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Frost tolerance, deacclimation and reacclimation traits in perennial ryegrass

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Introduction
The ability of perennial grasses to harden and maintain frost tolerance throughout the winter is crucial for winter survival. This includes the ability to resist deacclimation during transient mild spells in winter, and the ability to reacclimate when cold temperatures return. The latter traits are especially critical in regions with cycles of freezing and thawing, and lack of a stable, insulating snow-cover that can protect the plants from extreme air temperatures. Such conditions are typical for many coastal areas in Northern Eurasia and America, such as the southwestern coast of Norway. The climate is changing and one of the consequences for Norway will be stronger fluctuations in winter temperatures. This might open up for increased use of perennial ryegrass (Lolium perenne L.) in Scandinavian forage grass production systems at the expense of timothy (Phleum pratense L.), the most commonly used forage grass today.

However, there are still important questions about the winter survival of perennial ryegrass under future climate conditions that needs to be addressed before a wider use of this grass species can be recommended. One is related to the risk of frost injury connected with more fluctuating temperatures at plant level in winter and spring. Thus, simulation studies using a grassland model parameterized for current winter-hardy cultivars of perennial ryegrass indicated an increased risk of frost injury in winter and spring in many areas of North Europe including Norway (Höglind et al., 2013). The increased risk was associated with a reduced snow cover, and earlier onset of spring growth followed by frosts. The simulation results indicate that cultivars that can resist deacclimation and/or that can reacclimate to a substantial degree will be needed for successful grass production under the projected future climate conditions. However, more information is needed about the genetic variation with respect to deacclimation resistance and reacclimation capacity.

The aim of the present work was to compare three cultivars of perennial ryegrass with respect to their resistance to dehardening and ability to reharde under fluctuating winter temperatures. The plants differed widely with respect geo-climatic origin. Plants were first hardened under controlled conditions, and then subjected to a period of mild temperatures followed by increase to pre-dehardening temperatures. Frost tolerance was estimated by freezing tests after completed hardening, twice during the mild episode and twice after the return to pre-hardening conditions. Our hypothesis was that the deacclimation and reacclimation characteristics would differ largely between the cultivars given their contrasting geo-climatic origin.

Materials and Methods
The experiment was carried out under controlled conditions at NIBIO, Klepp st., Norway. Three cultivars of perennial ryegrass was included: the Norwegian cultivars Fagerlin (Synthetic polycross of Russian, Swedish, Norwegian, and Finnish material, adapted for North Norwegian conditions), FuRa9805 (Fura) (Based on Irish material, adapted for Southern Norwegian conditions by polycrossing surviving plants in Southern Norway), and the Polish cultivar Arka. Fagerlin is the most winter hardy of these cultivars under current Norwegian field conditions.

Plants were propagated from seeds in pots (pot volume 1l) filled with sand placed in a greenhouse at ca. 18°C. In January, when the plants were 1½ months old, they were transferred to growth chambers for subsequent treatments. Plants were first hardened for three weeks at +2°C; then subjected to dehardening conditions for 10 days at +10°C; followed by rehardening conditions for 14 days at +2°C. Light during the hardening and dehardening was given as 150-200 µmol m⁻² h⁻¹ for 10 h day⁻¹ using high-pressure sodium lamps.

Frost tolerance was estimated as in Höglind et al. (2010). Bundles of tillers were placed in moist sand in programmed freezers. Temperature was lowered from 2°C to -2°C by 1°C h⁻¹ and kept at this level for 12 hours, after which the temperature was lowered by 1°C h⁻¹ to -10°C and thereafter by 3°C h⁻¹. Bundles of tillers were removed intervals of 3°C for each of five test temperatures, thawed and transplanted into sand in a heated greenhouse. After 3 weeks of regrowth, the number of surviving tillers was counted, and the frost tolerance (LT50; temperature required to kill 50% of the tiller population) of the individual treatments and replicates was estimated using the probit procedure of Minitab, followed by ANOVA.

Results and Discussion
All cultivars hardened to a similar LT50, -16°C. Only minor, non-significant differences were observed between the cultivars with respect to rates of dehardening and rehardening. The average loss of frost tolerance during the dehardening period was 5.4°C, whereas the average increase in frost tolerance during rehardening was 4.5°C. The results indicate that the genetic variation for deacclimation resistance and reacclimation ability in perennial ryegrass is relatively small. However, more research is needed to verify this observation with more cultivars and different hardening conditions.
**Conclusion**
The three cultivars did not differ with respect to deacclimation resistance and reacclimation capacity under the study conditions, in spite of their contrasting geo-climatic origin, indicating limited genetic variation for these traits in *L. perenne*.

**References**


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