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Rapid increases in β-1,3-glucanase and chitinase activities are markers of resistance to *Microdochium nivale* in grasses of the *Lolium-Festuca* complex

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Abstract

Microdochium nivale causes pink snow mould - a destructive disease of seedlings, stem bases, and ears of winter grasses and cereals. Glucanase and chitinase belong to pathogenesis-related proteins and exhibit a specific activity in response to pathogens. The aim of this study was to investigate changes in the activity of these enzymes in the leaves of chosen forage grasses during *Microdochium nivale* infection. Different cultivars of *Festuca arudinacea*, *F. pratensis*, *Festulolium brauni*, *Lolium multiflorum,* and *L. perenne* were prehardened at 12 °C and hardened at 2 °C and then inoculated with *M. nivale* mycelium. Leaf samples were collected before infection, as well as two, four, and eight days after inoculation. Each cultivar showed a specific pattern of changes in the enzymatic activities in response to *M. nivale* infection. We conclude that a rapid increase in glucanase and chitinase activities after *M. nivale* attack enhanced resistance to this pathogen and may be recognized as a physiological marker of grass resistance to this snow mould.

Additional key words: forage grasses; pathogenesis-related proteins; snow mould.

Introduction

Microdochium nivale (Fr) Samuels & Hallet is a hemibiotrophic fungus causing pink snow mould (Płażek *et al*. 2018). The disease affects many species of winter cereals, turf, and forage grasses in Poland and other countries of the Northern Hemisphere (Gaudet *et al*. 1999). The pathogen destroys seedlings, stem bases and ears, especially when the air temperature in winter drops below 0 ºC and plants are under snow cover for several months (Jamalainen 1974, Prończuk and Zagdańska 1993, Wiśniewski *et al*. 1997). The damage caused by *M. nivale* is visible shortly after snow thawing – the leaves are dry, pinkish, and pressed down against the soil (Jamalainen 1974). However, long periods of consistently cold (4 - 15 ºC) and rainy weather, typical for maritime climates, are also favourable for the development of the pathogen. Such cases have been observed in North American Pacific Northwest, Canadian British Columbia, Great Britain, and northern Europe (Dahl 1934, Strömqvist and Jarvis 2005, Watschke *et al*. 2013). In the absence of snow cover, *Microdochium* patches are usually pink or reddish-brown, occasionally grey rings of *M. nivale* mycelium around patches may occur (Watschke *et al*. 2013).

Plant defence responses include a number of physiological and biochemical processes triggering synthesis of intermediary compounds that induce expression of the resistance genes or stimulate expression of structural genes. This results in the synthesis of new specific molecules such as reactive oxygen species (ROS) (Marzec-Schmidt *et al*. 2018), phenolic compounds, phytoalexins, and pathogenesis-related (PR) proteins (Hammond-Kosack and Jones 1996). The most important defensive mechanisms include hypersensitive reactions (HR), gene-to-gene resistance, and signal transduction (Hammond-Kosack and Jones 1996). Pathogenesis-related proteins are divided into 17 classes (Okushima *et al*. 2000), and each of them exhibits specific activity against pathogens. Endo-β-1,3-glucanase belongs to the PR-2 class of proteins, and it hydrolyzes β-1,3-glucan. Endochitinases are hydrolases belonging to classes PR-3, 4, 8, and 11 that degrade chitin, which is the main component of a fungal cell wall (Mauch *et al*. 1988). β-1,3-Glucanase and chitinase protect plants against pathogen attack in two

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Abbreviations: ARI - average regrowth index; ASI - average severity index; CHT - chitinase; dai – days after inoculation; GLC - β-1,3-glucanase; PPFD - photosynthetic photon flux density; PR - pathogenesis-related.

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ways. Firstly, their hydrolytic activity towards the pathogen cell walls may release signalling molecules that activate various defence mechanisms (Ryan 1988). Secondly, these enzymes can also directly affect the fungal cell walls and destroy the fungus (Yun *et al*. 1997, Garcia-Olmedo *et al*. 1998). PR proteins induced by cold and then stored in the apoplast may be also important for the development of resistance to the fungus causing snow mould in grasses and winter cereals (Ergon *et al*. 1998, Gaudet *et al*. 2000, Kuwabara *et al*. 2002). Some PR proteins synthesized during frost hardening may promote resistance to both factors, *i.e*., low temperature and snow mould (Gaudet *et al*. 2000, Kuwabara *et al*. 2002). Plant response to snow mould attack is similar to plant reaction to other biotic and abiotic stresses. In late autumn, winter, and early spring, winter cereals enhance amount of lipid transfer proteins (LTPs) and phenylalanine ammonia-lyase (PAL) – a key enzyme of the phenolic pathway (Gaudet *et al*. 2000). Glucanases and chitinases are commonly found in plant tissues. Some of them participate in various metabolic processes, other in plant defence against pathogen attack, or protection against other biotic and abiotic stresses. The presence of these proteins positively correlates with disease resistance (Stintzi *et al*. 1993). Yu and Griffith (1999, 2001) confirmed the presence of antifreeze proteins in rye and wheat apoplast. These proteins are structurally similar to glucanase, chitinase, and thaumatin. Hardened winter rye plants accumulate β-1,3-glucanase and chitinase, both preventing the freezing of water in the apoplast and showing antifungal properties (Hon *et al*. 1994, 1995). Contrary to that, β-1,3-glucanase and chitinase produced after inoculation of unhardened spring rye plants with *M. nivale* exhibited only antibiotic properties and did not prevent freezing (Nakajima and Abe 1996, Ergon *et al*. 1998). PR proteins are encoded by small gene families, and possibly different isoforms are produced in various stress situations (Collinge *et al*. 1993, Margis-Pinheiro *et al*. 1993, Stintzi *et al*. 1993).

Italian ryegrass, perennial ryegrass, and meadow fescue are commonly grown in Poland and they are of great importance among forage grasses. Their significance for agriculture makes them the objects of numerous research studies, *e.g.*, on breeding new cultivars more resistant to biotic and abiotic stresses or with better quality in terms of biochemical composition and health-promoting qualities. *Festuca* and *Lolium* species were used for crossbreeding to obtain interspecific *Festulolium* hybrids, combining the most desirable features of both parental species, *i.e.*, good overwintering ability or drought resistance (from fescue) and tastiness, high sugar content and digestibility (from ryegrass) (Perlikowski *et al*. 2013, Ghesquière *et al*. 2016, Masajada *et al*. 2018, Płażek *et al*. 2018). Species from *Lolium-Festuca* complex also differ in their susceptibility to *M. nivale*, *e.g*., *Festuca pratensis* is more resistant to snow mould than *F. arundinacea* and *Lolium multiflorum*, while *Festulolium* hybrid (*F. pratensis* × *L. multiflorum*) demonstrates pathogen tolerance similar to meadow fescue. Moreover, differences in susceptibility to snow mould are observed even within the same species, *e.g.*, *L. perenne* cv. Taya is more resistant to *M. nivale* than *L. perenne* cv. Darius (Prończuk *et al*. 2003).

The aim of this study was to find out if the activity of β-1,3-glucanase and chitinase affects the resistance of the studied forage grasses to *M. nivale* infection, and if it could be identified as a physiological marker of their resistance.

Materials and methods

Plants and growth conditions: The experiment involved plants of tall fescue (*Festuca arundinacea* Schreb.) cv. Kord, meadow fescue (*Festuca pratensis* Hack.) cv. Skra, Festulolium (*Festulolium braunii Richt.*) cv. Felopa, Italian ryegrass (*Lolium multiflorum* Lam.) cv. Tur, and perennial ryegrass (*Lolium perenne* L.) cvs. Darius and Taya differing in their susceptibility to *M. nivale*. Seeds of cvs. Kord, Skra, Felopa, and Tur were obtained from *Danko Hodowla Roślin*, Szelejewo, Poland, while seeds of cvs. Darius and Taya were obtained from the Institute of Plant Breeding and Acclimatization, Radzików, Poland. Seeds were sown into 4 dm^3-pots filled with commercial gardening soil (pH = 6.0) (*EKO-Ziem*, *Kronen, Lasland*, Cerkwica, Poland). Plants (20 seedlings per pot) were grown in October at 18 °C in a greenhouse in natural irradiance for two weeks. After that, they were prehardened for two weeks at a temperature of 12 °C, a 10-h photoperiod, and photosynthetic photon flux density (PPFD) of 250 µmol m^{-2} s^{-1}, and hardened for three weeks at 2 °C, a 8-h photoperiod, and PPFD of 250 µmol m^{-2} s^{-1} in a phytotronic chamber (*Agro Philips Euronext*, Amsterdam, Netherland). Next, they were inoculated with *M. nivale* mycelium and incubated with the fungus at 2 °C in darkness for 30 d.

Inoculation with *Microdochium nivale*: *Microdochium nivale* (Fr) Samuels &Hallet mycelium (highly aggressive isolate 3/98), originally isolated from perennial ryegrass in Norway in 1998, was used to prepare the inoculum. The mycelium was grown on potato dextrose agar (PDA, Sigma-Aldrich, St. Louis, MO, USA), in Petri dishes at 20 °C in darkness for seven days. Then the fungus was cultured on soil medium (soil/peat/sand; 2/2/1; v/v/v), containing 5 % (m/m) of grounded wheat kernels, at 18 - 20 °C in darkness for the next 7 d. The soil was macerated after being colonized by the mycelium. One gram of the inoculum per plant was used for inoculation as previously described by Prończuk and Zagdańska (1993). The non-inoculated (control) and inoculated plants were covered with moistened blotting paper and black plastic foil to keep high humidity and imitate the conditions under the snow cover. Afterwards, all plants were incubated at 2 °C in darkness for 35 d. Subsequently, the blotting paper and foil were removed and the plants were grown at 2 °C and PPFD of 100 µmol m^{-2} s^{-1} for 3 d and next at 12 °C and the same PPFD for 7 d. The resistance to snow mould was evaluated based on the disease severity, assessed directly after removing the cover, and regrowth ability rated after 10 d.

Evaluation of snow mould resistance: Disease severity symptoms were assessed after 35 d of incubation at 2 °C in darkness, according to the visual rating system (0 - 5) previously described by Hartman *et al*. (1984), and slightly modified by Płażek and Żur (2003), where '0 means a healthy plant without any disease symptoms and '5 means an entirely infected plant. Next, the average severity index (ASI) was calculated according to a formula: ASI = $(n \times 0) + (n \times 1) + ... + (n \times 5)] \times N^{-1}$, where n is the number of plants corresponding to each disease severity category (0 - 5), and *N* is the total number of tested plants. The higher ASI value the greater susceptibility to the pathogen.

Plant ability to regrow after *M. nivale* inoculation was visually evaluated after 10 d of regrowth, using a 0 - 4 scale described by Prończuk and Zagdańska (1993), where 0 means a healthy plant without any disease symptoms and 4 means a dead plant without any signs of regrowth ability after infection. The average regrowth index (ARI) was calculated according to the formula: ARI = $(n \times 0) + (n \times 1) + ... + (n \times 4)] \times N^{-1}$, where n is the number of plants corresponding to each disease category (0 - 4), and N is the total number of all tested plants. The higher ARI value, the greater susceptibility to the pathogen.

The calculations represented an average from 5 replicates, where one pot means one replicate, with 20 plants per pot.

Glucanase activity: The activity of β-1,3-glucanase (GLC) was measured using Finks *et al.* (1988) method modified by Mohase and Westhuizen (2002). Leaf samples were collected prior to the infection, and 2, 4, and 8 d after inoculation (dai). Frozen leaves were ground in liquid N_2 to a fine powder with a chilled mortar and pestle. Then, the powder was homogenized with 100 mM acetic sodium buffer (pH 5.6) with 1 mM EDTA at 4 °C and centrifuged at 16 000 *g* for 15 min. The reaction mixture consisted of 336 mm^3 of a measuring buffer (50 mM acetic sodium buffer, pH 5.6), 350 mm^3 of laminarin (2 mg laminarin in 1 cm^3 of the measuring buffer) and 14 mm^3 of plant extract. The mixture was heated at 37 °C for 30 min. After that, 700 mm^3 of Somogyi reagent (Somogyi 1952) were added and incubated at 95 °C for 10 min. The mixture was cooled down and 700 mm^3 of Nelson reagent (Nelson 1944) were added. The amount of glucose detached from laminarin by the enzyme in plant extract was measured at λ = 540 nm with a spectrophotometer (*Ultrospec 2100 pro Biosciences*, Amersham, Sweden). A standard curve obtained by measuring the absorbance of different glucose concentrations was used to calculate glucanase activity. Glucanase activity was measured for five replicates per each studied cultivar.

Chitinase activity: The activity of chitinase (CHT) was measured using Legrand *et al*. (1987) method modified by Hiilovaara-Teijo *et al*. (1999). Leaf samples were collected prior to infection, and 2, 4, and 8 dai. Frozen leaves were ground in liquid N_2 to a fine powder with a chilled mortar and pestle. Then the powder was homogenized with 0.5 M acetic sodium buffer (pH 5.2) at 4 °C and centrifuged at 10 000 *g* for 10 min. The reaction mixture consisted of 0.5 mg of colloidal chitin, 400 mm^3 of 0.1 M acetic sodium buffer (pH 5.2) and 100 mm^3 of plant extract. The mixture was incubated overnight at 37 °C using a rotatory mixer and centrifuged at 10 000 *g* for 10 min. Then, the supernatant (300 mm^3) was supplied with 20 mm^3 of 3 % (m/v) glucuronidase (in 0.1 M acetic sodium buffer, pH 5.2) and incubated at 37 ºC for 60 min. After that, 100 mm^3 of 0.6 M potassium tetraborate were added and the mixture was boiled for 3 min. It was then cooled down and 1 cm^3 of 4-(dimethylamino)benzaldehyde (DAMB) (10 %, m/v) in glacial acetic acid and 11.5 M hydrochloric acid, 87.5:12.5, v/v) diluted (1:2) with glacial acetic acid were added. The mixture was incubated at 37 ºC for 20 min. The amount of N-acetyl-glucosamine released from chitin by the enzyme in plant extract was measured at λ = 585 nm using the same spectrophotometer. Enzyme activity was expressed as catal, where 1 catal defines the activity of enzyme releasing 1 M N-acetyl-glucosamine after 1 s. Chitinase activity was measured for five replicates per each studied object.

Soluble protein content: Soluble protein content was determined according to Bradford (1976). A reaction mixture consisted of 500 mm^3 of distilled water, 75 mm^3 of an extraction buffer, 50 mm^3 of Bradford reagent and 25 mm^3 of plant extract. The mixture was shaken and incubated for 15 min at room temperature. Absorbance was measured at λ = 595 nm. A standard curve obtained by measuring the absorbance for different concentrations of bovine serum albumin was used to calculate the protein content. The measurement was done in five replicates per each studied object.

Statistical analysis: Statistical analyses *ANOVA*/*MANOVA* and Duncan's multiple range test (at α = 0.05) were performed using the *Statistica 12.0* software (*Statsoft*, Tulsa, OK, USA). Data are presented as means ± SEs.

Results

The most visible leaf damage after 35 d of incubation occurred in Festulolium plants cv. Felopa (Table 1), followed by a slightly lower ASI of cvs. Skra, Darius, and Kord. However, no statistically significant differences were observed between cultivars mentioned above. Leaf damages of cvs. Tur and Taya were significantly lower than the ASI of the rest of studied plants.

Ten days after finishing the incubation with *M. nivale*, the plant ability to regrowth was evaluated (Table 1). Cultivar Skra regrew most effectively, even though it was among the cultivars showing the most severe damage caused by the pathogen. Also, cultivars Tur and Taya exhibited high regrowth ability. Regrowth in cvs. Darius and Felopa was significantly weaker, and the plants of cv. Kord demonstrated significantly lower ability to regrow after *M. nivale* attack in comparison to other studied cultivars.

In the leaves of cv. Kord, the activity of GLC was lower in infected plants *vs*. control at 4 dai but the difference

Table 1. The resistance of grass cultivars to *Microdochium nivale* on the basis of average severity index (ASI) assessed after 35 days of incubation at 2 °C in darkness, and average regrowth index (ARI) evaluated 10 days after finishing the plant incubation with the pathogen. Studied species: tall fescue (*Festuca arundinacea*) cv. Kord, meadow fescue (*Festuca pratensis)* cv. Skra, Festulolium (*Festulolium braunii*) cv. Felopa, Italian ryegrass (*Lolium multiflorum*) cv. Tur and perennial ryegrass (*Lolium perenne*) cvs. Darius and Taya. Means ± SEs, $n = 5$; different letters in the same column indicate significant differences at $P < 0.05$.

Cultivar	ASI	ARI
Kord	3.92 ± 0.06a	2.16 ± 0.08c
Skra	4.07 ± 0.04a	0.19 ± 0.02a
Felopa	4.29 ± 0.04a	0.87 ± 0.07b
Tur	3.12 ± 0.26b	0.21 ± 0.20a
Darius	4.05 ± 0.11a	0.76 ± 0.10b
Taya	3.05 ± 0.23b	0.32 ± 0.04a

was not significant (Fig. 1). In cv. Skra, GLC activity was lower in control plants than in the inoculated ones on the second day of the experiment. In the case of cv. Felopa, a significant increase in GLC at 8 dai was observed in inoculated plants, while in the control and infected leaves of cv. Tur, an unspecific pattern of enzyme activity occurred with the highest increase in glucanase activity on 4 dai. Both Darius and Taya showed a gradual increase in GLC activity in control and infected plants. Control plants of cv. Darius showed higher GLC activity after two days of the experiment, whereas in plants of cv. Taya there was no difference in the activity of this enzyme between control and infected variants. Response of studied plants to *M. nivale* infection in regard to GLC activity was cultivar specific.

In the leaves of cv. Kord, the activity of chitinase (CHT) decreased at 2 and 4 dai but it increased after 8 dai (Fig. 1). In cv. Skra, chitinase activity in the inoculated plants was higher than in the controls at 2 and 8 dai. In the case of cv. Felopa, the difference between treatments in favour of the infected plants was visible only at 4 dai. In cv. Tur, the highest chitinase activity was recorded in the inoculated plants at 4 dai. However, total CHT activity in Italian ryegrass was very low, when compared with the other studied species. In infected plants of cv. Darius, the activity of CHT increased two times after 4 dai and four times at 8 dai, as compared with the controls, and was the highest among all studied plants. In cv. Taya, the activity of CHT was significantly higher in the inoculated plants at 2 and 8 dai. In general, activity of CHT remained rather low in both inoculated and non-inoculated leaves of cvs. Kord, Skra, Felopa, and Tur, while two cultivars of *L. perenne*, Darius and Taya, exhibited significantly higher CHT activity, especially in inoculated leaves of cv. Darius.

Discussion

We evaluated the response of forage grasses with varying degrees of susceptibility to *M. nivale* based on the activity of glucanase and chitinase that belong to pathogenesis related proteins. Our findings demonstrated highly specific response of all studied species to the pathogen attack.

After the inoculation, the activity of glucanase and chitinase increased. Some studies suggest that the presence of these proteins in plants is associated with disease resistance (Stintzi *et al*. 1993). Hydrolysis of β-1,3-glucan and chitin in the pathogen cell wall by endo-β-1,3-glucanase and chitinase may result in direct destruction of infectious filaments (Ryan 1988). At the same time, as a result of activity of hydrolytic enzymes secreted by fungi (pectinases, cellulases), oligomers like chitosan or glucan fragments are formed that also work as signal molecules triggering the plant defence system. Some studies advocate synergistic effects of β-1,3-glucanase and chitinase in response to pathogen attack (Mauch *et al*. 1988, Jongedijk *et al*. 1995). Expression of the *glucanas*e gene in barley after powdery mildew infection is significantly lower in susceptible genotypes than in the resistant ones whereas the activity of glucanase in susceptible and resistant non-infected plants is low and do not change significantly over time (Jutidamrongphan *et al*. 1991). An experiment with barley, wheat, rice, and sorghum infected with *Bipolaris sorokiniana* (Jutidamrongphan *et al*. 1989) identified the expression of *β-1,3-glucanase* gene as a typical response to fungal infection in cereals; resistant and susceptible sorghum exhibit an increase in chitinase and β-1,3-glucanase activity after fungal infection, although, the amount of β-1,3-glucanase is higher in resistant plants. Wheat inoculated with *Puccinia graminis* f.sp. *tritici* shows no changes in activities of most enzymes as compared to control plants, however, an increase in glucanase and chitinase is observed. Moreover, resistant cultivars respond more rapidly to the infection than susceptible ones (Münch-Garthoff *et al*. 1997). A significant increase in β-1,3-glucanase as well as chitinase in rice infected with *Rhizoctonia solani vs*. non-infected plants has been also reported (Anuratha *et al*. 1996). Glucanase and chitinase may accumulate in winter rye apoplast in response to the fungus causing snow mould, and supposedly inhibit development of the pathogen (Hiilovaara-Teijo *et al*. 1999).

In the present study, the resistance of five species of forage grasses: tall fescue, meadow fescue, Festulolium, Italian ryegrass, and perennial ryegrass to pink snow mould was examined. Cultivar Kord (tall fescue) was moderately damaged by *M. nivale*, with ASI not significantly different from cvs. Skra, Felopa, and Darius, but its leaf regrowth was significantly weaker than that of other studied cultivars. Based on these observations, cv. Kord was considered the most susceptible to the pathogen. Cultivar Skra (meadow fescue) was among cultivars significantly damaged by *M. nivale* but also showed the best leaf regeneration. Statistically, cv. Skra was not significantly different in terms of leaf emergence from cvs. Tur and Taya, but definitely different from cvs. Kord, Felopa and Darius. These results indicate that cvs. Skra, Tur, and Taya are the most resistant to snow mould, while cvs. Kord, Felopa, and Darius are more susceptible. Even though the

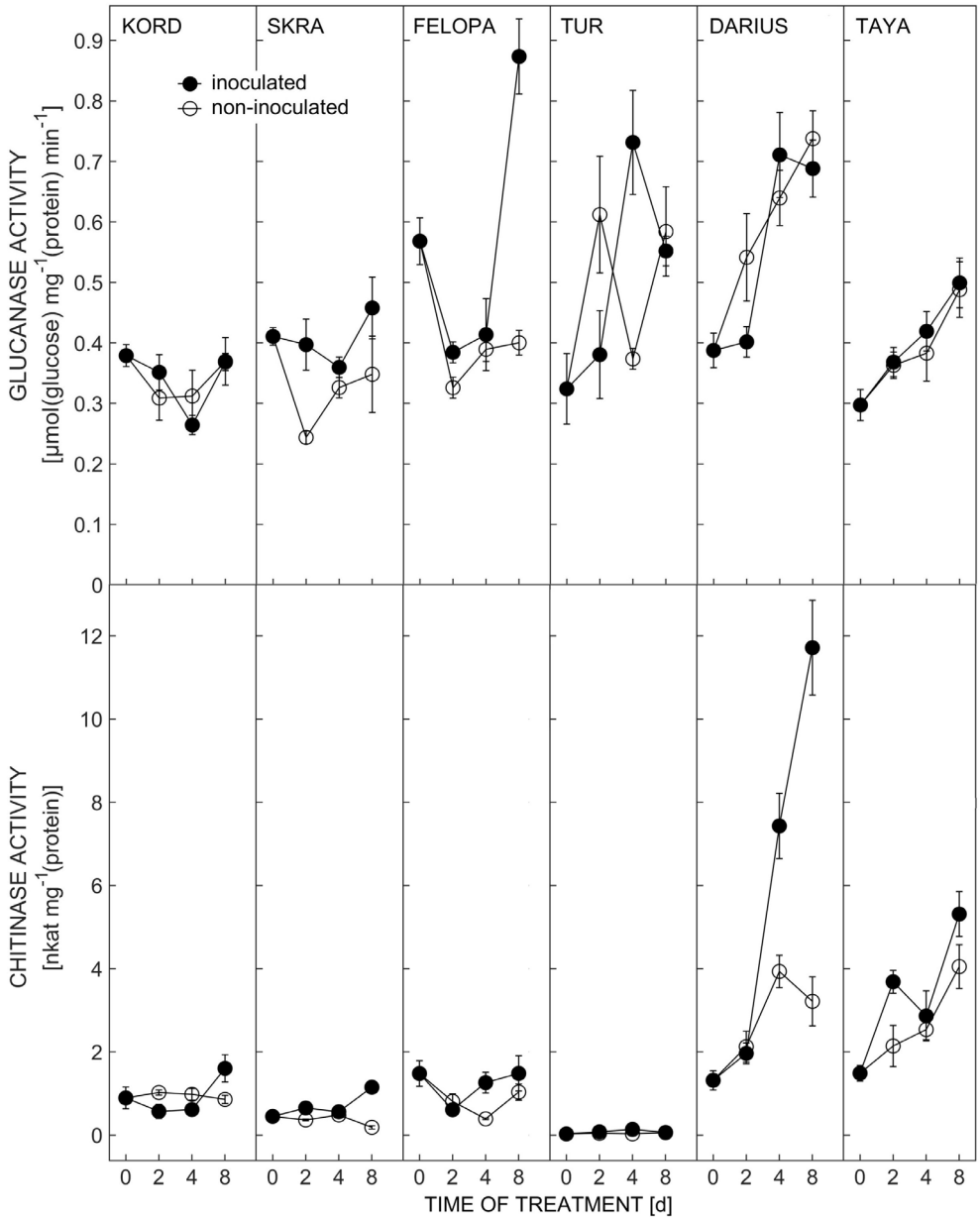

Fig. 1. Glucanase and chitinase activities in the leaves of grass cultivars: before inoculation (0) and 2, 4, and 8 days after *Microdochium nivale* inoculation. Studied species: tall fescue (*Festuca arundinacea*) cv. Kord, meadow fescue (*Festuca pratensis)* cv. Skra, Festulolium (*Festulolium braunii*) cv. Felopa, Italian ryegrass (*Lolium multiflorum*) cv. Tur, perennial ryegrass (*Lolium perenne*) cvs. Darius and Taya. Means ± SEs, $n = 5$.

leaves of all tested cultivars were significantly damaged by the pathogen, we observed huge differences in their ability to regrow. Our findings corroborate previous evaluations made during field trials by Prończuk *et al*. (2003), showing that cv. Darius was less resistant to snow mould than cv. Taya. According to Żur *et al*. (2011), winter rye plants with root-shoot junction and root system not seriously affected by *M. nivale* infection regenerated better and plants were able to recover. Therefore, we assumed that this could explain the strong regrowth ability of cvs. Skra, Tur, and Taya, even after severe leaf damage by the pathogen.

For cvs. Kord, Darius, and Taya, we found no significant differences in GLC activity after *M. nivale* attack as compared to the control plants. Cultivars Skra and Felopa showed an increase in GLC activity but at different times after infection, while cv. Tur exhibited an initial decline followed by a strong increase in glucanase activity in response to the pathogen attack. Hiilovaara-Teijo *et al*. (1999) claimed that rye plants infected with *M. nivale* spores enhanced glucanase activity *vs*. the control plants. Among all studied cultivars, only Skra, Felopa, and Tur responded in this way to *M. nivale* infection. Cultivar Skra, considered resistant to snow mould, responded to the inoculation with a rapid increase (at 2 dai) in glucanase activity. Perhaps, this is one of the elements of the effective defence strategy against fungal

infection. Jutidamrongphan *et al*. (1991), who studied the response of barley to *Erysiphe graminis*, showed a drop in GLC activity in sensitive plants withstanding the pathogen attack, and an increase in resistant plants. We found no such relationship in the cultivars investigated in our study. Glucanase activity remained unaffected by the pathogen in cvs. Darius and Kord (susceptible to *M. nivale*), and Taya (resistant to *M. nivale*). The results on increasing GLC activity in cv. Skra were partially consistent with Jutidamrongphan *et al*. (1991) studies.

In the case of Skra, Tur, Darius, and Taya, chitinase activity increased in the infected plants *vs*. controls. In cvs. Kord and Felopa, the initial decrease was followed by an increase in CHT activity in the infected plants. The lowest activity of this enzyme was observed in the leaves of cv. Tur. Cultivars Kord, Skra, and Felopa exhibited similar chitinase activity, higher than in cv. Tur, while the highest activity of the enzyme was seen in both cultivars of perennial ryegrass (Darius and Taya). Hiilovaara-Teijo *et al*. (1999) demonstrated that hardened rye plants infected with *M. nivale* spores exhibited lower chitinase activity than non-infected hardened plants, however, the difference was not statistically significant. In our cultivars, the decline in CHT activity in *M. nivale* infected plants was perceptible only in cvs. Kord and Felopa. This differs from the results of Legrand *et al*. (1987), who showed a gradual increase in chitinase activity from the first day after infection in tobacco inoculated with *Tobacco mosaic virus*. Kuźniak and Urbanek (1997) also showed increasing chitinase activity in pea plants in response to *Botrytis cinerea* infection. We found the same pattern of chitinase activity in cvs. Skra, Tur, Darius, and Taya. Żur *et al*. (2013) demonstrated that low temperature during hardening suppressed β-1,3-glucanase and chitinase activity in winter triticale cv. Magnat (susceptible to *M. nivale*), while in resistant cv. Hewo only a decrease in CHT activity was observed. In this work, an increase in GLC occurred during pathogenesis in both resistant and susceptible cultivars. In our study, resistant cvs. Skra and Tur enhanced their GLC activity relatively early after inoculation with *M. nivale* mycelium, whereas in susceptible cv. Felopa GLC activity did not rise until the eighth dai. Żur *et al*. (2013) reported that CHT activity increased and remained high even 7 d after inoculation, but only in the resistant cv. Hewo. This is partially consistent with our study, where CHT activity in the resistant Skra, Tur, and Taya rose in the infected plants *vs*. the control ones, but it also did in susceptible cv. Darius.

In conclusion, improved resistance to *M. nivale* infection seems to depend on more than a single mechanism. In meadow fescue cv. Skra, plant resistance to the snow mould is due to a rapid increase in the activity of glucanase and chitinase after the pathogen attack. A similar but slightly delayed reaction was also typical of the relatively resistant Italian ryegrass cv. Tur. In perennial ryegrass cv. Taya, resistance may result from the rapid growth of chitinase activity caused by pathogen inoculation, but also as a side effect of the elevated glucanase activity. Therefore, we suggest that an increase in both glucanase and chitinase activity after snow mould infection could be recognized as one of markers of grass resistance to *M. nivale*.

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