Abstract

Dormancy is an adaptive mechanism that enables woody plants to survive the freezing temperatures of winter. This complex process is characterized by the cessation of meristem activity, which is accompanied by winter bud set, extensive metabolic remodelling, an acquired high tolerance to cold and, in deciduous trees, by leaf senescence and abscission. The induction of dormancy occurs in response to seasonal environmental signals. In most woody plants, shortening of the photoperiod induces growth cessation, bud set, and some degree of cold acclimation. The subsequent drop in temperature then leads to a greater tolerance to cold and leaf fall. Experimental evidence indicates that the phytochrome system plays an important role as a day length sensor, and it has been recently reported that in poplar (Populus tremula x tremuloides), the photoperiodic control of dormancy induction is driven by a molecular mechanism that shares components with the mechanism of the photoperiodic control of flowering time in Arabidopsis. In contrast, the effects of low temperatures are less well understood. Nonetheless, it has been established that the chestnut (Castanea sativa Mill.) circadian molecular clock is disrupted both during winter and in response to cold, with presumable consequences on the general physiology of the plant. However, there is no direct evidence so far for its role in dormancy regulation.

Additional key words: bud set, circadian clock, cold acclimation, endodormancy, photoperiodism, phytochrome.

Resumen

La dormancia es un mecanismo adaptativo que capacita a las plantas leñosas para sobrevivir a las bajas temperaturas invernales. Este complejo proceso se caracteriza por el cese de la actividad de los meristemos, y va acompañado del desarrollo de las yemas de otoño, de notables modificaciones metabólicas, de la adquisición de una elevada tolerancia al frío y, en las especies caducifolias, de la senescencia y abscisión de las hojas. La inducción de la dormancia responde a señales medioambientales. En la mayoría de las plantas leñosas el acortamiento del fotoperiodo induce el cese del crecimiento, la formación de las yemas de otoño y una moderada aclimatación al frío. Después, la bajada de las temperaturas induce una mayor tolerancia al frío y la caída de las hojas. La evidencia experimental indica que el sistema de los fitocromos juega un papel importante como sensor de la duración del día y recientemente se ha comprobado que el control fotoperiódico de la inducción de la dormancia en chopo ocurre mediante un mecanismo molecular que tiene elementos comunes con el que controla la transición floral en respuesta al fotoperiodo en Arabidopsis. La influencia de las bajas temperaturas es menos conocida. El reloj circadiano del castaño (Castanea sativa Mill.) se altera durante el invierno y en respuesta al frío, lo que debe tener importantes consecuencias sobre la fisiología general de la planta. Sin embargo, no hay todavía evidencia directa sobre su incidencia en la regulación de la dormancia.

Palabras clave adicionales: aclimatación al frío, endodormancia, fitocromo, fotoperiodismo, phytochrome.
Introduction

The stressful environmental conditions that woody plants growing in temperate or cold regions endure each winter are combatted by a survival mechanism known as winter dormancy. The dormancy process determines to what extent fruit crops will survive the winter and early spring without shoot and flower bud damage and, in long-lived forest species, the length of dormancy limits the growing season and thus affects wood production and quality. Consequently, the times at which dormancy begins and ends are critical ecological variables. In fact, a thorough understanding of the molecular physiology of dormancy induction will have numerous applications for the genetic improvement of plants. Several features of dormancy have been well revised in recent reports (Rowland and Arora, 1997; Rohde et al., 2000; Arora et al., 2003; Horvath et al., 2003; Tanino, 2004; Olsen, 2006; Welling and Palva, 2006; Rohde and Bhalerao, 2007), and for this reason, in this review we will be mainly focused in the control of winter dormancy onset, while dormancy maintenance and release will not be addressed.

The term dormancy, as applied to plants, implies that growth is arrested and the plant enters a state in which the meristems of buds and/or the vascular cambium are at rest. Lang (1987) described three states of dormancy: ecodormancy, in which growth arrest is determined by environmental factors; paradormancy, in which growth is inhibited by factors originating in parts of the plant other than the dormant bud; and a state of endodormancy provoked by endogenous plant factors within the dormant tissue. Once endodormancy is established, no growth can restart until a chilling requirement has been satisfied. Thus, for bud break to occur, endodormant plants need to be exposed to low temperatures (LT) for a cumulative number of hours (chilling requirement). In contrast, during ecodormancy, growth can resume if the environmental conditions become favourable (Lang, 1987; Howe et al., 1999). Rohde and Bhalerao (2007) recently proposed a more restricted definition of dormancy as «the inability to initiate growth from meristems (and other organs and cells with the capacity to resume growth) under favourable conditions». Evidently, the ecodormancy state is not covered by this definition.

Winter dormancy not only involves growth arrest and bud dormancy

Winter dormancy is a phenomenon whose seasonality is well defined (Fig. 1) (Lang, 1987; Crabbé and Barnola, 1996; Rohde et al., 2000). In the temperate climates of the Northern Hemisphere, most woody plants enter into a state of ecodormancy at the end of summer or beginning of autumn. A shortened photoperiod causes the cessation of growth and a change in the development of terminal shoots, which pass from leaf shoots to form primordia with protective scales, giving rise to autumn buds (Wareing, 1956; Nitsch, 1957). Simultaneously, the plants develop a moderate resistance to cold (first cold acclimation stage) (Weiser, 1970; Nissila and Fuchigami, 1978; Sakai and Larcher, 1987). Later on, induced by exposure to even lower temperatures, this resistance to cold increases considerably (second cold acclimation stage) and leaf senescence and abscission occur in deciduous trees (Weiser, 1970; Perry, 1971; Arora et al., 2003). The tree is said to be endodormant. In this state, the plant reaches its maximum adaptation to the cold and may tolerate extreme temperatures of −50°C to −100°C (Weiser, 1970; Howe et al., 1995; Li et al., 2002). Subsequently, when the chilling requirement has been satisfied, the tree returns to its endodormant state and finally with the return of warm temperatures and longer day lengths, growth resumes and the plant loses its cold acclimation (Fig. 1).

In this description of winter dormancy, the term dormancy has a wider meaning than the definitions mentioned earlier. Although the path to endodormancy is a continuum, during the period in which plants are endodormant, two physiological states may be clearly distinguished. To induce the first of these states, exposure to short days (SD) is sufficient, but to reach the second state, besides SD, LT are also required. Plants in which endodormancy is induced exclusively under controlled SD conditions have often been used as experimental models of winter dormancy (see for example Qamaruddin et al., 1993; Jeknic and Chen, 1999; Li et al., 2003a). In fact, these models have been proven as useful tools although the leaves of these deciduous endodormant plants fail to show any obvious marks of senescence and leaf fall does not occur.

Abbreviations used: ABA (abscisic acid), ABI (abscisic acid insensitive), CBF (C-repeat binding factor), CCA (circadian clock associated), CO (constans), Cs (Castanea sativa), ETR (ethylene receptor), FLC (flowering locus C), FT (flowering locus T), GA (gibberellic acid), LD (long days), LHY (long elongated hypocotyl), LT (low temperatures), PHY (phytochrome), PRR (pseudo-response regulator), QTL (quantitative trait loci), RNAi (RNA interference), SD (short days), TOC1 (timing of CAB1).
Moreover, they only acquire moderate cold acclimation (Howe et al., 1999; Jeknic and Chen, 1999). Accordingly, if we consider winter dormancy as an adaptive survival strategy, the features of the first state of endodormancy would not be sufficient to fit this description. Further experimental observations have reinforced the idea that the establishment of winter dormancy cannot be considered as strictly photoperiodic. Thus, apple (Malus pumila Mill.), pear (Pyrus communis L.) and other species of the Rosaceae family are insensitive to photoperiod (Wareing, 1956) and, until recently, their dormancy was attributed entirely to endogenous plant factors (Battey, 2000). However, recent data indicate that low temperatures control growth cessation and dormancy induction in these species independently of photoperiodic conditions (Cook et al., 2005; Heide and Prestrud, 2005). In addition, temperature may affect the rate of dormancy acquisition and depth of dormancy in very different woody species (Westergaard and Eriksen, 1997; Juntila et al., 2003; Fennell et al., 2005; Svendsen et al., 2007). For example, high autumn temperatures during SD dormancy induction significantly increase the chilling requirement for dormancy release in alder [Alnus glutinosa (L.) Moench.] and Betula spp. (Heide, 2003). Moreover, the analysis of quantitative trait loci (QTL) associated with dormancy in hybrid poplars has indicated that genetic differences in photoperiodic responses only partly explain genetic differences in bud set timing under natural field conditions, suggesting that responses to other environmental factors, such as temperature, could help to complete the emerging picture (Howe et al., 1999, 2000; Chen et al., 2002). Finally, SD and LT have a synergistic effect in enhancing the freeze tolerance of buds and stem tissues of birch (Betula pendula Roth) (Li et al., 2002). This synergistic effect of SD and LT has also been observed at the molecular level in a study where the expression of a C repeat-binding factor-controlled dehydrin gene was examined (Puhakainen et al., 2004).

**Molecular control of winter dormancy establishment**

Until fairly recently, knowledge at the molecular level of the induction of winter dormancy by SD was scarce, despite the fact that the key role of phytochromes in perceiving the length of the photoperiod has long been established (Williams et al., 1972). The role of these photoreceptors has been widely demonstrated at the physiological level and information has also been provided by genetic and molecular approaches (Olsen,
2006). *Populus trichocarpa* has three phytochrome genes, *PHYA*, *PHYB1*, and *PHYB2* (Howe et al., 1998). One of these, *PHYB2*, has been mapped to a linkage group containing QTL for bud set and bud flush in several experiments (Frewen et al., 2000; Chen et al., 2002). In addition, an adaptive response in this gene to local photoperiodic conditions has been suggested (Ingvarsson et al., 2006). Studies on transgenic hybrid aspen (*Populus tremula × tremuloides*) overexpressing the oat *PHYA* gene have added support to the role of phytochrome A in day length sensing (Olsen et al., 1997). Lines of *PHYA* overexpressing plants are insensitive to day length when grown at constant temperature. In these plants, SD does not induce growth cessation, bud set, cold acclimation or entry into dormancy. Interestingly, exposure to LT resulted in cold acclimation of transgenic plants to a degree comparable to the wild type (Welling et al., 2002). These results suggest the independent activation of cold acclimation by LT and SD in hybrid aspen.

The recently reported work of Böhlenius et al. (2006) has been pivotal to our knowledge of the signalling pathway triggered in response to shortening day length in aspen trees. These authors have demonstrated that a mechanism of external coincidence similar to that described for photoperiodic control of flowering in Arabidopsis (Hayama and Coupland, 2003; Yanovsky and Kay, 2003; Jarillo and Piñeiro, 2006) also comes into play in the regulation of seasonal growth cessation in aspen. The *CO (CONSTANS)/FT (FLOWERING LOCUS T)* module that controls flowering also drives SD dormancy induction (Böhlenius et al., 2006). Expression of *P. trichocarpa FT1* gene, a putative ortholog of *AtFT*, is a critical determinant of the timing of growth cessation and bud set in the SD response. Transgenic aspens (*P. tremula × P. tremuloides*) overexpressing *FT1* do not stop growing upon exposure to SD, and plants in which *FT1* expression is down-regulated by RNA interference (RNAi) show growth cessation and bud set independently of day length (Böhlenius et al., 2006). Poplar seems to share the *CO/FT* module’s mode of action described for Arabidopsis (revised in Kobayashi and Weigel, 2007). In the presence of light, a high level of *CO* gene expression, controlled by the circadian molecular clock, activates the *FT* transcription. In long day (LD) conditions, *CO* transcription peaks at dusk when there is still light, which induces expression of the *FT* gene and the consequential induction of flowering. Under SD conditions, *CO* mRNA levels remain low during daylight hours and do not rise until nighttime, reasons why *FT* is not expressed (Suárez-López et al., 2001; Yanovsky and Kay, 2002; Valverde et al., 2004; Imaizumi et al., 2005; Sawa et al., 2007). In poplar, when the days get shorter as autumn sets in, *CO* expression levels remain low during the light period, and thus *FT* is not expressed, which causes growth cessation and bud set (Böhlenius et al., 2006). Notwithstanding, it is interesting that after transferring poplar plants to a SD photoperiod in controlled conditions, the down-regulation of *FT1* expression could be detected within 3 d, yet it took several weeks for the plants to reach a state of endodormancy in these conditions.

This control mechanism could also explain the fact that ecotypes of several species show varying critical photoperiods (the longest photoperiod inducing growth cessation), depending on the latitude of their place of origin (Heide, 1974; Junnila, 1982; Howe et al., 1995; Qamaruddin et al., 1995; Clapham et al., 1998; Li et al., 2002). In *P. tremula* of European provenances, *CO* gene expression starts to rise earlier after dawn in the most southern ecotypes (Böhlenius et al., 2006). Accordingly, day lengths have to be shorter in the more southern compared to the more northern places of origin for *CO* transcripts to be produced only in the dark and thus not induce *FT* expression. In addition, the model described is consistent with the finding that transgenic *Populus* plants overexpressing the oat *PHYA* gene continue to grow in SD conditions (Olsen et al., 1997), since *PHYA* regulates *FT* transcription by modulating *CO* (Yanovsky and Kay, 2002). In fact, this overexpression prevents *FT* repression in SD conditions (Böhlenius et al., 2006). Surprisingly, a strong correlation has been detected in *Picea abies* between the expression profile of an *FT*-like gene and photoperiodic induction of bud set (Gyllenstrand et al., 2007). In addition, Hanzawa et al. (2005) have shown in Arabidopsis that substitution of a single amino acid can transform an *FT* protein from being an activator to a suppressor of flowering. Therefore, further work is needed to clarify the role of *FT* genes in the endodormancy of angiosperms and gymnosperms.

In a recent systems biology approach to unravel the underlying molecular program of apical bud development in poplar, combined transcript and metabolite profiling has been applied to a high-resolution time course from SD induction to dormancy (Ruttink et al., 2007). Analysis of metabolite and gene expression dynamics have allowed to reconstruct the temporal sequence of events during bud development. Importantly, to each
of the following processes, bud formation, acclimation to dehydration and cold, and dormancy, specific sets of regulatory and marker genes and metabolites have been associated, which can provide a reference frame for future functional studies and for genetic approaches to assess adaptation of trees to climate change. Interestingly, the identification of a large set of genes commonly expressed during the growth-to-dormancy transitions in poplar apical buds, cambium, or Arabidopsis seeds suggests parallels in the underlying molecular mechanisms in different plant organs (Ruttink et al., 2007).

The molecular features of the role played by LT in the establishment of winter dormancy are also gradually being elucidated. Transgenic Populus constitutively expressing C-repeat binding factor 1 (CBF1) from Arabidopsis (AtCBF1), which contributes to the acclimation to freezing temperatures in annual herbaceous species, develop increasing freezing tolerance of non-acclimated leaves and stems relative to wild-type plants (Benedict et al., 2006). Genes up-regulated by ectopic AtCBF1 expression in Populus have been determined, demonstrating a strong conservation of the CBF regulon between Populus and Arabidopsis and identifying differences between leaf and stem regulons (Benedict et al., 2006). Moreover, studies involving induction kinetics and tissue specificity of four CBF paralogues identified from the Populus balsamifera subsp. trichocarpa indicate that the pivotal role played by the CBF family in cold acclimation of Arabidopsis has been maintained in Populus (Benedict et al., 2006). However, the differential expression of the PtCBFs and differing clusters of CBF-responsive genes in leaf and stem tissues suggest that the perennial-driven evolution of winter dormancy may have given rise to specific roles for these «master-switches» in the different annual and perennial tissues of woody species (Benedict et al., 2006).

A winter disruption of the circadian clock in chestnut has been reported in another study (Ramos et al., 2005). The chestnut genes TIMING OF CAB1 (CsTOC1) and LONG ELONGATED HYPOCOTYL (CsLHY), which are homologous to essential components of the central circadian oscillator in Arabidopsis, have been noted to cycle daily during vegetative growth as expected. However, during winter dormancy, the presence of high non-oscillating CsTOC1 and CsLHY mRNA levels indicates alteration of the circadian clock. A similar disruption could be induced by chilling (4°C) chestnut seedlings (Ramos et al., 2005). It has been recently proposed that the circadian oscillator in Arabidopsis is comprised of several interlocking feedback loops, in which several PSEUDO-RESPONSE REGULATOR genes (PRR5, PRR7, and PRR9) participate besides the genes LHY, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and TOC1 (Mizuno and Nakamichi, 2005; Gardner et al., 2006; Locke et al., 2006; McClung, 2006; Zeilinger et al., 2006). Expression analysis of chestnut genes homologous to Arabidopsis PRR5, PRR7 and PRR9 has indicated that the disruption of the circadian clock, reflected by CsTOC1 and CsLHY expression levels, observed in winter and in response to cold similarly affects the genes CsPRR5, CSPRR7 and CsPRR9 (Ibáñez, 2007). Although this general modification to the circadian clock is not linked to dormancy maintenance since the normal cycling of all these genes resumed when winter endodormant plants were returned to 22°C, the initial changes induced by the first low autumn temperatures could trigger subsequent steps in the establishment of dormancy. Evidently, disruption of the molecular oscillator would modify the action of the CO/FT module induced by SD, since clock genes are known to directly play a part in regulating the CONSTANS-dependent photoperiodic pathway (Searle and Coupland, 2004; Nakamichi et al., 2007; Niwa et al., 2007). Further, it should be highlighted that: a) the circadian clock regulates plant rhythmic growth (Nozue and Maloof, 2006; Nozue et al., 2007); b) it acts in cold signalling pathways in Arabidopsis by gating the low-temperature induction of CBFs and modulates low-temperature Ca^{2+} signals (Fowler et al., 2005; Dodd et al., 2006); and c), stopping of the clock in response to winter LT could in part explain the extensive remodelling of meristem transcriptome observed during the transition from growth to dormancy in the vascular cambium of poplar (Schneider et al., 2004; Druart et al., 2007).

Interestingly, the ruin lizard (Podarcis sicula), a hibernating ectothermal vertebrate, shows similar clock disruption in response to cold (Chiara Magnone et al., 2005; Vallone et al., 2007). The basic mechanisms of clock function in plants and animals are similar, although their oscillator genes are unrelated. This parallelism between two such evolutionarily distinct organisms suggests that the stopping of the circadian clock in response to cold could be part of a general adaptive strategy that enables living organisms that undergo dormancy or hibernation to survive the winter. Recently, the interruption of the molecular circadian clock in the European hamster during hibernation has also been described (Revel et al., 2007).
The role played by phytohormones in winter dormancy establishment

Our understanding of the role played by phytohormones in the onset of winter dormancy is still highly fragmented (Tanino, 2004; Olsen, 2006; Rohde and Bhalerao, 2007). The contributions of several hormones, particularly gibberellins (GA) and abscisic acid (ABA), have been subject of many research efforts.

The exposure of woody plants to SD induces a drop in their GA contents (Junttila and Jensen, 1988; Olsen et al., 1995, 1997). This reduction in GA is correlated with the time of growth cessation and with cell division arrest in the subapical meristem area of the stem. Further, the application of GA to SD-induced terminal buds rapidly stimulates cell division in apices (Hansen et al., 1999). Transcriptional down regulation by SD of GA-20 oxidase, a gene encoding a key gibberellin biosynthetic enzyme, has also been observed in Populus leaves, and plants overexpressing this enzyme have been shown to exhibit a delay in growth cessation (Eriksson and Moritz, 2002). This regulation of GA biosynthesis could occur through interaction with the phytochrome system. In hybrid aspen overexpressing the oat PHYA gene, no drop in GA levels or reduction in GA-20 oxidase activity occurs (Olsen et al., 1997; Mølmann et al., 2003). These findings indicate that GA biosynthesis inhibition is involved in growth cessation and bud set in the SD response, although microarray analyses suggest that it is a negative regulator of GA signalling who restricts growth in the autumn (Druart et al., 2007).

The role played by ABA in dormancy development is controversial. Although results are contradictory, it seems that endogenous ABA levels increase under SD conditions (Qamaruddin et al., 1993; Rinne et al., 1994; Welling et al., 1997; Rohde et al., 2002; Li et al., 2003b). However, attempts to induce dormancy by exogenous ABA application have been unsuccessful (Welling et al., 1997; Li et al., 2003a,b), yet SD conditions were able to induce dormancy in an ABA-deficient birch (Rinne et al., 1998). Nevertheless, in these conditions the mutant type had reduced tolerance to LT compared to wild birch (Rinne et al., 1998). These and similar results have prompted the idea of the more direct involvement of ABA in the photoperiodic control of cold acclimation than in the induction of endodormancy (Arora et al., 2003). Recent investigations have tried to readress this issue (Rohde and Bhalerao, 2007). Thus, it is thought that induction of poplar homologues of the FCA gene during apical dormancy (Rohde and Bhalerao, 2007) and the fact that the FCA protein may be an ABA receptor (Razem et al., 2006) could be connected. In Arabidopsis, FCA regulates the expression of FLOWERING LOCUS C (FLC), which in turn, represses the FT gene (see Searle and Coupland, 2004; Searle et al., 2006). In fact, the presence of ABA prevents the action of FCA (Razem et al., 2006), such that ABA could even affect the action of the CO/FT module mentioned above.

The role of ethylene in bud set and dormancy has also been investigated using transgenic ethylene-insensitive birches that express the Arabidopsis ethylene receptor gene ETR1 carrying the dominant-negative mutation etr1-1. Under SD conditions, transgenic trees ceased elongation growth yet terminal bud formation was abolished. However, although delayed, these trees eventually attained a state of endodormancy (Ruonala et al., 2006). Interestingly, a similar finding has been described in poplars overexpressing the ABI3 homologue: the trees failed to form buds and yet they became dormant (Rohde et al., 2002). It therefore seems that bud development is independent of dormancy onset.

Recently, it has been proposed that light, ethylene, and ABA signal transduction pathways consecutively control poplar bud formation, acclimation to dehydration and cold, and dormancy by setting, modifying, or terminating these processes (Ruttink et al., 2007). Ethylene signal transduction is positioned temporally between light and ABA signals and is putatively activated by transiently low hexose pools (Ruttink et al., 2007).

Future perspectives

The path to winter dormancy is a continuum in which the plant passes through different physiological states. The rate of dormancy development and depth of dormancy vary according to the environmental signals that induce the process, especially day length and temperature. It is clearly a complex process that probably results from the interaction of several subprocesses with their corresponding signalling pathways. Identifying and independently analysing these subprocesses is likely to be especially revealing. The efficient approaches of functional genomics that have started to be used for the study of winter dormancy (Andersson et al., 2004; Schrader et al., 2004; Druart et al., 2007) will help discern and characterize the different stages. The
results of Böhlenius et al. (2006) have represented a breakthrough in our present understanding of the influence of the photoperiod on growth cessation and bud set, but there are still many issues that remain unclear. It would be interesting to determine which measurement mechanisms of flowering time control persist in the regulation of dormancy seasonality. In addition, studies focused on plants in which dormancy is exclusively induced by LT, such as the apple or pear, will provide clues to explain the role of temperature in the process.

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