</tr>
<tr>
<td>Green sugar beet leaves</td>
<td>2416</td>
<td>162</td>
<td>403</td>
<td>15.5</td>
</tr>
</tbody>
</table>

Results and Conclusions
- The method we have developed has been used for the analysis of these compounds in plants with different levels of chlorosis.
- A chromatogram example of green and chlorotic sugar beet leaf extracts is shown in Fig. 2. The insets at the times indicated with arrows show zoomed mass spectra at 2.4 and 2.9 were ASA and GSH coelute with their internal standards, ASA* and GSH*, respectively.
- Concentration of the different analytes is shown in table 1.
- We did not observed significant differences in the analyte concentrations between chlorotic and green leaves in tomato and pear leaf extracts
- Sugar beet results agree with previous results in our lab were analyte concentrations were measured by enzymatic reactions [6].
- ASA, GSH and GSSG levels in chlorotic and green leaves vary between species. It is difficult to infer a general behavior.

References

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