Defence reactions in Norway spruce toward the pathogenic root-rot causing fungus *Heterobasidion annosum*

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Abstract

The root-rot causing fungus *Heterobasidion annosum* can attack both spruce and pine trees and is the economically most damaging pathogen in northern European forestry. We have monitored the *H. annosum* S-type (fairly recently named *H. parviporum*) colonization rate and expression of host chitinases and other host transcripts in Norway spruce material with differing resistances using quatitative real-time PCR. Transcript levels of three chitinases, representing classes I, II and IV, were monitored. Ramets of two 33 -year-old clones differing in resistance were employed as host material and inoculation and wounding was performed. clones in the area immediately adjacent to inoculation. Fourteen days after infection, pathogen colonization was restricted to the area immediately adjacent to the site of inoculation for the strong clone (589), but had progressed further into the host tissue in the weak clone (409). Transcript levels of the class II and IV chitinases increased following wounding or inoculation, while the transcript level of the class I chitinase declined following these treatments. Transcript levels of the class II and class IV chitinases were higher in areas immediately adjacent to the inoculation site in 589 than in similar sites in 409 three days after inoculation, suggesting that the clones differ in the rate of pathogen perception and host defense signal transduction. This an earlier experiments using mature spruce clones as substrate indicate that it is the speed of the host response and not maximum amplitude of the host response that is the most crucial component in an efficient defense in Norway spruce toward pathogenic fungi such as *H. annosum*.

Introduction

The root and butt rot fungus *Heterobasidion annosum* (Fr.) Bres. *s. lat.* can attack both spruce and pine trees and is economically the most damaging tree pathogen in northern Europe. Suberized bark tissues form a strong barrier to penetration by this pathogen (Lindberg & Johansson 1991). However, bark wounds caused by wind, animals, insects and timber extraction expose the trees to this pathogen, which is characterized by a high spore deposition rate and long spore viability in bark.

Norway spruce, among other conifers, has been screened with stem inoculations to identify clones that differ in resistance towards *H. annosum*. Based on lesion length and fungal isolations, considerable clonal variation in genetic resistance has been recorded for Norway spruce. However, the mechanisms contributing to variation in resistance against *H. annosum* remain unknown.

Chitinases, PR proteins produced particularly upon pathogen attack, hydrolyze the 1,4-*N*-acetyl-D-glucosamine (GlcNAc) linkages of chitin, a component of cell walls of higher fungi. Hydrolysis of chitin results in the swelling and lysis of the hyphal tips and the chitinolytic breakdown products generated can act as elicitors of further defense reactions in plants (Schlumbaum *et al.* 1986). The objectives of the present study were to monitor *H. annosum* colonization rate and expression of class I, II and IV host chitinases in Norway spruce upon infection by *H. annosum* (S-type) in order (i) to identify defense related chitinases, and (ii) to evaluate whether trees displaying variation in host resistance show differences in the expression of chitinases.

Material and methods

Ramets of two 33-year-old Norway spruce clones differing in resistance were employed as host material. Following bark inoculation with an agar plug containing pathogen mycelia, a rectangular strip containing phloem and cambium, with the inoculation site in the middle, was removed at the start and 3, 7 and 14 days after inoculation. Prior to sampling, the rhytidome and the periderm were removed. The tissue was then divided into 50mg sections (length, 2 mm; width, 5 mm; depth, approximately 3 mm), which were processed individually (Fig. 1).

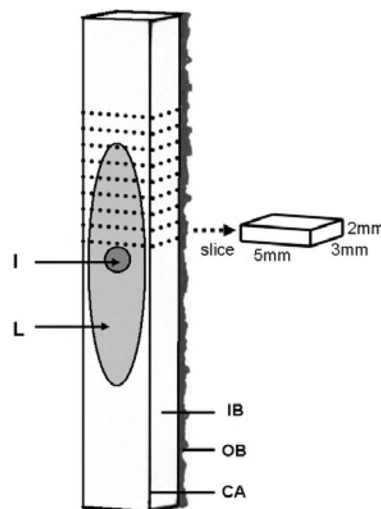

Fig. 1 Example of sampling from lesions. Inoculation point (I), lesion (L), outher bark (OB), Cambium (CA) and inner bark (IB) are marked. Two 33-year-old rametes of each clone were used in this inoculation experiment. DNA and RNA was extracted from the same section in each case to compare the colonization (genomic DNA of H. annosum *and Norway spruce) and the transcript level of the class I, II and IV chitinases.*

Chitinase expression levels were monitored with singleplex real-time PCR by using cDNA obtained from sampled sections and synthesised from total RNA as template (Hietala *et al*. 2004). Multiplex real-time PCR detection of host and pathogen DNA was performed on RNA prior to Dnase treatment (Hietala *et al*. 2003) in order to establish the colonization levels in each sampled section.

Results

Three days after inoculation, comparable colonization levels were observed in both clones in the area immediately adjacent to inoculation. Fourteen days after infection, pathogen colonization was restricted to the area immediately adjacent to the site of inoculation for clone 589, whereas it had progressed further into the host tissue in clone 409 (Fig. 2). Transcript levels of the class II and IV chitinases increased following wounding or inoculation, but the transcript level of the class I chitinase declined following these treatments. Transcript levels of the class II and class IV chitinases (Fig. 2) were higher in areas immediately adjacent to the inoculation site in clone 589 than in similar sites in clone 409 three days after inoculation. This difference was even more pronounced 2 to 6 mm away from the inoculation point, where no infection was yet established, and suggests that the clones differ in the rate of chitinase-related signal perception/transduction. Fourteen days after inoculation, these transcript levels were higher in clone 409 than in clone 589, suggesting that the massive upregulation of class II and IV chitinases (Fig. 2) after the establishment of infection comes too late to reduce or prevent pathogen colonization.

Discussion

On day 3 clone 589 had higher transcript levels of class II and IV chitinases than did clone 409 in areas adjacent to the inoculation site. This observation suggests that the time from signal perception and transduction to the induction of these genes was shorter in the more resistant clone. Chitinase enzyme activity and protein and transcript levels often are higher in resistant cultivars than in susceptible ones shortly after inoculation, when a lower level of chitinases may suffice to prevent or reduce hyphal penetration.

The higher class II and IV chitinase transcript levels in clone 589 during the early stages of infection also could result in earlier production of exogenous elicitors from the fungal cell wall, and an earlier triggering of other host defense reactions, *e.g.* increased lignification. To test the hypothesis that the rapidity of the overall response and the degree of coordination of the different defense strategies contribute to the level of resistance, studies of transcriptional activation of phenylalanine lyase and genes related to lignification at an early stage of *H. annosum* infection could be helpful. To allow an efficient screening of a larger amount of clones, sampling of bark inoculations could be restricted to the first 6 mm away from the inoculation point, an area where the clones now studied showed pronounced differences in chitinase expression.

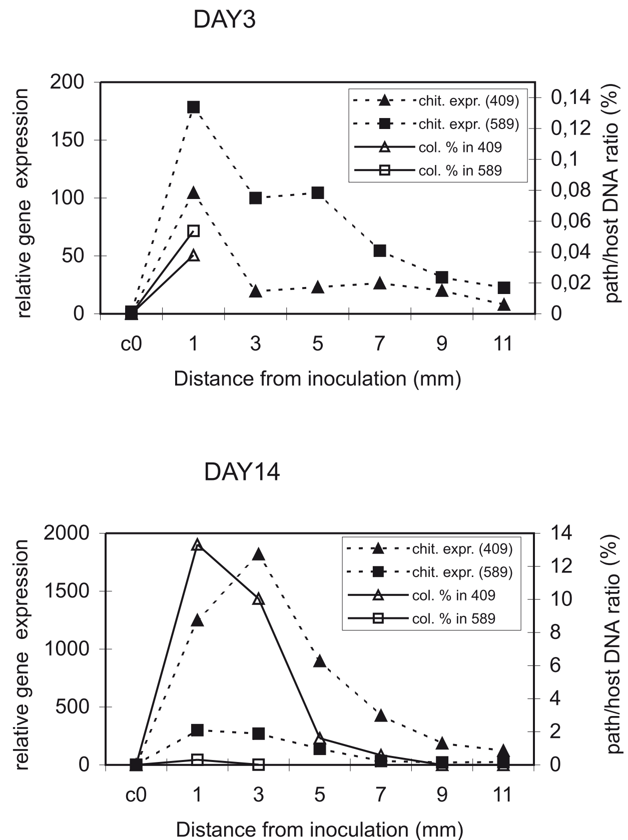

Fig. 2. Pathogen colonization levels and relative gene expression profiles of PaChi4, a class IV chitinase, in bark of two Norway spruce clones following inoculation with Heterobasidion annosum *(Hietala et al. 2004). The bark around the inoculation site was spatially sampled (see Fig. 1) 3 days (upper panel) and 14 days (lower panel) after inoculation. The basal transcript levels of the chitinase in clone 409 at the time of inoculation were used as a reference transcript level and defined as the 1x expression level, and the transcript levels of all the other samples are expressed as the fold change over this reference level. (Figure reproduced from Schmidt et al. 2005).*

References

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