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Changes in plasmalemma K^+Mg^{2+}-ATPase dephosphorylating activity and H^+ transport in relation to seasonal growth and freezing tolerance of *Festuca pratensis* Huds

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Summary

Changes in plasmalemma K^+Mg^{2+}-ATPase dephosphorylating activity and H^+ transport were examined in freezing-tolerant and non-tolerant genotypes of the perennial grass species *Festuca pratensis* Huds. Enzyme activity and $\Delta\mu H^+$ were measured in plasmalemma fractions isolated from basal nodes and roots. Three types of experiments were undertaken: (i) a field experiment, utilizing the seasonal growth and cessation cycle of a perennial plant; (ii) a cold acclimation experiment in hydroponics; and (iii) an instant freezing test. A specific fluctuation in K^+Mg^{2+}-ATPase activity was found throughout the seasonal growth of the plants (i). The K^+Mg^{2+}-ATPase activity peaks for both the basal node and the root plasmalemma were determined early in the spring before the renewal of growth. The lowest activity values in roots occurred at the time approaching flowering, and in basal nodes at the transition into the growth cessation. The K^+Mg^{2+}-ATPase activity was approximately 50% lower in the basal node plasmalemma of freezing-tolerant plants than of non-tolerant ones, when assessed at the optimal growth stage in hydroponics. In hydroponics (ii) and in the freezing test (iii), temperature stress was followed by a more pronounced change in the level of K^+Mg^{2+}-ATPase activity than in that of H^+

Abbreviations: FT, freezing-tolerant genotypes; NT, freezing non-tolerant genotypes

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transport, and this change was more clearly differentiated in the basal node plasmalemma of contrasting genotypes than in the roots. Stress response was manifested differently in freezing-tolerant and non-tolerant plants at cold acclimation (4–2 °C) and at freezing (−8 °C) temperatures. Proton transport regulation via coupled changes in the hydrolysed ATP/transported proton ratio, as an attribute of freezing-tolerant plants, is discussed.

Introduction

Festuca pratensis (meadow fescue) is a perennial grass species widely distributed throughout Europe. It is used in agriculture as a forage grass, and is generally valued for its adaptiveness to climatic stress in Northern Europe. As a species colonizing wide and differentiated areas, *F. pratensis* exhibits variation in its adaptiveness to low temperatures. Freezing-tolerant and non-tolerant genotypes used in our study were previously selected by the Norwegian group of Alm et al. (2006). Meadow fescue has an annual cycle of development, which consists of intensive growth (summer), cold acclimation, which for herbaceous perennial plants is commonly defined as cold hardening (autumn), growth cessation (winter) and de-hardening (early spring). The upper parts, stems and leaves, desiccate before growth cessation, but the lower parts, basal nodes and roots, which are located close to the soil surface and in the soil, respectively, undergo significant physiological changes and are maintained throughout the winter. Our experiment was carried out in Lithuania, where the growth of *F. pratensis* extends over 7 months, between April and October. Winter conditions are severe for plants here; freezing temperatures below 0 °C are common, while more extreme temperatures can fall as low as −25 to −30 °C. The soil stays frozen up to 4–5 months, which also imposes heavy stress on the plants.

Environmental stress signals induce a plant response that starts with the perception of a signal followed by its transduction, changes in gene expression and cell metabolism, and finally results in changes in plant growth and productivity. The response to stress depends on the species and the nature and magnitude of the stress signal (Aarts and Fiers, 2003; Kacperska, 2004). In the case of cold stress, two types of plant cell responses are known, depending on whether the temperature is positive or negative. As a general rule, plants demonstrate a cold acclimation response at lowered temperatures, and this may reduce the harmful impact of freezing (Uemura et al., 2006).

The cell plasmalemma is not only the site of stress-induced damage but, due to rapid changes in its physical state and microdomain organization, it also affects stress signal transduction and the expression of stress-response genes. Under the effects of cold, the physical properties of glycerolipids and the expression of response genes may undergo changes accompanied by enhanced levels of unsaturated fatty acids and membrane protein modification (Glatz et al., 1999; Kawamura and Uemura, 2003; Uemura et al., 2006). All of these changes inevitably alter the functional state of the plasmalemma, including the ion-transport systems. The question therefore arises as to the functional state of the plasmalemma, which is related with freezing tolerance. Taking a broad view of this topic, we focused our analysis on the processes that are obligatory for every living cell, i.e. transmembrane potential support and cell homeostasis maintained by H^+-ATPases (proton pumps), which mediates H^+ extrusion from the cell. In the plant cell, plasmalemma ATP-dephosphorylation and ATP-dependent H^+ transport functions are attributed to K^+, Mg^{2+}-ATPase (Maksimov, 1989; Jurin et al., 1991; Medvedev, 1998). The aim of the present study was thus to elucidate changes in K^+Mg^{2+}-ATPase activity and H^+ transport in the genotypes of *F. pratensis* with large differences in freezing tolerance, documented at the whole-plant level.

We focused on changes in the basal nodes and roots, since these are the vital parts of perennial grasses that withstand freezing throughout the winter. The experimental conditions ranged from the natural growth cycle in the field to controlled cold acclimation temperatures in hydroponics, as well as an instant freezing test. The field experiment was set up to investigate the relationship between plasmalemma K^+Mg^{2+}-ATPase activity and natural developmental changes throughout the annual growth cycle. The experiment in hydroponics was conducted in order to provide a more detailed study of the responses of the plasmalemma assessed by K^+Mg^{2+}-ATPase dephosphorylation activity and H^+ transport throughout the process of cold acclimation at lowered temperatures (8 °C → 4 °C → 2 °C). Finally, an instant freezing test at −8 °C was included in this