THE MOLECULAR STRUCTURE AND EVOLUTIONARY RELATIONSHIPS OF A 16.9 kDa HEAT SHOCK PROTEIN FROM NORWAY SPRUCE [*PICEA ABIES* (L.) KARST.]

Roland Schubert[1], Gerhard Müller-Starck[1], Heinrich Sandermann Jr[2], Dieter Ernst[2] & Klaus-Peter Häger[3]

[1)] Ludwig Maximilians University of Munich, Faculty of Forest Sciences, Section of Forest Genetics, Am Hochanger 13, D–85354 Freising, Germany

[2)] GSF–National Research Center for Environment and Health, Institute of Biochemical Plant Pathology, Ingolstädter Landstr. 1, D–85764 Neuherberg, Germany

[3)] University of Bayreuth, Department of Plant Ecology and Systematics, Universitätsstr. 30, D–95440 Bayreuth, Germany

Received March 17, 1997; accepted July 17, 1997

ABSTRACT

Based on an elicitor-induced cDNA library from *Picea abies* (L.) Karst. (Norway spruce), clone pPA0010 encoding a small heat shock protein of 16.91 kDa molecular weight was identified. Comparison of the deduced amino acid sequence with gene bank data revealed significant similarities to cytosolic class II small heat shock proteins. Evolutionary relationships to small heat shock proteins from *Picea glauca* and *Pseudotsuga menziesii* and to those from angiosperms were analysed using distance matrix- and parsimony-methods, respectively. The different classes of small heat shock proteins appear to have already existed in the last common ancestor of extant seed plants, and further diversification has occurred within class II small heat shock proteins from *Picea*. Phosphorylation motifs for serine protein kinases were detected in the heat shock domains of *Picea* small heat shock proteins.

Key words: *Picea abies* (L.) Karst., elicitor, small heat shock proteins, evolution, gymnosperms, angiosperms

INTRODUCTION

Norway spruce is wide spread in Central Northern and Eastern Europe. It is a carrier species of various forest ecosystems and covers a wide range of habitats from the mountainous to the subalpine regions (*e.g.* SCHMIDT-VOGT 1977).

Like other long lived tree species, populations of Norway spruce are exposed to a great heterogeneity of environmental conditions in time and in space. Environmental stress conditions consist of a large variety of abiotic and biotic stress factors. Norway spruce is subjected to an increasing extent to forest dieback following acidification due to air pollution. Studies on genetic response of Norway spruce populations to air pollution verified changes in genetic structures, losses of genetic variation and viability selection (BERGMANN & SCHOLZ 1989; ROTHE & BERGMANN 1995). Global warming will expose forest trees such as Norway spruce to abiotic and biotic stress conditions. In responses to stress, heat shock proteins play a major role and have been recognized long as an important factor affecting survival of forest tree populations (*e.g.* MAGUIRE 1955).

Eukaryotes synthesize a characteristic set of heat shock proteins (HSPs) to protect living cells against damages caused by elevated temperatures and certain other environmental stimuli (for detailed information see for instance VIERLING 1991). According to their approximate molecular weights in kDa they are designated as HSP 110, HSP90, HSP70, HSP60, and small HSPs (~17–30 kDa molecular mass), and in several cases homologous proteins have been identified in prokaryotes as well (LINDQUIST & CRAIG 1988). *In vitro* studies indicate that HSPs may function as molecular chaperones that bind to partially folded or denatured proteins, preventing heat-induced irreversible protein inactivation and aggregation (reviewed by LANDRY & GIERASCH 1994). Large HSPs are generally present at significant levels in most normal cells and do exhibit increasing expression in response to stress and endogenous signals (YABE *et al.* 1994). In contrast, small HSPs are apparently not involved in basal cell functions. Their cellular accumulation is strongly induced by a variety of stress factors or as a specific part of ontogenic development (KOBAYASHI *et al.* 1994).

All small HSPs share a conserved C-terminal heat

shock region which is related to the α-crystallin proteins of the vertebrate eye lens. In non-plant eukaryotes, only one or very few small HSPs are found, and they are localized in the cytosol (ARRIGO & LANDRY 1994). These proteins are related to but are quite distinct from the more abundant and more diverse small HSPs from higher plants (PLESOFSKY-VIG *et al.* 1992). In angiospermous taxa there are at least five nuclear small gene-families coding for a specific class of small HSPs, each. The proteins are targeted to different cellular compartments, including the cytosol (cytosolic class I and II small HSPs), the chloroplast, the mitochondria and the endoplasmic reticulum (reviewed by WATERS *et al.* 1996). More than 45 complete small HSP sequences from different angiosperms are currently available in electronic databases. Evolutionary analysis previously performed suggests that the small HSP gene-families have evolved from a common ancestral gene, however both the order and the time point of the gene duplications involved have remained unknown. It has been assumed, that the respective gene duplications and the divergence of small HSP sequences occurred prior to the radiation of angiosperms (WATERS 1995), certainly before the evolutionary split of the monocotyledonous and dicotyledonous species which has been estimated with different molecular clocks at about 200 million years ago (WOLFE *et al.* 1989).

In order to extend our knowledge of small HSP structure and evolution in coniferous tree species, an elicitor-induced cDNA library from photomixotrophic suspension cultures of *Picea abies* has been used to identify several genes (SCHUBERT *et al.* 1997). We report here on the isolation and characterization of a cDNA encoding a cytosolic class II small HSP of 16.91 kDa. The results from sequence comparisons and phylogenetic analyses including small HSPs from two additional Pinaceae species and from angiosperms are presented. Implications for small HSP-evolution are discussed. Moreover, the conserved kinase recognition sequence Arg-X-X-Ser, the serine-residue of which is known to be phosphorylated in certain mammalian small HSPs (GAESTEL *et al.* 1991), has been detected within *Picea* small HSP sequences.

MATERIALS AND METHODS

Cell suspension cultures/elicitor treatment

Photomixotrophic cell suspension cultures of Norway spruce were grown in continuous light. After 7 days of subculture cells were treated with 50 $\mu g \cdot ml^{-1}$ elicitor from the fungus *Rhizosphaera kalkhoffii* Bub. and further incubated in the light for 24 hours as described (MESSNER *et al.* 1991).

cDNA library construction

The construction of a spruce cDNA library in the plasmid pSPORT 1 has been previously published (GALLIANO *et al.* 1993). In brief, poly(A)$^+$ RNA was isolated from elicitor-treated cells and used for the enzymatic synthesis of cDNA. Directional cloning of the double-stranded cDNA was carried out with the Superscript Plasmid System (GIBCO/BRL).

Nucleic acid isolation and sequencing

The DNA of randomly picked recombinant pSPORT 1 plasmid derivatives was isolated with QIAGEN-tip 20 columns according to the manufacturer's instructions. The sequence of both DNA strands was determined by an oligonucleotide walking strategy using the Cy^5-AutoRead sequencing Kit (PHARMACIA). Fluorescently labelled dideoxy chain terminated fragments were detected by an Alfexpress automated sequencer (PHARMACIA). Clone-specific oligo-nucleotides were obtained from GIBCO/BRL.

Alignment search

DNA similarity searches were performed using the BLAST network service at the National Centre for Biotechnology Information (Cambridge, UK) which utilizes the basic local alignment search tool developed by ALTSCHUL *et al.* (1990). Maximum matching of amino acid alignments was calculated using the HIBIO DNASIS version 2.1 programme from HITACHI according to the Needleman-Wunsch algorithm (NEEDLEMAN & WUNSCH 1970).

Phylogenetic analysis

Sequences included in this study have been obtained from the EMBL data library, specified by the accession numbers given in brackets: *Arabidopsis thaliana* HSP 17.6′ (X63443), HSP 17.6 (X16076), HSP 21 (X54102); *Chenopodium rubrum* HSP 23 (X15333); *Glycine max* HSP 17.6 (M11317), HSP 22 (X07188), HSP 22′ (X63198); *Ipomoea nil* HSP 17.2 (M99429); *Picea glauca* HSP 17.O (L47717), HSP 17.1 (L47740), HSP 23.5 (L47741); *Pisum sativum* HSP 17.7 (M33901), HSP22.7 (M33898); *Zea mays* HSP 17.5 (X54076); *Pseudotsuga menziesii* HSP 18.2A (X92983) and HSP 18.2B (X92984).

The derived amino acid sequences were aligned in an interactive multiple sequence alignment editor (Sequence Editor and Analysis Programme, OLSEN 1990), essentially according to the alignment of angiosperm small HSPs given in WATERS (1995). Two

regions displaying unambiguously alignable sites were selected for analyses: residue positions 94–240 and 256–267 (positions refer to Fig. 2). Within these intervals, a few positions where the majority of sequences had deletions were omitted. The corresponding regions of the nucleotide sequences were used for phylogenetic analyses, only first and second codon positions were included (DNA alignment is available upon reqest). Using the PHYLIP programme package (version 3.5c; J. Felsenstein, Department of Genetics, University of Washington, 1993), distances were calculated on the basis of the Kimura-two parameter-model, and trees were constructed with the neighbor joining method. In addition, parsimony analyses were conducted on the same data sets (PAUP version 3.1.1, D. L. Swofford, Phylogenetic Analysis Using Parsimony, Illinois Natural History Survey, Champaign, 1993). Computing was carried out on a VAX 6000–310 and on an Apple Macintosh IIfx.

RESULTS AND DISCUSSION

The molecular structure of the *Picea abies* small heat shock protein hsp 16.9

Clone pPA0010 was isolated from an elicitor-induced spruce cDNA library and chosen for sequence analysis. The nucleotide sequence and the corresponding amino acid sequence are presented in Fig. 1. The cDNA is 775 bp in length and contains a 5` non-coding region of 72 bp and a 3` non-coding region of 250 nucleotides including the poly(A) tail. The longest open reading frame begins at an ATG codon at nucleotide 73 and ends with a TAA termination codon at position 526.

```
GAGCATTTCGCATTACTCTGCAACAAATCCGCCATTCTGCGATTGTTGAAATCCGGGAAA     60

              M  A  M  D  P  L  L  S  T  V  Q  Q  L  L  G  V      16
GATATTTGAAAGATGGCAATGGATCCTCTGTTGAGCACCGTGCAGCAGCTGCTGGGTGTG    120

 P  D  D  L  E  R  I  L  H  A  P  T  R  S  Y  M  R  D  T  E      36
CCGGACGACCTGGAGAGGATCCTCCACGCCCCGACTCGCTCTTATATGCGCGACACCGAG    180

 A  T  A  S  T  P  V  D  V  K  E  Y  P  N  S  Y  V  F  I  V      56
GCCACGGCTTCGACTCCCGTAGATGTTAAAGAATACCCCAACTCTTATGTCTTTATAGTC    240

 D  M  P  G  L  K  S  N  D  I  K  V  Q  V  E  D  E  N  V  L      76
GACATGCCCGGCCTTAAATCCAACGACATCAAGGTTCAAGTGGAAGACGAGAACGTTCTG    300

 N  I  S  G  E  R  K  R  N  E  K  E  E  G  E  V  K  Y  I  R      96
AACATCAGTGGCGAGCGGAAGAGGAACGAGAAGGAAGAAGGGGAAGTGAAATACATCCGC    360

 M  E  R  R  V  A  K  F  M  R  K  F  S  L  P  A  D  C  N  L     116
ATGGAGCGCAGAGTGGCAAAGTTCATGAGGAAATTCAGCTTGCCTGCTGACTGCAACCTG    420

 E  A  I  S  A  A  C  Q  D  G  V  L  T  V  T  V  P  K  L  P     136
GAGGCCATCTCTGCTGCGTGCCAGGATGGAGTGCTGACCGTCACTGTTCCCAAGCTTCCC    480

 P  P  E  P  K  K  P  K  T  I  A  V  K  I  G                    151
CCACCGGAACCGAAGAAACCTAAGACGATTGCAGTCAAAATTGGATAAATCCTCCGCTGT    540

GTATGCAGGGATTGAAATCTTTGGTGAATTTTGTAGGGTTTTGTTGCCTGTGTCCTATCT    600

AGATTTTGCACGATTTTACTTCCAGGCTTGAGAAATATATCCAACCTTATTGCGCATAAA    660

TAAAGGTTCTCTACATTTCTGTCATGTATCGAGCTTCCTGTTAATGGTACAACAGAGCTC    720

TGCATTTAAATATAATTTCTGTATTTGAAAGCTTTTACTGTACCAAAAAAAAAAA         775
```

Figure 1. Nucleotide sequence (numbered 1–775) and deduced amino acid sequence (numbered 1–151) of the clone pPA0010 encoding a small HSP from *Picea abies*. Nucleotides coding for the conserved carboxy-terminal consensus I region (corresponding amino acid residues 106 to 134) and the conserved consensus II region (corresponding amino acid residues 57 to 83) of the heat shock domain are underlined. Two possible poly(A) addition signals are indicated in bold letters. The sequence data reported in this figure have been submitted to the EMBL Database under the accession number X99346.

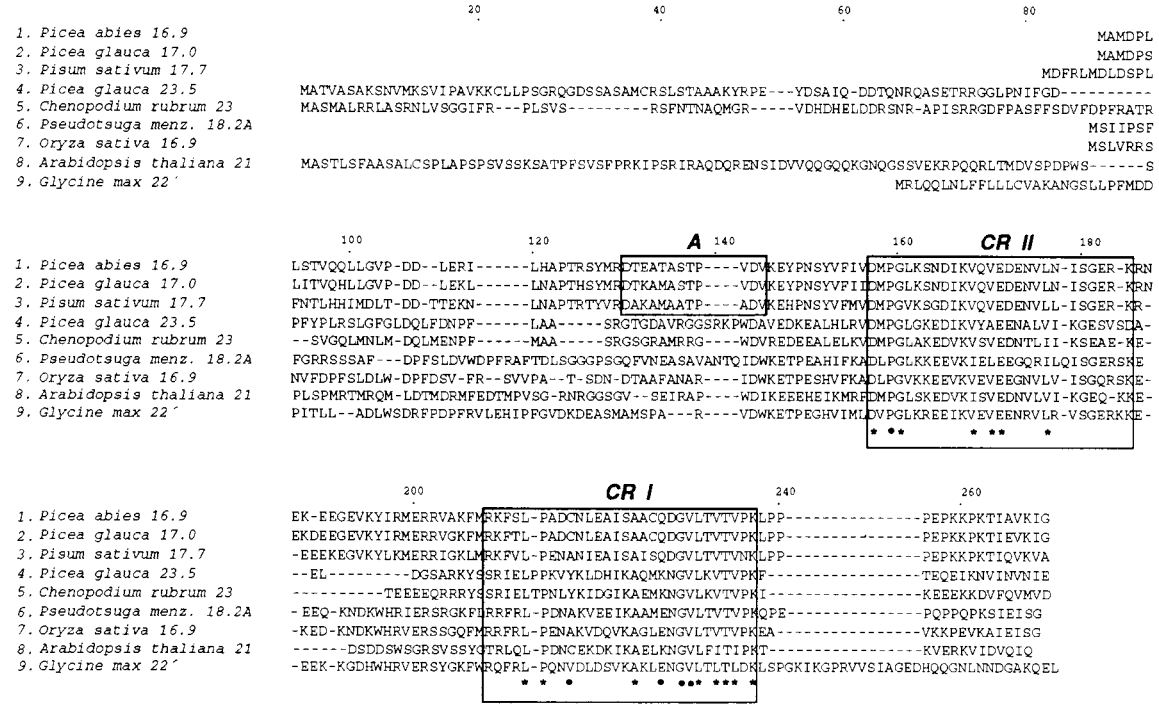

Figure 2. Amino acid sequence alignment of small HSPs from *Picea abies*, *Picea glauca* and *Pseudotsuga menziesii*, together with angiosperm sequences representing the five different small HSP gene-families. Sequences 1–3: cytosolic class II; 4,5: mitochondria–localized; 6,7: cytosolic class I; 8: chloroplast-localized; 9: ER-localized small HSPs. Boxes indicate the consensus regions diagnostic for cytosolic class II small HSPs (A) and the „heat shock domain"-located consensus regions II and I (CRII, CRI). Within the boxes filled circles and asterisks mark positions of completely and highly conserved amino acid residues, respectively, as determined from sequences of small HSPs from angiosperms (WATERS 1995). Gaps (dashes) have been introduced to maximize alignments. For corresponding EMBL-database accession numbers see topic Materials and Methods.

The sequence AAAGATGGC surrounding the ATG codon is very similar to the AACAATGGC translation initiation consensus from angiosperms (ELLISTON & MESSING 1988). There is an AATAAA polyadenylation signal at nucleotide position 659. A related AATATA motif at position 729 may also function as a poly(A) addition signal analogous as has been observed from angiosperm mRNAs (ELLISTON & MESSING 1988). Similarity searches in the EMBL/GenBank/ DDBJ databases using the BLAST network service revealed significant homologies between the estimated cDNA and various nucleotide sequences which are coding for different classes of plant small HSPs (data not shown).

The open reading frame of pPA0010 encodes a polypeptide 151 amino acids in length with a predicted molecular weight of 16.91 kDa. As compared to the amino acid sequences of angiosperm HSPs, pPA0010 shares 28–36 % identity with the chloroplast-localized small HSPs, 30–32 % identity with the mitochondria-localized small HSPs, 36 % identity with the endoplasmic reticulum-localized small HSPs, 40–42 % identity with the cytosolic class I small HSPs, and 58–66 % identity with the cytosolic class II small HSPs (calculated on the basis of the Needleman-Wunsch algorithm using DNASIS software, see topic Materials and Methods). Maximum values of identity ranging from 90–96 % are estimated in comparisons with the recently published cytosolic class II small HSP sequences from the gymnospermous tree species *Picea glauca*. Altogether these sequence similarities agree with the previous observation, that the different classes of small HSPs share structural similarities and may have derived from a common ancestral gene. From the identities observed it is clear that the pPA0010 coding sequence represents a member of the plant small HSP families, and the protein therefore is termed as hsp 16.9.

An amino acid alignment of the *Picea abies* hsp 16.9 sequence with representative members of the different plant small HSP classes is depicted in Fig. 2. Two sequences from small HSPs of *Picea glauca* which had been tentatively assigned to the cytosolic class II and to the mitochondria-localized type (DONG & DUNSTAN 1996), respectively, as well as a cytosolic class I small HSP sequence from *Pseudotsuga menziesii* (KAUKINEN *et al.* 1996) have been included. The proteins show considerable differences in sequence and size, however this heterogeneity is restricted to their N-

terminal domains. In contrast, the hsp 16.9 sequence and the reference sequences share conserved C-terminal regions (position 157–237, Fig. 2), sometimes termed as the „heat shock domain". With respect to this domain, plant small HSPs are structurally related to small HSPs from other organisms and to the α-crystallin proteins of vertebrate eye lens. The Pro-X14-Gly-Val-Leu motif present in the hsp 16.9 sequence (position 214–231, Fig. 2) is highly conserved at homologous positions of the heat shock domain in all eukaryotic small HSPs (LINDQUIST & CRAIG 1988). Within angiosperms, this motif is embedded in the consensus I region which is additionally characterized by six highly conserved amino acids (see asterisks Fig. 2; WATERS 1995); the respective residues are conserved in the gymnosperm sequences as well. An additional conserved region of the heat shock domain, the consensus II sequence containing the Pro-X14-X-Val/Leu/Ile- Val/ Leu/Ile motif (position 159–176, Fig. 2), is specifically found in plant small HSPs (VIERLING 1991); this motif and a further five residues are highly conserved within angiosperms (see asterisks Fig. 2; WATERS 1995). Despite the phylogenetic distance of the taxa concerned, the same residues are conserved in the *Picea abies* hsp 16.9 sequence, too. Notably, this conservation is found in all the gymnosperm small HSPs as well, indicating high selective pressure laying on this region.

As a diagnostic character, the first N-terminal amino acids of the chloroplast-, mitochondrial- and endoplasmic reticulum-localized small HSPs represent organelle-specific transit sequences, not present in the hsp 16.9 sequence described here. Comparisons of cytosolic class II small HSPs from multiple angiosperms have revealed a conserved domain of 12 amino acid residues in length being located in the central position of the molecule (position 130– 145, Fig. 2) and not present in the other small HSP classes (WATERS 1995). The conservation of this sequence (DAXAMA-ATPADV) suggests it to play an essential role in type II-specific functions. The motif DTEATASTPVDV found at a corresponding position of the hsp 16.9 sequence is clearly homologous to the above domain and identifies the *Picea abies* small HSP as a member of the cytosolic class II family. It is interesting to note that some of the amino acid substitutions relative to the conserved domain resemble cytosolic class I sequences: The Thr- and Ser-residues at position 131 and 136 (Fig. 2) are preferentially (Thr) or exclusively (Ser) confined to homologous positions of various class I sequences from angiosperms. This finding may suggests that cytosolic class II and cytosolic class I small HSPs are more closely related to each other than to any of the remaining classes.

Phosphorylation signals are present within gymnosperm small HSPs

The putative protein kinase phosphorylation site motif Arg-X-X-Ser (PEARSON *et al.* 1985) can be recognized in our study within the sequences of hsp 16.9 from *Picea abies* (position 208–211, Fig. 2) and at a homologous position in the hsp 17.1 from *Picea glauca* (data not shown; see DONG & DUNSTAN 1996). An Arg-Arg-Ser-Ser-Ser sequence has been recently identified at a different position within the cytosolic class I small HSPs from *Pseudotsuga menziesii* (position 10–14; see KAUKINEN *et al.* 1996). These differences in positions indicate an independend evolution of putative phosphorylation signals in both classes of conifer genes.

Reversible phosphorylation of serine and/or threonine- and tyrosine sites is a major strategy for the regulation of protein activity in the transduction of environmental signals in all living organisms. It has been assumed that phosphorylation plays a crucial role in the function of mammalian small HSPs. Phosphorylation changes drastically in response to different stress factors, and protein kinases involved in small HSP phosphorylation have been described (GAESTEL *et al.* 1991; BENNDORF *et al.* 1992). To date it can not unambigously be decided whether or not phosphorylation is significant for plant small HSP functions. Although enzymes representing members of the eukaryotic kinase superfamily have been identified in plants (reviewed by STONE & WALKER 1995), a previous study failed to detect phosphorylation of plant small HSPs (NOVER & SCHARF 1984). In particular, it has been stated that the plant small HSPs known so far are lacking the conserved protein kinase phosphorylation site Arg-X-X-Ser (WATERS *et al.* 1996). Further studies are awaited to investigate the functional significance of these amino acid motifs present in gymnosperm small HSPs.

Evolutionary relationships of the *Picea abies* hsp 16.9 protein

To date, the molecular evolution and phylogenetic relationships of nucleotide sequences encoding plant small HSPs have been analyzed in great detail only from angiosperms. In order to determine the relationships of *Picea abies* hsp 16.9 to the small HSPs from *Picea glauca*, *Pseudotsuga menziesii* and from angiosperms, the amino acid sequences from representative members of each small HSP class were aligned essentially as described in WATERS (1995). Nucleotide alignments from selected regions were used to infer relationships on the basis of distance matrix

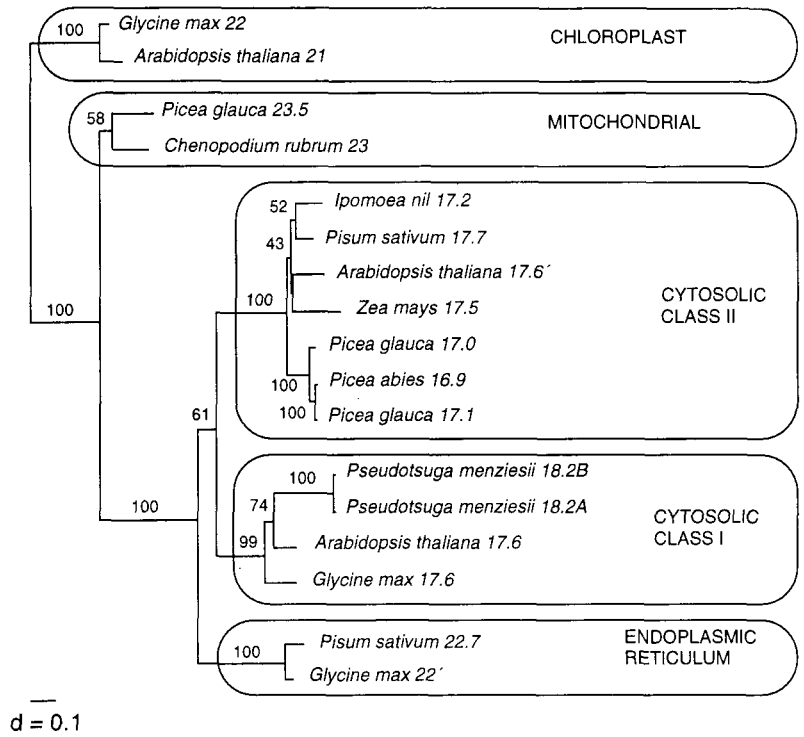

Figure 3. Evolutionary relationships of *Picea abies* hsp 16.9 to small HSPs from gymnosperms and angiosperms. The dendrogram is based on neighbor-joining analysis of nucleotide sequences coding for the entire heat shock-domain. For included sequence positions, alignment and references see text. Numbers adjacent to the nodes are bootstrap values indicating frequencies of respective furcations found in 100 replications of subset tree calculations; the bar at the bottom denotes 10% dissimilarity.

analysis (PHYLIP) and of parsimony analysis (PAUP) as well (see topic Materials and Methods). At present, there are no sequences of non-plant small HSPs available which could clearly resolve interrelationships between the plant small HSP classes; therefore the chloroplast small hsp 21 sequences from *Glycine max* (VIERLING *et al.* 1988) and *Arabidopsis thaliana* (CHEN & VIERLING 1991) were used as arbitrary outgroup sequences. The resulting neighbor-joining tree is shown in and its topology indicates that a series of gene duplications and subsequent sequence divergence has given rise to those gene families which represent the different classes of small HSPs. The members of the respective families form monophyletic groups that are highly supported by maximal bootstrap values, with the exception of the mitochondrial clade (58 %).

The interrelationships between the two cytosolic and the ER-type sequence groups could not conclusively be determined: in 61 out of 100 replicate subset-trees the ER-type small HSPs are in a sister-group relation to the cytosolic class I and cytosolic class II proteins, in 39 subset-trees ER- plus cytosolic class I proteins appear as a sister-group of class II proteins. This latter topology is supported with 88 % bootstrap value in the PAUP-tree (not shown). *Picea abies* hsp 16.9 described here falls unambiguously into the cytosolic class II cluster, and it forms a monophyletic group together with hsp 17.0 and hsp 17.1 from *Picea glauca* (which had already been assumed as cytosolic class II sequences; DONG & DUNSTAN 1996). A basal dichotomy separates this group from the poorly resolved cytosolic class II proteins of monocotyledonous and dicotyledonous angiosperms. Notably, a gene duplication has given rise to paralogous class II genes in *Picea*, with *Picea abies* 16.9 appearing as the ortholog of *Picea glauca* 17.1; the *Picea abies*-ortholog of *Picea glauca* 17.0 still remains to be detected. The coding sequences of the orthologs share 96 % identity at the amino acid level, and the respective polypeptide sequences both contain the Arg-X-X-Ser signal not present in hsp 17.0. Actually it can not be decided whether the gene lineages are unique to *Picea* or whether they result from a less recent duplication traceable throughout Pinaceae or even throughout conifers. Intra-class II diversification is also known from small HSP genes of angiosperms. Thus, there are 2-3 cytosolic class II genes in species from *Ipomoea*, *Zea* and *Lilium*; and some of

them are differentially expressed. This suggests that functional divergence occurred within class II small HSPs from angiosperms (WATERS 1995), and the same might be true for gymnosperms.

An other small HSP from *Picea glauca*, namely hsp 23.5, clearly belongs to the mitochondrial-type small HSPs; it is to be expected that a cytosolic class I member is also present in this genus. Two small HSP genes from *Pseudotsuga menziesii* (hsp 18.2A and hsp 18.2B, Fig. 3) share 97 % sequence identity within their coding regions and form a monophyletic group with cytosolic class I representants from angiosperms, confirming the recent investigation of KAUKINEN *et al.* (1996).

It has been assumed that the diversification of plant small HSP occurred prior to the radiation of angiosperms, certainly before the divergence of monocotyledonous and dicotyledonous species. The identification and phylogenetic analysis of a small HSP from *Picea abies* presented here together with the recent reports on small HSPs from *Picea glauca* and *Pseudotsuga menziesii* indicate that the gene duplications giving rise to the respective gene families are even older and can be predated to the last common ancestor of extant seed plants, about 300 million years ago (SAVARD *et al.* 1994). If plant small HSP diversification was related to stress encountered during transition to growth on land (WATERS *et al.* 1996), then investigations of small HSPs from pteridophytes and bryophytes should significantly improve our knowledge of small HSP evolution and should contribute to evaluate relationships to small HSPs from non-plant eukaryotes.

CONCLUDING REMARKS

The present study proceeds in the characterization of a small HSP from the forest tree species *Picea abies*. Diagnostic sequence motifs and phylogenetic analysis identified hsp 16.9 from Norway spruce as a cytosolic class II member of the small HSP superfamily. The occurrence of cytosolic class I and mitochondria-localized small HSPs from a further two Pinaceae indicate that the respective gene families were already present within the progymnosperms which are accepted as the ancestral group to all seed plants. Additional diversification has occurred within cytosolic class II small HSPs of *Picea* in the course of evolution and the ortholog cytosolic class II small HSPs from *Picea* contain the conserved serine kinase recognition sequence. The evident sequence similarities of small HSPs among angiosperms and gymnosperms may signify universal plant strategies in responses to stress. The identified small HSP sequences will allow us to design PCR-based gene markers for use in monitoring genetic variation within forest tree populations subject to different abiotic and biotic stresses.

ACKNOWLEDGEMENT

Parts of this work were supported financially by EUROSILVA.

REFERENCES

ALTSCHUL, S. F., GISH, W., MILLER, W., MYERS, E. W. & LIPMANN, D. J. 1990: Basic local alignment search tool. *J. Mol. Biol.* **215**: 403–410.

ARRIGO, A. P. & LANDRY, J. 1994: Expression and function of the low-molecular weight heat shock proteins. *In*: The biology of heat shock proteins and molecular chaperones. (eds. R. Morimoto, A. Tissieres, C. Georgopolous). pp. 335–373. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

BENNDORF, R., HAYEß, K., STAHL, J. & BIELKA, H. 1992: Cell-free phosphorylation of the murine small heat-shock protein hsp25 by an endogenous kinase from Ehrlich ascites tumor cells. *Biochimica et Biophysica Acta* **1136**:203–207.

BERGMANN, F. & SCHOLZ, F. 1989: Selection effects of air pollution in Norway spruce (*Picea abies*) populations. In: Genetic effects of air pollutants in forest tree populations. (eds. F. Scholz, H.R. Gregorius, D. Rudin). pp. 143–160. Springer Verlag, Berlin.

CHEN, Q. & VIERLING, E. 1991: Analysis of conserved domains identifies a unique structural feature of a chloroplast heat shock protein. *Mol. Gen. Gen.* **226**(3):425–431.

DONG, J.-Z. & DUNSTAN, D. I. 1996: Characterization of three heat-shock-protein genes and their developmental regulation during somatic embryogenesis in white spruce [*Picea glauca* (Moench) Voss]. *Planta* **200**: 85–91.

ELLISTON, K. & MESSING, J., 1988: The molecular architecture of plant genes: A phylogenetic perspective. *In*: Architecture of eukaryotic genes. (ed. G. Kahl). pp. 21–56. VCH Verlagsgesellschaft.

GAESTEL, M., SCHRÖDER, W., BENNDORF, R., LIPPMANN, C., BUCHNER, K., HUCHO, F., ERDMANN, V. A. & BIELKA, H. 1991: Identification of the phosphorylation sites of the murine small heat shock protein hsp25. *The J. of Biol. Chem.* **266**(22):14721–14724.

GALLIANO, H., CABANE', M., ECKERSKORN, C., LOTTSPEICH, F., SANDERMANN, H. & ERNST, D. 1993: Molecular cloning, sequence analysis and elicitor-/ozone-induced accumulation of cinnamyl alcohol dehydrogenase from Norway spruce (*Picea abies* L.). *Plant Mol. Biol.* **23**:145–156.

KAUKINEN, K. H., TRANBARGER, T. J. & MISRA, S. 1996: Post-germination-induced and hormonally dependent expression of low-molecular-weight heat shock protein genes in Douglas fir. *Plant Mol. Biol.* **30**:1115–1128.

KOBAYASHI, T., KOBAYASHI, E., SATO, S., HOTTA, Y., MIYAJIMA, N., TANAKA, A. & TABATA, S. 1994: Characterization of cDNAs induced in meiotic prophase in lily microsporocytes. *DNA Research* **1**:15–26.

LANDRY, S. J. & GIERASCH, L. M. 1994: Polypeptide interactions with molecular chaperones and their relationship to *in vivo* protein folding. *Annual Review of Biophysics and Biomol. Structure* **23**.645 –669.

LINDQUIST, S. & CRAIG, E. A. 1988: The heat shock proteins. *Annu. Rev. of Gen.* **22**:631–677.

MAGUIRE, W. P. 1955: Radiation, surface temperature, and seedling survival. *For. Sci.* **1**:277–285.

MESSNER, B., BOLL, M. & BERNDT, J. 1991: L-Phenylalanine ammonialyase in suspension culture cells of spruce (*Picea abies* L.). *Plant Cell Tiss. Organ. Cult.* **27**: 267–274.

NEEDLEMAN, S. B. & WUNSCH, C. D. 1970: Description of the method used in PCOMPARE and SCANISM. *J. Mol. Biol.* **48**:443–453.

NOVER, L. & SCHARF, K. 1984: Synthesis, modification and structural binding of heat shock proteins in tomato cell cultures. *European J. of Biochem.* **139**:303–313.

PEARSON, R. B., WOODGETT, J. R., COHEN, P. & KEMP, B. E. 1985: Substrate specifity of a multifunctional calmodulin-dependent protein kinase. *The J. of Biol. Chem.* **260**(27):14471–14476.

PLESOFSKY-VIG, N., VIG, J. & BRAMBL, R. 1992: Phy-logeny of the α-crystallin-related heat-shock proteins. *J. of Mol. Evol.* **35**:537–545.

ROTHE, G. M. & BERGMANN, F. 1995: Increased efficiency of Norway spruce heterozygous phosphoenolpyruvate carboxylase phenotype in response to heavy air pollution. *Angewandte Botanik* **69**:27–30.

SAVARD, L., LI, P., STRAUSS, S. H., CHASE, M. W., MICHAUD, M. & BOUSQUET, J. 1994: Chloroplast and nuclear gene sequences indicate Late Pennsylvanian time for the last common ancestor of extant seed plants. *Proc. Natl. Acad. Sci. USA* **91**:5163 –5167.

SCHMIDT-VOIGT, H., 1977: Die Fichte. Band I. Taxonomie – Verbreitung – Morphologie – Ökologie – Waldgesellschaften, Verlag Paul Parey, Hamburg und Berlin, 647 pp.

SCHUBERT, R., ERNST, D., SANDERMANN, H. & MÜLLER-STARCK, G. 1997: Gene discovery in Norway spruce based on cDNA sequencing in *Abstracts of the Joint Meeting S.04–07 and S. 04–06* „Somatic Cell Genetics and Molecular Genetics of Trees", Quebec, Canada, August 12–16 (in press).

STONE, J. M. & WALKER, J. C. 1995: Plant protein kinase families and signal transduction. *Plant. Physiol.* **108**: 451–457.

VIERLING, E., NAGAO, R. T., DEROCHER, A. E. & HARRIS, L. M. 1988: A heat shock protein localized to chloroplasts is a member of a eukaryotic superfamily of heat shock proteins. *EMBO J.* 7:575–581.

VIERLING, E. 1991: The roles of heat shock proteins in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**: 579–620.

WATERS, E. R. 1995: The molecular evolution of the small heat-shock proteins in plants. *Genetics* **141**: 785–795.

WATERS, E. R., LEE, G. J. & VIERLING, E. 1996: Evolution, structure and function of the small heat shock proteins in plants. *J. Exp. Bot.* **296**: 325–338.

WOLFE, K. H., GOUY, M., YANG, Y. W., SHARP, P. M. & LI; W. H. 1989: Date of the monocot-dicot divergence estimated from chloroplast DNA sequence data. *Proc. Natl. Acad. Sci. USA* **86**: 6201–6205.

YABE, N., TAKAHASHI, T. & KOMEDA, Y. 1994: Analysis of tissue–specific expression of *Arabidopsis thaliana* HSP90–family gene HSP81. *Plant Cell Physiology* **35**:1207–1219.