Assessment of the electron partitioning between the cytochrome and alternative oxidases in wheat seedling mitochondria *in vivo*: effects of cold treatment

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It is well-known that plant mitochondria possess the cyanide-resistant alternative oxidase (AOX) pathway which might play a role in preventing the production of toxic oxygen species. As generally accepted, AOX activity is thought to increase under unfavorable conditions, including low temperatures. For testing this hypothesis, effects of cold treatment on the activities of AOX and the main cytochrome oxidase (COX) pathway were studied using mass-spectrometry. Gas-phase systems of oxygen isotope fractionation (the ratio $^{18}O/^{16}O$) technique allow to assess the electron partitioning between the terminal pathways of respiratory chain in intact plant tissues. This is based on the differential fractionation of the two terminal oxidases being between 18% and 20% for the COX, while the fractionation of the AOX is more variable, from 24% to 31% (Ribas-Carbo et al., 2005). Etiolated seedlings of bread wheat *Triticum aestivum* L. (winter cv Mironovskaya 808) were grown in the dark at 25 °C for 3 d (control), and a part of seedlings were placed for 3 d at 4 °C (chilled). Oxygen isotope discrimination in samples (coleoptiles with first unfolding of primary leaves inside) in the absence of inhibitors (Δn) was 23.4% (control) and 22.7% (chilled), and the electron partitioning between the AOX and COX pathways ($\tau_a = \nu_{alt}/\nu_{cyt}$) diminished from 0.35 to 0.3. The discrimination values determined in the presence of KCN were similar in control (29.7%) and chilled (29.15%) samples. Total respiratory activities were 64–69 nmol O_2 g^{-1} dry weight (DW) s^{-1} and did not change significantly after cold treatment, but the values of chilled plants became higher (on 18%) because of increased ν_{cyt} in case of their calculating on fresh weight (FW). Respiration through the AOX pathway (ν_{alt}) did not change (on FW) or decreased (on DW) in cold-treated plants. The differences among results on FW- and DW-basis reflect a significant difference on DW/FW ratios between control (0.076) and cold-treated (0.095) seedlings. These facts indicate that the activity and capacity of AOX might decrease in tissues of wheat etiolated seedlings after cold treatment and depend on calculation units (on FW or DW).

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