Molecular responses to thermal stress in woody plants

L. Gómez, I. Allona, A. Ramos, P. Núñez, C. Ibáñez, R. Casado and C. Aragoncillo*

Departamento de Biotecnología, E.T.S. de Ingenieros de Montes. Universidad Politécnica de Madrid. Ciudad Universitaria s/n. E-28040 Madrid. Spain

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.

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.
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 Roseae 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 hardness, 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 DHN 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; Plã et al., 1998; Ukai 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 endormant 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 response 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 thermostolerance 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.

### Acknowledgments

This work was supported by grants (BIO2002-805 and BIO2003-7801) from the Spanish Ministerio de Ciencia y Tecnología.

### References


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

