Original Paper Plant Biotechnology, **21** (1), 9-14 (2004)

Low Sugar Status Promotes Endogenous ABA Level and ABA Sensitivity in *Arabidopsis*

Chizuko MORITA-YAMAMURO[1], Paolo VERNIERI[2] and Junji YAMAGUCHI[1]*

[1]*Division of Biological Sciences, Graduate School of Science, Hokkaido University, Kita-ku N10-W8, Sapporo 060-0810, Japan*
[2]*Dip. Biologia Piante Agrarie, University of Pisa, via Mariscoglio 34, 56124 Pisa, Italy*
**Corresponding author* E-mail: jjyama@sci.hokudai.ac.jp

Received 22 July 2003; accepted 18 August 2003

Abstract

Antagonistic interaction between endogenous sugar status and abscisic acid (ABA) signaling was examined in *Arabidopsis*. Endogenous ABA content in suspension-cultured cells and seedlings grown in the dark increased following reduction in endogenous sugar content. Expression of *Arabidopsis* 9-cis epoxycarotenoid dioxygenase (*AtNCED*) genes, which encode key enzymes for ABA biosynthesis and *ABSCISIC ACID INSENSITIVE 3 (ABI 3)* which controls ABA sensitivity was enhanced by sugar starvation. GUS activity analysis in transgenic *Arabidopsis* carrying *GUS* controlled by the *ABI3* promoter showed that *ABI3* was expressed, when sugar was depleted and thereby leaf development was terminated, in the shoot apical meristem. We conclude that sugar depletion promotes ABA signaling to terminate leaf development in *Arabidopsis* seedlings. Antagonistic effects of sugar on ABA signaling in the process of vegetative quiescence processs are discussed.

Key words: ABA, ABA biosynthesis, *ABI3*, sugars, vegetative quiescence process.

Abbreviations

ABA, abscisic acid; *NCED*, 9-cis epoxycarotenoid dioxygenase; SAM, shoot apical meristem.

Introduction

In higher plants, sugars play important physiological and developmental roles, for example in shoot development, flowering, seed development and senescence. Abundance or depletion of endogenous sugars can enhance or repress the expression of many genes (Koch, 1996; Sheen *et al.*, 1999; Wobus and Weber, 1999). Physiological and genetic analyses pointed to a crosstalk between sugar- and abscisic acid (ABA)-signaling. ABA is a plant hormone that regulates physiological and developmental processes including stomatal closure, protection from a variety of environmental stresses, and maintenance of seed dormancy which is believed to be a strategy to survive severe environmental conditions. In rice and barley embryos, application of glucose at 25-90 mM reduces endogenous ABA content and represses expression of the ABA-inducible gene, *Rab16A*, by decreasing transcription rate and mRNA stability (Perata *et al.*, 1997; Toyofuku *et al.*, 2000). During germination, glucose at 35 mM prevents the ABA-induced inhibition of radicle emergency in *Arabidopsis* (Finkelstein *et al.*, 2000).

Seedlings germinated in the dark show long hypocotyls, closed small cotyledons, and an arrest of the shoot apical meristem (SAM). These morphological peculiarities are due to adaptive quiescence process. The relationships between seed dormancy and adaptive quiescence process in the shoot apex have long been discussed. Recent molecular genetic research on *Arabidopsis* mutants revealed that the *ABSCISIC ACID-INSENSITIVE 3 (ABI3)* gene which is related to ABA sensitivity may play an important role in both processes. Rohde *et al.* (1999) demonstrated that *ABI3* is expressed in an arrested shoot apices during vegetative quiescence to terminate cell division. Surprisingly, sugar application breaks vegetative quiescence and represses expression of *ABI3* indicating that sugars and ABA may act antagonistically in the vegetative quiescence process. The possibility that the endogenous sugar status controls ABA biosynthesis and sensitivity remains to be evaluated.

In this study, we attempt to clarify the relationship between the endogenous sugar status and ABA

signaling using suspension-cultured cells and seedlings of *Arabidopsis*. Furthermore, we discuss the antagonistic effects of sugar on ABA signaling in the vegetative quiescence process and its physiological significance.

Materials and Methods

Plant materials and growth conditions

Arabidopsis thaliana (L. Heynh. ecotype Landsberg *erecta*) was grown at 22℃ Suspension-cultured cells of *Arabidopsis* were grown in Murashige and Skoog medium (Murashige and Skoog, 1962) supplemented with vitamins, 3% sucrose and 2,4-D (0.5 mg l^{-1}). Suspension cultured cells were kept in the dark at 22℃ with shaking at 125 rpm. Seeds of P*ABI3*-GUS transgenic plants (*Arabidopsis thaliana*, Landsberg *erecta*) that carried a chimeric gene consisting of the *ABI3* promoter with the GUS gene were provided by Dr. Jérôme Giraudat (CNRS, France). For germination, seeds were surface-sterilized and placed on Murashige and Skoog medium supplemented with 10 g l^{-1} sucrose. After an overnight cold treatment for synchronized germination, seeds were grown at 22℃, 50% relative humidity, and a 16 h light: 8 h dark cycle. For dark treatments, plates were wrapped twice with aluminum foil and placed in a dark container in the same culture room.

ABA assay

ABA content in *Arabidopsis* suspension-cultured cells was determined by radioimmunoassay using a highly specific monoclonal antibody as previously described (Vernieri *et al.*, 1989; Walker-Simmons *et al.*, 1990). The absence of cross-reacting material in the extracts was verified by HPLC fractionation of the crude extracts (Perata *et al.*, 1997).

Sugar assay

Extraction of plant material and the glucose assay were performed as previously described (Guglielminetti *et al.*, 1995).

RT-PCR analysis

A single-stranded cDNA was synthesized from RNA isolated from suspension-cultured cells. First-stranded cDNA synthesis was performed with reverse transcriptase using 1 μg of total RNA and oligo(dT) primer (RNA PCR kit, TaKaRa SHUZO CO., LTD. Siga, Japan). The PCR (total volume: 20 μl) was performed using 0.2 units of Taq DNA polymerase (Ex Taq, TaKaRa SHUZO CO., LTD. Siga, Japan). The gene-specific primers were designed to produce DNA fragments from *AtNCED2*, 3, 5, 6, and *ABI3*. *NCED* cDNAs of *Arabidopsis* were isolated previously (Iuchi *et al.*, 2001). The primers used (see **Table 1**) were *AtNCED2-1* and *AtNCED2-2* (Set 1); *AtNCED3-1* and *AtNCED3-2* (Set 2); *AtNCED5-1* and *AtNCED5-2* (Set 3); *AtNCED6-1* and *AtNCED6-2* (Set 4); *ABI3-1* and *ABI3-2* (Set 5). DNA fragments of *AtNCED2, 3, 5, 6* and *ABI3* were amplified from cDNAs by PCR using the synthetic oligonucleotide set 1, 2, 3, 4, and 5, respectively (**Table 1**). The amount of template cDNA required and the number of PCR cycles necessary were determined in preliminary experiments to ensure that amplification occurred in the linear range and allowed good quantification of the amplified products. The amplified DNA products (10 μl of each reaction) were separated on a 1.2% (w/v) agarose gel, transferred to a nylon menbrane (Hybond-N^+, Amersham Pharmacia Biotech, Buckinghamshire, U.K.), and hybridized with [^{32}P]-labelled cDNA fragments at 65℃. The filter was washed twice with 2x SSC, 0.1% SDS at 65℃ for 15 min and autoradiographed.

Table 1 The PCR primer name and sequence of synthetic oligonucleotides

Primer name	Sequence(5′~3′)
AtNCED 2-1	CCCGGGATCCCTCAAGCCTCTCTACT
AtNCED 2-2	CCCGGGATCCTTTATACGGATTCTGA
AtNCED 3-1	ATGGCCAGTCGTGTCACACGTA
AtNCED 3-2	ACCGGTTCCGTGTGCCGGGTCG
AtNCED 5-1	ATGACGATAATAACCATTATTTCT
AtNCED 5-2	GTCTAAAACCATAGCGGCGGTTC
AtNCED 6-1	ATGCAACACTCTCTTCGTTCTGAT
AtNCED 6-2	AACCTGGTTATCAAAACCGATACT
ABI 3-1	ATGAAAAGCTTGCATGTGGC
ABI 3-2	TTCTTGCCGCTGATTCAATC

β-Glucuronidase (GUS) assays

GUS activity was assayed as described by Jefferson (1987). After the histochemical reaction, samples were fixed with 3% glutaraldehyde in phosphate buffer (50 mM, pH 7.0) for 1 h, washed twice with the same phosphate buffer, and passed through an ethanol series to remove chlorophyll prior to observation under a light microscope.

Quantitation of sugar by electrophoresis

Sugar separation was carried out on fused-silica capillaries with 104 cm effective length and 50 μm I.D. The electrolyte solution contained 20 mM 2,6-pyridinedicarboxylic acid and 0.5 mM n-hexadecyltrimethylammonium hydroxide which was used to reverse the direction of the electroosmotic flow. The electrolyte pH was adjusted to 12.3 with NaOH. Prior to first use, each new capillary was pretreated with the electrolyte solution for 20 min. Before each injection, the capillary was preconditioned for 7 min by flushing with the electrolyte. The sample was injected with a pressure of 50 mbar for 15.0 s. The applied voltage was 30 kV, and the capillary temperature was thermostated at 15℃. Detection was carried out by indirect UV detection using a diode-array detector. The signal wavelength was 350 nm with the reference at 230 nm.

Results

Sugar depletion promotes endogenous ABA level in suspension-cultured cells

We examined the correlation of sugar content and endogenous ABA level in suspension-cultured cells of *Arabidopsis* grown in a standard medium (Treatment 1 in **Fig. 1**), as well as after 2 and 3 days incubation in sucrose-free medium (sugar starvation: treatment 2 and 3, respectively), and after 2 days incubation in a medium containing 3% sucrose following 2 days of sugar starvation (Treatment 4). The ABA content in treatment 1 (control) was less than 30 ng per gram fresh weight (ng g^{-1} FW; **Fig. 1A**). During sugar starvation (Treatments 2 and 3), the ABA content increased up to 220 ng g^{-1} FW (approximately 8-fold), and endogenous sugar level was almost depleted (Treatments 2 and 3 in **Fig. 1B**). Addition of sucrose (Treatment 4) restored the control levels of sucrose, glucose, and ABA (**Fig. 1A, B**). Since sucrose and glucose are metabolizable, non-metabolic sugars and their derivatives will be useful to distinguish the sugar-specific effects from the osmotic effect. Mannitol was also used to apply to the cells at the same conditions as sucrose to evaluate the possibility of osmotic effects. However, ABA level was similar in sugar-starved and mannitol-treated cells (**Fig. 1A**), ruling out osmotic effects on endogenous ABA level. These results indicate that sugar depletion promotes endogenous ABA level, and that high sugar content suppresses endogenous ABA in suspension-cultured cells.

Sugar depletion enhances expression of genes for ABA biosynthesis and sensitivity in suspension-cultured cells

NCED encodes a 9-cis-epoxycarotenoid dioxygenase that catalyzes the rate-limiting step of ABA biosynthesis, whereas the *ABI3* gene product is

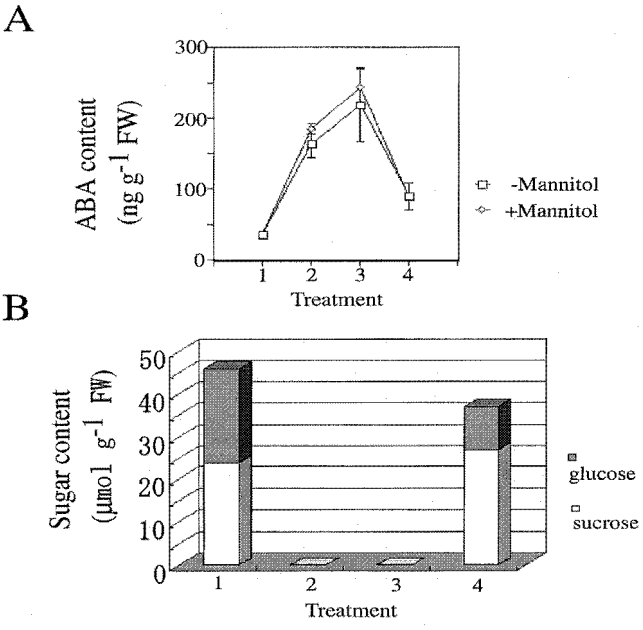

Fig. 1 Effect of sucrose application on endogenous ABA (A) and sugar (B) content of suspension-cultured cells of *Arabidopsis*.

The suspension-cultured cells were grown in a standard medium (Treatment 1), or were incubated for 2 or 3 days in sucrose-free medium (sugar starvation; treatment 2 and 3, respectively), or were cultivated in a medium containing 3% sucrose for 2 days after they had undergone 2 days of sucrose starvation (Treatment 4). Mannitol application (◇) instead of sucrose was carried out to evaluate osmotic effect in **Fig. 1A**. ABA content was quantified using a monoclonal antibody (see Material and Methods). Glucose and sucrose contents were estimated by an enzyme coupling assay (Guglielmineti *et al.*, 1995). FW, fresh weight. Data shown are the means (± SE) of 3 replicates.

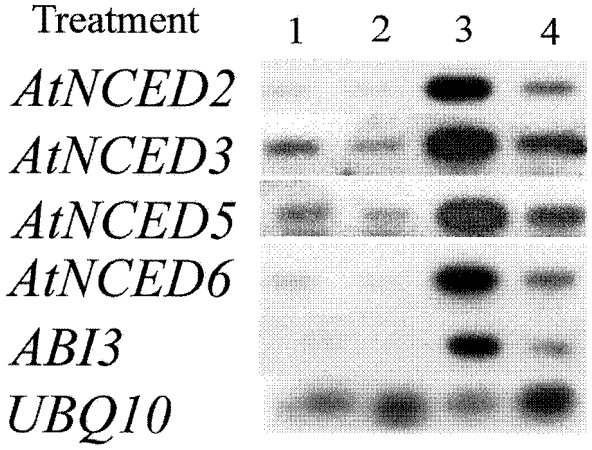

Fig. 2 Semi-quantitative detection of transcripts of *AtNCED 2, 3, 5, 6,* and *ABI3* by RT-PCR in suspension-cultured cells.

Total RNA from cells of Treatment 1, 2, 3, and 4 (compare **Fig. 1**) was extracted and analyzed. *UBIQUITIN 10 (UBQ10)* indicates a reference used for normalization.

related to ABA sensitivity. To evaluate the effect of sugars on ABA biosynthesis and sensitivity, we examined the expression of *AtNCED2, 3, 5, 6,* and *ABI3*. Total RNA from the cells after Treatments 1, 2, 3, and 4 was extracted and analyzed by semi-quantitative RT-PCR (**Fig. 2**). Sugar depletion as experienced by the cells of Treatment 3 dramatically enhanced transcription levels for *AtNCED2, 3, 5,* and *6* as compared with treatments 1 and 2. Expression of *ABI3* also increased dramatically in cells incubated in sucrose-free medium for 3 days (Treatment 3). These results suggested that sugar depletion promotes both ABA biosynthesis and ABA sensitivity in *Arabidopsis* suspension-cultured cell. This conclusion was further supported by the finding that the elevated expression levels of the ABA-related genes in the sugar-starved cells declined again after sucrose application (Treatment 4).

Endogenous sugar status regulates ABA signaling and leaf development in Arabidopsis seedlings in the dark

We demonstrated anti-parallel relationship between endogenous sugar status and ABA signaling in the suspension-cultured cells. To evaluate the significance of the putative crosstalk of endogenous sugar status and ABA signaling in seedling development, we examined etiolated transgenic *Arabidopsis* seedlings that carried a chimeric P*ABI3-GUS* construct (Parcy *et al.*, 1994).

Seedlings were grown in the dark either on vertical (the etiolated hypocotyl was mostly in contact with the plate; sugar-abundant Condition 1) or oblique (hypocotyls not in contact with the

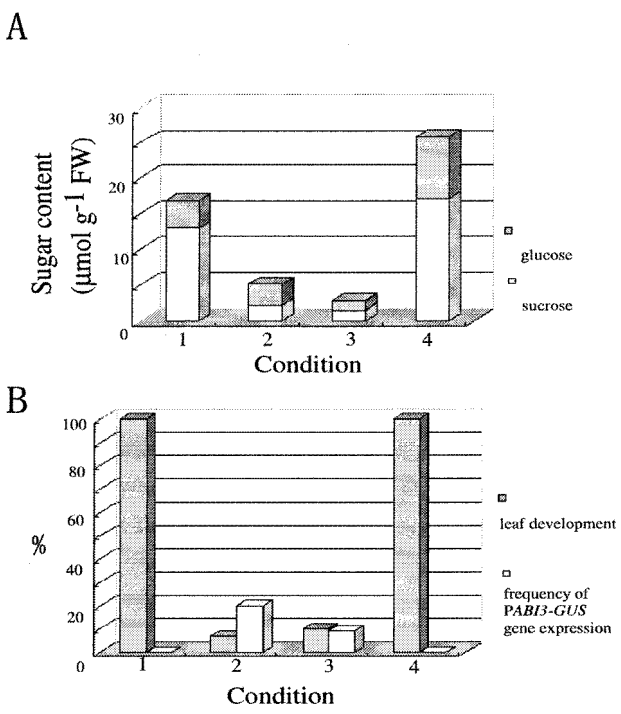

Fig. 3 Effect of endogenous sugar status on frequency of P*ABI3-GUS* gene expression and leaf development.

Seedlings grown on vertical (Condition 1; sugar abundance) or oblique (Condition 2; sugar depletion) dishes containing 2% sucrose were examined. Remainders on Condition 1 were transferred to new medium with sucrose (Condition 3; conversion from sugar abundance to depletion) and those on Condition 2 also to new medium with sucrose (Condition 4; conversion from sugar depletion to abundance) and grown with close contact to the medium for 10 days. (A) Endogenous sugar content of each condition. (B) Frequency of P*ABI3-GUS* gene expression and leaf development. Number of observations = 50. FW, fresh weight.

substrate; sugar-depleted Condition 2) dishes that contained 2% sucrose. Endogenous sugar contents were approximately 4-fold higher under Condition 1 than under Condition 2 (**Fig. 3A**). Seedlings grown under Condition 1 developed leaves at the SAM (**Fig. 3B, 4A**), and GUS activity driven by the *ABI3* promoter could not be observed in the shoot apex (**Fig. 3B, 4A**). In contrast, sugar-depleted seedlings under Condition 2 developed closed small cotyledons and an arrested SAM (**Fig. 4B**). GUS staining was detectable in around 20% of all SAMs and cotyledons of Condition 2 plants (n=50; **Fig. 3B, 4B**). To examine the reversibility of the effects described in Condition 1, seedlings were transferred to a medium without sugars (Condition 3; conversion from sugar abundance to depletion), while seedlings raised under Condition 2 were

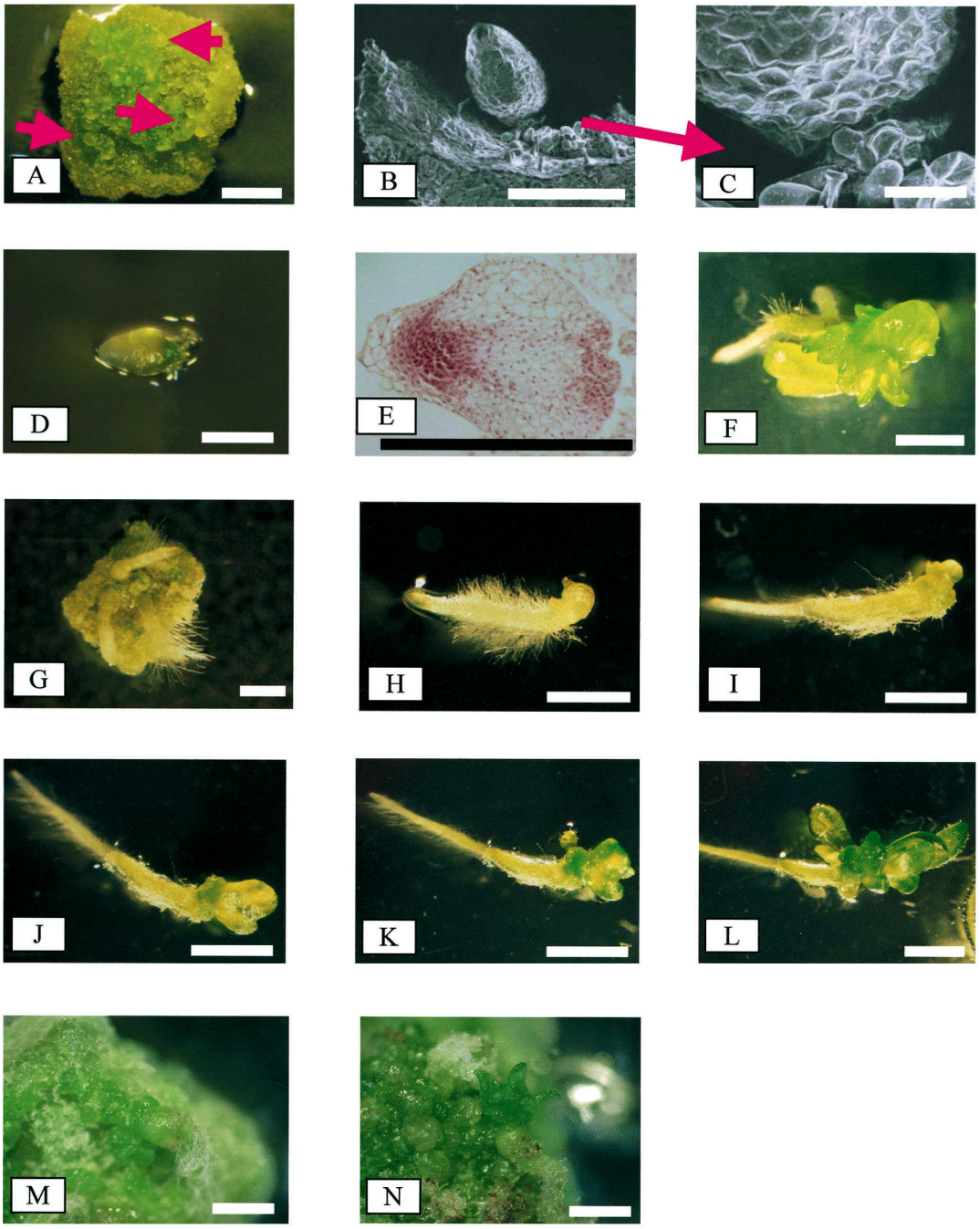

Fig. 1 Somatic embryogenesis and plant regeneration in chrysanthemum cv. Shuho-no-chikara.
A ; The leaf segment on the D2K1 medium for 15 days. Arrows indicate globular-shaped somatic embryos.
B ; The globular shaped somatic embryo observed by SEM.
C ; A magnified SEM image of a part of the suspensor shown in Fig. 1B (×5).
D ; The somatic embryo on phytohormone free MS medium for 10 days.
E ; The globular shaped somatic embryo observed by histological assay.
F ; The regenerated somatic embryo on phytohormone free MS medium for 20 days.
G ; The somatic embryo on the D2K1 medium.
H ; Transferred to phytohormone free MS medium for 3 days, I ; 5 days, J ; 7 days, K ; 20 days, and L ; 30 days.
M ; The somatic embryo on the D2K1 medium for 40 days and N ; for 50 days.
Scale bar indicates 10 mm at A, G, H, I, J, K and L, 0.2 mm at C. Those in other pictures are 2 mm.

allowed to grow on vertical (the etiolated hypocotyls was mostly in contact with plate) dishes containing 2% sucrose (Condition 4; conversion from sugar depletion to abundance). Under Condition 3, sugar content and the frequency of leaf emergence were dramatically decreased (**Fig. 3A, B**). GUS activity was observed in 8% of the shoot apices tested (n=50), indicating that the expression of P*ABI3-GUS* was induced by decreased endogenous sugar content (**Fig. 4A, B**). Under Condition 4, endogenous sugar content increased approximately 6-fold as compared to Condition 2, and there was no GUS-activity and developing leaves at the SAM (Condition 4). These results indicated that sugar depletion in *Arabidopsis* seedlings under dark conditions promoted *ABI3* expression and suppressed leaf development.

Discussion

Crosstalk between sugar and ABA signaling

Endogenous ABA level and expression of genes related to the biosynthesis and sensitivity for ABA were controlled by sugar depletion in suspension-cultured *Arabidopsis* cells. This was demonstrated by the following results: (i) ABA content increased 8-fold in the cells after sugar starvation (**Fig. 1A**); (ii) enhanced expression of *AtNCED 2, 3, 5, 6,* and *ABI3* was detectable in the cells after sugar starvation (**Fig. 2**). Although elevated ABA levels were observed in Treatment 2 (**Fig. 1**), no up-regulation of *AtNCED2, 3, 5,* and *6* was detected in these cells (**Fig. 2**). Since the *Arabidopsis* genome contains at least seven *AtNCED* genes (Iuchi *et al.*, 2001), isoforms other than those examined here might be up-regulated under the conditions of Treatment 2. In fact, Iuchi *et al.* (2001) reported that expression of *AtNCED 3* and *9* were induced by drought stress but other *AtNCEDs* were not. Moreover, the possibility that degradation of ABA was suppressed instead of up-regulation of the ABA biosynthesis could not be ruled out.

Crosstalk between sugar and ABA signaling has been studied frequently. Elevated sugar level in *Arabidopsis* seedlings leads to distinctive disorders such as inhibition of chlorophyll synthesis, accumulation of anthocyanin, and developmental arrest of leaves. These symptoms are known as the "sugar response". Genetic screening have resulted in the isolation of many sugar insensitive mutants. Cloning studies revealed that most mutants for sugar insensitivity are identical to mutants previously characterized as being defective in genes related to ABA biosynthesis or ABA sensitivity. In *Arabidopsis,* artificially elevated sugar level promoted ABA biosynthesis (Arenas-Huertero *et al.*, 2000; Huijser *et al.*, 2000; Laby *et al.*, 2000). While these findings imply a synergistic relationship between sugar and ABA levels, our present results suggest an antagonistic interdependence, i.e. endogenous ABA level increases in response to sugar starvation. Thus it seems that excess amounts as well as a lack of endogenous sugars induce increased ABA content. Since ABA level is generally enhanced in the context of responses to severe stress conditions, we suggest that both antagonistic and synergistic effects of sugar on ABA biosynthesis form elements for the adaptation of plants to environmental changes.

Sugar sensing in vegetative quiescence process.

In this study, we demonstrated the antagonistic correlation between endogenous sugar level and ABA signaling in etiolated seedlings using P*ABI3-GUS* transgenic plants (**Fig. 3B, 4**). These results are consistent with those obtained from suspension-cultured cells. In the dark, seedlings terminate leaf development by arresting the SAM to save energy. We demonstrated that sugar depletion in etiolated seedlings resulted in arrested SAMs, leading to vegetative quiescence process. This process is probably controlled by *ABI3*. Our results are in accord with the idea that ABA sensitivity in the SAM is enhanced by sugar depletion. Considering our findings in suspension-cultured cells, sugar depletion may also promote ABA biosynthesis in the seedling. ABA is known to play a role in the termination of cell division that is mediated by the induction of *ICK1*, a cyclin-independent kinase inhibitor which modulates the decrease in Cdc2a-like activity, leading to cell cycle arrest at the check-point of transition from the G_1 to the S phase (Finkelstein *et al.*, 2002). In fact, deficient and insensitive ABA mutants show de-etiolated phenotypes that were unable to stop cell division in the SAM under dark conditions (Rhode *et al.*, 2000). We propose that plants sense the cellular sugar status, and that an increased ABA signal during phases of sugar depletion leads to developmental arrest (**Fig. 5**). Under sugar-abundance, however, plants constantly produce new organs from the SAM. Presumably, these are the two alternative routes of the default program for vegetative development. ABA, which is promoted by sugar depletion, may play a critical role in the decision which of these pathways to be followed. Further studies are needed to test this hypothesis.

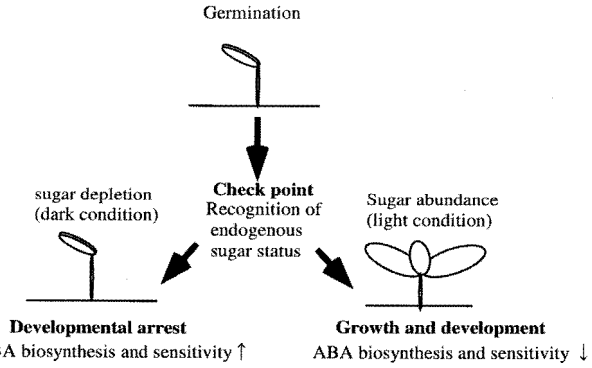

Fig. 5 Hypothetical model for the relationship between endogenous sugar status and ABA signaling in *Arabidopsis* seedling.

There is a check point that senses endogenous sugar status. Sugar-depletion promotes ABA biosynthesis and increases ABA sensitivity, triggering the vegetative quiescence process.(↑) and (↓) indicate up- and down-regulation of ABA biosynthesis and sensitivity, respectively.

Acknowledgements

The seeds of the transgenic P*ABI3-GUS* in Ler ecotype were kindly provided by Dr. Jérôme Giraudat. This work was supported by CREST of the JST (Japan Science and Technology Corporation), by and Research for the Future (JSPS-00L01603) of the Japan Society for the Promotion of Sciences (JSPS), and partially by the 21st century COE Hokkaido University. C. M.-Y. acknowledges a Research Fellowship from the Japan Society for the Promotion of Science for Young Scientist (2002-2003).

Reference

Arenas-Huertero, F., Arroyo, A., Zhou, L., Sheen, J., Leon, P., 2000. Analysis of *Arabidopsis* glucose insensitive mutants, *gin5* and *gin6*, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. Genes Dev., **14**: 2085-2096.

Finkelstein, R. R., Gampala, S. S. L., Rock, D. C., 2002. Abscisic acid signaling in seeds. Plant Cell., **14**: S15-S45.

Finkelstein, R. R., Lynch, T. J., 2000. Abscisic acid inhibition of radicle emergency but not seedling growth is suppressed by sugars. Plant Physiol., **122**: 1179-1186.

Guglielminetti, L., Perata, P., Alpi, A., 1995. Effect of anoxia on carbohydrate metabolism in rice seedlings. Plant Physiol., **108**: 735-741.

Huijser, C., Kortstee, A., Pego, J., Weisbeek, P., Wisman, E., Smeekens, S., 2000. The *Arabidopsis SUCROSE UNCOUPLED-6* gene is identical to *ABSCISIC ACID INSENSITIVE-4*: involvement of abscisic acid in sugar responses. Plant J., **23**: 577-585.

Iuchi, S., Kobayashi, M., Taji, T., Naramoto, M., Seki, M., Kato, T., Tabata, S., Kakubari, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., 2001. Regulation of drought tolerance by gene manipulation of 9-cis epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. Plant J., **27**: 325-333.

Jefferson, R. A., 1987. Assaying Chimeric gene in plants: the GUS gene fusion system. Plant Mol. Biol., **5**: 387-405.

Koch, K. E., 1996. Carbohydrate-modulated gene expression in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., **47**: 509-540.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assay with tobacco tissue cultures. Physiol. Plant., **15**: 473-497.

Laby, R. J., Kincaid, M. S., Kim, D., Gibson, S. I., 2000. The *Arabidopsis* sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. Plant J., **23**: 587-596.

Parcy, F., Valon, C., Raynal, M., Gaubier-Comella, P., Delseny, M., Giraudat, J., 1994. Regulation of gene expression programs during *Arabidopsis* seed development: rol of the *ABI3* locus and of endogenous abscisic acid. Plant Cell., **6**: 1567-1582.

Perata, P., Matsukura, C., Vernieri, P., Yamaguchi, J., 1997. Sugar repression of gibberellin-dependent signaling pathway in barley embryos. Plant Cell., **9**: 2197-2208.

Rohde, A., De Rycke, R., Beeckman, T., Engler, G., Van Montagu, M., Boerjan, W., 2000. *ABI3* affects plastid differentiation in dark-grown *Arabidopsis* seedlings. Plant Cell., **12**: 35-52.

Rohde, A., Van Montagu, M., Boerjan, W., 1999. The *ABSCISIC ACID-INSENSITIVE 3 (ABI3)* gene is expressed during vegetative quiescence processes in *Arabidopsis*. Plant Cell Environ., **22**: 261-270.

Sheen, J., Zhou, L., Jang, J. C., 1999. Sugars as signaling molecules. Curr. Opin. Plant Biol., **2**: 410-418.

Toyofuku, K., Loreti, E., Vernieri, P., Alpi, A., Perata, P., Yamaguchi, J., 2000. Glucose modulates the abscisic acid-inducuble *Rab16A* gene in cereal embyos. Plant Mol. Biol., **42**: 451-460.

Vernieri, P., Perata, P., Armellini, D., Bugnoli, M., Presentini, R., Lorenzi, R., Ceccarelli, N., Alpi, A., Tognoni, F., 1989. Solid phase radioimmunoassay for the quantitation of abscisic acid in plant crude extracts using a new monoclonal antibody. J. Plant Physiol., **134**: 441-446.

Walker-Simmons, M., Reaney, M., Quarrie, S., Perata, P., Vernieri, P., Abram, S., 1990. Monoclonal antibody recognition of abscisic acid analogs. Plant Physiol., **95**: 46-51.

Wobus, U., Weber, H., 1999. Seedmaturation: genetic programmes and control signals. Curr. Opin. Plant Biol., **2**: 33-38.