Plant Physiology and Biochemistry 47 (2009) 105–115

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Identification of *PaCOL1* and *PaCOL2*, two *CONSTANS*-like genes showing decreased transcript levels preceding short day induced growth cessation in Norway spruce

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ARTICLE INFO

Article history:
Received 17 October 2008
Available online 17 November 2008

Keywords:
CONSTANS-like gene
Growth cessation
Norway spruce
Picea abies
Woody plant

ABSTRACT

In woody plants of the temperate zone short photoperiod (SD) leads to growth cessation. In angiosperms *CONSTANS* (*CO*) or *CO*-like genes play an important role in the photoperiodic control of flowering, tuberisation and shoot growth. To investigate the role of *CO*-like genes in photoperiodic control of shoot elongation in gymnosperms, *PaCOL1* and *PaCOL2* were isolated from Norway spruce. *PaCOL1* encodes a 3.9 kb gene with a predicted protein of 444 amino acids. *PaCOL2* encodes a 1.2 kb gene with a predicted protein of 385 amino acids. Both genes consist of two exons and have conserved domains found in other *CO*-like genes; two zinc finger domains, a CCT and a COOH domain. *PaCOL1* and *PaCOL2* fall into the group 1c clade of the *CO*-like genes, and are thus distinct from *Arabidopsis CO* that belongs to group 1a. Transcript levels of both *PaCOL*-genes appear to be light regulated, an increasing trend was observed upon transition from darkness to light, and a decreasing trend during darkness. The increasing trend at dawn was observed both in needles and shoot tips, whereas the decreasing trend in darkness was most prominent in shoot tips, and limited to the late part of the dark period in needles. The transcript levels of both genes decreased significantly in both tissues under SD prior to growth cessation and bud formation. This might suggest an involvement in photoperiodic control of shoot elongation or might be a consequence of regulation by light.

1. Introduction

Unlike annuals, perennials must be able to survive seasonal changes. In juvenile woody species of temperate and boreal regions photoperiods shorter than a critical length result in growth cessation and formation of a terminal bud [1–5]. For northern populations longer photoperiods are required to maintain growth compared to southern populations [3–7]. Perception of the photoperiod is known to involve the phytochrome system [8,9].

Although little information is available on the molecular mechanisms underlying photoperiodic control of shoot elongation in trees, especially in gymnosperms, a substantial amount of information has accumulated on molecular mechanisms of photoperiodic control of flowering in herbaceous plants. In the facultative long day (LD) plant *Arabidopsis thaliana* a photoperiodic pathway for flowering has been well characterised. This pathway acts through the *GIGANTEA* (*GI*), *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) genes. *CO* encodes a zinc finger transcription factor critical for flowering. Overexpression of *CO* accelerates flowering, whereas mutations in CO delay flowering [10]. PhyA together with cryptochrome 2 (cry2) stabilise the CO protein, whereas phyB promotes degradation [11]. The coincidence of high *CO* mRNA levels and light is important to promote flower induction in LD [12,13]. The CO protein activates the transcription of *FT* and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) [14–17]. High levels of *FT* induce flowering by up regulation of meristem and flower identity genes. *SOC1* encodes a MADS box transcription factor that promotes flowering [16,18]. High transcript levels of *CO*-like genes also stimulate flowering in the LD plants wheat (*Triticum aestivum*) and perennial ryegrass (*Lolium perenne*), as well as the SD plant Japanese morning glory (*Pharbitis nil*) [19–21]. In the SD plant rice (*Oryza sativa*) genes homologous to the *Arabidopsis GI*, *CO* and *FT* genes have also been isolated and characterised [22–24]. In rice the

Abbreviations: CO, CONSTANS; FT, FLOWERING LOCUS T; GI, GIGANTEA; iPCR, inverse polymerase chain reaction; LD, long day; SD, short day; SOC 1, suppressor of overexpression of CO 1; CCT, CO CO-like TOC1.

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doi:10.1016/j.plaphy.2008.11.003

CO-homologous gene *Hd1* suppresses transcription of the *FT* homologue *Hd3a* under non-inductive photoperiods [22,24].

Apart from playing a key role in the photoperiodic response to flowering *CO*-like genes have also been shown to be involved in other photoperiodically regulated developmental processes. In potato (*Solanum tuberosum*) SD induced tuberisation is inhibited by overexpression of the *Arabidopsis CO* gene [25]. In the moss *Physcomitrella patens PpCOL1* appears to be involved in the photoperiodic regulation of reproduction [26]. Recently it was shown that in the woody angiosperm *Populus* a CO-FT regulon controls flower induction and photoperiodic control of growth cessation [27,28]. Similar to the situation in *Populus*, in Sitka spruce (*Picea sitchensis*) an *FT* gene showing higher transcript levels in LD than SD was recently reported [29]. On the other hand, in Norway spruce SD-induced growth cessation was shown to correlate with increased transcript levels of another *FT*-like gene [30]. In *Arabidopsis* the *CO*-like gene *COL3* is a positive regulator of photomorphogenesis [31].

CO-like genes have been characterised mainly in annual and biannual herbaceous plants as well as in the moss *P. patens* [19–22,25,26,32–38]. In *Arabidopsis* 17 *CO*-like genes including *CO* have been identified [38]. The *CO*-like genes have been grouped into four, groups I–IV [35]. Group 1-genes, which include *CO* in *Arabidopsis* and *CO*-like genes involved in photoperiodism in a number of other plants, have two zinc finger boxes near the amino terminus [35]. *Arabidopsis CO*, *COL1* and *COL2*, rice *Hd1*, Japanese morning glory *PnCO*, barley *HvCO1* and *HvCO2*, as well as black cottonwood *PtCO1* and *PtCO2* belong to group 1a of the *CO*-like genes, whereas *Arabidopsis COL3* and *COL4* as well as *Physcomitrella PpCOL1-3* belong to the group 1c. *CO*-like genes in woody species have so far to our knowledge only been characterised in the angiosperms eastern cottonwood (*Populus deltoides*), black cottonwood (*Populus trichocarpa*) and apple (*Malus domestica*) ([27,28]; GenBank accession nos. AAC99309, AAC99310). Recently a *CO*-like gene has also been cloned in the gymnosperm Norway spruce (*Picea abies*), but no information about gene expression patterns is available ([39]; GenBank accession no. AM267532).

Here we report on molecular cloning and characterisation of two *CO*-like genes, *PaCOL1* and *PaCOL2*, in Norway spruce. Both genes classify to the group 1c of the *CO*-like gene family, and are thus distinct from *Arabidopsis* CO and black cottonwood *PtCO1* and *PtCO2* that belong to group 1a. The transcript levels of *PaCOL1* and *PaCOL2* appear to be light regulated with an increasing and decreasing trend in transcript levels at dawn and during darkness, respectively, both in LD and SD. The increasing trend at dawn was observed in needles as well as shoot tips whereas the decreasing trend in darkness was most prominent in shoot tips and limited to the late part of the dark period in needles. The transcript levels of *PaCOL1* and *PaCOL2* in both tissues decreased significantly under SD prior to the SD-induced growth cessation and winter bud formation. This might suggest an involvement of both genes in photoperiodic control of shoot elongation in Norway spruce or might be a consequence of regulation by light.

2. Materials and methods

2.1. Plant materials and growth conditions

CO homologues in Norway spruce (*Picea abies* (L.) Karst) were cloned from plant material from two 35-year-old trees (clone 409 and 589) grown outdoors at Ås, Norway (Norwegian Forest and Landscape Institute, 59°N). For gene expression and growth studies seedlings of two provenances were used. The provenances originated from 66°N (P1 from Rana, Norway, seed lot 4145, Norwegian Forest Seed Centre, Hamar, Norway) and 59°N (CØ1 from Halden, Norway, seed lot 98063). The critical photoperiod for trees originating from these latitudes is approximately 20–22 and 16–17 h, respectively [3,4,7].

Seedlings were grown at 18 °C in peat: perlite (3:1) mixture. Humidity was adjusted to give 0.5 kPa water vapour pressure deficit. The photon flux density of the main light period of 12 h was 180 $\mu mol\ m^{-2}\ s^{-1}$ at 400–750 nm from high-pressure sodium lamps (General Electric, Fairfield, CT, USA) supplemented with 8 $\mu mol\ m^{-2}\ s^{-1}$ from incandescent lamps (Osram 75 W, Munchen, Germany). To obtain long days (LD), longer than the critical photoperiod for both provenances, the light period was extended to 24 h with low-intensity light from incandescent lamps (8 $\mu mol\ m^{-2}\ s^{-1}$). Such a day extension was used to avoid substantial increase in the daily light integral under LD as compared to short days (SD). Thus, confounding photoperiodic effects with effects of higher total irradiance could then be avoided. After 8 weeks a subset of plants was transferred to SD. The conditions for SD were the same as for LD except that no day extension was given (12 h light/12 h dark). Stem length was measured once a week in 10 randomly selected reference plants.

To investigate the diurnal rhythm of *PaCOL1* and *PaCOL2* expression, tissues from the provenances from 66°N and 59°N were harvested every third or fourth hour for 48 h. To obtain a photoperiod longer than the critical one, the provenance from 66°N was exposed to LD of 24 h (as described above). To study the effect of LD conditions with light–dark cycles, 8 weeks old plants of the provenance from 59°N were transferred to an 18 h photoperiod (12 h light/6 h day extension/6 h dark) for 1 week. The plants were then retained in an 18 h photoperiod or transferred to SD of 12 h photoperiod. To further investigate the effect of continuous light, a subset of plants was transferred to conditions with 12 h photosynthetic active radiation and 12 h day extension as described. For further investigating the effect of photoperiod on *PaCOL* gene expression, tissues of the 66°N provenance were harvested every second day for 28 days (24 days in replicate 2) 12 h after start of the light treatment. For comparison of the distribution of transcripts, tissues were harvested from 8 weeks old plants grown in LD 3 h after the start of the light period.

In all cases needles and 6 mm of the shoot tip were harvested separately from a bulk of 4 randomly selected plants in each of two replicate experiments. The shoot tips contained immature needle tissue and needle primordia. Although mature leaves have been shown to be locus of day-length perception in induction of growth cessation and dormancy of a variety of woody species, there are also several reports on day-length sensing of shoot tips and buds [40,41]. Thus, although generation of a transmissible signal (FT) due to action of one or more *CO*-like genes in mature leaves appears to be the case in *Populus* [28], an action of *CO*-like genes also in shoot tips in woody species cannot be excluded. Thus, both needles and shoot tips were analysed for *PaCOL* gene expression in the present study.

2.2. DNA and RNA isolation

DNA was extracted from needles of Norway spruce according to Hodgetts et al. [42]. RNA was extracted using Ambion RNAqueous RNA isolation kit (Ambion, Austin, TX, USA) or plant isolation reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturers instructions. Residual DNA was removed through a RNase free DNase treatment using DNA-FreeTM (Ambion). Quality and quantity of RNA were analysed by spectrophotometric measurements and gel electrophoresis.

2.3. Isolation of two CO-like genes from Norway spruce

BLAST searches using the *A. thaliana* CO amino acid sequence to an EST library from Norway spruce (http://nisk-15.nisk.no) were