

## EXPRESSION ANALYSIS OF GENE INVOLVED IN THE IRON DEFICIENCY OF CITRUS ROOTSTOCKS

LICCIARDELLO C.\*, TORRISI B.\*, TONONI P.\*\*\*, ALLEGRA M.\*, SCIACCA F.\*, DELLEDONNE M.\*\*\*, INTRIGLIOLO F.\*, REFORGIATO RECUPERO G.\*

\*) CRA-Research Centre for Citriculture and Mediterranean Crops, Corso Savoia 190, 95024 Acireale (Italy)

\*\*\*) Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona (Italy)

*Citrus rootstock, differential gene expression, iron chlorosis, CombiMatrix Array*

At least 20 to 50% of fruit trees in the Mediterranean basin suffer from iron deficiency, due to high concentrations of calcium and bicarbonate in soils. The most common symptoms in plants consist of interveinal chlorosis in young leaves, a decrease in leaf net photosynthetic rate, leaves reduced in size, fragile and very thin. Then a growth retardation followed by death in more severe conditions, associated to the loss of yield, delayed fruit ripening and impaired fruit quality is also observed. It's possible to recover the iron deficiency adding inorganic iron salts, synthetic chelates, and natural organic compounds, even if it's difficult to correct because of the rapid transformation of iron contained in fertilizers into an unavailable form. So the easiest way to avoid iron chlorosis on calcareous soils is the use of tolerant rootstocks. In citrus even if the sour orange (*Citrus aurantium* L.) was the most diffused rootstock in the Mediterranean area, considering its tolerance to calcareous soils, the sensitivity to Citrus Tristeza Virus (CTV), one of the most serious citrus biotic stress, is producing its replacement. However the search of alternatives rootstocks remains the only strategy to react to iron deficit problem.

The aim of the work was to isolate differentially expressed genes involved in the iron deficiency using a CombiMatrix platform, made up of around 8,000 ESTs of roots extracted from NCBI database of citrus and its relatives. At first we worked on Carrizo citrange (quite tolerant) and Swingle citrumelo (sensitive) rootstocks, grown on pots with two different soils, one calcareous (9% of active lime) and another volcanic (0% of active lime). Microarray experiments were carried out with RNA samples extracted from tip roots. Foliar analysis was also performed on leaves. Expression analysis, based on the comparison between Swingle citrumelo growing on calcareous soil respect to volcanic one, showed five differential genes up-regulated, which glutathione peroxidase (GPX) is the only annotated. The GPX, such as superoxide dismutase, ascorbate peroxidase, catalase and glutathione reductase, is an antioxidant enzyme strictly involved in biotic and environmental stresses. In particular way GPXs are diverse enzymes catalyzing the reduction of hydrogen peroxide, organic and lipid hydroperoxides by reduced glutathione, helping to protect cells against oxidative damage. In a second experiment GPX was used as marker to evaluate its expression level correlated to iron deficiency on seven different rootstocks (3 sensitive and 4 tolerant), growing on soil characterized by a medium-high calcium level. The expression analysis, conducted through the Real time PCR on root and leave samples, confirmed the higher expression of GPX in citrumelo roots respect to Carrizo ones. It could be due to the reaction of sensitive rootstock against stressed and oxidant conditions. Anyway the opposite trend was evidenced in leaves. These data are comparable to peroxidase, Fe concentration, SPAD index and chlorophyll

content performed on leaves, and to Fe chelate reductase conducted on roots. The down expression of GPX on leaves of sensitive rootstocks could be explained considering that GPX is an Fe-dependent enzyme, unable to work causing the low iron availability, and taking, as consequence, to the accumulation of free radicals. Further analysis will be addressed in array experiment on comparison of tolerant and sensitive rootstocks growing in the volcanic soil, with the aim to isolate genes involved in the protection of iron deficiency.