Characterization of Pin m III cDNA in western white pine

XUESHU YU,1 ABUL K. M. EKRAMODDOULLAH1,3 and SANTOSH MISRA2

Received July 9, 1999

Summary Maximum accumulation of Pin m III protein in western white pine (Pinus monticola Doug. ex D. Don) needles occurred during the winter months. To characterize Pin m III, an expression cDNA library from poly(A)+ mRNA of needles was immunoscreened and the full length cDNA was cloned. An open reading frame of 486 bases encodes a protein of 161 amino acid residues with a molecular mass of 18 kD and a predicted isoelectric point of 5.5. The deduced amino acid sequence had some similarities (37%) with an intracellular protein, named Pin m III (Ekramoddoullah et al. 1995), was identified in western white pine foliage. The N-terminal amino acid sequence of Pin m III was 89% similar to that of Pin l I needles infected with blister rust compared with healthy needles.

Keywords: intracellular PR protein, PR-10 family, seasonal regulation, sugar pine, white pine blister rust.

Introduction

Western white pine (Pinus monticola Doug. ex D. Don) is a commercially important, fast-growing species widely distributed in the Pacific Northwest, Idaho and Montana. Although this species has been devastated by the white pine blister rust, it has recently been reintroduced as a commercial species because of its high wood quality and resistance to laminated root rot (Phellinus weirii Murr.).

White pine blister rust is caused by the fungus Cronartium ribicola J.C. Fisch. ex Rabenh, which has five spore stages on two unrelated hosts, western white pine and Ribes spp. (currants, gooseberries). Basidiospores are produced on Ribes in the fall and are dispersed by wind to western white pine foliage, where they germinate and infect the needles through the stomata, producing an infection spot. Hyphae grow down the needles into the bark, causing perennial cankers that eventually kill the tree by the formation of canker girdles around the main stem. Although some large older trees can survive the infection, younger trees almost always die.

Several types of plant proteins are induced in response to both abiotic and biotic stresses including vegetative storage proteins, heat shock proteins and pathogenesis-related proteins (Hightower 1991, Linthorst 1991, Mason and Mullet 1990, Ekramoddoullah et al. 1995, Sabehat et al. 1996). Pathogenesis-related proteins (PRs) are a heterogeneous group of host-encoded proteins that are induced following exposure to pathogens such as viruses, bacteria and fungi or certain chemicals (van Loon et al. 1994). On initiation of plant systemic acquired resistance, PRs are induced and account for part of the multicomponent resistance system (Ward et al. 1991). Van Loon et al. (1994) classified PRs into 11 families. Among them, PR-10 is defined as a family of intracellular defense-related proteins with a ribonuclease-like structure, typified by parsley (Petroselinum crispum (Mill.) Nyman ex A.W. Hill) “PR1” (van Loon et al. 1994). The PR-10 is a ubiquitous family of conserved proteins found as intracellularly localized, acidic, low molecular mass (16–19 kDa) proteins (Walter et al. 1990). Members of PR-10 include STH-2 in potato (Solanum tuberosum L.) (Matton and Brisson 1989), garden asparagus (Asparagus officinalis L.) PR1 (Warner et al. 1992), birch (Betula sp.) major pollen allergen BET V I (Breiteneder et al. 1989), and bean (Phaseolus vulgaris L.) PR1 and PR2 (Walter et al. 1990). Some members of PR-10 have ribonuclease activities (Moiseyev et al. 1994, Swoboda et al. 1993). Members of the PR-10 family are transcriptionally upregulated following pathogen infection, treatment with elicitors, wounding or in response to various stresses (Walter et al. 1990, Paakkonen et al. 1998).

Recently, a 19-kDa protein Pin l I was detected in sugar pine (Pinus lambertiana Doug.) foliage in increasing amounts in autumn (Ekramoddoullah et al. 1995). A homolog of this protein, named Pin m III (Ekramoddoullah et al. 1995), was identified in western white pine foliage. The N-terminal amino acid sequence of Pin m III was 89% similar to that of Pin l I...
The accumulation of Pin m III in western white pine seedlings is seasonally regulated, increasing during the fall and reaching a maximum in winter, then declining to a minimum in the summer (Ekramoddoullah et al. 1995). The amount of Pin m III is also correlated with frost hardness of western white pine (Ekramoddoullah et al. 1995). Because Pin m III accumulates in western white pine trees inoculated with blister rust fungus (Ekramoddoullah et al. 1998), we investigated its possible relationship to known pathogenesis-related proteins, by cloning and characterizing the gene encoding Pin m III. An expression cDNA library from poly(A)+ mRNA of winter needles was generated and the Pin m III cDNA was cloned by antibody screening. BLAST search and subsequent sequence comparisons indicate that Pin m III may be a member of the intracellular PR-10 protein family. We report the complete sequence of the cDNA encoding Pin m III from western white pine, its possible phylogenetic relationship to other angiosperm PR-10 proteins, analysis of its temporal and spatial expression, and its expression pattern in infected and healthy foliage of resistant and susceptible plants.

**Materials and methods**

**Plant material collection and treatment**

Total RNA was extracted from foliage samples of western white pine originating from Seedlots 2881, 2888 and 3159 as previously described (Ekramoddoullah et al. 1995). Current-year needle samples used for the expression cDNA library were collected in January 1996 from a western white pine tree growing at the University of Victoria (Victoria, British Columbia, Canada). For the analysis of tissue-specific expression, current-year and 1-year-old needles and their corresponding twigs and roots were harvested from 2-year-old white pine seedlings. Samples were collected in September 1996 from an open-sided greenhouse (shelter house) at the Pacific Forestry Centre (Seedlot 3144, Victoria, British Columbia, Canada), frozen in liquid nitrogen and lyophilized.

For the analysis of disease-specific expression, 4-year-old sugar pine seedlings that were inoculated with blister rust fungus in August 1995 were used. Samples were collected in September 1996 for the extraction of total RNA and protein. Sugar pine needles from resistant (R4) or susceptible (S4) seedlings were collected from the shelter house and treated as described for the 2-year-old western white pine seedling. Needles were classified as healthy if they had no visible infection spots. Infection spots were collected from the infected needles by cutting out the localized infection spots (about 2 mm long) from the resistant tree, or the expanding spots (more than 5 mm long) from the susceptible tree, and pooled for extraction of RNA and protein. The remaining spotless portions of the infected needles were also pooled and analyzed. Pycnia andaecia were visible on the susceptible tree (S4) in 1998, whereas no cankers were observed on the stem of the resistant tree (R4).

**RNA extraction**

To construct the cDNA library, total RNA was extracted from 10 g of current-year western white pine needles as previously described (Schultz et al. 1994). Needles were ground to a fine powder in liquid nitrogen with a mortar and pestle. The powder was transferred to cooled centrifugation tubes with extraction buffer and further homogenized in a polytron. Poly(A)+ mRNA was isolated by using the mRNA Purification Kit from Pharmacia Biotech according to the manufacturer’s instructions (Amersham Pharmacia Biotech, Inc., Piscataway, NJ). For northern blot analysis, total RNA was extracted by the method of Wang and Vodkin (1994). The RNA concentration was determined spectrophotometrically and confirmed by agarose gel electrophoresis.

**Construction of expression cDNA library and antibody screening of the library**

An expression cDNA library was constructed in λgt22A from poly(A)+ mRNA of western white pine needles, according to the manufacturer’s protocol (Gibco BRL, Life Technologies, Inc., Gaithersburg, MD). The library was screened with a polyclonal antibody against a synthetic N-terminal (17 amino acid residue) peptide of the sugar pine protein Pin l I that cross reacted with Pin m III (Ekramoddoullah et al. 1995). In the first 17 amino acid residues, Pin m III and Pin l I differed in a single amino acid at position 13 (Ekramoddoullah et al. 1995, Ekramoddoullah and Taylor 1996). Immunoscreening was conducted following Stratagene’s picoBlueTM immuno-screening instruction manual (Stratagene, La Jolla, CA) with some modifications: 5% skim milk powder (5% w/v in TBS) was used as a blocking agent and incubated overnight. Three initial positive plaques were identified and one was purified through three rounds of screening. The cDNA insert was subcloned into pBluescript KS+ (Stratagene).

**Northern blot analysis**

For northern blot analysis, 20 µg of total RNA per lane was separated on 1.2% agarose gels containing 2.2 M formaldehyde (Sambrook et al. 1989), photographed, and transferred to Zeta-Probe GT membranes according to the manufacturers instructions (BIO-RAD, Missisauga, Ontario, Canada). The Pin m III cDNA insert and the 18S rRNA gene insert were labeled with the random primers DNA labeling system with [α-32P]dCTP and hybridized according to the manufacturer’s instructions (Gibco BRL). The membranes were washed under high stringency according to the manufacturer’s instructions (BIO-RAD). Membranes were exposed to BioMax film (Kodak, Rochester, NY) for 3.5 hours for the 18S rRNA gene probe, and for 3–7 days for the Pin m III probe with intensifying screens at −80 °C. Northern blot analysis was repeated three times for the analysis of the seasonal expression and repeated twice for the analyses of tissue specificity and disease response. The autoradiograms of northern blots were scanned with a laser scanner (Model 110A, Molecular Dynamics, Sunnyvale, CA) interfaced with a workstation (SPARK 1, Sun Microsystems of Canada Inc., Vancouver, British Columbia,
Canada) and Protein + DNA ImageWare Systems (PDI, Huntington Station, NY) for blot processing with the software program ONED. Scanning, detection, and quantification were performed according to instructions of the manufacturer (PDI). The mRNA was quantified as described by Kaukinen et al. (1996).

**Genomic DNA extraction and southern analysis**

Genomic DNA was extracted from freeze-dried current-year foliage samples by the CTAB method (Wagner et al. 1987). Thirty µg of DNA was digested with restriction enzyme overnight at 37 °C, fractionated through 0.8% agarose gels and blotted onto Zeta-Probe GT membranes. The Pin m III cDNA insert (EcOR I fragment) was labeled, hybridized and washed as described for the northern blot analysis. Southern analysis was repeated three times.

**DNA sequencing**

Lambda DNA was purified by the QIAGEN Lambda Maxi Kit (QIAGEN GmbH, Hilden, Germany). The inserts from the λgt22A vector were purified by fractionating the digestion mixture by electrophoresis on 1% agarose and extracting the insert by QIAquick Gel Extraction Kit (QIAGEN GmbH). The cDNA insert was subcloned into pBluescript KS+ vector (Stratagene, La Jolla, CA). Inserts were sequenced on both strands by the dideoxy method (Sequenase Version 2.0, US Biochemical, Cleveland, OH) with T3 and T7 primers and other internal primers that were based on derived sequences (Stratagene, La Jolla, CA). Inserts from the EcoRI fragment (QIAGEN GmbH, Hilden, Germany). The inserts from the cDNA expression library with the polyclonal antibody against the Pin l I N-terminal 17 amino acid residues was used in the synthesis of peptide because the synthetic N-terminal Pin l I peptide identified three positive clones in the first round. The second and third rounds of immunoscreening of one of the three clones confirmed a specific signal. After three rounds of consecutive screening, the sequence of a cDNA encoding the Pin m III protein was obtained. The Pin m III cDNA sequence was 806 bp in length with a 5’ untranslated region of 54 bp, a 486-bp open reading frame encoding the Pin m III protein, and a 266-bp 3’ untranslated region including the poly(A) tail (Figure 1). The deduced open reading frame codes for 161 amino acids and the partial N-terminal protein sequence confirmed that the cDNA encoded the Pin m III protein. The predicted molecular mass of Pin m III is 18 kD, which is in agreement with the molecular mass of 18.4–18.8 kD estimated by SDS-PAGE (Ekramoddoullah et al. 1996). The 3’ noncoding region contained a putative polyadenylation signal (AATAAA) located at position 590 and the poly(A) tail was located 221 nucleotides downstream from the TAG translation termination codon. The deduced amino acid sequence of Pin m III had two more strongly acidic (negatively charged) residues (D, E) than strongly basic (positively charged) amino acids (K, R) (22 acidic versus 20 basic amino acids). It had 50 hydrophobic amino acids (A, I, L, F, W, V) and 44 polar amino acids (N, C, Q, S, T, Y). The predicted isoelectric point is 5.5, which is consistent with the pI value of 5.4 observed in 2-dimensional gel electrophoresis analysis of Pin m III (Ekramoddoullah et al. 1996). At pH 7.0 the predicted charge is ~ -2.024. Hydrophobicity analysis by the Kyte-Doolittle method (Kyte and Doolittle 1982) predicted that the protein is hydrophilic (data not shown).

**Results**

**Cloning and characterization of Pin m III cDNA**

Immunoscreening of the λgt22A western white pine needle cDNA expression library with the polyclonal antibody against synthetic N-terminal Pin l I peptide identified three positive clones in the first round. The second and third rounds of immunoscreening of one of the three clones confirmed a specific signal. After three rounds of consecutive screening, the sequence of a cDNA encoding the Pin m III protein was obtained. The Pin m III cDNA sequence was 806 bp in length with a 5’ untranslated region of 54 bp, a 486-bp open reading frame encoding the Pin m III protein, and a 266-bp 3’ untranslated region including the poly(A) tail (Figure 1). The deduced open reading frame codes for 161 amino acids and the partial N-terminal protein sequence confirmed that the cDNA encoded the Pin m III protein. The predicted molecular mass of Pin m III is 18 kD, which is in agreement with the molecular mass of 18.4–18.8 kD estimated by SDS-PAGE (Ekramoddoullah et al. 1996). The 3’ noncoding region contained a putative polyadenylation signal (AATAAA) located at position 590 and the poly(A) tail was located 221 nucleotides downstream from the TAG translation termination codon. The deduced amino acid sequence of Pin m III had two more strongly acidic (negatively charged) residues (D, E) than strongly basic (positively charged) amino acids (K, R) (22 acidic versus 20 basic amino acids). It had 50 hydrophobic amino acids (A, I, L, F, W, V) and 44 polar amino acids (N, C, Q, S, T, Y). The predicted isoelectric point is 5.5, which is consistent with the pI value of 5.4 observed in 2-dimensional gel electrophoresis analysis of Pin m III (Ekramoddoullah et al. 1996). At pH 7.0 the predicted charge is ~ -2.024. Hydrophobicity analysis by the Kyte-Doolittle method (Kyte and Doolittle 1982) predicted that the protein is hydrophilic (data not shown).

**Pin m III is similar to AoPR1 and Bet v I and is a member of the PR-10 family**

A BLAST search (Altschul et al. 1997) of the protein databases revealed similarities among the sequences of Pin m III and a group of intracellular pathogenesis-related (PR) proteins and major tree pollen allergens (Figures 2 and 3). Pin m III shares 22 to 36.7% amino acid similarity with the representative intracellular PR and allergen family members from angio-
Northern analysis of the same foliage samples showed that the seasonal variation in amount of Pin m III was barely detectable in July and August (Figure 5). Western analysis of the monthly collections of western white pine foliage samples showed that Pin m III accumulation was detectable in September and gradually increased in amounts as fall progressed, reaching a maximum in January. It was seasonally regulated (Figure 5, Ekramoddoullah et al. 1995). It was found that the western white pine genome has a small family of PR-10-related sequences. Eastern white pine (Pinus strobus) genome had a similar banding pattern to white pine (Pinus ponderosa) genomic DNA did not cross-hybridize with the Pin m III probe (Figure 4, Lane 7).

**A small multigene family may code for PR-10 in western white pine**

Thirty µg of genomic DNA extracted from foliage samples was digested with the restriction enzymes Xba I, Bam HI, Hind III or EcoR I and analyzed by Southern blot hybridization with the PR-10 sequence. On DNA blots the Xba I digestion gave at least seven bands (Figure 4, Lane 1), whereas Hind III and EcoR I digestion showed at least four distinct bands (Figure 4, Lanes 3 and 4). Bam HI digestion also had at least four bands (on another repeated blot). Thus, we have preliminary evidence that the western white pine genome has a small family coding for PR-10-related sequences. Eastern white pine (Pinus strobus L.) genome had a similar banding pattern to western white pine when digested with Hind III or EcoR I (Figure 4, Lane 5) had twice as much transcript (based on the densitometric analysis, data not shown). The expression of Pin m III transcripts and protein in healthy and diseased tissues

To study the expression of Pin m III in different tissues of western white pine seedlings, 2-year-old seedlings from Seedlot 3144 were harvested in September 1996. Foliar and twig samples (stems) from the current-year and previous year (1 year older) and the mixed root systems from the seedlings were collected and analyzed for amounts of transcript and protein. Because the samples were collected in September, the expression of Pin m III was low in the foliage (Figures 5 and 6). There was a large accumulation of Pin m III transcripts in the root system and the corresponding Pin m III protein concentration was higher in the root system than in the other tissues analyzed (Figure 7, Lane 5; Figure 8, Lane 5). The amounts of transcript and protein in the foliage and stem were low compared with the root system (Figures 7 and 8). From the same seedling sample, the amounts of transcript and protein in stem samples (Figure 7, Panel A, Lanes 3 and 4; Figure 8, Lanes 3 and 4) were higher than in foliage samples (Figure 7, Panel A, Lane 1; Figure 8, Lanes 1 and 2). Compared with the October foliage sample of Seedlot 2888 collected in 1992 (Figure 7, Panel A, Lane 6), which had the highest amount of transcript (Figure 6, Lane 3), the seedling root system (Figure 7, Panel A, Lane 5) had twice as much transcript (based on the densitometric analysis, data not shown). The expression of Pin m III was highest in roots, followed by stems and then foliage.

**Seasonal regulation of the transcription of Pin m III**

Western analysis of the monthly collections of western white pine foliage samples showed that Pin m III accumulation was seasonally regulated (Figure 5, Ekramoddoullah et al. 1995). It was detectable in September and gradually increased in amount as fall progressed, reaching a maximum in January. It was seasonally regulated (Figure 5, Ekramoddoullah et al. 1995). It was detectable in July and August (Figure 5). Western analysis of foliage samples from Seedlots 2881, 2888 and 3159 confirmed the seasonal variation in amount of Pin m III. Northern analysis of the same foliage samples showed that the amount of transcript also changed seasonally, preceding parallel changes in the amount of Pin m III protein. The amount of transcript in the summer months was low. Transcripts started to accumulate at the onset of fall and reached a maximum in October (Figure 5). The amount of transcript stayed high until December and then gradually decreased.

Expression of the Pin m III transcripts and protein in healthy and diseased tissues

To study the expression of Pin m III in different tissues of western white pine seedlings, 2-year-old seedlings from Seedlot 3144 were harvested in September 1996. Foliar and twig (stems) samples from the current-year and previous year (1 year older) and the mixed root systems from the seedlings were collected and analyzed for amounts of transcript and protein. Because the samples were collected in September, the expression of Pin m III was low in the foliage (Figures 5 and 6). There was a large accumulation of Pin m III transcripts in the root system and the corresponding Pin m III protein concentration was higher in the root system than in the other tissues analyzed (Figure 7, Lane 5; Figure 8, Lane 5). The amounts of transcript and protein in the foliage and stem were low compared with the root system (Figures 7 and 8). From the same seedling sample, the amounts of transcript and protein in stem samples (Figure 7, Panel A, Lanes 3 and 4; Figure 8, Lanes 3 and 4) were higher than in foliage samples (Figure 7, Panel A, Lane 1; Figure 8, Lanes 1 and 2). Compared with the October foliage sample of Seedlot 2888 collected in 1992 (Figure 7, Panel A, Lane 6), which had the highest amount of transcript (Figure 6, Lane 3), the seedling root system (Figure 7, Panel A, Lane 5) had twice as much transcript (based on the densitometric analysis, data not shown). The expression of Pin m III was highest in roots, followed by stems and then foliage.

To investigate the role of Pin m III in disease resistance, the sugar pine–white pine blister rust pathosystem was used. In
sugar pine, resistant trees carry the dominant R gene, as homozygous (RR) or heterozygous (Rr), which confers resistance to white pine blister rust (Kinloch et al. 1970). Healthy or in-

Figure 3. Phylogenetic tree of 30 representative members of the intra-

Figure 4. DNA blot analysis of genomic DNA from western white pine, related pine species and tobacco. Thirty µg of genomic DNA was used in each overnight digestion. Lane 1, western white pine DNA cut with EcoRI; Lane 2, BamHI; Lane 3, HindIII; Lane 4, HindIII; Lane 5, eastern white pine genomic DNA digested with HindIII; Lane 6, ponderosa pine genomic DNA digested with HindIII; Lane 7, tobacco genomic DNA digested with HindIII; Lane 8, western white pine genomic DNA cut with HindIII (same as Lane 3).
fectected needles from 4-year-old sugar pine seedlings were collected and used in northern or western analysis. In both susceptible and resistant sugar pine seedlings, the amounts of protein and transcript of Pin l I (homologue of Pin m III) were higher in infected needles than in healthy needles (Figure 7, Panel B; Figure 8, Lanes 6–11). The susceptible sugar pine showed higher amounts of Pin l I transcript and protein than the resistant sugar pine, indicating that the expression of Pin l I and the amounts of Pin l I transcript and protein were correlated with the severity of the fungal infection.

**Discussion**

During investigations of the host–pathogen interaction of the white pine blister rust pathosystem (Ekramoddoullah and Hunt 1993), a sugar pine protein, Pin l I, was detected in in-

Figure 5. Western analysis of Seedlot 2888 foliage proteins showing seasonal variation. Five µg of total protein was loaded in each lane. The blot was probed with polyclonal antibodies against the N-terminal region of Pin l I.

Figure 6. RNA gel blot analysis of seasonal expression of Pin m III transcripts in western white pine foliage. Total RNA was isolated, separated by electrophoresis, and transferred to membranes as described in Materials and methods. Pin m III transcripts of about 0.9 kb were detected with a 32P radiolabeled probe of the full-length Pin m III insert. The membranes were exposed for one week. All needle samples were collected from Seedlot 2888.

Figure 7. RNA gel blot analyses. Panel A: Pin m III transcript in western white pine seedling tissues. Tissues were collected from healthy western white pine seedlings (Seedlot 3144) in September 1996. Lane 1, current-year foliage; Lane 2, blank (originally, 1-year-old foliage but the sample failed); Lane 3, current-year twig; Lane 4, 1-year-old twig; Lane 5, root system; Lane 6, October 1992 foliage sample of Seedlot 2888 as a positive control. Panel B: Pin l I transcript in sugar pine needles infected with white pine blister rust. Lane 1, sugar pine Seedlot S4 (susceptible) infection spots of infected needles; Lane 2, Seedlot S4 healthy needles; Lane 3, Seedlot R4 (resistant) infection spots of infected needles; Lane 4, Seedlot R4 healthy needles.
creasing amounts in the fall (Ekramoddoullah et al. 1995). Its homolog, Pin m III, was detected in western white pine using an anti-Pin l I antibody. Furthermore, the concentration of Pin m III, which reached its maximum in winter months, was significantly correlated with frost hardiness of western white pine foliage (Ekramoddoullah et al. 1995). To characterize the Pin m III protein, we constructed an expression cDNA library and isolated full-length cDNAs. Sequence analysis revealed some similarities between the deduced Pin m III polypeptide and the PR-10 family of ribonuclease-like PR proteins, including major tree pollen allergens such as BV1A-BETVE (Bet v I) from birch pollen (Breiteneder et al. 1989) and asparagus intracellular PR proteins PR1-ASPOF (Warner et al. 1992). The intracellular PRs grouped together, whereas the allergens formed a second group, suggesting a possible divergence of functions inherent to the groups as shown in the phylogenetic tree in Figure 3. Pin m III is most closely related to the PR1-ASPOF (Warner et al. 1992) from asparagus (36.7% similarity).

Intracellular PR proteins are known to be up-regulated in plants by pathogens or by treatment of cell cultures with microbial elicitors (Warner et al. 1992). In Medicago sativa L., accumulation of MsPR10-1 transcript increased in leaf blades during an incompatible interaction and the expression of this gene was induced by salicylic acid treatment of the leaves (Breda et al. 1996). Furthermore, during the incompatible interaction with P. syringae pv. pisi, several genes or allelic variants of PR-10 class were expressed. These findings are indicative of a close correlation between PR mRNA accumulation and the disease-resistance response of the plant and the restriction of fungal growth.

In the sugar pine model, infection analysis suggested that Pin l I is upregulated at the mRNA and protein levels in both resistant and susceptible sugar pine seedlings (Figures 7 and 8). Ekramoddoullah et al. (1998) also observed a high amount of Pin m III protein in infected spots and cankered tissues of a large number of infected mature and young western white pine trees. Although there is no evidence that Pin m III or its homolog provides protection against fungal infection, there is sequence similarity with known intracellular PR proteins, such as the PR protein from potato (Matton and Brisson 1989). Furthermore, the expression of many PR proteins is temporal and spatial, particularly in infection sites, suggesting a defensive role of Pin m III in white pine blister rust pathosystem. It is worth noting that chitinase, a well-known PR protein, was detected in large amounts in a susceptible rice cultivar (Anuratha et al. 1996) and in susceptible western white pine (Davidson and Ekramoddoullah 1997).

The timing of seasonal Pin m III accumulation parallels the accumulation of vegetative storage proteins, which store nitrogen over winter for mobilization for new growth in the spring (Wetzel et al. 1989, Wetzel and Greenwood 1991, Roberts et al. 1991). However, Pin m III lacks the properties of vegetative storage proteins, which are usually basic, 30–32 kDa in size and are rich in glutamine and asparagine. The hydrophilicity of Pin m III is an important characteristic of cryoprotectants. Thus the accumulation of Pin m III in winter (Ekramoddoullah et al. 1995, 1996) may reflect its role as an antifreeze protein.

The finding that Pin m III, which normally accumulates in winter months, can also be induced by a fungal pathogen is similar to some pathogenesis-related proteins that are induced by both biotic and abiotic stress (Lin et al. 1996). There may be a common receptor that perceives signals by two different stresses. For example, in overwintering plants that have to survive frost, the ability to limit water potential could be important for survival. It has been suggested that regulation of water potential by host plants restricts microbial colonization (Pearce 1996). Overwintering grasses that have to survive freezing temperature and desiccation are known to have increased resistance to fungal diseases (Tronsmo 1984, 1985, Tronsmo et al. 1993). Based on the genotypic correlation between freezing tolerance and resistance to the snow molds Typhula ishikariensis Imai (Tish) and Fusarium nivale (Fr.)
Ces. (Tronsmo et al. 1993), Hon et al. (1995) suggested that the same genetic trait(s) may be involved in both disease and freezing tolerance.

We postulate that the Pin m III gene encodes a member of the PR-10 family of intracellular PR proteins. It is seasonally regulated and is upregulated by Cronartium ribicola.

Acknowledgments and notes

This research was supported by Canadian Forest Service & T Opportunities Fund. We thank Y. Tan, D. Taylor, T. Tranbarger and J. Van Raamdsok for their technical support. The nucleotide sequence data reported in this paper have been submitted to the GenBank Nucleotide Sequence Database under the Accession no. GenBank AF038949.

References


TREES PHYSIOLOGY VOLUME 20, 2000

Downloaded from https://academic.oup.com/treephys/article-abstract/20/10/663/1706507 by guest on 03 April 2019


