AN IN VITRO SYSTEM TO IDENTIFY AND STUDY GENE EXPRESSION IN APPLE UNDER COLD STRESS CONDITIONS

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Growth and development are strongly influenced by environmental factors as productivity of agricultural species. Among them cold, with rigour of winter and spring, is highly recurrent in relation to climate global change. Apple is considered tolerant cold specie, however, rapid changes in temperature during the spring period can induce severe injuries to many organs of plant, compromising the productivity. Aim of this research was to assess an in vitro system able to permit the isolation and the study in cv ‘Mondial Gala’ apple genes related to adaptation to low temperature. Using this system diverse genes have been isolated and sequenced that have been already submitted to Gene Bank, and some of these are codifying gene for protein with unknown functions. Three MYB candidate genes have been found, together with one catalase gene, two ADP-glucose phosphorylase genes, which resulted temperature regulated. In particular, we have found a glycosyltransferase-like gene with a high homology to a 4-LGT gene of A. thaliana. Moreover, 18S, elongation factor (EF) and actin genes have been used as housekeeping genes to analysed the quantitative gene expression by Lyght Cycler Real-Time PCR.

Gene expression experiments have been conducted through two thermal modification trials: in the first trial, temperature was immediately decreased from 24°C to 8°C, and plant materials was sampled after a overnight period at 8°C constant temperature; in the second trial, temperature was decreased, every two hrs, with a ramp of 2 °C from 24°C to 8°C, and after overnight at this temperature plants were moved to 2 °C. In latter experiment plant material was sampled at temperature step of 24, 16, 12, 8 (2 hrs), 8 (over-night) and 2 °C. Ion leakage measurement have been done to evaluated the stress degree of plantlets, and a following pattern of progress injury was determined: 7.1% of ion leakage at 24°C, 9.4% at 12°C, 7.7% at 8 °C after 2 hrs, 10.9% ad 8°C after over-night period, and 85,7% at 2°C after over-night period.

In the first experiment MYB-related genes increased their levels of expression in the plant after they were moved at 8 °C, whereas glycosyltransferase-like and catalase genes decrease their levels of expression. During the gradual decrease of temperature MYB-related genes did not change their levels of expression, and a light decrement in gene expression was detected only for glycosyltransferase-like and catalase genes. A cold regulation of actin and EF housekeeping was also found. All of the isolated genes shown to be regulated by temperature, and we
hypothesized a regulative role for the isolated *MYB*-related genes. Data will be discussed in relation with a possible cold regulative pathway of *MYB* gene.

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