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Research article

Mechanisms involved in the regulation of photosynthetic efficiency and carbohydrate partitioning in response to low- and high-temperature flooding triggered in winter rye (*Secale cereale*) lines with distinct pink snow mold resistances

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ABSTRACT

In terms of climate changes and global warming, winter hardiness could be determined by unfavorable environmental conditions other than frost. These could include flooding from melting snow and/or rain, coincident with fungal diseases. Therefore, we designed an experiment to identify potential common mechanisms of flooding tolerance and snow mold resistance, involving the regulation of photosynthetic efficiency and carbohydrate metabolism at low temperatures. Snow mold-resistant and susceptible winter rye (*Secale cereale*) plants were characterized by considerably different patterns of response to flooding. These differences were clearer at low temperature, thus confirming a possible role of the observed changes in snow mold tolerance. The resistant plants were characterized by lower PSII quantum yields at low temperature, combined with much higher energy flux for energy dissipation from the PSII reaction center. During flooding, the level of soluble carbohydrates increased in the resistant plants and decreased in the susceptible ones. Thus increase in resistant line was connected with a decrease in the energy dissipation rate in PSII/increased photosynthetic activity (energy flux for electron transport), a lower rate of starch degradation and higher rates of sucrose metabolism in leaves. The resistant lines accumulated larger amounts of total soluble carbohydrates in the crowns than in the leaves. Irrespective of flooding treatment, the resistant lines allocated more sugars for cell wall composition, both in the leaves and crowns. Our results clearly indicated that studies on carbohydrate changes at low temperatures or during anoxia should investigate not only the alterations in water-soluble and storage carbohydrates, but also cell wall carbohydrates.

The patterns of changes observed after low and high-temperature flooding were different, indicating separate control mechanisms of these responses. These included changes in the photosynthetic apparatus, starch accumulation and cell wall carbohydrate accumulation.

1. Introduction

Winter survival in the northern hemisphere depends mainly on winter temperatures and the duration of snow cover. However, in terms of climate changes and global warming, freezing tolerance, understood as the ability to acclimate to cold, will be progressively less important than resistance to other unfavorable environmental conditions during the winter (Rapacz et al., 2014). These conditions include flooding by water from melting snow and/or rain, and an increase in temperature leading to plant de-acclimation. Temperature fluctuations during the winter, resulting in excess soil moisture and even temporary flooding, also favor the occurrence of fungal diseases. In turn, snow mold fungi have wide host ranges, and despite a lack of snow cover, they can live saprophytically in the soil for years (Hsiang et al., 1999). The main sources of *Microdochium nivale* (Fr) Samuels & Hallet infection, the causative agent of pink snow mold disease, are mycelium in the soil or plant debris (Gaudet et al., 1999).

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One of the strategy that allows the plant to withstand the snow mold infection for a longer period of time is an accumulation of carbohydrate reserves in the crowns (Gaudet et al., 1999). Carbohydrates, particularly fructans, are essential for surviving in the presence of snow mold pathogen, frost and flooding (Hsiang et al., 1999; Gaudet et al., 1999; Li et al., 2013; Schlüter et al., 1996). More intensive accumulation of soluble sugars, particularly fructans, was recorded under hypoxia-inducing conditions in the shoots and roots of many plants (Schlüter et al., 1996). Fructans are synthesized from sucrose when the supply of photoassimilates exceeds the plant's demand (Pollock and Cairns, 1991). For this reason, those plants that increase their photosynthetic activity during cold acclimation accumulate large reserves of carbohydrates during the winter (Hüner et al., 1993). Thus, it may be hypothesized that carbohydrate management during cold acclimation might be crucial for the resistance to snow mold pathogens, freezing, cold-induced photoinhibition of photosynthesis and low-temperature flooding. A significant increase in frost tolerance after low-temperature flooding, observed in a *Festuca pratensis* genotype, was related to a higher carbohydrate concentration in the leaves (Jurczyk et al., 2013). Low-temperature flooding may also improve the resistance to cold by inducing photoinhibition of photosynthesis in some *F. pratensis* and *Lolium perenne* genotypes. The genotypes activating non-photochemical mechanisms of photosynthetic acclimation to cold during low-temperature flooding might show increased freezing tolerance. By contrast, a decrease in freezing tolerance observed after low-temperature flooding in some *F. pratensis* genotypes was probably connected with lowered photochemical activity and photoinhibition of photosynthesis (Jurczyk et al., 2013, 2015).

In *Arabidopsis*, low temperature and anoxia strongly induced the expression of the sucrose synthase (SS) encoding *SUS1* gene (Baud et al., 2004). SS and sucrose phosphate synthase (SPS) play major roles in sucrose biosynthesis. SPS catalyzes the formation of sucrose-6-phosphate from UDP-glucose and fructose-6-phosphate, while SS catalyzes the reversible reaction UDP-glucose + fructose ⇔ sucrose + UDP (Castonguay and Nadeau, 1998). SPS activity is associated with phloem loading (Huber and Huber, 1996), and it is markedly higher at low temperatures (Castonguay and Nadeau, 1998). The activity of SS is critical for surviving flooding, especially in the cold, because sucrose-synthase-deficient mutants are unable to survive flooding (Ricard et al., 1998).

To the best of our knowledge, the relationship between cold flooding tolerance and the snow mold resistance has not been studied. Carbohydrate changes at low temperatures and/or anoxia are well known; however, they are regarded mainly as alterations in water-soluble and storage, but not structural, carbohydrates. The role of synthesis and degradation of cell wall polysaccharides during low temperature and anoxia stress is less recognized. The cell wall is a dynamic structure having structural, protective and growth-regulating functions. Appropriate and timely modification of the cell wall is vital during plant-environmental interactions (Sasidharan et al., 2011). Changes in the cell wall composition are an important part of plant survival strategies in unfavorable conditions, involving growth enhancement or retardation under abiotic stress. Accumulation of β-D-glucans correlate with rapid elongation of maize coleoptiles (Carpita et al., 2001), while arabinose polymers prevent strong interactions between pectin polysaccharides, thereby increasing cell wall flexibility (Moore et al., 2008). Moreover, the cell wall can act as a strong barrier against pathogens, releasing products that can induce a plant defense response or be a source of valuable carbohydrates (Sasidharan et al., 2011).

The probability of pathogen infection is higher during or after flooding, and the occurrence of snow cover brings about a risk not only of infection, but also oxygen deficiency; therefore, we hypothesized the existence of common tolerance mechanisms to both stresses. We attempted to verify this hypothesis using winter rye (*Secale cereale*), which is generally resistant to various unfavorable environmental factors, excluding flooding and *M. nivale* infection. As a possible explanation, we investigated the flooding-induced changes in carbohydrate metabolism in inbred lines of winter rye with different resistances to snow mold. In this study, photosynthetic activity, the activity of key sucrose-synthesizing enzymes, soluble sugar accumulation in the leaves and crowns, as well as sugar accumulation in insoluble forms, i.e. starch or cell wall components, were considered.

2. Materials and methods

2.1. Experimental design

The study was carried out using 3-week-old seedlings of six inbred lines of winter rye, which differed in their level of pink snow mold resistance (Pociecha et al., 2013b). Lines 1, 2 and 4 had higher resistance to pink snow mold than lines 21, 24 and 25. The seeds were sown in pots (20 plants per 25-cm pot), containing a mixture of soil and sand (2:1 v/v), and cultured for 3 weeks in a greenhouse at 18 °C (day/night) in daylight (the experiment was conducted in September in Krakow, Poland, 50°03′ N, 19°55′ E). The plants were fertilized weekly with Hoagland's liquid medium.

The experiments were arranged in a completely randomized design, and performed in two independent series. Plants in pots belonging to the two independent series were grown in separate growth chambers. Each of the treatments in each series comprised four pots (25 plants per pot).

At the stage of three leaves, the plants were divided into two groups. Those in the first group were cold-acclimated in a growth chamber for 3 weeks at 4 °C under an 8-h photoperiod with a light intensity of 250 μmol m^{-2} s^{-1} Photosynthetic Photon Flux Density (PPFD) (using AGRO Philips sodium lamps). The plants belonging to the second group were not acclimated, but continued their growth at 20 °C also for 3 weeks under a 12-h photoperiod and 250 μmol m^{-2} s^{-1} PPFD. After 3 weeks plants belonging to both groups were flooded by placing the pots up to their rims in a tap water-containing box for 10 days. The plants of both groups were at similar developmental stage, before generative transition and before tillering.

The quantum efficiency of energy trapping in the PSII reaction center (Fv/Fm) was measured before flooding (control) and after 6 and 10 days of flooding. Shoot length and total soluble carbohydrate content (TSC) were determined after 10 days of flooding. Starch accumulation and sugar composition of the cell wall matrix in the leaves and crowns, as well as SS and SPS activities, were determined for two lines (the most and the least resistant to snow mold) after 10 days of flooding. Specific parameters of phenomenological energy fluxes were also calculated during the flooding period.

2.2. Measurements of photosystem II (PSII) photochemical activity (chlorophyll ‘a’ fluorescence transient)

PSII efficiency was measured using a Plant Efficiency Analyzer (Handy PEA; Hansatech Ltd., King's Lynn, Norfolk, UK), with excitation light intensity of 3 mmol m^{-2} s^{-1}. The measurements were performed on the youngest fully expanded leaves after 30 min of adaptation to the dark in leaf clips (Hansatech). The quantum efficiency of energy trapping in PS II reaction center (F_v/F_m), as well as the following parameters of phenomenological energy fluxes, were calculated based on Strasser et al. (Strasser et al., 2000): ABS/CS –