In vitro physiological responses to select new hybrid pear rootstocks tolerant iron chlorosis.

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Physiological responses under in vitro culture conditions of different interspecific Pyrus hybrids were compared in this work. Hybrids derived from crosses between the INRA pear rootstock selection ‘Pyriam’ and four Pyrus species of the Mediterranean region, a Pyrus communis var. cordata (Desv.) Hook. f. hybrid, P. amygdaliformis Vill., P. amygdaliformis var. persica Bornme., and P. elaeagrifolia Pall., which are known for their tolerance to iron-chlorosis, drought, hot summers, sandy soil and various pests and diseases. These hybrids were cloned and rooted, and plantlets were used for their in vitro characterization as well as for their field evaluation after acclimatization. Rooted plantlets were transferred to a paper bridge in a Magenta flask containing a liquid medium with 2 µM Fe³⁺DTPA and 10 and 20 mM NaHCO₃. Physiological responses were analyzed at four different times (0, 3, 7 and 28 days) during the in vitro assay. Chlorophyll level, phenolic acid exudation, Fe²⁺ reduction and medium pH were analyzed in each date. The responses of all clones, derived from the same inter-specific hybrid, were pooled together to analyze the results. Along the 4-week-long assay, and for both bicarbonate treatments, the leaf chlorophyll level for the clones derived from P. amygdaliformis and Pyrus communis var. cordata were similar, and always higher than those for clones derived from P. elaeagrifolia. These results are in accordance to what was previously observed in field plots, after grafting with a pear variety, as well as in previous in vitro assays. The same pattern was observed with the exudation of phenolic acids into the culture medium. During the first two weeks of the in vitro assay, the exudation of phenolic acids was higher for P. amygdaliformis and Pyrus communis var. cordata, when the pH was buffered with 10mM bicarbonate. At the highest bicarbonate level, P. amygdaliformis also maintained the highest level of phenolic acids, while the other two species inverted their behavior. The ability to acidify the culture medium was only observed for the 10mM bicarbonate concentration, during the first week of culture, and only significantly for clones derived from the Pyrus communis var. cordata. When
bicarbonate was at 20mM, none of the clones was able to lower the medium pH, and only the clones derived from the *Pyrus communis var. cordata* were able to maintain the pH to the initial level. After the 14 day of culture the pH decreased but never to the initial values. On the other hand, clones derived from *P. elaeagrifolia* showed a higher Fe$^{3+}$ reduction activity than clones derived from *P. amygdaliformis* or *Pyrus communis var. cordata*. This was observed during the four weeks of assay and for both bicarbonate concentrations. While a higher ability to reduce iron at the root level should be related with a higher tolerance iron deficiency in calcareous soils, it might not be sufficient to have a higher availability of reduced iron in the leaf cells, and avoid the chlorosis symptoms. In consequence, shortening the assay to the first two weeks of culture and using a 10mM bicarbonate concentration seem to be sufficient to select the best adapted clones. Besides the leaf chlorophyll level, the exudation of phenolic acids and acidification of the culture medium, seem to be the best physiological responses to predict the tolerance to lime induced chlorosis.

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