View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Servicio de Coordinación de Bibliotecas de la Universidad Politécnica de Madrid

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar

Spanish Journal of Agricultural Research 2008, 6 (Special issue), 201-210
ISSN: 1695-971-X

Review. Molecular control of winter dormancy establishment in trees

I. Allona, A. Ramos, C. Ibáñez, A. Contreras,
R. Casado and C. Aragoncillo*

Centro de Biotecnología y Genómica de Plantas UPM-INIA. Departamento de Biotecnología.
ETS de Ingenieros de Montes. Universidad Politécnica de Madrid.
Ciudad Universitaria, s/n. 28040 Madrid. Spain

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

Revisión. Control molecular del establecimiento de la dormancia invernal en los árboles

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, reloj circadiano, yemas de otoño.

* Corresponding author: cipriano.aragoncillo@upm.es
Received: 30-08-07; Accepted: 05-12-07.

Introduction[1]

The stressful environmental conditions that woody plants growing in temperate or cold regions endure each winter are combated 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° 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 ecodormant 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.

[1] 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).

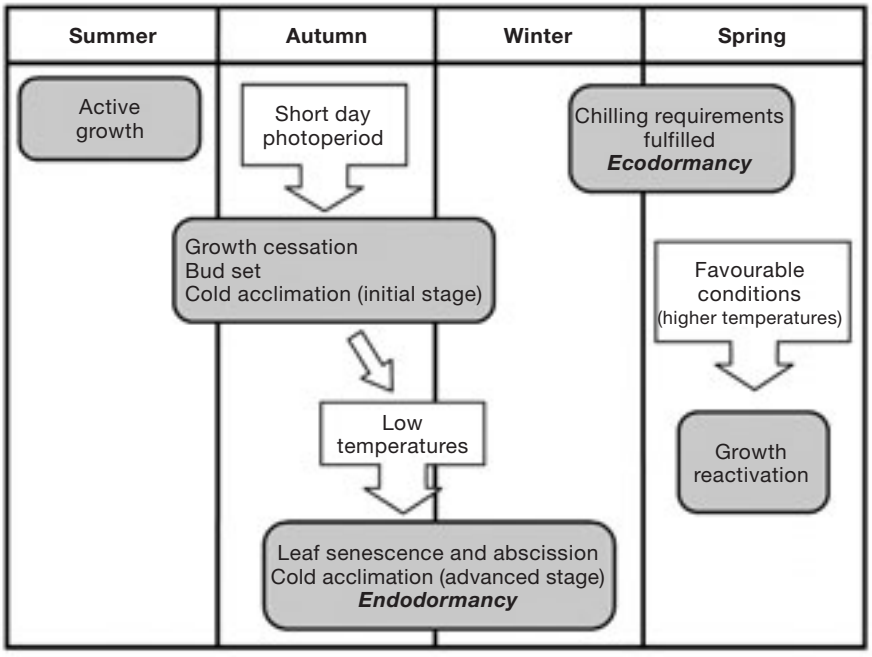

Figure 1. Diagram showing seasonal transition from the induction to the establishment, maintenance and release of winter dormancy in woody plants in temperate climates (Northern Hemisphere).

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; Junttila *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 consequent 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; Junttila, 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 asses 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 (Schrader *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 evolutionary 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 readdress 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.

Acknowledgments

This work was supported by grant AGL2005-00080 from the Spanish Ministerio de Educación y Ciencia.

References

ANDERSSON A., KESKITALO J., SJODIN A., BHALERAO R., STERKY F., WISSEL K., TANDRE K., ASPEBORG H., MOYLE R., OHMIYA Y. *et al.,* 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.

BATTEY N.H., 2000. Aspects of seasonality. J Exp Bot 51, 1769-1780.

BENEDICT C., SKINNER J.S., MENG R., CHANG Y., BHALERAO R., HUNER N.P., FINN C.E., CHEN T.H., HURRY V., 2006. The *CBF-1*-dependent low temperature signalling pathway, regulon and increase in freeze tolerance are conserved in *Populus spp*. Plant Cell Environ 7, 1259-1272.

BÖHLENIUS H., HUANG T., CHARBONNEL-CAMPAA L., BRUNNER A.M., JANSSON S., STRAUSS S.H., NILSSON O., 2006. CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312, 1040-1043.

CHEN T.H.H., HOWE G.T., BRADSHAW H.D., 2002. Molecular genetic analysis of dormancy-related traits in poplars. Weed Sci 50, 232-240.

CHIARA MAGNONE M., JACOBMEIER B., BERTOLUCCI C., FOÀ A., ALBRECHT U., 2005. Circadian expression of the clock gene Per2 is altered in the ruin lizard (*Podarcis sicula*) when temperature changes. Brain Res Mol Brain Res 133, 281-285.

CLAPHAM D., DORMLING I., EKBERG I., ERIKSSON G., QAMARUDDIN M., VINCE-PRUE D., 1998. Latitudinal cline for requirement for far-red light for the photoperiodic control of bud set and extension growth in *Picea abies*. Physiol Plant 102, 71-78.

COOK N.C., BELLEN A., CRONJÉ P.J.R., DE WIT I., KEULEMANS W., VAN DEN PUTTE A., STEYN W., 2005. Freezing temperature treatment induces bud dormancy in «Granny Smith» apple shoots. Sci Hortic 106, 170-176.

CRABBÉ J., BARNOLA P., 1996. A new conceptual approach to bud dormancy in woody plants. In: Plant dormancy: physiology, biochemistry and molecular biology (Lang G.A., ed), CAB International. pp. 83-113.

DODD A.N., JAKOBSEN M.K., BAKER A.J., TELZEROW A., HOU S.W., LAPLAZE L., BARROT L., POETHIG R.S., HASELOFF J., WEBB A.A., 2006. Time of day modulates low-temperature Ca signals in Arabidopsis. Plant J 48, 962-973.

DRUART N., JOHANSSON A., BABA K., SCHRADER J., SJÖDIN A., BHALERAO R.R., RESMAN L., TRYGG J., MORITZ T., BHALERAO R.P., 2007. Environmental and hormonal regulation of the activity-dormancy cycle in the cambial meristem involves stage-specific modulation of transcriptional and metabolic networks. Plant J 50, 557-573.

ERIKSSON M., MORITZ T., 2002. Daylength and spatial expression of a gibberellin-20-oxidase isolated from hybrid aspen (*Populus tremula ×P. tremuloides* Michx). Planta 214, 920-930.

FENNELL A., MATHIASON K., LUBY J., 2005. Genetic segregation for indicators of photoperiod control of dormancy induction in *Vitis* species. Acta Hort 689, 533-540.

FOWLER S.G., COOK D., THOMASHOW M.E., 2005. Low temperature induction of Arabidopsis *CBF1*, *2*, and *3* is gated by the circadian clock. Plant Physiol 137**,** 961-968.

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.

GARDNER M.J., HUBBARD K.E., HOTTA C.T., DODD A.N., WEBB A.A.R., 2006. How plants tell the time. Biochem J 397, 15-24.

GYLLENSTRAND N., CLAPHAM D., KÄLLMAN T., LAGERCRANTZ U., 2007. A Norway spruce *FLOWERING LOCUS T* homolog is implicated in control of growth rhythm in conifers. Plant Physiol 144, 248-257.

HANSEN E., OLSEN J.E., JUNTTILA O., 1999. Gibberellins and subapical cell divisions in relation to bud set and bud break in *Salix pentandra*. J Plant Growth Regul 18, 167-170.

HANZAWA Y., MONEY T., BRADLEY D., 2005. A single amino acid converts a repressor to an activator of flowering. Proc Natl Acad Sci USA 102, 7748-7753.

HAYAMA R., COUPLAND G., 2003. Shedding light on the circadian clock and the photoperiodic control of flowering. Curr Opin Plant Biol 6, 13-19.

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., 2003. High autumn temperature delays spring bud burst in boreal trees, counterbalancing the effect of climatic warming. Tree Physiol 23, 931-936.

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.

HORVATH D.P., ANDERSON J.V., WUN S.C., FOLEY M.E., 2003. Knowing when to grow: signals regulating bud dormancy. Trends Plant Sci 8, 534-540.

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., BUCCIAGLIA P.A., FURNIER G.R., HACKETT W.P., CORDONNIER-PRATT M.M., GARDNER G., 1998. Evidence that the phytochrome gene family in black cottonwood has one *PHYA* locus and two *PHYB* loci, but lacks members of the *PHYC/F* and *PHYE* subfamilies. Mol Biol Evol 15, 160-175.

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., 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.

IBÁÑEZ C., 2007. Caracterización y expression de genes implicados en la dormancia invernal del castaño (*Castanea sativa* Mill.). Doctoral thesis. Polytechnic University, Madrid. [In Spanish].

IMAIZUMI T., SCHULTZ T.F., HARMON F.G., HO L.A., KAY S.A., 2005. FKF1 F-box protein mediates cyclic degradation of a repressor of *CONSTANS* in *Arabidopsis*. Science 309, 293-297.

INGVARSSON P.K., GARCÍA M.V., HALL D., LUQUEZ V., JANSSON S., 2006. Clinal variation in *phyB2*, a candidate gene for day-length-induced growth cessation and bud set, across a latitudinal gradient in European aspen (*Populus tremula*). Genetics 172, 1845-1853.

JARILLO J.A., PIÑEIRO M.A., 2006. The molecular basis of photoperiodism. Biol Rhythms Res 37, 353-380.

JEKNIC Z., CHEN T.H.H., 1999. Changes in protein profiles of poplar tissues during the induction of bud dormancy by short-day photoperiods. Plant Cell Physiol 40, 25-35.

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., JENSEN E., 1988. Gibberellins and photoperiodic control of shoot elongation in Salix. Physiol Plant 74, 371-375.

JUNTTILA O., NILSEN J., IGELAND B., 2003. Effect of temperature in the induction of bud dormancy in various ecotypes of *Betula pubescens* and *B. pendula*. Scan J For Res 18, 208-217.

KOBAYASHI Y., WEIGEL D., 2007. Move on up, it's time for change mobile signals controlling photoperiod-dependent flowering. Genes Dev 21, 2371-84.

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

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.

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 pendula* to exogenous ABA application. Tree Physiol 23, 481-487.

LOCKE J.C.W., KOZMA-BOGNÁR L., GOULD P.D., FEHÉR E., NAGY F., TURNER M.S., HALL A., MILLAR A.J., 2006. Experimental validation of a predicted feedback loop in the multi-oscillator clock of *Arabidopsis thaliana*. Mol Syst Biol 2, 59.

McCLUNG C.R., 2006. Plant circadian rhythms. Plant Cell 18**,** 792-803.

MIZUNO T., NAKAMICHI N., 2005. Pseudo-response regulators (PRRs) or true oscillator components (TOCs). Plant Cell Physiol 46**,** 677-685.

MØLMANN J.A., BERHANU A.T., STORMO S.K., ERNSTSEN A., JUNTTILA O., OLSEN J.E., 2003. Metabolism of gibberellin A_{19} is under photoperiodic control in *Populus*, *Salix* and *Betula*, but not in daylength-insensitive *Populus* overexpressing phytochrome A. Physiol Plant 119, 278-286.

NAKAMICHI N., KITA M., NIINUMA K., ITO S., YAMASHINO T., MIZOGUCHI T., MIZUNO T., 2007. Arabidopsis clock-associated pseudo-response regulators PRR9, PRR7 and PRR5 coordinately and positively regulate flowering time through the canonical CONSTANS-dependent photoperiodic pathway. Plant Cell Physiol 48, 822-832.

NISSILA P.C., FUCHIGAMI L.H., 1978. The relationship between vegetative maturity and the first stage cold acclimation. J Am Soc Hort Sci 103, 710-711.

NITSCH J.P., 1957. Photoperiodism in woody plants. Proc Am Soc Hort Sci 70, 526-544.

NIWA Y., ITO S., NAKAMICHI N., MIZOGUCHI T., NIINUMA K., YAMASHINO T., MIZUNO T., 2007. Genetic linkages of the circadian clock-associated genes, TOC1, CCA1, and LHY, in the photoperiodic control of flowering time in *Arabidopsis thaliana*. Plant Cell Physiol 48, 925-937.

NOZUE K., MALOOF J.N., 2006. Diurnal regulation of plant growth. Plant Cell Environ 29, 396-408.

NOZUE K., COVINGTON M.F., DUEK P.D., LORRAIN S., FANKHAUSER C., HARMER S.L., MALOOF J.N., 2007. Rhythmic growth explained by coincidence between internal and external cues. Nature 448, 358-361.

OLSEN J.E., 2006. Mechanisms of dormancy regulation. Acta Hort 727, 157-165.

OLSEN J.E., JUNTTILA O., MORITZ T., 1995. A localised decrease of GA_1 in shoot tips of *Salix pentandra* seedlings precedes cessation of shoot elongation under short photoperiod. Physiol Plant 95, 627-632.

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.

PERRY T.O., 1971. Dormancy of trees in winter. Science 17, 29-36.

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.

RAZEM F.A., EL-KEREAMY A., ABRAMS S.R., HILL R.D., 2006. The RNA-binding protein FCA is an abscisic acid receptor. Nature 439, 290-294.

REVEL F.G., HERWIG A., GARIDOU M.L., DARDENTE H., MENET J.S., MASSON-PÉVET M., SIMONNEAUX V., SABOUREAU M., PÉVET P., 2007. The circadian clock stops ticking during deep hibernation in the European hamster. Proc Natl Acad Sci USA 104, 13816-13820.

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., BHALERAO R.P., 2007. Plant dormancy in the perennial context. Trends Plant Sci 12, 217-223.

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 (Jain S.M., Minocha S.C., eds.), Vol. 1. 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.

ROWLAND L.J., ARORA R., 1997. Proteins related to endodormancy (rest) in woody perennials. Plant Sci 126, 119-144.

RUONALA R., RINNE P.L.H., BAGHOUR M., MORITZ T., TUOMINEN H., KANGASJARVI J., 2006. Transitions in the functioning of the shoot apical meristem in birch (*Betula pendula*) involve ethylene. Plant J 46, 628-640.

RUTTINK T., AREND M., MORREEL K., STORME V., ROMBAUTS S., FROMM J., BHALERAO R.P., BOERJAN W., ROHDE A., 2007. A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell 8, 2370-90.

SAKAI A., LARCHER W., 1987. Frost survival of plants: responses and adaptation to freezing stress. Springer Verlag, Berlin.

SAWA M., NUSINOW D.A., KAY S.A., IMAIZUMI T., 2007. FKF1 and GIGANTEA complex formation is required for day-length measurement in Arabidopsis. Science 318(5848), 261-265.

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

SEARLE I., COUPLAND G., 2004. Induction of flowering by seasonal changes in photoperiod. EMBO J 23, 1217-1222.

SEARLE I., HE Y., TURCK F., VINCENT C., FORNARA F., KROBER S., AMASINO R.A., COUPLAND G., 2006. The transcription factor *FLC* confers a flowering response to vernalization by repressing meristem competence and systemic signalling in Arabidopsis. Genes Dev 20, 898-912.

SUÁREZ-LÓPEZ P., WHEATLEY K., ROBSON F., ONOUCHI H., VALVERDE F., COUPLAND G., 2001. CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature 410, 1116-1120.

SVENDSEN E., WILEN R., STEVENSON R., LIU R., TANINO K.K., 2007. A molecular marker associated with low-temperature induction of dormancy in red osier dogwood (*Cornus serica*). Tree Physiol 27, 385-397.

TANINO K., 2004. Hormones and endodormancy induction in woody plants. J Crop Improv 10, 157-199.

VALLONE D., FRIGATO E., VERNESI C., FOÀ A., FOULKES N.S., BERTOLUCCI C., 2007. Hypothermia modulates circadian clock gene expression in lizard peripheral tissues. Am J Physiol Regul Integr Comp Physiol 292, R160-166.

VALVERDE F., MOURADOV A., SOPPE W., RAVENSCROFT D., SAMACH A., COUPLAND G., 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Nature 303, 1003-1006.

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., PALVA E.T., 2006. Molecular control of cold acclimation in trees. Physiol Plant 127, 167-181.

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.

WESTERGAARD L., ERIKSEN E.N., 1997. Autumn temperature affects the induction of dormancy in first-year seedlings of *Acer platanoides* L. Scand J For Res 12, 11-16.

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.

YANOVSKY M.J., KAY S.A., 2002. Molecular basis of seasonal time measurement in *Arabidopsis*. Nature 419, 308-312.

YANOVSKY M.J., KAY S.A., 2003. Living by the calendar: how plants know when to flower. Nature Rev 4, 265-275.

ZEILINGER M.N., FARRÉ E.M., TAYLOR S.R., KAY S.A., DOYLE F.J., 2006. A novel computational model of the circadian clock in Arabidopsis that incorporates PRR7 and PRR9. Mol Syst Biol 2, 58.