

Regulation of potato tuberization by daylength and gibberellins

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ABSTRACT Tuberization of *S. tuberosum* ssp. *andigena* is induced by short days (SD) and prevented by long days (LD) or by interruption of the long nights with a 30 min night break (SD+NB). Antisense inhibition of the *StGA20ox1* gene, encoding the GA 20-oxidase biosynthetic enzyme results in early tuberization in SD, but does not overcome the need of SD conditions for tuber induction. Using RT-PCR differential display of leaves from plants grown under inductive (SD) and non-inductive (SD+NB) conditions we have isolated clone PHOR1 encoding a novel arm-repeat containing protein with homology to *Drosophila* armadillo. *PHOR1* mRNA oscillates with a diurnal rhythm and is up-regulated in plants induced for tuberization. Transgenic lines inhibited in *PHOR1* expression are semi-dwarf and show a reduced response to GAs, which is consistent with a function of PHOR1 in GA signaling. These results indicate that changes in GA sensitivity are involved in mediating tuber induction.

Introduction

Tuberization of potato plants is strongly influenced by daylength, with short days (SD) in general promoting tuber formation. Strength of the photoperiodic response varies among different genotypes (Ewing, 1995). While commercial potato varieties are relatively insensitive to daylength conditions, potato wild species like *Solanum demissum* or some lines of *S. tuberosum* ssp. *andigena*, are completely dependent on photoperiod for tuber induction. These species tuberize only in SD conditions, and do not form tubers when grown in long-days (LD) or SD supplemented with a night-break (SD+NB; Ewing and Struick, 1992). Therefore, they are an excellent model system in which to study of photoperiodic control of tuberization. Because of their strict photoperiodic response, it is possible to have sets of plants induced and non-induced for tuber formation just by growing them under different light regimes. Although these species are designated as SD potato species, it has been shown that it is the overall length of the night period what determines the tuberization response. Long night interruptions with a red light night break are more effective than night break treatments with lights of other wavelengths. The red light inhibitory effect on tuberization can be, in its turn, reversed by a far red light treatment given immediately afterwards, thus providing evidence for the involvement of the photoreceptor phytochrome in this response (Batutis and Ewing, 1982).

Work in our laboratory has demonstrated that the light stable phytochrome *PHYB* is the major photoreceptor involved in regulation of tuber induction. Using an antisense approach in the SD species *S. tuberosum* ssp. *andigena* we have shown that plants

with reduced levels of expression of *PHYB* have lost photoperiodic control of tuber formation and form tubers both under SD and LD or SD+NB conditions (Jackson *et al.*, 1996). Loss of the photoperiodic response in these plants suggests that *PHYB* exerts a negative control on tuberization, by inhibiting tuber induction under non-favorable conditions. Grafting experiments have demonstrated that this photoreceptor controls the synthesis of a graft-transmissible inhibitory signal that is produced in LDs, and is absent or inactive in the *PHYB*-antisense plants (Jackson *et al.*, 1998). Several lines of evidence implicate gibberellins (GAs) as components of this inhibitory signal. GAs were reported to have an inhibitory effect on tuber induction and GA activity was shown to decrease when leaves are exposed to SD conditions (Ewing, 1995; Kumar and Wareing, 1974). Furthermore, treatments with the inhibitors of GA-biosynthesis, ancymidol and paclobutrazol, are able to promote tuberization of the *S. tuberosum* ssp. *andigena* plants under non-inductive LD conditions (Jackson and Prat, 1996).

Results

Changes in the levels of expression of GA 20-oxidase and 3 β -hydroxylase affect tuber induction and tuber yield

To investigate the possible role of GAs in the control of tuber induction, we did isolate clones encoding the three last steps of the GA biosynthetic pathway. By using a PCR approach we have isolated three cDNA clones encoding the enzyme GA 20-oxidase, two cDNA clones encoding 3 β -hydroxylase, and one cDNA clone encoding GA 2-oxidase (Carrera *et al.*, 1999; Bou *et al.*, manuscript in preparation). These genes were found to be under negative feedback (the GA 20-oxidase and 3 β -hydroxylase genes) and feed-forward (GA 2-oxidase) regulation by the end-product of the pathway GA₁, and their transcript levels were diurnally regulated, with opposite patterns of expression detected for the GA 20-oxidase and 3 β -hydroxylase genes.

Antisense inhibition of the GA 20-oxidase gene resulted in potato plants with reduced stem length and darker green leaves. These plants tuberized under SD conditions earlier than the controls and showed an increased tuber yield, which establishes a positive correlation between decreased GA activity and tuber induction (Carrera *et al.*, 2000). Over-expression of this cDNA resulted in taller plants that tuberized later than controls. In opposite to this, over-expression of the 3 β -hydroxylase gene, resulted in only slightly taller plants that under SD conditions tuberized earlier than the controls. This result was largely unexpected and can be in part explained by a differential mobility of the

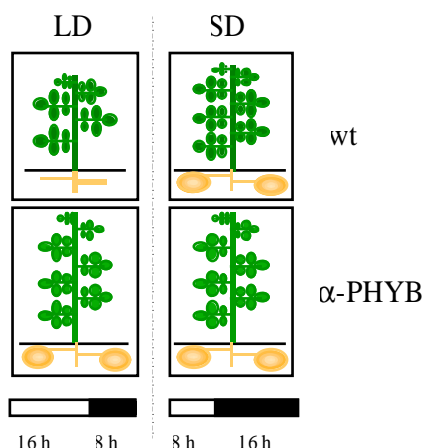


Fig. 1. Phytochrome B mediates the photoperiodic control of tuber formation in potato. *S. tuberosum ssp. andigena* antisense plants inhibited in *PHYB* expression tuberize under both SD and LD or SD+NB conditions.

GA₂₀ precursor as compared to the GA₁ product. Whereas the precursor GA₂₀ would be readily transported throughout the plant, GA₁ might be a non mobile product active only in the tissues where it is synthesized. In northern analysis we detected relatively high levels of GA 20-oxidase gene expression in the leaves as compared to other plant tissues, which indicates that this tissue is the main source of GA₂₀ within the plant. In the GA 20-oxidase over-expressers, transport of GA₂₀ from the leaves to the stolons is increased, and thus tuber induction is delayed. However, in the GA 3 β -hydroxylase over-expresser lines, GA₂₀ produced in the leaves would be rapidly converted into GA₁, which can not be transported out of the leaves. Increased conversion of GA₂₀ into GA₁ in these plants would result in a decreased export of GA₂₀ from the leaves and therefore decreased synthesis of GA₁ in the stolons, thus leading to early tuberization.

Together, these results establish a role of GAs in the control of tuber induction in potato. The fact that down-regulated expression of the GA 20-oxidase gene does not overcome the need for SD conditions, however, suggests that in addition to a decrease in GA biosynthesis, SD conditions activate an additional inductive signal that is required for tuber induction.

SD conditions induce accumulation of PHOR1, an armadillo-related protein with a function in GA signaling

Grafting experiments have shown that the principal site of perception of the photoperiodic signal is in the leaves. Under favorable SD conditions, the leaves produce a mobile inductive signal that is transported to the stolons to induce tuber formation. To gain an insight on the mechanisms implicated in this signaling process, we

have used RNA display to examine the expression patterns of leaves from plants grown under inducing (SD) and non-inducing (SD+NB) conditions. Using this approach we have isolated clone *PHOR1* (*photoperiod responsive 1*) encoding an arm-repeat protein with homology to the *Drosophila* segment polarity protein *armadillo* (Monte *et al.*, submitted). Expression of the *PHOR1* gene oscillates with a diurnal rhythm characterized by two peaks of transcript at dawn and dusk. Antisense inhibition of *PHOR1* produces a semi-dwarf phenotype similar to that of GA-deficient plants. The antisense *PHOR1* lines exhibit shorter internodes, reduced petiole length and broader leaves, and under SD conditions tuberize earlier than controls. Dose-response curves to exogenous GA showed that GA-response is reduced in these plants. Measurements of endogenous GA levels evidenced a higher GA content in the antisense lines compared to the non-transformed controls. As evidence of an impaired GA response, they exhibit altered feed-back regulation of the GA 20-oxidase and GA 2-oxidase transcripts. Levels of expression of the *GAST1* transcript were also found to be reduced in the antisense lines, indicating an altered GA-regulated gene expression in these plants. Subcellular localization studies using a translational fusion of the PHOR1 protein to GFP showed that GAs induce a rapid translocation of the protein from the cytoplasm to the nucleus, this migration being blocked by treatment with the GA-biosynthesis inhibitor ancymidol. These results are consistent with a role of PHOR1 in GA signal transduction, and suggest a positive function of this protein in the GA signaling cascade, in contrast to GAI or SPY which were reported to act as GA response repressors.

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