# CONSTANS delays flowering and affects tuber yield in potato

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## Abstract

CONSTANS (CO) has a central role in the photoperiodic regulation of flowering in *Arabidopsis thaliana*. We show here that potato (*Solanum tuberosum* ssp. *andigena*) plants constitutively expressing *Arabidopsis CO* (pACo plants) flower late under all photoperiodic conditions tested. Exogenous application of gibberellic acid to pACo plants corrected their short stem phenotype but not their late flowering. To further understand the effect of CO in potato, we used three photoperiodic conditions: short days (SD), which strongly induce tuberisation of wild type plants, SD supplemented with a night break (SD+NB), which are moderately inductive, and tuberisation-inhibiting long days. Tuberisation of pACo plants was delayed under SD and very strongly delayed or completely inhibited under SD+NB, suggesting that CO affects an autonomous pathway controlling potato tuberisation. In addition, tuber yield, a trait of high agronomic relevance, was significantly increased in pACo plants expressing moderate CO levels. Our results indicate that CO affects flowering and stem elongation through distinct mechanisms and suggest that its effects on flowering and tuberisation in potato are photoperiod-independent.

*Additional key words*: flowering time, photoperiod, *Solanum tuberosum* ssp. *andigena*, tuberisation.

## Introduction

Flowering and tuber development can be regulated by endogenous and environmental factors. Photoperiod, temperature and nutrition affect the time of tuber induction and flowering in many species (Jackson 1999, Bernier and Périlleux 2005). The photoperiodic control of flowering has been extensively studied in *Arabidopsis thaliana*, whose flowering is accelerated by long days (LD) and delayed by short days (SD; Searle and Coupland 2004). The effect of photoperiod on flowering in potato (*Solanum tuberosum*), however, has not been clearly established so far, probably because it varies among genotypes and growth conditions (Almekinders and Struik 1996). Although several studies have provided information on the environmental regulation of potato flowering (Macháčková *et al.* 1998, Konstantinova *et al.* 1999, Markarov 2002), the molecular bases of flowering time control in this species are still unknown.

CONSTANS (CO) is a key regulator of the photoperiodic flowering response in *Arabidopsis*. Mutations in *CO* delay flowering specifically under LD conditions and constitutive CO expression accelerates flowering under LD and SD conditions (Searle and Coupland 2004). CO allows the plant to determine seasonal time by integrating signals from the circadian clock and photoreceptors to induce genes that accelerate flowering under LD (Suárez-López *et al.* 2001, Yanovsky and Kay 2002, Valverde *et al.* 2004). Two recent reports indicate that CO regulates the production or transport of a leaf-generated signal that moves to the shoot apex to control flowering onset (An *et al.* 2004, Ayre and Turgeon 2004). *CO* encodes a zinc-finger nuclear protein (Putterill *et al.* 1995, Robson *et al.* 2001) and belongs to the *CONSTANS-LIKE* (*COL*) gene family (Lagercrantz and Axelsson 2000). In several plant species, but not in potato, *CO* homologues performing the same function as *CO* have been identified (Robert *et al.* 1998, Yano *et al.*

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*Abbreviations*: CO - CONSTANS; COL - CONSTANS-like; GA - gibberellin; $GA_3$ - gibberellic acid; Hd1 - heading date 1; LD - long day; PHYB - phytochrome B; SD - short day; SD+NB - short day plus night break; WT - wild type.

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2000, Liu *et al.* 2001, Nemoto *et al.* 2003, Martin *et al.* 2004). The case of rice (*Oryza sativa*), a SD plant, illustrates how the same genetic pathway can lead to opposite responses to photoperiod. The rice orthologue of CO, Heading date 1 (Hd1), regulates flowering time in response to day-length through the same floral activator as CO, but Hd1 represses the expression of the floral activator in LD, thus inhibiting flowering under LD (Kojima *et al.* 2002, Hayama *et al.* 2003).

In SD species, plants grown under SD in which the night is interrupted by a short light period or night break (SD+NB) behave similarly to LD-grown plants (Thomas and Vince-Prue 1997). In potato, SD promote tuber formation and LD delay or inhibit it depending on the species or cultivar (Jackson 1999). *Solanum tuberosum* ssp. *andigena* is usually described as a qualitative SD plant that cannot tuberise under LD, although there are reports of tuberisation in plants grown under LD in medium with high sucrose content (Rosin *et al.* 2003) and in old plants grown in pots (Kumar and Wareing 1973). Under SD+NB, the inhibitory effect of the night break on potato tuberisation varies with the length of the exposure to light in the middle of the night and with light quality (Batutis and Ewing 1982, Ewing and Struik 1992). It is generally assumed that potato *andigena* does not form tubers under SD supplemented with a white light night break, based on experimental results (Jackson *et al.* 1996, Macháčková *et al.* 1998). However, since other environmental and internal factors also influence tuberisation (Jackson 1999, Fernie and Willmitzer 2001), the inhibitory effect of SD+NB may sometimes be partial (Batutis and Ewing 1982).

*Solanum tuberosum* ssp. *andigena* plants that express constitutively the *Arabidopsis CO* gene (pACo plants) tuberise later than wild type (WT) plants under SD conditions (Martínez-García *et al.* 2002b). This led to the interpretation that CO affects the photoperiodic regulation of tuberisation (Martínez-García *et al.* 2002b). However, since WT plants do not tuberise under LD, it is unclear whether CO causes a general photoperiod-independent delay of tuberisation or it has a specific effect in SD. The late tuberisation phenotype of pACo plants is graft-transmissible (Martínez-García *et al.* 2002b), suggesting that CO interferes with long-distance signalling processes that regulate tuberisation.

The growth regulators gibberellins (GAs) influence tuberisation, flowering time and stem elongation in numerous species (Ewing and Struik 1992, Fleet and Sun 2005). In potato, GAs inhibit tuberisation (Ewing and Struik 1992, Van den Berg *et al.* 1995, Carrera *et al.* 2000) and have a positive effect on internode length (Van den Berg *et al.* 1995), but to our knowledge studies on their effect on flowering in this species have not been reported.

Due to the relevance of CO in the regulation of flowering and tuberisation, we carried out a detailed characterization of the effects of this gene in potato. The aims of this work were: *1*) to determine whether flowering of *S. tuberosum* ssp. *andigena* is regulated by daylength; *2*) to study whether CO affects flowering time in potato; *3*) to identify conditions that would allow to distinguish photoperiod-specific effects from general effects on tuberisation; and *4*) to determine whether the effect of CO on tuberisation is photoperiod-dependent or independent.

## Materials and methods

**Plants and growth conditions:** *Solanum tuberosum* L. ssp. *andigena* line 7540, obtained from Salomé Prat (Jackson *et al.* 1996), was used as the WT. Transgenic *S. tuberosum* ssp. *andigena* plants overexpressing *Arabidopsis CO* (pACo lines) were kindly provided by Salomé Prat and have been described previously (Martínez-García *et al.* 2002b). Plants were vegetatively propagated from apical cuttings and single-node stem cuttings on MS medium containing 2 % (m/v) sucrose and 0.2 % (m/v) *Gelrite*® (*Duchefa Biochemie*, Haarlem, The Netherlands) at 22 ºC under LD conditions (16 h light/8 h darkness). Two weeks after propagation, the plants were planted in soil and grown in the greenhouse at 23 ºC under LD conditions. During the SD of autumn and winter, the light period was extended to 16 h with high-pressure sodium vapour lamps (*SON-T Agro* 400 W, *Philips Ibérica*, Madrid, Spain). Whenever natural irradiance fell below 65 μmol $m^{-2}$ $s^{-1}$ during the 16-h light period, natural irradiance was supplemented with the same lamps. Plants were watered daily with modified Hoagland's solution (Johnson *et al.* 1957) diluted 1/60.

**Measurement of flowering time:** Plants grown under LD in greenhouse were transferred to controlled environment chambers at the 10 - 15 leaf stage, 4 weeks after potting. The photoperiods were SD (8 h light/16 h darkness) and SD+NB (SD supplemented with a 30 min white light night break given 8 h after the start of the dark period). Lighting was provided by high pressure sodium vapour lamps (irradiance of 200 μmol $m^{-2}$ $s^{-1}$). The shoot apex was carefully checked for visible signs of flowering every two days. Flowering time was measured as the number of days from planting in soil to the appearance of the floral bud. Leaf number was also recorded at the time when the floral bud was visible.

**GA treatments:** After 3 - 4 weeks in the greenhouse, plants were sprayed to run-off every three days with water or 10 μM gibberellic acid ($GA_3$; *Sigma-Aldrich*, Tres Cantos, Spain). Flowering time was determined as described above. Stem height was measured as the distance from soil surface to the shoot apex.

**Tuberisation assays:** Plants were moved from the LD greenhouse to SD and SD+NB growth chambers, as described for the flowering time assays. Tuberisation was analysed once per week. Tuberisation time was measured as the number of days from transfer to SD or SD+NB conditions to the appearance of tubers. Leaf number was also recorded when tubers were first visible.

To measure tuber yield, we stopped watering WT plants when they showed unequivocal symptoms of senescence, about 5 - 7 weeks after tuberising in SD. At this stage, WT tubers do not grow further because plants die after a few days even if they are watered. Since WT plants grown in SD+NB and pACo plants in SD senesce much later than WT plants in SD or do not senesce, we stopped watering those 7 - 9 weeks after tuberisation to allow their tubers to grow. We followed this strategy because measuring tuber yield at the same time for the WT and pACo in SD and the WT in SD+NB would show decreased tuber yield in WT in SD+NB and pACo plants simply because they tuberise later than the WT in SD. Two weeks after stopping watering, tuber number and tuber fresh mass per plant were determined.

## Results and discussion

*S. tuberosum* ssp. *andigena* plants were grown under three different photoperiods and the time to flowering was analysed. Our results showed that flowering time is not significantly different under SD, SD+NB and LD conditions (Table 1).

Table 1. Flowering time (number of days from potting to flowering) and number of leaves at flowering of *S. tuberosum* ssp. *andigena* under SD, SD+NB and LD. Means ± SE from 3 independent experiments ($n$ = 11 - 12).

| | Flowering time [d] | Leaf number |
|---|---|---|
| SD | 39.6 ± 0.8 | 20.2 ± 0.7 |
| SD+NB | 40.5 ± 0.7 | 21.0 ± 0.5 |
| LD | 39.8 ± 0.9 | 20.2 ± 0.5 |

*Arabidopsis co* mutants flower later than the WT under inductive LD and plants with increased CO levels flower earlier than the WT under LD and SD (Searle and Coupland 2004). Therefore, we analysed the flowering time of potato pACo plants, which express constitutively the *Arabidopsis CO* gene, under several photoperiods. Since all the pACo lines showed similar phenotypes (late tuberisation in SD, reduced internode length and increased anthocyanin content; Martínez-García *et al.* 2002b), we chose two lines for this study, pACo-7 and pACo-20, with moderate and high levels of *CO* expression, respectively. Under SD, SD+NB and LD, pACo plants flowered significantly later than the WT, with pACo-20 being later than pACo-7 (Fig. 1). Other pACo lines tested in one experiment also showed late flowering (data not shown). Like in the WT, the flowering time of the pACo-7 and pACo-20 lines was not affected by photoperiod (Fig. 1).

Several characteristics of pACo plants, *i.e.* shorter internodes, late tuberisation in SD (Martínez-García *et al.* 2002b) and late flowering (Fig. 1) are similar to those in plants with altered GA biosynthesis or GA response (Fleet and Sun 2005). To examine whether GAs could be involved in pACo phenotypes, the effect of exogenous $GA_3$ on pACo plants was analysed. $GA_3$ did not affect the time to flowering of WT or pACo plants (not shown). We

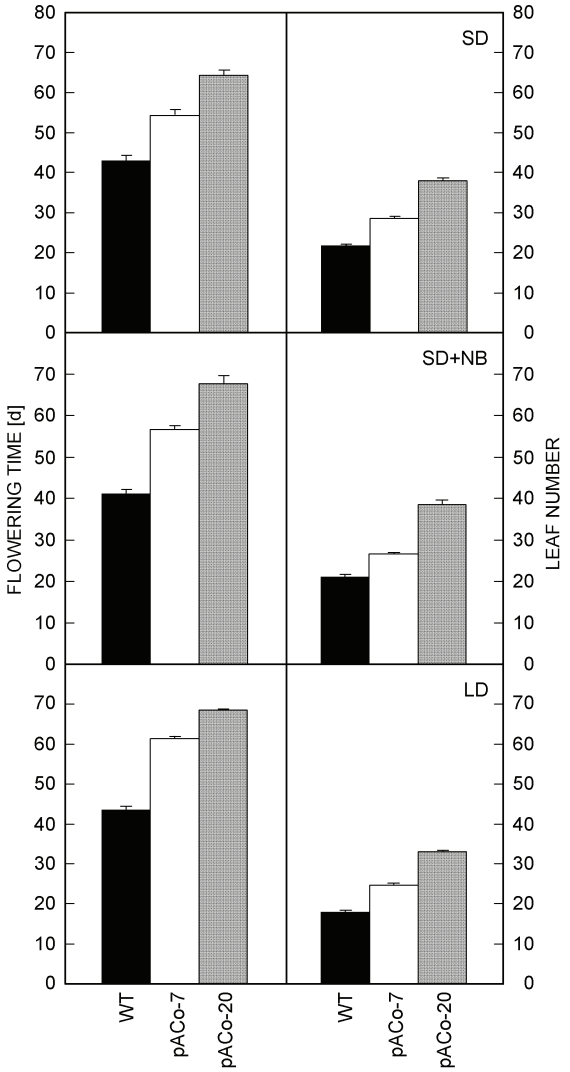
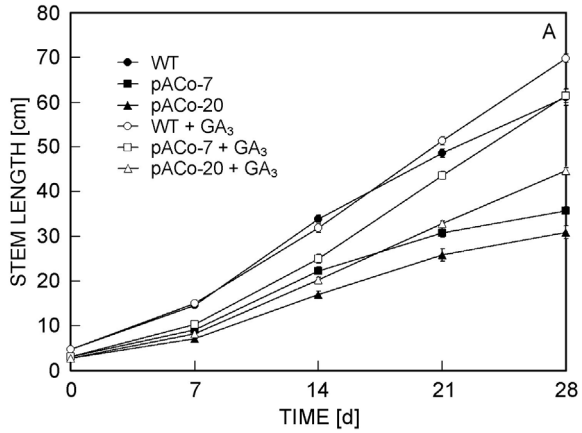



Fig. 1. Flowering time and number of leaves of *CO*-expressing potato plants under different photoperiodic conditions. Means ± SE in SD for 22 WT, 11 pACo-7 and 9 pACo-20 plants, in SD+NB for 15 WT, 15 pACo-7 and 13 pACo-20 plants, and in LD for 19 WT, 20 pACo-7 and 20 pACo-20 plants. Data are representative of at least 3 independent experiments, except for pACo-20 in SD+NB, which was analysed twice.

then tested the effect of GAs on stem elongation of the WT and pACo plants. The stem growth rate of the pACo-7 and pACo-20 lines was slower than that of the WT, with pACo-20 having a more severe phenotype than pACo-7 (Martínez-García *et al.* 2002b and Fig. 2*A*). When these plants were treated with $GA_3$, the growth rate of the WT and pACo lines increased (Fig. 2*A*). We observed that pACo-7 and WT plants treated with $GA_3$ grew at the same rate (after 2 weeks of treatment the slope of the curves becomes almost identical) and the height of pACo-7 plants treated with the $GA_3$ reached that of untreated WT plants after 4 weeks (Fig. 2*A*,*B*). $GA_3$ application corrected partially the reduced height of the pACo-20 line (Fig. 2*A*,*B*).

*S. tuberosum* ssp. *andigena* plants were grown under SD, SD+NB and LD conditions to study the response of tuberisation to photoperiod. Plants formed tubers under SD and SD+NB conditions (Table 2) and, as expected, did not tuberise under LD for at least 4 months. These results were highly reproducible in 6 independent experiments and plants tuberised on average about 17 d later under SD+NB than under SD. The number of leaves produced by the plants until they started to tuberise was higher in SD+NB (28.7 ± 0.3, $n$ = 9 plants) than in SD (22.6 ± 0.3, $n$ = 22), also indicating that SD+NB delays tuberisation compared to SD.



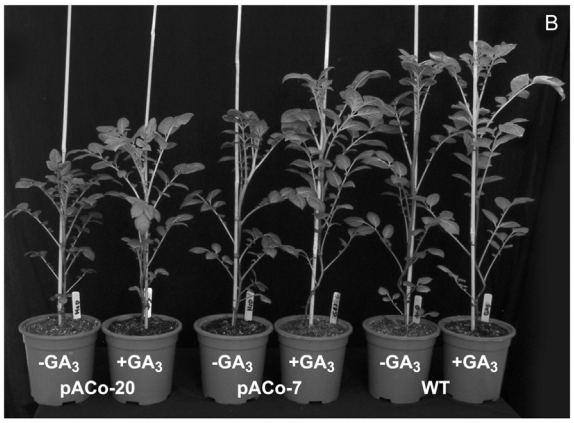




Fig. 2. Effect of exogenous $GA_3$ on potato plants that express the *Arabidopsis CO* gene. *A* - Stem growth of WT and pACo plants treated with water (-$GA_3$) or 10 μM $GA_3$ (+$GA_3$). Data represent the mean ± SE for 10 plants of each line. *B* - Photograph showing WT and pACo plants 3 weeks after the start of $GA_3$ treatment. Data are representative of 3 independent experiments.

Potato pACo plants tuberise later than the WT under SD and, like the WT, do not tuberise under LD (Martínez-García *et al.* 2002b). Tuberisation of pACo-7 and pACo-20 was therefore tested under moderately inducing, SD+NB conditions. These two pACo lines tuberised later than the WT under SD (Table 2), as expected. Under SD+NB, pACo-7 formed tubers much later than the WT and pACo-20 did not tuberise during the experiment (Table 2). Therefore, the effect of CO on tuber induction is not specific of SD. The pACo-20 line tuberised later than the pACo-7 line in SD (Table 2) and neither of them formed tubers even after growing in LD for 3 to 4 months, as reported previously (Martínez-García *et al.* 2002b). Consistent with their tuberisation times, pACo-7 produced more leaves than the WT and fewer than pACo-20 before starting to tuberise (not shown).

Table 2. Tuberisation time [d] of potato plants expressing *CO*. Means ± SE of at least 4 independent experiments, except for pACo-20 in SD+NB, which was analysed only once. [a] - plants were maintained under SD+NB for 4 months. 7 out of 15 plants formed tubers; [b] - plants were maintained under SD+NB for 3.5 months.

| | SD | SD+NB |
|---|---|---|
| WT | 14.6 ± 0.4 ($n$ = 22) | 33.4 ± 1.0 ($n$ = 9) |
| pACo-7 | 32.5 ± 1.1 ($n$ = 11) | >108 ($n$ = 15)[a] |
| pACo-20 | 42.8 ± 2.5 ($n$ = 9) | no tubers ($n$ = 13)[b] |

Tuber yield was higher for WT plants grown under SD+NB than under SD conditions (Table 3). This increase in tuber yield could be a consequence of the weaker tuber induction in SD+NB than in SD, because moderate induction can enhance tuber yield relative to very strong induction (Ewing and Struik 1992). Since pACo-7 tuberises in SD at about the same time as the WT in SD+NB (Table 2), we examined the tuber yield of this pACo line. Table 3 shows an increase in tuber yield in pACo-7 compared to WT plants. The increase in tuber fresh mass per plant, which varies between 5 and 57 % among different experiments, is due to a higher tuber number (Table 3), since the average mass per tuber was not increased. It has to be noted that the period left between the start of tuberisation and the measurement of tuber yield of the WT under SD+NB and pACo-7 was shorter in experiment 3 than in the other experiments. This may account for the small differences in tuber mass observed in this experiment, which nevertheless showed differences in tuber number similar to the other experiments (Table 3). Although the pACo-20 line produced more tubers (10.2 ± 2.2, $n$ = 9) than the WT (5.3 ± 0.2, $n$ = 18) under SD, it did not show an increase

Table 3. Tuber yield of WT and pACo plants determined in 6 independent experiments. Means ± SE (number of plants in parentheses); n.d. - not determined.

| | Tuber mass [g plant$^{-1}$] | | | Tuber number | | |
|---|---|---|---|---|---|---|
| | WT SD | WT SD+NB | pACo-7 SD | WT SD | WT SD+NB | pACo-7 SD |
| Exp. 1 | 53.88 ± 2.95 ( 5) | 51.47 ± 4.74 ( 5) | 66.97 ± 3.51 ( 5) | 6.6 ± 0.7 ( 5) | 9.2 ± 1.1 ( 5) | 8.6 ± 1.1 ( 5) |
| Exp. 2 | 46.69 ± 1.63 (21) | 76.04 ± 2.04 (21) | 58.20 ± 2.53 (21) | 6.9 ± 0.3 (21) | 12.0 ± 0.8 (21) | 7.5 ± 0.6 (21) |
| Exp. 3 | 41.27 ± 1.18 ( 7) | 44.61 ± 2.48 ( 7) | 43.19 ± 5.68 ( 9) | 5.4 ± 0.6 ( 7) | 10.9 ± 1.0 ( 7) | 7.0 ± 1.2 ( 9) |
| Exp. 4 | 65.13 ± 2.04 ( 7) | n.d. | 83.35 ± 3.13 ( 7) | 8.9 ± 0.3 ( 7) | n.d. | 11.9 ± 0.6 ( 7) |
| Exp. 5 | 37.10 ± 0.63 (18) | 61.98 ± 1.63 ( 9) | 58.10 ± 2.10 (11) | 5.3 ± 0.2 (18) | 11.4 ± 0.6 ( 9) | 8.7 ± 0.7 (11) |
| Exp. 6 | 28.93 ± 1.63 (11) | 48.06 ± 1.62 (11) | n.d. | 4.3 ± 0.5 (11) | 9.0 ± 1.0 (11) | n.d. |

in tuber mass per plant (data not shown).

The effect of photoperiod on the timing of flowering is very well documented in numerous plant species, especially in *Arabidopsis* (Searle and Coupland 2004, Bernier and Périlleux 2005). In potato, however, the scarce studies on the role of photoperiod in flowering control have led to contrasting conclusions (Almekinders and Struik 1996, Macháčková *et al.* 1998, Jackson *et al.* 2000). In our experiments, *S. tuberosum* ssp. *andigena* behaves as photoperiod insensitive for floral induction since flowering time is essentially identical under all photoperiods tested (Table 1). In agreement with our results, Jackson and Thomas (1997) mention that flowering is not photoperiodically regulated in some lines of this subspecies. However, Macháčková *et al*. (1998) and Konstantinova *et al*. (1999) reported that flowering of potato *andigena* was induced by LD and SD+NB, but did not occur under SD conditions. Although we cannot rule out an effect of differences in growth conditions, the discrepancy with Macháčková *et al*. (1998) and Konstantinova *et al*. (1999) might perhaps be due to the use of a different *andigena* line.

According to Martínez-García *et al*. (2002b), pACo plants do not differ in flowering time from the WT, although data that supported this conclusion were not shown. In this work, we present consistent and reproducible evidence that pACo plants flower later than the WT under three photoperiods (Fig. 1). This indicates that CO delays flowering in potato in a daylength-independent manner. Since *Arabidopsis* plants overexpressing *CO* flower early (Simon *et al.* 1996, Onouchi *et al.* 2000), it may seem unexpected that potato pACo plants show delayed flowering (Fig. 1). However, *COL* genes can have either a positive or negative effect on flowering. CO promotes flowering in *Arabidopsis* (Putterill *et al.* 1995), whereas Hd1, the closest CO homologue from rice, promotes flowering under SD and inhibits it under LD conditions (Yano *et al.* 2000). Other *COL* genes from *Arabidopsis* delay flowering (Cheng and Wang 2005, Datta *et al.* 2006). Two potato *COL* genes have been reported (Martínez-García *et al.* 2002b, Drobyazina and Khavkin 2006), but their function is still unknown. It is possible that CO delays potato flowering by interfering with a potato COL protein that would presumably induce flowering.

To further understand the effects of *Arabidopsis* CO in potato, we tested the hypothesis that GAs could be involved in the phenotypes of pACo plants. Reduced plant height and late flowering are very often associated with low GA content or response (Fleet and Sun 2005), whereas increased GA content can delay tuberisation (Ewing and Struik 1992, Carrera *et al.* 2000). We found that $GA_3$ corrected the stem elongation (Fig. 2) but not the flowering time of pACo plants (not shown), suggesting that separate pathways mediate both effects. The pACo plants respond to the addition of GAs, which suggests that their reduced stem elongation could be caused by a reduction in GA content. However, we do not have evidence that flowering time is affected through this mechanism, since flowering of WT and pACo plants was not affected by $GA_3$.

*S. tuberosum* ssp. *andigena* is regarded as strictly dependent on SD to form tubers (Jackson 1999). However, our experiments show that this potato subspecies also tuberises under SD+NB, although later than in SD, with very consistent results (Table 2). Our results are different from those of Macháčková *et al.* (1998) and Jackson *et al.* (1996), who reported that SD+NB does not induce tuber formation. While the potato *andigena* line and growth conditions used by Jackson *et al*. (1996) and in the present work were essentially the same, Macháčková *et al*. (1998) used 10 h SD with a 1 h night break, conditions less inductive than the 8 h SD plus 30 min night break that we used. This could explain the difference between our results and those of Macháčková *et al*. (1998), although we cannot exclude that different *andigena* lines were used.

SD+NB is a moderately inductive conditions for tuberization. Thus it can help distinguish between photoperiod-dependent and -inde-pendent effects on tuberisation. Therefore we used SD+NB to investigate further the late tuberisation of pACo plants (Martínez-García *et al.* 2002b). These plants tuberised late in both SD and SD+NB. This might be caused by lower level of the tuberisation inducing signal or higher level of the inhibitory signal in pACo than in WT plants. The difference in tuberisation time between SD and SD+NB was much more pronounced in pACo than WT plants (Table 2), indicating that constitutive expression of *CO* increases the response of tuberisation to photoperiod.

This effect is similar to that observed in *Arabidopsis* mutants affected in the autonomous pathway controlling flowering time: they show a higher difference in flowering time between LD and SD than the WT (Koornneef *et al.* 1991). In contrast, mutations or overexpression of genes affecting the photoperiodic flowering pathway in *Arabidopsis* reduce the response of flowering to photoperiod (reviewed by Searle and Coupland 2004). At least two genetic pathways regulate tuberisation in potato, a photoperiodic and an autonomous pathway (Martínez-García *et al.* 2002a). Our results indicate that CO probably interferes with the autonomous rather than the photoperiodic pathway. Martínez-García *et al*. (2002b) had suggested that CO acts in the photoperiodic tuberisation pathway after analysing its effect only in two extreme daylengths: strongly inducing SD and noninducing LD. By including SD+NB, we have obtained a more complete picture of the response of pACo plants that allows a different interpretation of their tuberisation phenotype.

We found that the WT under SD+NB and the pACo-7 line under SD showed higher tuber yields than the WT under SD (Table 3). However, tuber yield was not increased in the pACo-20 line, which shows later tuberisation (Table 2) and higher levels of *CO* expression (Martínez-García *et al.* 2002b) than pACo-7. Our interpretation is that a slight delay in tuberisation or a weaker tuber induction caused either by the night break or by moderate expression of *CO* leads to an increase in tuber yield. This is in agreement with previous findings showing that the highest tuber yields are usually associated with moderate levels of induction (Ewing and Struik 1992). In pACo-20, despite a significant increase in tuber number, the long delay in tuberisation seems to limit tuber growth, as has been reported previously for weakly inductive conditions (Ewing and Struik 1992). Furthermore, in SD-grown WT plants, tubers stop growing because the plants senesce and die. In contrast, senescence is much delayed, or does not occur, in pACo plants (Martínez-García *et al.* 2002b) and in the WT grown under SD+NB (not shown). This allows the tubers to develop for a longer period, resulting in a higher tuber yield.

Potato plants that carry an antisense construct of the photoreceptor phytochrome B (PHYB) tuberise under SD, SD+NB and LD conditions and show elongated stems (Jackson *et al.* 1996). These phenotypes are opposite to those of pACo plants (Table 2 and Fig. 2). *PHYB* antisense plants have increased $GA_1$ content in leaves (Martínez-García *et al.* 2002a), which can explain their elongated stems. Correspondingly, the presumed reduction of GAs could account for the short stems of pACo plants. However, the tuberisation phenotypes of *PHYB* antisense and pACo plants are in apparent contradiction with their GA levels because increased GA abundance inhibits or delays tuberisation and decreased GAs promote it (Bamberg and Hanneman 1991, Van den Berg *et al.* 1995, Carrera *et al.* 2000). One possibility to explain this apparent inconsistency may be that GAs are increased in the leaves but reduced in the stolons of *PHYB* antisense plants and, conversely, reduced in the leaves but increased in stolons of pACo plants. This might occur by differential regulation of GA biosynthesis and catabolism in different parts of the plant (Fleet and Sun 2005). Alternatively, transport of GAs from the shoot to the stolons could be reduced in *PHYB* antisense plants and increased in pACo plants. Consistent with this hypothesis, there is strong evidence that GAs are part of the transmissible signals that regulate flowering in *Lolium temulentum* (King and Evans 2003), GAs are probably transported between different organs in other plant species (Fleet and Sun 2005), CO regulates the production of a long-distance signal that induces flowering in *Arabidopsis* (An *et al.* 2004, Ayre and Turgeon 2004) and the transmissible signals for flowering and tuberization are probably identical or very similar (reviewed by Suárez-López 2005).

We propose that CO plays at least three different roles: regulation of flowering whether or not it is photoperiodic, control of tuberisation through a pathway that is not responsive to photoperiod, and a role in stem elongation possibly involving GAs. Our results contribute to the knowledge of flowering time regulation in potato. Potato plants that express *CO* can be used to identify potato genes regulating flowering and, therefore, can provide new tools to improve the production of true potato seeds, a valuable system to propagate pathogen-free potato (Simmonds 1997). This work also gives clues to study possible functions of CO that had not been identified in *Arabidopsis*, like effects on GA levels, and strongly suggests the existence of a potato *CO* homologue that will presumably affect flowering time and tuberisation. Furthermore, it reveals the potential of CO for biotechnological applications to increase crop yield.

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In comparison to the previous volume 57 containing 33 reviews, the reviewed book is evidently thinner. It brings only 20 review papers written by 45 scientists that work in eight countries. As usual, authors from the U.S.A. prevail (16), followed by Japan (10), the U.K. (7), Denmark, Korea, and Switzerland (3 each), Canada (2), and Sweden (1).

The introductory review was prepared by Diter von Wettstein whom I know as an excellent scientist in chloroplast field (his previous review dealt with this topic and with chromosome pairing). Here he discusses his engagement in genetic engineering of barley (synthesis and mobilization of endosperm proteins, improved production of feed and malt, proanthocyanidin biochemistry in barley and other plant species). One may be surprised seeing photographs of chickens, but these birds were fed with diet supplemented with small addition of transgenic grain containing (1,3;1,4)-β-glucanase. Phototropins as blue radiation receptors are the next topic (J.M. Christie). These kinases are present in higher and lower (ferns, mosses, algae) plants, they sense radiant energy, are autophosphorylated, and control photosynthetic efficiency, signal phototropism, leaf movements, stomatal opening, chloroplast movement, hypocotyl growth, *etc*. Another type of sensing and signalling is analysed in the following review (D.P. Schachtman and R. Shin): it detects the deprivation of macronutrients such as phosphorus, nitrogen, potassium, and sulphur in soils. The respective signal transduction pathways and networks are elucidated as well as the increase in content of reactive oxygen species (ROS) accompanying the deprivation. A special topic are arabinogalactan glycoproteins of cell surface (G.J. Seifert and K. Roberts) that serve as signals, modulators, or co-receptors, mediating between cell wall, plasma membrane, and cytoplasm; they may also function in organ abscission. These substances are studied using trihydroxybenzene derivatives (Yariv reagents) or monoclonal antibodies. Gibberellin signalling in plants (M. Ueguchi-Tanaka *et al*.) includes action of different receptors; they are gene encoded and depend on specific gibberellin-binding proteins in various plant species. Nevertheless, *Arabidopsis* and rice are very often studied from this point of view.

M.L. Ghirardi *et al*. analyse literature on the hydrogenases and hydrogen photoproduction in photosynthetic microorganisms (green algae and cyanobacteria). They deal with enzyme structures, genetics, mechanisms of action, oxygen inhibition, *etc*. Leaf senescence is explained (O.O. Lim *et al*.) from the point of view of structural and biochemical changes in tissues, programmed cell death, and molecular and genetic approaches and regulatory mechanisms (internal and environmental factors). The review on stomatal development (D.C. Bergmann and F.D. Sack) describes mechanisms and genetics of stomata formation, the participating signals, receptors, kinases, and proteases, and stomatal control of gas exchange affected by environmental factors. Another specialized review is on regulation of stomatal movements (opening) by blue and red radiation (K. Shimazaki *et al*.): signalling, receptors, $H^+$ pump, $K^+$ uptake, energy sources *etc*. are discussed. Cyclic electron transport around photosystem 1 (T. Shikanai) has essential function in both photoprotection and photosynthesis. Genetic studies helped rediscover this basic mechanism of photosynthetic electron transfer, its components, subunits, and machinery. Biosynthesis of tetrapyrroles (chlorophylls, hemes, siroheme, phytochromobilin) has a common biosynthetic pathway and is important for plant photosynthetic activities, plastid-to-nucleus signal transduction, organ abscission, cell-death program, acclimation to irradiance, *etc*. (R. Tanaka and A. Tanaka). Genetic modification of this pathway may help in agricultural and horticultural applications (reduction in green colour contamination, formation of herbicide-tolerant plants, "stay-green" phenotype, *etc*.).

The review on the architecture of root systems (K.S. Osmond *et al.*), root responses to endogenous and exogenous factors, and formation of root meristem includes also approaches to studying root branching (microarray analysis, proteomics, isolation of modifiers).

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