Regulation in Rhizome Transition to Storage Organ in Lotus (*Nelumbo nucifera* Gaertn.) with Exogenous Gibberellin, Gibberellin Biosynthesis Inhibitors or Abscisic Acid

Jun-ichiro Masuda*, Yukio Ozaki and Hiroshi Okubo

Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

Effects of exogenous gibberellin (GA), gibberellin biosynthesis inhibitors, and abscisic acid (ABA) on rhizome morphogenesis (transition to storage organ) were examined in seed-derived lotus (*Nelumbo nucifera* Gaertn.) plants. Exogenous 1 mg·L\(^{-1}\) GA\(_3\) promoted rhizome elongation without swelling under short-day conditions, whereas uniconazole with 0.1 mg·L\(^{-1}\) and paclobutrazole with 0.1, 1, and 10 mg·L\(^{-1}\) stimulated rhizome enlargement under long-day conditions. Rhizomes of the plants grown with 10 and 25 mg·L\(^{-1}\) ABA also enlarged under long-day conditions. Rhizome enlargement was closely related to cell enlargement and starch grain accumulation. In all the experiments, starch grain accumulation in expanded cells was recognized in the enlarged rhizomes, but not in the elongated rhizomes. It was suggested that rhizome transition to the storage organ was regulated via biosynthesis of GA and/or ABA.

**Key Words:** Abscisic acid, gibberellin, *Nelumbo*, rhizome growth, storage organ.

**Introduction**

Storage organs from modified stems are classified into tubers, rhizomes, and corms among geophytes. Mechanisms of storage organ formation in rhizomatous plants are little understood, because there are no plants in which storage organ formation can be strictly regulated by environmental factors, to our knowledge.

Enlarged rhizomes are found in lotus plants (*Nelumbo nucifera* Gaertn.). Enlarged rhizomes sprout as temperature rises in early spring, and the rhizome elongates underground in a single direction with the appearance of a few floating leaves and many upright leaves from each node of the rhizomes above ground. Axillary rhizomes subsequently appear from each node in the main rhizome, which elongate underground with many upright leaves from the ground. The upright leaves become larger in more advanced nodes. Rhizome girth and length become large and short in late summer, respectively, with ceasing leaf proliferation. Thus, the rhizomes and leaves develop under- and aboveground, respectively, and the rhizomes elongate underground by about 11 m in length. One plant resultantly requires a large field (more than 2 m\(^2\)/plant) for cultivation; thereby, detailed investigation of the rhizome growing underground consumes time and labor. We previously reported that rhizome enlargement (storage organ formation) and rhizome elongation of the lotus is induced by short days (SD) and long days (LD), respectively, irrespective of temperature (Masuda et al., 2006), and phytochrome plays an important role in the photoperiodic response to rhizome enlargement (Masuda et al., 2007). This suggested that lotus can be suitable as a model plant to study storage organ formation in rhizomatous plants. More detailed investigation can help to improve our understanding of the mechanisms of rhizome transition to storage organ formation.

The potential role in gibberellin (GA) has been extensively studied in SD-induced tuberization in some tuberous plants. Application of exogenous GA inhibited tuber formation, whereas treatment with GA biosynthesis inhibitors accelerated tuberization in *Solanum tuberosum* (Jackson and Prat, 1996; Kumar and Wareing, 1974; Okazawa, 1959) and *Begonia evansiana* (Nagao and Okagami, 1966; Okagami et al., 1977). A dwarf mutant of potato with a block in the GA biosynthetic pathway tuberized under LD as well as under SD (Van den Berg et al., 1995).

Abscisic acid (ABA), another phytohormone, has also
been implicated in storage organ formation in *S. tuberosum*. The effects of exogenous ABA were confirmed in soil- (El-Antably et al., 1967) and in vitro-growing plants (Xu et al., 1998). It was also shown that application of ABA stimulates tuber formation under tuber non-inducing conditions.

We report here the effects of GA, GA biosynthesis inhibitors, and ABA on storage organ formation in lotus plants.

**Materials and Methods**

**Plant materials, germination, and growth investigation**

Open pollinated seeds of *N. nucifera* ‘Chugoku’ were used in this study. The seeds were prepared for germination by soaking in conc. H$_2$SO$_4$ for 3 h and rinsed with distilled water. They were then soaked in distilled water for one day at 25°C. After removing softened seed coats, the seeds were incubated in distilled water at 25°C under continuous fluorescent light (approximately 40 μmol·m$^{-2}$·s$^{-1}$ photosynthetic photon flux density (PPFD)) until germination (about one week). Seedlings were transplanted into sandy soil containing 30 g slow-release fertilizer (N:P:K = 16:5:10) per plastic container (45 × 32 × 23.5 cm).

The number of leaves in main stems was counted, and growth of rhizomes was investigated by calculating the rhizome enlargement index (REI) (= maximum internode diameter/internode length) in each internode of main rhizomes after culture. The number of leaves indicates whether rhizome growth continued without swelling or ceased to transit to storage organs, because leaf proliferation continues with rhizome elongation and ceases with transition to storage organs developing a leaf bud from each node. The value of 0.2 in REI was used to judge whether an internode of rhizomes elongated or enlarged as reported previously (Masuda et al., 2006, 2007). The distal internodes were sliced in cross-section by hand with a razor blade as thin as possible, and the slices were stained with an iodine-potassium iodide solution. Starch grain accumulation in cells was observed under a light microscope soon after staining.

**Effect of GA on leaf number and rhizome growth under SD**

The seedlings were transplanted to plastic containers on January 15, 2003, and the respective container was filled with water or different concentrations (0, 0.01, 0.1, and 1 mg·L$^{-1}$) of gibberellic acid (GA$_3$) (Kyowa Hakko Bio Co., Ltd., Japan) solution up to 5 cm above soil level. They were grown under SD (natural photoperiod: 10–11 h photoperiod) at 30°C in the phytotron glass room of the Biotron Institute, Kyushu University for four weeks. The solution was replaced twice a week.

**Effect of GA biosynthesis inhibitors on leaf number and rhizome growth under LD**

The seedlings were transplanted on July 12, 2004, and grown for two weeks in containers filled with water in an unheated greenhouse. The photoperiod was 14 h by extending with a fluorescent light (approximately 40 μmol·m$^{-2}$·s$^{-1}$ PPFD) for plant growth (Toshiba Lighting and Technology Co., Japan) for 3 h both before and after 8 h natural light. They were subsequently cultivated in containers filled with water or ABA (S-abscisic acid, Lomon Bio Technology Co., Ltd., China) of different concentrations (0, 1, 10, and 25 mg·L$^{-1}$) for two weeks in the greenhouse. The fluctuation range of ambient maximum and minimum temperature in Fukuoka was from 31.6°C to 34.6°C and from 24.0°C to 27.8°C, respectively. The solution in each container was replaced twice a week.

**Results and Discussion**

**Effect of ABA on leaf number and rhizome growth under LD**

The seedlings were transplanted on July 12, 2004, and grown for two weeks in containers filled with water in an unheated greenhouse. The photoperiod was 14 h by extending with a fluorescent light (approximately 40 μmol·m$^{-2}$·s$^{-1}$ PPFD) for plant growth (Toshiba Lighting and Technology Co., Japan) for 3 h both before and after 8 h natural light. They were subsequently cultivated in containers filled with water or ABA (S-abscisic acid, Lomon Bio Technology Co., Ltd., China) of different concentrations (0, 1, 10, and 25 mg·L$^{-1}$) for two weeks in the greenhouse. The fluctuation range of ambient maximum and minimum temperature in Fukuoka was from 31.6°C to 34.6°C and from 24.0°C to 27.8°C, respectively. The solution in each container was replaced twice a week.

**Effect of GA on leaf number and rhizome growth under SD**

Plants treated with 0, 0.01, 0.1, and 1 mg·L$^{-1}$ GA$_3$ had four to five leaves (data not shown). There was no significant difference in the number of leaves between GA$_3$-treated plants and control plants, probably due to the shortness of the cultivation period. REIs from first to fourth internodes were unable to be calculated in all plants because their internodes were always extremely short. REIs in control plants were in the range of 0.83–0.87 (Fig. 1). GA$_3$-treated plants showed low values in REIs depending on the concentration, and the REIs were lower than 0.2 in fifth to eighth internodes at 1 mg·L$^{-1}$ GA$_3$. These results indicate that rhizomes elongated without their enlargement under SD by treating with GA$_3$, as shown in Figure 2A and 2B. It was recognized that cells of the distal internode of elongated rhizomes in GA$_3$-treated plants were smaller than those in control plants (Fig. 2C, D). The control plants had many starch grains in the cells. The cells in GA$_3$-treated plants, on the other hand, contained fewer starch grains according to the concentration of GA$_3$, and no starch accumulation was observed at 1 mg·L$^{-1}$ GA$_3$. These observations prove that the application of 1 mg·L$^{-1}$ GA$_3$ inhibited starch

with water on May 26, 2002 and cultivated under LD (natural photoperiod: about 14 h photoperiod) in an unheated greenhouse for four weeks. The ambient maximum and minimum temperature in Fukuoka fluctuated from 24.0°C to 34.5°C and from 15.6°C to 27.2°C, respectively. They were then grown in the containers filled with water or GA biosynthesis inhibitors; 0.1, 1, or 10 mg·L$^{-1}$ of uniconazole (Sumitomo Chemical Co., Ltd., Japan) and paclobutrazol (Nihon Nohyaku Co., Ltd., Japan, Nissan Chemical Industries, Ltd., Japan, and Takeda Pharmaceutical Co., Ltd., Japan) for four weeks in the greenhouse. The replaced solution was each week.
grain accumulation in rhizome cells. Thus, it is suggested that GA is involved in the inhibition of rhizome transition to storage organs.

**Effect of GA biosynthesis inhibitors on leaf number and rhizome enlargement under LD**

The number of leaves in plants grown in 0.1 (eight leaves), 1 (eight leaves), and 10 (seven leaves) mg·L⁻¹ paclobutrazol was significantly lower than in control plants (ten leaves) (data not shown). Uniconazole treatment had similar effects to paclobutrazol. The plants treated with 0.1 mg·L⁻¹ uniconazole produced fewer leaves (eight). All plants treated with 1 and 10 mg·L⁻¹ uniconazole died. Although plants grown in water (control plants) showed low values (<0.2) of REIs in all the internodes, those in 0.1, 1, and 10 mg·L⁻¹ paclobutrazol gave high values from eighth, ninth, and ninth internodes, respectively (Figs. 3 and 4A, B). The number of internodes with a high value of REI at 1 and 10 mg·L⁻¹ paclobutrazol was fewer than at 0.1 mg·L⁻¹ paclobutrazol. This was probably due to the strong inhibitory action of the high concentration of GA biosynthesis inhibitor in rhizome elongation. A high value of REIs from the ninth internode was also shown in plants treated with 0.1 mg·L⁻¹ uniconazole, and the value in the 11th internode reached 1.73 (Figs. 3 and 4C). These results indicate that newly produced rhizomes enlarged under LD by treating with these inhibitors, but already elongated rhizomes did not. A difference in the
number of internodes with a high value of REI between plants grown with 0.1 mg·L$^{-1}$ paclobutrazol and 0.1 mg·L$^{-1}$ uniconazole solution was also observed. The plants treated with 0.1 mg·L$^{-1}$ uniconazole had fewer internodes with a high value of REI than those with 0.1 mg·L$^{-1}$ paclobutrazol. It was shown that the inhibitory activity of uniconazole in rhizome elongation was stronger than that of paclobutrazol. The cells of the distal internode in all plants treated with GA biosynthesis inhibitors were larger than in control plants (Fig. 4D, E, F). The cells in all plants treated with inhibitors contained many starch grains in comparison to control plants. These observations demonstrate that treatment with GA biosynthesis inhibitors stimulated rhizome transition to storage organs accompanied with cell enlargement and starch grain accumulation; thus, these results also suggest the involvement of GA in rhizome transition to storage organs.

Our results share similarities in that applying GA and GA biosynthesis inhibitor affected tuber formation in *S. tuberosum* (Jackson and Prat, 1996; Kumar and Wareing, 1974; Okazawa, 1959) and *B. evansiana* (Nagao and Okagami, 1966; Okagami et al., 1977), SD-tuberized species. Further investigation demonstrated that SD decreases the endogenous level of GA in tuberization in *S. tuberosum* as expected (Railton and Wareing, 1973; Xu et al., 1998); however, bulbous plants showed no effects of GA on bulb swelling. S-3307, an inhibitor of GA biosynthesis, did not swell leaf sheaths in young seedlings of *Allium cepa* of bulbous plants under SD (non-bulb-swelling condition) (Mita and Shibakoa, 1984a, b). In addition, endogenous GA levels in plants grown under SD were lower than those under LD (Nojiri et al., 1993), indicating that GA is not involved in bulb formation in *A. cepa*. This may be because GA regulates the transition to storage organs originating in stems, but not in leaves.

**Effect of ABA on leaf number and rhizome growth under LD**

The number of leaves in plants treated with 1 (seven leaves) and 10 (seven leaves) mg·L$^{-1}$ ABA was similar to that in control plants (seven leaves), whereas leaves in plants with 25 (six leaves) mg·L$^{-1}$ ABA were significantly smaller than in control plants (data not shown). Control plants had low REIs of 0.2 in all
internodes under LD (Fig. 5). Treatment with 10 and 25 mg·L⁻¹ ABA, on the other hand, resulted in high REI of 0.29 (eighth internode) and 0.54 (seventh and ninth internodes), respectively. It was revealed that treating with ABA promoted rhizome enlargement, as shown in Figure 6A and 6B. Cells in rhizomes of the distal internode in treated plants enlarged depending on the concentration of ABA, and much starch grain accumulation was observed in cells of plants treated with 10 and 25 mg·L⁻¹ ABA (Fig. 6D). By contrast, no or few starch grains were accumulated in the cells of the distal internode in control plants (Fig. 6C). These results indicate that exogenous 10 and 25 mg·L⁻¹ ABA stimulated rhizome transition to storage organs, suggesting that ABA is involved in rhizome transition to storage organs.

The effect of exogenous ABA on tuberization in S. tuberosum has been investigated similarly. Tuberization was promoted when ABA was applied to the leaves of potato plants growing under LD (El-Antably et al., 1967). Similar effects of ABA on tuberization under tuber non-inducing conditions (1% sucrose) were confirmed using an in vitro system, in which tuber formation was controlled by sucrose concentration; culture with 10 and 25 mg·L⁻¹ ABA promoted rhizome enlargement, as shown in Figure 6A and 6B. Cells in rhizomes of the distal internode in treated plants enlarged depending on the concentration of ABA, and much starch grain accumulation was observed in cells of the distal internode in treated plants with 10 and 25 mg·L⁻¹ ABA (Fig. 6D). By contrast, no or few starch grains were accumulated in the cells of the distal internode in control plants (Fig. 6C). These results indicate that exogenous 10 and 25 mg·L⁻¹ ABA stimulated rhizome transition to storage organs, suggesting that ABA is involved in rhizome transition to storage organs.

ABA level is required to clarify whether ABA is involved in rhizome transition to storage organs in lotus. This is the first report suggesting the involvement of GA and/or ABA regulation of rhizome transition to storage organs in rhizomatous plants forming storage organs by environmental factors.

**Literature Cited**


![Image](image-url)