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Overexpression of *Arabidopsis thaliana LOV KELCH REPEAT PROTEIN 2* promotes tuberization in potato (*Solanum tuberosum cv.* May Queen)

Hideyuki Inui [a,1], Yasunobu Ogura [b,1], Tomohiro Kiyosue [b,*]

[a] *Research Center for Environmental Genomics, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan*
[b] *Department of Life Science, Faculty of Science, Gakushuin University, 1-5-1 Mejiro, Toshima-ku, Tokyo 171-8588, Japan*

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ABSTRACT

Potato tuberization is induced under short-day conditions and repressed under long-day conditions. In this study, we produced transgenic potatoes overexpressing either *Arabidopsis thaliana LOV KELCH PROTEIN 2* (35S:LKP2) or *CONSTANS* fused with a transcription repressor motif (35S:CO-Rep). In an in vitro tuberization assay, the average number of tubers per plant was greater in 35S:LKP2 plants than in vector-control plants, but lower in 35S:CO-Rep plants. Under long-day conditions in soil, all 35S:LKP2 plants tuberized, whereas most control plants and 35S:CO-Rep plants did not. These results suggest genes involved in flowering time regulation can be used to control potato tuber production.
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1. Introduction

Plants utilize light both as a signal for various physiological responses and as the source of energy for photosynthesis; therefore, light is one of the most important factors in their environment. One of the light-related environmental factors important for the regulation of plant development is the seasonal change in photoperiod (day length). Flowering is influenced by photoperiod through the induction of florigen, a non-cell-autonomous signal that promotes flowering. In the facultative long-day (LD) plant *Arabidopsis thaliana*, the expression of *FLOWERING LOCUS T* (*FT*), a florigen gene, is induced under LD conditions and leads to flowering [1]. *FT* expression is induced by CONSTANS (CO), a transcription factor containing a zinc finger domain: expression of *CO* is induced late in the day under LD conditions [2]. Similar genes and regulatory mechanisms exist in the short-day (SD) plant rice (*Oryza sativa*): rice Heading date 1 (Hd1), an ortholog of CO, induces the expression of *Hd3a*, an ortholog of *FT* that functions as a florigen gene [3] under SD conditions. However, Hd1 represses *Hd3a* expression in rice under LD conditions [4].

CO/*FT*-dependent photoperiodic regulation is also believed to be involved in potato tuberization, which is promoted under SD conditions and delayed under LD conditions [5]. In wild potatoes, such as *Solanum tuberosum* ssp. *andigena*, tuberization is strictly dependent on SD conditions [6]. In modern potato cultivars, the degree of dependence on photoperiod as an environmental cue differs among species. Both photoperiod and levels of endogenous hormones are important factors regulating potato tuberization [5,7,8]. Potato tubers contain high levels of nutrients such as starch, protein, and vitamin C; hence, an understanding of tuberization control has practical applications for agriculture and human nutrition.

A grafting experiment suggested that tobacco florigen could promote potato tuberization [9]. Overexpression of the microRNA *miR172*, which accelerated flowering in *A. thaliana*, promoted potato tuberization under LD conditions [10]. In contrast, overexpression of *A. thaliana CO* (*AtCO*) suppressed tuberization in transgenic *andigena* potato under SD conditions [11].

We are interested to know how factors with an effect on flowering in *A. thaliana* opposite to that of CO would affect potato tuberization. The overexpression of either *LOV KELCH PROTEIN 2* (*LKP2*), a member of the ADO/FKF/LKP/ZTL family, or *CO* fused with a dominant transcription repressor motif (*CO-Rep*) delays flowering in *A. thaliana* under LD conditions [12–14]. In this study, we investigated the effect of *A. thaliana LKP2* and *CO-Rep* overexpression on potato tuberization.

* Corresponding author. Fax: +81 3 5992 1029.
E-mail address: tomohiro.kiyosue@gakushuin.ac.jp (T. Kiyosue).
[1] These authors contributed equally to this work.

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2. Materials and methods

2.1. Plant materials, plasmids, and transformation

S. tuberosum cv. May Queen was used for transformation. Microtubers were grown and *Agrobacterium*-mediated transformation was performed as described previously [15]. Tuber disks were inoculated with *Agrobacterium tumefaciens* strain LBA4404 harboring the chosen transformation plasmid. For overexpression, the plasmids consisted of *AtLKP2* or *AtCO-Rep* cDNAs cloned into the pBE2113 vector, which contains the cauliflower mosaic virus 35S promoter; the resultant plasmids were named pBE2113-*LKP2* [12] and pBE2113-*CO-Rep* [14]. pBE2113 was included in the experiment as a vector control. The regenerated transformants were selected on the basis of their growth in MS (Murashige and Skoog) medium containing 100 mg/L kanamycin and 200 mg/L cefotaxime; transformation was confirmed by polymerase chain reaction (PCR) amplification of the kanamycin-resistance gene. Transformants were propagated in vitro from cuttings grown on MS medium containing 2% sucrose under LD conditions (16-h light/8-h dark) [16].

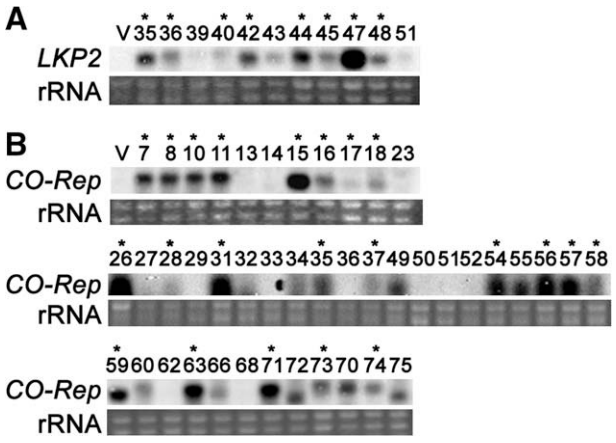

Fig. 1. RNA gel blot analysis of transgenic potato. Asterisks indicate the transformants used in the in vitro tuberization experiment. (A) Accumulation of *LKP2* mRNA in 35S:LKP2 transformants. (B) Accumulation of *CO-Rep* mRNA in 35S:CO-Rep transformants. rRNAs as a loading control were visualized by ethidium bromide staining.

2.2. RNA blot analysis

Leaves from selected transformants were harvested and powdered in liquid nitrogen. Total RNA was isolated by using an RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions, separated in 1% agarose gel containing formaldehyde, and transferred onto a nylon membrane. A digoxigenin-11-dUTP (DIG-dUTP)-labeled RNA fragment was prepared from the full-length coding region of *AtLKP2* or *AtCO* and used for hybridization according to the manufacturer's instructions for DIG labeling (Roche Diagnostics). Chemiluminescence signals were generated with alkaline-phosphatase-conjugated anti-DIG antibody and CDP-Star reagent (GE Healthcare) and detected by using a light capture system (Model AE-6955: Atto).

2.3. Tuberization assays

Transformants propagated as above for 1 month were cultured in MS liquid medium containing 15% sucrose for 2 months in the dark. The number of tubers per plant and the total weight of tubers per plant were measured and statistically analyzed using Student's *t*-test. We examined tuberization in plants grown in soil under LD conditions. Plants from 8 lines per vector were grown in vitro for 4 weeks, transferred to soil in 15-cm pots, and grown under LD conditions (16-h light/8-h dark) for an additional 6 weeks. The number of tubers on each plant was counted and statistically analyzed using Student's *t*-test.

3. Results

3.1. Expression of AtLKP2 or AtCO-Rep in transgenic potatoes

We used *S. tuberosum cv.* May Queen, which is a very popular potato cultivar in Japan and easy to transform [15,16]. May Queen plants produce higher numbers of tubers under SD conditions than under LD.

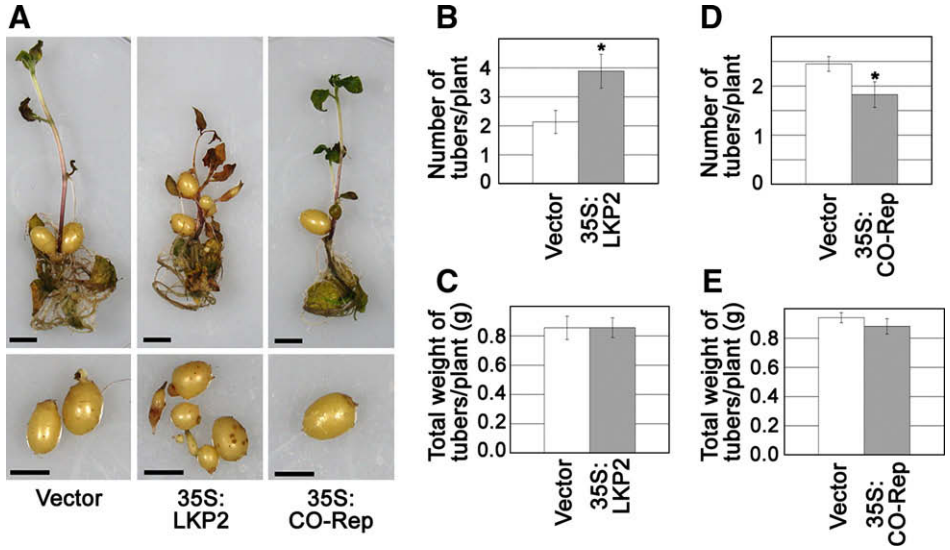

Fig. 2. In vitro tuberization of 35S:LKP2 and 35S:CO-Rep plants. (A) Whole plants (upper) and tubers (lower) of vector control, 35S:LKP2, and 35S:CO-Rep transformants are shown. Bars indicate 10 mm. (B and D) The average number of tubers per plant is compared between vector control and (B) 35S:LKP2 and (D) 35S:CO-Rep plants. Asterisks indicate a significant difference between vector control and transformants ($P < 0.05$) by Student's *t*-test. (C and E) The average total weight of tubers per plant is compared between vector control and (C) 35S:LKP2 and (E) 35S:CO-Rep plants. Bars in B–E indicate standard errors. For 35S:LKP2, $n = 8$; for 35S:CO-Rep, $n = 22$; for vector control, $n = 70$.

Fig. 3. Tuberization in soil under long-day conditions. (A) Whole plants representing vector control, (B) 35S:LKP2 transformants, (C) tubers of 35S:LKP2 transformants shown in (B), (D) 35S:CO-Rep transformants. Bars indicate 50 mm (A, B and D) and 10 mm (C). (E) Average number of tubers per plant. (F) Average total weight of tubers per plant. Bars indicate standard error ($n = 8$ for each of the three vectors). An asterisk indicates a significant difference between the vector control and 35S:LKP2 plants ($P < 0.05$) by Student's *t*-test.

Multiple transgenic lines of *S. tuberosum cv*. May Queen containing each of the three vectors, pBE2113 (vector control), pBE2113-*LKP2* (35S:LKP2), and pBE2113-*CO-Rep* (35S:CO-Rep), were obtained. Accumulation of *AtLKP2* mRNA or *AtCO-Rep* mRNA in transgenic plants (11 lines of 35S:LKP2 and 43 lines of 35S:CO-Rep) was analyzed by RNA gel blot analysis. We selected 8 lines of 35S:LKP2 (Fig. 1A) and 22 lines of 35S:CO-Rep (Fig. 1B) for the in vitro tuber assay. Several lines in which the transgene mRNA band showed unexpectedly slow migration were excluded from further analysis.

3.2. Tuber formation in vitro was promoted in 35S:LKP2 potatoes

Because tuber formation can be induced in vitro by treatment with high levels of sucrose under continuous dark [17], we examined tuber formation of the transgenic potatoes under these conditions. Representative plants derived from each of the three vectors are shown in Fig. 2A. The average number of tubers per plant in 35S:LKP2 lines was approximately double that in vector control lines (Fig. 2B), and the difference was statistically significant ($P < 0.05$). However, because several of the 35S:LKP2 tubers were very small, there was no significant difference in the total weight of tubers per plant between the control and 35S:LKP2 lines (Fig. 2C). The average number of tubers per plant was significantly lower in 35S:CO-Rep lines than in vector control lines ($P < 0.05$; Fig. 2D), but again there was no significant difference in the total weight of tubers per plant (Fig. 2E).

3.3. Tuber formation in soil was promoted in 35S:LKP potatoes

As described above, our in vitro study showed promotion of tuberization in *LKP2*-overexpressing potato plants. LKP2 affects photoperiodic responses in Arabidopsis under LD conditions [12], and potato tuber formation is controlled by photoperiod. Thus, we expected that tuberization might be able to occur efficiently in *LKP2*-overexpressing potato plants in soil under LD conditions, which do not normally induce tuber formation efficiently.

Representative plants containing each of the three vectors are shown in Fig. 3A, B, and D. Under these LD conditions, the vector control and 35S:CO-Rep transgenic plants rarely formed tubers (only 1 of 8 independent lines from each of these vectors formed tubers), whereas all 8 independent lines of 35S:LKP2 transgenic plants formed tubers (Fig. 3C). The average number of tubers per plant in the 35S:LKP2 lines was approximately five times that in the vector control lines (Fig. 3E), and the difference was statistically significant ($P < 0.05$). The total weight of tubers per plant showed a significant difference between the control and 35S:LKP2 lines, but not between the control and 35S:CO-Rep lines (Fig. 3F).

4. Discussion

The promotion of tuberization in 35S:LKP2 transgenic potato lines and its repression in 35S:CO-Rep lines indicated that potato tuberization could be regulated by expression of *A. thaliana LKP2* and *CO-Rep*. To our knowledge, this is the first study of the promotion of tuberization in cultivated species of potato caused by a gene (*LKP2*) whose overexpression causes late flowering in the LD plant *A. thaliana*. The total weight of tubers in the soil experiment was greater in the 35S:LKP2 lines than in either the vector control lines or the *CO-Rep* lines. This result raises hope for the use of genes involved in flowering regulation to expand the area and growing season of potato cultivation. However, some of the 35S:LKP2 tubers grown in soil were smaller than normal. It is possible that the conditions used in this study did not allow the tubers to develop to maturity. Further experiments in the field could clarify this.

Tuberization was more frequent in 35S:LKP2 plants than in vector-control plants, whether grown in vitro on high-sucrose medium or in soil under LD conditions. These results indicate that *AtLKP2* overexpression induced tuberization. *AtLKP2* overexpression and *CO* overexpression [11] had opposite effects on tuberization. A possible mechanism for the function of LKP2 is suggested by experiments with the Arabidopsis gene *ZEITLUPE* (*ZTL*), or *LKP1* (*LOV KELCH PROTEIN 1*), which encodes a protein with a structure highly similar to that of LKP2 and causes a similar phenotype when overexpressed. Overexpression of *ZTL*/*LKP1* resulted in a decrease in *CO* and *FT* mRNA levels, which led to late flowering [18,19]. The similarities between ZTL/LKP1 and LKP2 suggest that LKP2 might also affect the photoperiodic regulation of flowering via the *CO*/*FT* pathway.

On the basis of previous studies of *phytochromeB* (*phyB*) antisense and *AtCO*-overexpressing transgenic plants [9,11,20] and

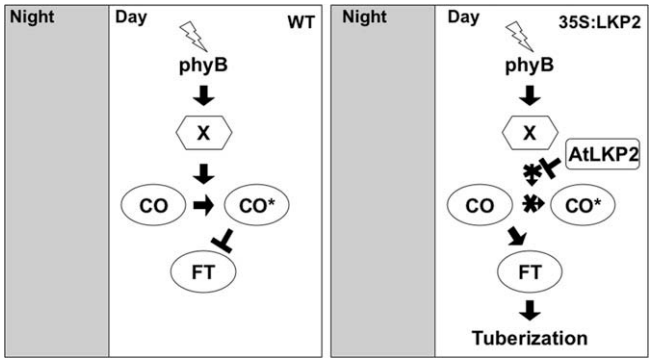

Fig. 4. Hypothesized mechanism for regulation of potato tuberization under long-day conditions. In wild-type potato (left panel), phyB acts directly or indirectly on CO to repress *FT* expression. In 35S:LKP2 potato (right panel), the effect of phyB on CO is blocked by AtLKP2. CO induces *FT* expression, leading to the promotion of tuberization.

on the photoperiodic regulation of flowering in rice, we postulate a model of photoperiodic regulation of potato tuberization (Fig. 4). Under LD conditions, phyB, a red/far-red light receptor, acts directly or indirectly on CO to repress *FT* expression, whereas under SD conditions, CO activates *FT* expression to induce tuberization [5]. If we apply the results of 35S:LKP2 transgenic potato in soil to this model, the phyB-mediated modification of CO activity leading to *FT* repression may be blocked by *AtLKP2* expression in 35S:LKP2, so that CO induces *FT* expression, leading to the promotion of tuberization. However, we do not exclude the possibility that AtLKP2 affects *FT* expression independent of CO. The results of the in vitro tuberization experiment (conducted in the dark with high sucrose) are not readily explained by this model. One possibility is that *AtLKP2* overexpression increases the effect of sucrose on the induction of tuberization, although the factors involved in this regulation remain unknown.

In 35S:CO-Rep transgenic plants, tuberization in vitro on high-sucrose medium under continuous darkness was less frequent than in vector-control plants. This result suggests that *AtCO-Rep* expression repressed tuberization. Potato CO is postulated to activate *FT* transcription in the night under SD and to repress *FT* transcription in daytime under LD [5]. *AtCO-Rep* may have mimicked the repressor type of potato CO, which led to a reduction in tuberization in the in vitro assay.

In this study, we produced transgenic potato lines expressing either *A. thaliana LKP2* (35S:LKP2) or *CO-Rep* (35S:CO-Rep). In an in vitro tuberization assay, 35S:LKP2 promoted tuberization, whereas 35S:CO-Rep repressed it. In addition, 35S:LKP2 promoted tuberization in plants grown in soil under LD conditions. These results suggest that potato tuberization can be regulated by a factor involved in photoperiodic flowering in *Arabidopsis*.

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