THE EXPRESSION OF CHLORIDE CHANNEL (clc) GENE DURING POST-GERMINATIVE GROWTH IN MAIZE POPULATIONS SELECTED FOR HIGH AND LOW COLD TOLERANCE

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Ion channels are known to regulate mitochondrial volume and respiratory activity in various physiological conditions. This work aims at the molecular characterization of the chloride channel (CIC) protein of maize mitochondria, and at the quantification of the clc gene expression with respect to the cold tolerance during post-germinative growth. For this purpose, maize populations selected for high (C4H) and low (C4L) cold tolerance during germination have been studied together to the original non-selected population (C0) (Landi et al, 1992; De Santis et al, 1999). First strand cDNAs isolated from leaf, mesocotile and root of etiolated maize seedlings have been amplified in PCR, using degenerate primers annealing to conserved regions of homologous ClCs. This DNA has been sequenced and proved to encode for a ClC protein. In order to study how low temperatures influence clc gene expression during maize post-germinative growth, Light Cycler PCR assay has been performed. This method allowed relative quantification of clc gene expression in the C0, C4H e C4L maize seedlings grown with different temperature treatments: 25°C (normal, N), 5°C (cold stressed, S), 14°C (acclimated, AC) and 14°C followed by 5°C (acclimated and cold stressed, ACS). In all the maize populations studied, clc gene expression was found to be temperature dependent. Low temperature treatments caused a general decrease in clc gene expression; however, the clc transcript level varies with the different maize population and temperature treatment. These results together with measurements, at different assay temperatures (from 5 °C to 25 °C), of chloride ion flux through the inner membrane of isolated mitochondria, allowed to calculate the specific activity of CIC protein for different experimental conditions. In particular, C4H population, selected for the high germination at low temperature, showed a higher relative increase of CIC protein specific activity at 5 °C with respect to the C0 and the C4L populations. Such increase was found in both the AC and ACS temperature treatments, and not in low temperature treatment without acclimation (S). These results suggest that CIC protein of maize mitochondria could be involved in the mechanisms of low temperature tolerance during post-germinative growth.