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Molecular responses to thermal stress in woody plants

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Abstract

Stress caused by high and low temperatures has been much less studied in woody plants than in herbaceous ones. The cold response shows specific traits in woody plants. Cold acclimation associated to winter dormancy develops in two sequential steps: the first is triggered by photoperiod shortening while the second requires temperatures to fall. The involvement of ABA in cold acclimation is beyond question, yet the roles played by this hormone are far better understood in herbaceous plants. The induction of protective proteins and changes in both glycan metabolism and circadian clock functioning are noteworthy among the molecular responses of woody species to low temperatures. Both herbaceous and woody plants seem to respond similarly to heat stress, with heat shock proteins (HSPs) dominating protein synthesis profiles. Certain HSPs are also induced in response to low temperatures, and recent data suggest that they are involved in cold acclimation.

Key words: cold acclimation, heat stress, dormancy, HSP, ABA.

Resumen

Respuestas moleculares de las plantas leñosas al estrés térmico

El estrés que producen las altas y las bajas temperaturas ha sido menos estudiado en las plantas leñosas que en las herbáceas. La respuesta al frío en las especies leñosas presenta características propias. La aclimatación al frío asociada a la dormancia invernal se desarrolla en dos etapas secuenciales: la primera es inducida por el acortamiento del fotoperiodo, y la segunda requiere una disminución de las temperaturas. Se ha comprobado una implicación del ABA en este proceso, sin embargo su papel es menos claro que el que tiene en la aclimatación de las plantas herbáceas. Entre las respuestas moleculares de los árboles al frío se deben destacar la inducción de proteínas protectoras, los cambios en el metabolismo de los glúcidos y la alteración en el funcionamiento del reloj circadiano. El comportamiento de las plantas leñosas frente al calor no parece tener peculiaridades específicas, siendo la síntesis de las proteínas de choque térmico (HSPs) la respuesta más significativa a nivel molecular. Algunas HSPs también se inducen en respuesta a las bajas temperaturas, y datos recientes sugieren que pueden estar implicadas en la aclimatación al frío.

Palabras clave: aclimatación al frío, choque de calor, dormancia, HSP, ABA.

Introduction

As sessile organisms, plants are necessarily exposed to changing environmental conditions, often unfavorable. This situation has led them to develop evolutive strategies to recognize different environmental stresses and activate appropriate responses. Although productivity of world ecosystems is probably limited more by water availability than by any other environmental factor, temperature is most limiting to plant distribution. In fact, global vegetation patterns are strongly related to temperature zones.

Studies on plant thermal stress have been mainly undertaken in herbaceous species. The effects of cold and heat stresses have been much less studied in woody species, although these plants must survive for decades in an environment in which temperatures fluctuate drastically with the change of seasons.

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Cold acclimation in woody plants

Many plants adapted to temperate and cold climates, when exposed to low temperatures (LT) (0-10 °C) develop physiological and biochemical responses which increase their freezing tolerance. This process is known as cold acclimation (Levitt, 1980). In annual herbaceous species ample knowledge has been acquired about the nature of the genes and the mechanisms responsible for freezing tolerance as well as sensing and regulatory mechanisms that activate the cold acclimation-response (Thomashow, 1999; Iba, 2002; Salinas, 2002; Shinozaki *et al.*, 2003; Yamaguchi-Shinozaki and Shinozaki, 2005). Much less research has been carried out concerning this process in woody plants, despite their adaptation to two different types of cold acclimation: acclimation to temperature fluctuations during the growing season, and the seasonal acclimation for overwintering (Li *et al.*, 2004). Forest species in cold and temperate climates are regularly exposed to freezing temperatures during winter months. Their ability to survive is based on adaptative mechanisms by which plants enter a state of dormancy and develop freezing tolerance. The onset of winter deep dormancy (endodormancy) is preceded by a stage of ecodormancy. Endodormancy is caused by plant endogenous factors and, once established, no growth can be achieved until a chilling requirement has been satisfied. In order for bud break to occur, plants need to be exposed to LT for a cumulative number of hours (chilling requirement). In contrast, during ecodormancy, growth is arrested by adverse environment and resumes when conditions become favourable (Lang, 1987; Howe *et al.*, 1999). The onset of endodormancy is one of the most frequently studied photoperiodic phenomena. Some important endodormancy-related traits such as growth cessation, bud set and the initial stages of cold acclimation, can be induced in many tree species by a short day photoperiod (SD) (Wareing, 1956; Thomas and Vince-Prue, 1997). Other changes, including leaf senescence and abscission, are not induced by SD alone but require exposure to LT (Weiser, 1970; Arora *et al.,* 2003).These stages coincide with sequential phases of cold acclimation of which the first is initiated by SD and the second by LT and freezing temperatures (Weiser, 1970). When fully hardened, the trees can tolerate exposure to extreme temperatures of –50 to –100 °C. There appears to be a third stage of acclimation in hardy woody species which is induced by low temperatures (–30 to –50 °C), that may not commonly be achieved in nature (Weiser, 1970). Thus, dormancy in a woody plant is superimposed on a seasonal development of cold hardiness. This makes it difficult to distinguish physiological and molecular changes associated with dormancy regulation from those underlying the seasonality of cold hardiness (Rohde *et al.*, 2000; Arora *et al.*, 2003).

Cold acclimation induction by SD and LT

Photoperiod is the most important environmental cue controlling the onset of dormancy in perennial plants. By responding to SD, plants are able to synchronize cold acclimation and dormancy induction with the end of the growing season and the onset of low temperatures in the fall. Because the length of the growing season varies latitudinally, photoperiodic responses often differ between northern and southern populations of the same species. In the northern hemisphere, trees from southern locations usually require shorter days to induce bud set than do northern trees. Differences have also been observed in the critical photoperiod length (the longest photoperiod inducing growth cessation) between ecotypes of different elevations. This behavior has been described in different forest species of several genera, such as *Betula, Picea, Populus o Salix* (Heide, 1974; Junttila, 1982; Junttila and Kaurin, 1990; Howe *et al.,* 1995; Qamaruddin *et al.,* 1995; Li *et al.*, 2002 and 2003a). Bud phenology is often found to be under strong genetic control (Bradshaw and Stettler, 1995). Quantitative trait locus (QTL) mapping experiments have shown that three major genes may be involved in the control of bud set in *Populus.* Two of these map respectively next to *PHYB1* gene*,* involved in photoperception of the photoperiod, and to *ABI1B* gene, related to the activity of abscisic acid (ABA) (Frewen *et al.*, 2000). However, other QTL analyses suggest that environmental factors other than day-length significantly influence genetic differences in the timing of bud set in the field (Howe *et al.*, 1999; Howe *et al.*, 2000).

Temperatures within normal growth range do not change the critical photoperiods for woody plant

dormancy induction significantly, whereas near-freezing night-time conditions may induce growth cessation even under continuous light (Heide, 1974). An important exception to the widely demonstrated SD control of dormancy has been reported for apple and some other genera of the Roseceae family, in which growth is unaffected by photoperiod (Wareing, 1956). It was thought that in these cases dormancy was controlled entirely by endogenous factors (Wareing, 1956); however, it has been recently proven that it is induced consistently by low temperatures and independently from the photoperiodic *régime* (Heide and Prestrud, 2005).

The basic molecular mechanisms of cold acclimation induction by SD in trees is not well understood. Phytochrome involvement in this process has been known for a long time (Williams *et al.*, 1972). More recent studies have strengthened the central role of phytochrome A in day-length sensing of woody plants. Overexpression of oat phytochrome A photoreceptor in hybrid aspen prevents cold acclimation in response to SD and significantly changes the critical photoperiod (Olsen *et al.*, 1997). In contrast, exposure to LT resulted in cold acclimation of transgenic plants to a degree comparable with wild type (Welling *et al.*, 2002). These results suggest an independent activation of cold acclimation by LT and SD in hybrid aspen. We should not forget, on the other hand, that woody plants can acclimate to the cold in response to the LT when they grow under conditions of long photoperiod, probably in a similar way to herbaceous plants (Welling *et al.*, 2002; Li *et al.*, 2002; Li *et al.*, 2004).

ABA in cold acclimation

ABA regulates diverse physiological and developmental processes in plants, including seed dormancy and germination, as well as mediating cellular adaptation to stresses such as freezing and drought (Giraudat *et al.*, 1994; Bewley, 1997; Thomashow, 1999; Shinozaki *et al.*, 2003; Yamaguchi-Shinozaki and Shinozaki, 2005). ABA has been shown to be involved in promoting cold hardiness, both in exogenously applied ABA to plants and cell cultures, and by measurement of endogenous ABA concentrations (Chen *et al.*, 1983; Lang *et al.*, 1989; Li *et al.*, 2004). In addition, Arabidopsis seedlings carrying either *aba1* or *abi1* mutations that, respectively, result in impairment of ABA synthesis and insensitivity to ABA, are less freezing tolerant than wild type plants (Heino *et al.*, 1990; Gilmour and Thomashow, 1991; Mäntylä *et al.*, 1995).

ABA role in woody plant cold acclimation is less clear. ABA levels in some woody plants increase under SD conditions and, therefore, it may be involved in signal transduction in cold acclimation (Odén and Dunberg, 1984; Qamaruddin *et al.*, 1993; Rinne *et al.*, 1994; Welling *et al.*, 1997). However, in other studies, endogenous ABA level was found to be unaffected by SD (Lenton *et al.*, 1972; Johansen *et al.*, 1986), and attempts to induce dormancy by exogenous ABA application has been unsuccessful (Welling *et al.*, 1997; Li *et al.*, 2003a and 2003b). Rinne *et al.* (1998) compared the behaviour of *Betula pubescens* and *Betula pubescens* f. *hibernifolia,* an ABA-deficient genotype. Wild type expressed elevated ABA levels before the onset of cold acclimation under SD, by contrast, the mutant type had reduced tolerance to LT. Nevertheless, SD conditions were still able to induce dormancy in ABA-deficient birch (Rinne *et al.*, 1998). These results indicate that involvement of ABA is more direct in the photoperiodic control of cold acclimation than in the induction of endodormancy (Arora *et al.*, 2003).

The function of PtABI3, the ABI3 homolog from poplar, in bud development and dormancy has also been investigated (Rohde *et al.*, 1998; Rohde *et al.*, 2002). Although the *abi3* mutant was isolated originally for its insensitivity to ABA during seed germination, ABI3 may have broader functions than ABA signaling (Koornneef *et al.*, 1984; Leung and Giraudat, 1998). The fact that ABI3 functions in vegetative Arabidopsis meristems, particularly during periods of growth-arresting conditions and quiescence (Rohde *et al.*, 1999), suggest to Rohde *et al.* (2002) that ABI3 could be involved in the regulation of bud dormancy in trees. *PtABI3* is expressed in buds during natural bud set, and expression occurs after perception of critical photoperiod. In SD, poplar trees transformed with sense and anti-sense constructs of *PtABI3* showed altered bud morphology (Rohde *et al.*, 2002). ABA levels peaked in buds concomitantly with *PtABI3* expression, suggesting that ABA and PtABI3 act simultaneously in bud set (Rohde *et al.*, 2002).

Molecular responses to low temperature

Molecular mechanisms by which trees respond to cold stress remain poorly understood despite their biological and practical importance (Rohde *et al.*, 2000; Kozlowski and Pallardy, 2002; Arora *et al.*, 2003). By far, more cold hardiness research has been carried out on herbaceous annuals like Arabidopsis than on woody perennials. However, two aspects of tree response to low temperature must be underlined: the induction of protective proteins and the change in carbohydrate metabolism.

Dehydrins (DHNs) are plant proteins belonging to the group 11 of late embryogenesis-abundant (LEA) proteins that are believed to play a protective role during cellular dehydration (Close, 1997; Campbell and Close, 1997). It has been suggested that DHNs stabilize membranes and rescue hydrolytic enzyme function under dehydrative conditions (Danyluk *et al.*, 1998; Rinne *et al.*, 1999). Cryoprotective and antifreeze activity has also been reported for a peach DHN (Wisniewski *et al.*, 1999), and Puhakainen *et al.* (2004) have shown that overexpression of multiple dehydrin genes enhances tolerance in freezing stress in Arabidopsis. Woody plants accumulate DHN during periods of cold acclimation in leaves, buds and bark, and the presence of these proteins is correlated with increased freeze tolerance (Arora and Wisniewski, 1994; Muthalif and Rowland, 1994; Cai *et al.*, 1995; Welling *et al.*, 1997; Rinne *et al.*, 1999; Núñez, 2003; Karlson *et al.*, 2003). In addition, seasonal dehydrin fluctuations have been observed in the xylem of multiple woody species. Different groups have carried out a series of experiments in an attempt to separate cold acclimation/deacclimation and dormancy transitions and evaluate changes in dehydrin levels in association with each phenomenon separately. Altogether, the results obtained indicate that different genes of DNH family behave differently, the response of the majority would be more closely associated with cold acclimation, whereas that of some others would be with dormancy status (Wisniewski *et al.*, 1996; Artlip *et al.*, 1997; Rinne *et al.*, 1998; Sauter *et al.*, 1999; Welling *et al.*, 2002; Karlson *et al.*, 2003; Welling *et al.*, 2004).

Members of another group of proteins called heat shock proteins (HSP) have been identified in herbaceous plants as being responsive to LT stress. The association of HSP, especially belonging to small HSP (sHSP) family, with cold acclimation (see below) and endodormancy (Wisniewski *et al.*, 1996; Plá *et al.*, 1998; Ukaji *et al.*, 1999; Lubaretz and zur Nieden, 2002; López-Matas *et al.*, 2004) has also been shown in some tree species.

Changes in tree carbohydrate metabolism in response to SD or LT, or during endodormancy has been observed (Sauter, 1988; Witt and Sauter, 1995; Renaut *et al.*, 2004). With the onset of autumn, starch is broken down while at the same time there is a build-up of oligosaccharides, predominantly sucrose, trehalose, raffinose and stachyose. The rise in sucrose levels occurs in direct response to SD, while raffinose and stachyose levels rise later in autumn, in response to temperature drop (Hinesley *et al.*, 1992; Ashworth *et al.*, 1993; Cox and Stushnoff, 2001). Under chilling conditions, sucrose, and trehalose accumulated rapidly, while raffinose content increased after one week at 4 °C (Renaut *et al.*, 2004). The protecting role suggested for these oligosaccharides in cold acclimation of herbaceous plants (Thomashow, 1999), can be important in woody plants. Indeed, sucrose and trehalose stabilize proteins and membranes during freezing (Leslie *et al.*, 1995; Oliver *et al.*, 1998), and the accumulation of raffinose may supplement sucrose in membrane stabilisation and might help to prevent sucrose crystallisation during glassy state (Caffrey *et al.*, 1988).

Cold acclimation and circadian clock

Recently, circadian clock performance during winter dormancy has been investigated in chestnut, using *CsTOC1* and *CsLHY* as marker genes, which are homologous to essential components of the central circadian oscillator in Arabidopsis (Ramos *et al.*, 2005). During vegetative growth, mRNA levels of these two genes in chestnut seedlings and adult plants cycled daily, as expected. However, during winter dormancy, *CsTOC1* and *CsLHY* mRNA levels were high and did not oscillate indicating that the circadian clock was altered. A similar disruption was induced by chilling (4°C) of chestnut seedlings. Normal cycling resumed when endodormant or cold-treated plants returned to 22°C (Ramos *et al.*, 2005). This behavior indicated that clock disruption is not intrinsic to the endodormancy state, but is a direct consequence of the response to cold. The effects of low temperature on Arabidopsis circadian oscillator's components have not been investigated, although mRNA levels

corresponding to genes under circadian control cycled as usually in seedlings grown for 5 days at 4°C (Kreps and Simon, 1997). The different behavior of Arabidopsis and chestnut circadian rhythms under cold temperatures indicates that cold acclimation in temperate woody plants may have significant specific features compared to annual herbaceous plants. Expression of at least 10% of Arabidopsis genes is under circadian clock control. The circadian clock is involved in the coordination and proper functioning of major metabolic pathways, as well as in the control of developmental processes (Harmer *et al.,* 2000; Schaffer *et al.,* 2001). Therefore, clock disruption observed in chestnut in response to LT could control physiological changes that take place specifically in woody perennials during cold acclimation.

The acclimation of woody plants to heat stress

Owing to its deleterious effects on plant growth and development, high temperature stress causes considerable annual losses in plant productivity. Furthermore, global warming will likely aggravate its impact on agriculture and forestry in forthcoming years (Walther *et al.,* 2002). In spite of substantial effort, breeding crops for higher thermotolerance has been much less successful than breeding for other agronomically relevant traits (Khush, 2001). Like other organisms, plants have evolved different mechanisms to withstand heat stress. These include a wide variety of long-term evolutionary adaptations affecting their morphology and ecophysiology, as well as shorter term acclimation mechanisms such as transpirational cooling, changes in leaf orientation, or alterations in membrane lipid composition (Larkindale *et al.,* 2005a).

Besides their inherent ability to withstand supra-optimal temperatures for short periods of time (basal thermotolerance), plants are able to become rapidly tolerant to otherwise lethal high temperatures (acquired thermotolerance). This reponse is triggered by prior exposure to a conditioning pretreatment, which can be a short, sublethal high temperature, a gradual temperature increase, or other moderate stress treatment (Boston *et al.,* 1996). As an example, it was shown forty years ago that exposure of European beech to 55°C resulted in elevated resistance to heat events (Wagenbreth, 1965). The adaptative value of acquired thermotolerance is clear: even plants growing in their natural habitat may experience diurnal heat conditions that would be lethal in the absence of a rapid protective response.

Heat-induced damage at the cellular level

Different lines of evidence indicate heat stress has a complex influence on cell function and homeostasis, suggesting that thermotolerance involves many processes. Some of them may be specific to either basal or acquired thermotolerance, but many will probably be involved in both. A recent survey of heat stress phenotypes of several Arabidopsis mutants showed that the acquisition of thermotolerance relies on multiple signaling pathways (Larkindale *et al.,* 2005b). High temperatures are known to affect plant membrane-associated processes as a consequence of alterations in membrane fluidity (e.g., Sangwan *et al.,* 2002; Iba, 2002). Protein stability and function are also affected by changes in temperature. Heat-induced alterations in enzyme activity, or even enzyme inactivation, translate into metabolic imbalance (Kampinga *et al.,* 1995; Boston *et al.,* 1996). Moreover, membrane and protein damage trigger the production of reactive oxygen species, which lead in turn to heat-induced oxidative stress (Larkindale and Knight, 2002; Larkindale *et al.,* 2005a,b). Heat stress has also been shown to activate programmed cell death (Swidzinski *et al.,* 2002). In plants, these types of damage cause reduced photosynthesis and carbon assimilation, impaired sugar translocation, and general cell misfunction, leading to altered growth, development and reproduction.

Heat stress proteins

Although the heat stress response has been described and studied in plants for decades, only a limited number of genes and proteins have been identified so far that contribute significantly to the development of thermotolerance. Many studies have shown that during heat stress plants induce massive transcription and translation of heat shock proteins (HSPs; for review, see Boston *et al.,* 1996). HSPs can be assigned to five families of proteins conserved among bacteria, plants and animals, based on sequence similarity and differences in molecular weight: HSP100, HSP90,

HSP70, HSP60, and small HSPs (sHSPs or low molecular weight HSPs). All major classes of HSPs are thought to act as molecular chaperones (see below) and a general protective role against heat-induced damage has been proposed for them. Nonetheless, stress conditions other than elevated temperatures can lead to HSP induction. These include osmotic and salt stress, stress from low oxygen, toxic compounds, dinitrophenol, UV radiation, and treatment with plant hormones such as abscisic acid or ethylene (Boston *et al.,* 1996; Iba, 2002).

In contrast to other eukaryotes, the most prominent heat-induced proteins of plants are the sHSPs, a structurally diverse family of polypeptides with sizes ranging from 15 to 30 kD. These proteins are encoded in higher plants by at least six multigene families and have been localized to the cytoplasm, ER, mitochondria, and chloroplast (van Monfort *et al.,* 2001). Considerably fewer sHSPs have been identified in animals, yeast, and prokaryotes, where the most important HSPs belong to the HSP60, HSP70, HSP90 and HSP110 families. In plants, only the HSP100 family has been shown to be essential for the acquisition of thermotolerance by genetic analysis (Hong and Vierling, 2000, 2001). Genetic data also indicate that different genes contribute to heat tolerance at different stages of plant life cycle, and that different genes may be essential for basal and acquired thermotolerance (Clarke *et al.,* 2004).

HSP gene expression is regulated mainly at the transcriptional level. Heat shock transcription factors, or HSFs, are regulatory proteins that exist as inactive proteins mostly found in the cytoplasm. Upon heat stress, HSFs become active through oligomerization and recompartmentation to the nucleus, where they bind to target sequences (heat shock elements, HSEs) present in the promoter region of HSP genes (Nover and Scharf, 1997). A peculiarity of plants is the unique complexity of the HSF family, with more than 20 members and the existence of heat stress-induced forms of HSFs which may play a major part in modulating transcription during long-term heat stress response (Nover *et al.,* 2001).

Molecular chaperones and stress tolerance

Many experiments have shown that plant HSPs have molecular chaperone activity (reviewed by Boston *et al.,* 1996). In woody plants such role was first demonstrated for a purified *Castanea sativa* (European chestnut) sHSP by Collada et al. (1997). Afterwards, Soto et al. (1999) found that overexpression of a chestnut protein in bacterial cells enhanced their tolerance to both high and low temperatures. Direct evidence for a chaperone function in cellular thermotolerance has been recently obtained for a cyanobacterial sHSP (Giese and Vierling, 2002). Other studies have reported that some HSPs have stabilizing effects on model membranes, suggesting a role in preserving membrane integrity during thermal fluctuations (e.g., Tsvetkova *et al.,* 2002). In any case, all HSPs analyzed are molecular chaperones that assist in folding, intracellular distribution, assembly and degradation of proteins, mainly by stabilizing partially unfolded states. The sHSPs have a high capacity to bind non-native proteins in an ATP-independent fashion, and current models propose that they act during heat stress by preventing irreversible protein aggregation (reviewed by van Montfort *et al.,* 2001). sHSP-bound proteins are subsequently refolded by ATP-dependent chaperones of the DnaK/HSP70 system (Lee *et al.,* 1997; Lee and Vierling, 2000).

An interesting outcome of studying sHSPs in trees is the recent finding that, besides protecting against high temperature stress, these proteins may be involved in cold acclimation (López-Matas *et al.,* 2004). Among proteins induced or up-regulated in plants by low temperatures there are high molecular weight HSPs (e.g., Sung *et al.,* 2001) and also sHSPs (Soto et al. 1999; Ukaji *et al.,* 1999). So far, woody species in which cold-specific accumulation of HSPs has been observed include *Prunus persica* (Wisniewski *et al.,* 1996), *Castanea sativa* (Soto *et al.,* 1999; López-Matas *et al.,* 2004). *Morus bombycis* (Ukaji *et al.,* 1999), *Acer platanoides, Sambucus nigra* (Lubaretz and zur Nieden, 2002), and *Cedrus atlantica* (authors' unpublished data). Chestnut sHSP is the first member of this ubiquitous protein family for which cryoprotective activity has been demonstrated (López-Matas *et al.,* 2004).

Future perspectives

It is now clear that woody plants alter significantly their gene activity profiles in response to environmental changes. Understanding these alterations is of capital

importance to improve or engineer forest tree tolerance to relevant environmental stresses, such as the ones reviewed here. Whereas a number of technical difficulties have hampered the study of trees at the molecular level for many years, things are changing rapidly with the implementation of DNA microarray techniques and high-throughput protein analysis. These tools have allowed rapid progress in our understanding of different aspects of tree biology such as wood formation (Schrader *et al.,* 2004b), tree leaf senescence (Andersson *et al.*, 2004), root development (Kohler *et al.*, 2003) or the abiotic stress response (Nanjo *et al.*, 2004; Renaut *et al.*, 2004; Schrader *et al.*, 2004a), to cite some examples. Arguably, *Pinus* and *Populus* (a draft of the complete genome of *P. trichocarpa* is available at http://genome.jgi-psf.org/Poptr1/Poptr1.home.html) will continue attracting much attention from tree biologists in the near future.

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References

ANDERSSON A., KESKITALO J., SJODIN A., BHALERAO R., STERKY F., WISSEL K., TANDRE K., ASPEBORG H., MOYLE R., OHMIYA Y., BHALERAO R., BRUNNER A., GUSTAFSSON P., KARLSSON J., LUNDEBERG J., NILSSON O., SANDBERG G., STRAUSS S., SUNDBERG B., UHLEN M., JANSSON S., NILSSON P., 2004. A transcriptional timetable of autumn senescence. Genome Biol 5, R24.

ARORA R., ROWLAND L. J., TANINO K., 2003. Induction and release of bud dormancy in woody perennials: a science come of age. HortScience 38, 911-921.

ARORA R., WISNIEWSKI M. E.., 1994. Cold acclimation in genetically related (sibling) deciduous and evergreen peach *(Prunus persica* [L.] Batsch.). Plant Physiol 105, 95-101.

ARTLIP T. S., CALLAHAN A. M., BASSETT C. L., WISNIEWSKI M. E., 1997. Seasonal expression of dehydrin gene in sibling deciduous and evergreen peach *(Prunus persica* [L.] Batsch.). Plant Mol Biol 33, 61-70.

ASHWORTH E. N., STIRM V. E., VOLENEC J. J., 1993. Seasonal variations in soluble sugars and starch within woody stems of *Cornus sericea* L. Tree Physiol 13, 379-388.

BEWLEY J. D., 1997. Seed germination and dormancy. Plant Cell 9, 1055-1066.

BOSTON R. S., VIITANEN P. V., VIERLING E., 1996. Molecular chaperones and protein folding in plants. Plant Mol Biol 32, 191-222.

BRADSHAW H. D., STETTLER R. F., 1995. Molecular genetics of growth and development in Populus. IV Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. Genetics 139, 963-973.

CAFFREY M., FONSECA V., LEOPOLD A. C., 1988. Lipid-sugar interactions: relevance of anhydrous biology. Plant Physiol 86, 754-758.

CAI Q., MOORE G. A., GUY C. L., 1995. An unusual group 2 LEA gene family in citrus responsive to low temperature. Plant Mol Biol 29, 11-23.

CAMPBELL S. A., CLOSE T. J., 1997. Dehydrins: genes, proteins, and associations with phenotypic traits. New Phytol 137, 61-74.

CHEN H. H., LI P. H., BRENNER M. L., 1983. Involvement of abscisic acid in potato cold acclimation. Plant Physiol 71, 362-365.

CLARKE S. M., MUR L. A., WOOD J. E., SCOTT I.M., 2004. Salicylic acid dependent signaling promotes basal thermotolerance but is not essential for acquired thermotolerance in *Arabidopsis thaliana.* Plant J 38, 432-447.

CLOSE T. J., 1997. Dehydrins: a commonality in the response of plants to dehydration and low temperature. Physiol Plant 100, 291-296.

COLLADA C., GOMEZ L., CASADO R., ARAGONCILLO C., 1997. Purification and in vitro chaperone activity of a class I small heat-shock protein abundant in recalcitrant chestnut seeds. Plant Physiol 115, 71-77.

COX S. E., STUSHNOFF C., 2001. Temperature related shifts in soluble carbohydrate content during dormancy and cold acclimation in *Populus tremuloides.* Can J For Res 31, 730-737.

DANYLUK J., PERRON A., HOUDE M., LIMIN A., FOWLER B., BENHAMOU N., SARHAN F., 1998. Accumulation of an acidic dehydrin in the vicinity of the plasma membrane during cold acclimation of wheat. Plant Cell 10, 623-638.

FREWEN B. E., CHEN T. H., HOWE G. T., DAVIS J., ROHDE A., BOERJAN W., BRADSHAW H. D., 2000. Quantitative trait loci and candidate gene mapping of bud set and bud flush in Populus. Genetics 154, 837-845.

GIESE K. C., VIERLING E., 2002. Changes in oligomerization are essential for the chaperone activity of a small heat shock protein in vivo and in vitro. J Biol Chem 277, 46310-46318.

GILMOUR S. J., THOMASHOW M. F., 1991. Cold acclimation and cold regulated gene expression in ABA mutants of *Arabidopsis thaliana.* Plant Mol Biol 17, 1233-1240.

GIRAUDAT J., PARCY F., BERTAUCHE M., GOSTI F., LEUNG J., MORRIS P. C., BOUVIER-DURAND M., VARTANIAN N., 1994. Current advances in abscisic acid signaling. Plant Mol Biol 26, 1557-1577.

HARMER S. L., HOGENESCH J. B., STRAUME M., CHANG H. S., HAN B., ZHU T., WANG X., KREPS J. A., KAY S. A., 2000. Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. Science 290, 2110-2113.

HEIDE O. M., 1974. Growth and dormancy in Norway spruce ecotypes *(Picea abies)*. I. Interaction of photoperiod and temperature. Physiol Plant 30, 1-12.

HEIDE O. M., PRESTRUD A. K., 2005. Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. Tree Physiol 25, 109-114.

HEINO P., SANDMAN G., LANG V., NORDIN K., PALVA E. T., 1990. Abscisic acid deficiency prevents development of freezing tolerance in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 79, 801-806.

HINESLEY L. E., PHARR D. M., SNELLING L. K., FUNDERBURK S. R., 1992. Foliar raffinose and sucrose in four conifer species: relationship to seasonal temperature. J Am Soc Hortic Sci 117, 852-855.

HONG S. W., VIERLING E., 2000. Mutants of *Arabidopsis thaliana* defective in the acquisition of tolerance to high temperature stress. Proc Natl Acad Sci USA 97, 4392-4397.

HONG S. W., VIERLING E., 2001. Hsp101 is necessary for heat tolerance but dispensable for development and germination in the absence of stress. Plant J 27, 25-35.

HOWE, G. T., DAVIS J., JEKNIC Z., CHEN T. H. H., FREWEN B., BRADSHAW H. D., SARUUL P., 1999. Physiological and genetic approaches to studying endodormancy-related traits in *Populus.* HortScience 34, 1174-1184.

HOWE G. T., HACKETT W. P., FURNIER G. R., KLEVORN R.E., 1995. Photoperiodic responses of a northern and southern ecotype of black cottonwood. Physiol Plant 93, 695-709.

HOWE G. T., SARUUL P., DAVIS J., CHEN T. H. H., 2000. Quantitative genetics of bud phenology, frost damage, and winter survival in an F-2 family of hybrid poplars. Theor Appl Genet 101, 632-642.

IBA K., 2002. Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. Annu Rev Plant Biol 53, 225-245.

JOHANSEN L. G., ODEN P. C., JUNTTILA O., 1986. Abscisic acid and cessation of apical growth in *Salix pentandra.* Physiol Plant 66, 409-412.

JUNTTILA, O., 1982. The cessation of apical growth in latitudinal ecotypes and ecotypes crosses of *Salix pentandra* L. J Exp Bot 33, 1021-1029.

JUNTTILA O., KAURIN A., 1990. Environmental control of cold acclimation in *Salix pentandra.* Scand J Forest Res 5, 195-204.

KAMPINGA H. H., BRUNSTING J. F., STEGE G. J., BURGMAN P. W., KONINGS A.W., 1995. Thermal protein denaturation and protein aggregation in cells made thermotolerant by various chemicals: role of heat shock proteins. Exp Cell Res 219, 536-546.

KARLSON D. T., ZENG Y., STIRM V. E., JOLY R. J., ASHWORTH E. N., 2003. Photoperiodic regulation of a 24-kD dehydrin-like protein in red-osier dogwood *(Cornus sericea* L.) in relation to freeze-tolerance. Plant Cell Physiol 44, 25-34.

KHUSH G. S., 2001. Green revolution: the way forward. Nat Rev Genet 2, 815-822.

KOHLER A., DELARUELLE C., MARTIN D., ENCELOT N., MARTIN F., 2003. The poplar root transcriptome: analysis of 7000 expressed sequence tags. FEBS Lett 542, 37-41.

KOORNNEEF M., REULING G., KARSSEN C. M., 1984. The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana.* Physiol Plant 61, 377-383.

KOZLOWSKI T. T., PALLARDY, S. G., 2002. Acclimation and adaptive responses of woody plants to environmental stresses. Bot Rev 68, 270-334.

KREPS J. A., SIMON A. E., 1997. Environmental and genetic effects on circadian clock-regulated gene expression in Arabidopsis. Plant Cell 9, 297-304.

LANG G. A., 1987. Dormancy: a new universal terminology. HortScience 22, 817-820.

LANG V., HEINO P., PALVA E. T., 1989. Low temperature acclimation and treatment with exogenous abscisic acid induce common polypeptides in *Arabidopsis thaliana* (l.) Heinh. Theor Appl Genet 77, 729-734.

LARKINDALE J., KNIGHT M. R., 2002. Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol 128, 682-695.

LARKINDALE J., MISHKIND M., VIERLING E., 2005a. Plant responses to high temperature. In MA Jenks, PM Hasegawa, eds, Plant Abiotic Stress. Blackwell Scientific Publications, Oxford (in press).

LARKINDALE J., HALL J. D., KNIGHT M. R., IERLING E., 2005b. Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiol 138, 882-897.

LEE G. J., ROSEMAN A. M., SAIBIL H. R., VIERLING E., 1997. A small heat-shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J 16, 659-671.

LEE G. J., VIERLING E., 2000. A small heat-shock protein cooperates with heat shock protein 70 systems to reactivate a heat-denatured protein. Plant Physiol 122, 189-198.

LENTON J. R., PERRY V. M., SAUNDERS P. F., 1972. Endogenous abscisic acid in relation to photoperiodically induced bud dormancy. Planta 106, 13-22.

LESLIE S. B., ISRAELI E., LIGHTHART B., CROWE J. H., CROWE L. M., 1995. Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. Appl Environ Microb 61, 3592-3597.

LEUNG J., GIRAUDAT J., 1998. Abscisic acid signal transduction. Annu Rev Plant Physiol Plant Mol Biol 49, 199-222.

LEVITT J., 1980. Responses of plants to environmental stresses. New York: Academic Press.

LI C., JUNTTILA O., ERNSTSEN A., HEINO P., PALVA, E. T., 2003a. Photoperiodic control of growth, cold acclimation and dormancy development in silver birch *(Betula pendula)* ecotypes. Physiol Plant 117, 206-212.

LI C., JUNTTILA O., HEINO P., PALVA, E. T., 2003b. Different responses of northern and southern ecotypes of *Betula pendulan* to exogenous ABA application. Tree Physiol 23, 481-487.

LI C., JUNTTILA O., HEINO P., PALVA E. T., 2004. Low temperature sensing in silver birch *(Betula pendula* Roth) ecotypes. Plant Sci 167, 165-171.

LI C., PUHAKAINEN T., WELLING A., VIHERÄ-AARNIO A., ERNSTSEN A., JUNTTILA O., HEINO P., PALVA, E. T., 2002. Cold acclimation in silver birch *(Betula pendula)*. Development of freezing tolerance in different tissues and climatic ecotypes. Physiol Plant 116, 478-488.

LÓPEZ-MATAS M. A., NUÑEZ P., SOTO A., ALLONA I., CASADO R., COLLADA C., GUEVARA M. A., ARAGONCILLO C., GOMEZ L., 2004. Protein cryoprotective activity of a cytosolic small heat shock protein that accumulates constitutively in chestnut stems and is up-regulated by low and high temperatures. Plant Physiol 134, 1708-1717.

LUBARETZ O., ZUR NIEDEN U., 2002. Accumulation of plant small heat-stress proteins in storage organs. Planta 215, 220-228.

MÄNTYLÄ E., LANG V., PALVA E. T., 1995. Role of abscisic acid in drought-induced freezing tolerance, cold acclimation and accumulation of LTI18 and RAB18 proteins in *Arabidopsis thaliana.* Plant Physiol 107, 141-148.

MUTHALIF M. M., ROWLAND L. J., 1994. Identification of dehydrin-like proteins responsible to chilling in floral buds of blueberry *(Vaccinium,* section *Cyanococcus)*. Plant Physiol 104, 1439-1447.

NANJO T., FUTAMURA N., NISHIGUCHI M., IGASAKI T., SHINOZAKI K., SHINOHARA K., 2004. Characterization of full-length enriched expressed sequence tags of stress-treated poplar leaves. Plant Cell Physiol 45, 1738-1748.

NOVER L., BHARTI K., DÖRING P., MISHRA S. K., GANGULI A., SCHARF K. D., 2001. Arabidopsis and the Hsf world: how many heat stress transcription factors do we need?. Cell Stress Chaperon 6, 177-189.

NOVER L., SCHARF K. D., 1997 Heat stress proteins and transcription factors. Cell Mol Life Sci 53, 80-103.

NÚÑEZ P., 2003. Caracterización genética y bioquímica de deshidrinas: respuesta molecular de las especies arbóreas frente al estrés abiótico. PhD Thesis. Universidad Politécnica de Madrid, 147 pp.

ODÉN P. C., DUNBERG A., 1984. Abscisic acid in shoots and roots of Scots pine *(Pinus sylvestris* L.) seedlings grown in controlled long-day and short-day environments. Planta 161, 148-155.

OLIVER A. E., CROWE L. M., CROWE J. H., 1998. Methods for dehydration-tolerance: Depression of the phase transition temperature in dry membranes and carbohydrate vitrification. Seed Sci Res 8, 211-222.

OLSEN J. E., JUNTTILA O., NILSEN J., ERIKSSON M. E., MARTINUSSEN I., OLSSON O., SANDBERG G., MORITZ T., 1997. Ectopic expression of oat phytocrome A in hybrid aspen changes critical daylength for growth and prevents cold acclimatization. Plant J 12, 1339-1350.

PLA, M., HUGUET,, G., VERDAGUER D., PUIGDERRAJOLS P., LLOMPART B., NADAL A., MOLINAS M., 1998. Stress proteins co-expressed in suberized and lignified cells and in apical meristems. Plant Sci 139, 49-57.

PUHAKAINEN T., HESS M. W., MAKELA P., SVENSSON J., HEINO P., PALVA E. T., 2004. Overxpression of multiple dehydrin genes enhances tolerance to freezing stress in Arabidopsis. Plant Mol Biol 54, 743-753.

QAMARUDDIN M., DORMLING I., EKBERG I., ERIKSSON G., TILLBERG E., 1993. Abscisic acid content at defined levels of bud dormancy and frost tolerance in two contrasting populations of *Picea abies* grown in a phytotron. Physiol Plant 87, 203-210.

QAMARUDDIN M., EKBERG I., DORMLING I., NORELL L., CLAPHAM D., ERIKSSON G., 1995. Early effects on long nights on budset, bud dormancy and abscisic acid content in two populations of *Picea abies.* Forest Genet 2, 207-216.

RAMOS A., PÉREZ-SOLÍS E., IBÁÑEZ C., CASADO R., COLLADA C., GÓMEZ L., ARAGONCILLO C., ALLONA I., 2005. Winter disruption of the circadian clock in chestnut. Proc Natl Acad Sci USA 102, 7037-7042.

RENAUT J., LUTTS S., HOFFMANN L., HAUSMAN J. F., 2004. Responses of poplar to chilling temperatures: proteomic and physiological aspects. Plant Biol 6, 1-10.

RINNE P. L. H., KAIKURANTA P. L. M., VAN DER PLAS L. H. W., VAN DER SCHOOT C., 1999. Dehydrins in cold-acclimated apices of birch *(Betuna pubescens* Ehrh.): production, localization and potential role in rescuing enzyme function during dehydration. Planta 209, 377-388.

RINNE P., SAARELAINEN A., JUNTTILA O., 1994. Growth cessation and bud dormancy in relation to ABA level in seedlings and coppice shoots of *Betula pubescens* as affected by a short photoperiod, water stress and chilling. Physiol Plant 90, 451-458.

RINNE P., WELLING A., KAIKURANTA P., 1998. Onset of freezing tolerance in birch *(Betula pubescens* Ehrh.) involves LEA proteins and osmoregulation and is impaired in an ABA-deficient genotype. Plant Cell Environ 21, 601-611.

ROHDE A., ARDILES-DIAZ W., VAN MONTAGU M., BOERJAN W., 1998. Isolation and expression analysis of an *ABSCISIC ACID-INSENSITIVE (ABI3)* homologue from *Populus trichocarpa.* J Exp Bot 49, 1059-1060.

ROHDE A., HOWE G. T., OLSEN J. E., MORITZ T., VAN MONTAGU M., JUNTTILA O., BOERJAN W., 2000. Molecular aspects of bud dormancy in trees. In:

Molecular Biology of Woody Plants vol. 1. S. M. Jain and S. C. Minocha, eds. Kluwer Academic Publishers, pp. 89-134.

ROHDE A., PRINSEN E., DE RYCKE R., ENGLER G., VAN MONTAGU M., BOERJAN W., 2002. PtABI3 impinges on the growth and differentiation of embryonic leaves during bud set in poplar. Plant Cell 14, 1885-1901.

ROHDE A., VAN MONTAGU M., BOERJAN W., 1999. The *ABSCISIC ACID_INSENSITIVE 3 (ABI3)* gene is expressed during vegetative quiescence processes in Arabidopsis. Plant Cell Environ 22, 261-270.

SALINAS J., 2002. Molecular mechanisms of signal transduction in cold acclimation. In: Frontiers in Molecular Biology. B. D. Hames and D. M. Glover, eds. Oxford: Oxford University Press, pp. 116-139.

SAUTER J. J., 1988. Temperature-induced changes in starch and sugars in the stem of *Populus* x *canadiensis* <robusta>. J Plant Physiol 132, 608-612.

SAUTER J. J., WESTPHAL S., WISNIEWSKI M., 1999. Immunological identification of dehydrin-related proteins in the wood of five species of *Populus* and in *Salix caprea* l. J Plant Physiol 154, 781-788.

SANGWAN V., ORVAR B. L., BEYERLY J., HIRT H., DHINDSA R. S., 2002. Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways. Plant J 31, 629-638.

SCHAFFER R., LANDGRAF J., ACCERBI M., SIMON V., LARSON M., WISMAN E., 2001. Microarray analysis of diurnal and circadian-regulated genes in Arabidopsis. Plant Cell 13, 113-123.

SCHRADER J., MOYLE R., BHALERAO R., HERTZBERG M., LUNDEBERG J., NILSSON P., BHALERAO R. P., 2004a. Cambial meristem dormancy in trees involves extensive remodelling of the transcriptome. Plant J 40, 173-187.

SCHRADER J., NILSSON J., MELLEROWICZ E., BERGLUND A., NILSSON P., HERTZBERG M., SANDBERG G., 2004b. A high-resolution transcript profile across the wood-forming meristem of poplar identifies potential regulators of cambial stem cell identity Plant Cell 16, 2278-2292.

SHINOZAKI K., YAMAGUCHI-SHINOZAKI K., SEKI M., 2003. Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6, 1-8.

SOTO A., ALLONA I., COLLADA C., GUEVARA M. A., CASADO R., RODRIGUEZ-CEREZO E., ARAGONCILLO C., GOMEZ L., 1999. Heterologous expression of a plant small heat-shock protein enhances *Escherichia coli* viability under heat and cold stress. Plant Physiol 120, 5213-5218.

SUNG D. Y., VIERLING E., GUY C. L., 2001. Comprehensive expression profile analysis of the Arabidopsis Hsp70 gene family. Plant Physiol 126, 789-800.

SWIDZINSKI J. A., SWEETLOVE L. J., LEAVER C. J., 2002. A custom microarray analysis of gene expression during programmed cell death in *Arabidopsis thaliana.* Plant J 30: 431-446.

THOMAS B., VINCE-PRUE D., 1997. Photoperiodism in Plants. 2nd Ed. London: Academic Press.

THOMASHOW M. F., 1999. Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol 50, 571-599.

TSVETKOVA N. M., HORVÄTH I., TÖRÖK Z., WOLKERS W. F., BALOGI Z., SHIGAPOVA N., CROWE L. M., TABLIN F., VIERLING E., CROWE J. H., Vý'GH L., 2002. Small heat shock proteins regulate membrane lipid polymorphism. Proc Natl Acad Sci USA 99, 13504-13509.

UKAJI N., KUWABARA C., TAKEZAWA D., ARAKAWA K., YOSHIDA S., FUJIKAWA S., 1999. Accumulation of small heat-shock protein homologs in the endoplasmic reticulum of cortical parenchyma cells in mulberry in association with seasonal cold acclimation. Plant Physiol 120, 481-489.

VAN MONTFORT R., SLINGSBY C., VIERLING E., 2001. Structure and function of the small heat shock protein/alpha-crystallin family of molecular chaperones. Adv Prot Chem 59, 105-156.

WAGENBRETH D., 1965. Das aufireten von zwei letalstufen bei hitzeeinwirkung auf pappelblätter. Flora 156A, 116-126.

WALTHER G. R., POST E., CONVEY P., MENZEL A., PARMESAN C., BEEBEE T. J. C., FROMENTIN J. M. HOEGH-GULDBERG O., BAIRLEIN F., 2002. Ecological responses to recent climate change. Nature 416, 389-395.

WAREING P. F., 1956. Photoperiodism in woody plants. Annu Rev Plant Physiol 7, 191-214.

WEISER C. J., 1970. Cold resistance and injury in woody plants. Science 169, 1269-1278.

WELLING A., KAIKURANTA P., RINNE P., 1997. Photoperiodic induction of dormancy and freezing tolerance in *Betula pubescens.* Involvement of ABA and dehydrins. Physiol Plant 100, 119-125.

WELLING A., MORITZ T., PALVA E. T., JUNTTILA O., 2002. Independent activation of cold acclimation by low temperature and short photoperiod in hybrid aspen. Plant Physiol 129, 1633-1641.

WELLING A., RINNE P., VIHERÄ-AARNIO A., KONTUNEN-SOPPELA S., HEINO P., PALVA E. T., 2004. Photoperiod and temperature differentially regulate the expression of two dehydrin genes during overwintering of birch *(Betula pubescens* Ehrh.). J Expl Bot 55, 507-516.

WILLIAMS B. J., PELLETT N. E., KLEIN R. M., 1972. Phytochrome control of growth cessation and initiation of cold acclimation in selected woody plants. Plant Physiol 50, 262-265.

WISNIEWSKI M., CLOSE T. J., ARTLIP T., ARORA R., 1996. Seasonal patterns of dehydrins and 70-kD

heat-shock proteins in bark tissues of eight species of woody plants. Physiol Plant 96, 496-505.

WISNIEWSKI M., WEBB R., BALSAMO R., CLOSE T. J., YU X., GRIFFITH M., 1999. Purification, immunolocalization, cryoprotective, and antifreeze activity of PCA60: a dehydrin from peach *(Prunus persica)*. Physiol Plant 105, 600-608.

WITT W., SAUTER J. J., 1995. *In vitro* degradation of starch grains by phosphorylases and amylases from poplar wood. J Plant Physiol 146, 15-21.

YAMAGUCHI-SHINOZAKI K., SHINOZAKI K., 2005. Organization of cis-acting regulatory elements in osmotic- and cold-stress-responsive promoters. Trends Plant Sci 10, 88-94.