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Induction of Plant Tubers and Flower Buds under Noninducing Photoperiod Conditions by a Natural Product, Theobroxide

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Abstract

Theobroxide is an epoxy cyclohexene compound isolated from the culture filtrate of the fungus *Lasiodiplodia theobromae* that induces potato microtuber formation in vitro (Nakamori and others 1994). When sprayed on potato (*Solanum tuberosum* L.) and morning glory (*Pharbitis nil*) plants, which require short days to induce tubers and flower buds, respectively, potato plants kept in noninducing conditions (long days) produced tubers. Theobroxide spray treatment also produced flower buds in morning glory plants kept under noninducing conditions (long days). Furthermore, under inducing conditions (short days), the number of flowers of seedlings sprayed with theobroxide was about 1.5 times that of controls.

Introduction

Formation of certain plant organs, including tubers, bulbs, and flower buds, is controlled by photoperiod. Potato (*Solanum tuberosum* L.) and Jerusalem artichoke (*Helianthus tuberosus* L.) tubers are formed under short days. Onion (*Allium cepa* L.) bulb formation occurs under long days. Morning glory (*Ipomea* and *Pharbitis*) produces flower buds under short days and vegetative growth under long days. These phenomena are controlled by several endogenous plant growth regulators. Tuberonic acid glucoside (Fig. 1), an inducer of tuber formation, has been isolated from the leaves of potato (*S. tuberosum* L.) (Koda and others 1988; Yoshihara and others 1989) and Jerusalem artichoke (*H. tuberosus* L.) (Matsuura and others 1993), using a bioassay consisting of in vitro culture of single-node segments of potato stolons (Koda and others 1988). Exogenous jasmonic acid also induces tuberization of potato stolons cultured in vitro (Yoshihara and others 1989; Koda and others 1991; Pelacho and others 1991) and stimulates shoot and bulb formation of garlic (*Allium sativum*) in vitro (Ravnikar and others 1993). In contrast, gibberellins are known to inhibit potato tuberization (Okazawa 1960; Racca and others 1968). Inhibitors of gibberellin biosynthesis such as ancymidol (Perl and others 1991) and tetcyclacis (Vreugdenhil and others 1994), have been used to induce tuberization of potato in vitro. In a previous

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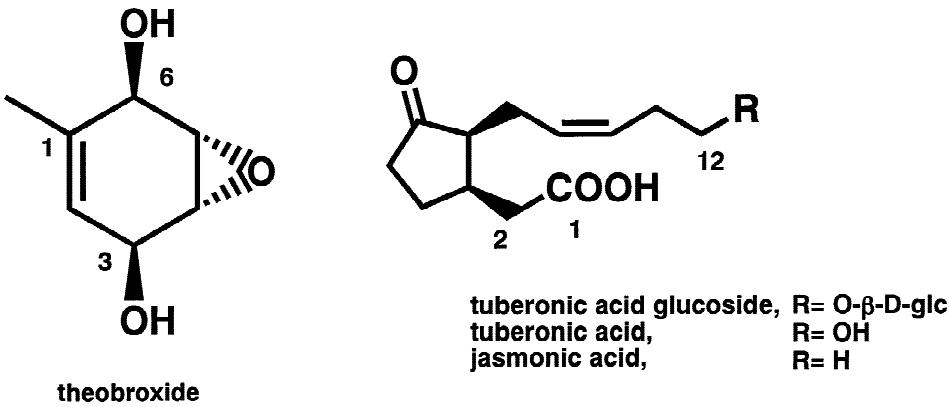


Figure 1. The structures of theobroxide, tuberonic acid glucoside, tuberonic acid, and jasmonic acid.

article (Yoshihara and others 1996), we presented information showing that formation of tubers and flower buds of potato may be the result of the same mechanism. In addition, other reports suggest that the signals involved in floral induction are similar to those involved in tuberization (Chailakhyan and others 1977, Martin and others 1982).

Theobroxide is an epoxy cyclohexene natural product (Fig. 1) isolated from the culture filtrate of the fungus *Lasiodiplodia theobromae* that induces potato microtubers in vitro (Nakamori and others 1994). Its activity is almost identical to that of (±) jasmonic acid. In this article, we describe spray applications of theobroxide to potato plants (*S. tuberosum* L.) to test for tuber formation under noninducing conditions. Moreover, we investigated whether the application of theobroxide would promote flower-bud formation in other plants such as morning-glory (*Pharbitis nil*).

Materials and Methods

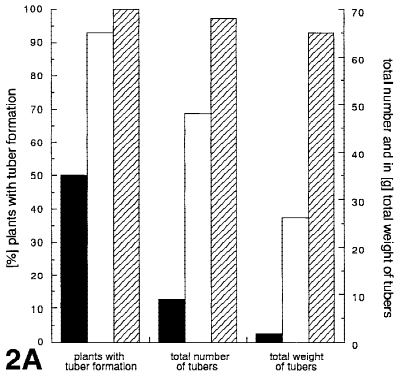
Plants were grown in growth chambers equipped with a 10^4Lx fluorescent light, a temperature of 25°C, and relative humidity of 60%. Theobroxide was isolated from the culture filtrate of *Lasiodiplodia theobromae* IFO 31059. A solution of 10^{-3} M theobroxide in 100 ppm Tween 20 in H_2O was applied with plastic spray bottles onto the surface of the plant leaves three times (total 3 mL) at intervals of 2 days.

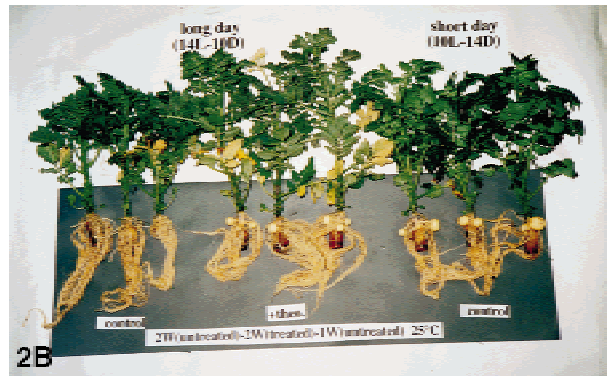
Potato cultivar used for this experiments was Irish Cobbler. Photoperiods for short and long day conditions were 10-h light–14-h dark and 14-h light–10-h dark, respectively. Cylinders (2-cm diameter × 2.5-cm long) containing a single sprout excised from potato tubers using a cork borer were planted in 2-L pots containing vermiculite. The plants were grown under long day conditions for 2 weeks in the growth chamber before treatments. The solution of theobroxide was sprayed onto the leaves of these potato plants grown under long day conditions. As a negative control, the leaves of one group of plants grown under long day conditions were sprayed with an equal volume of 100 ppm Tween 20 solution, and as a positive control, one group of plants was transferred to short day conditions without theobroxide treatments. After 3 weeks from the beginning of this treatment, all plants were harvested, and the numbers and weights of tubers of each treatment group were measured. In these experiments, the definition of the tuber was established as follows: the diameter of the tip of the stolon was more than two times wider than that of the stolon.

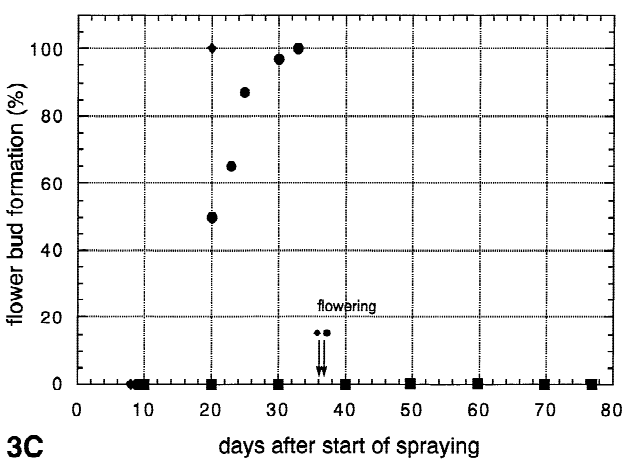
Morning glory (*Pharbitis nil* cv. Sun Smile dwarf type) seedlings were grown 33 days in a mixture of peat moss and perlite (2:1, v/v) in 400-mL pots under long day (18-h light–6-h dark) conditions before treatment. Theobroxide was sprayed onto the leaves of the plants at intervals of 2 days. As a negative control, a group of plants grown under long day conditions was sprayed with an equal volume of 100 ppm Tween 20 solution as the treated group. As a positive control, the plants were transferred to short day (10-h light–14-h dark) conditions without theobroxide treatments. The numbers of flower buds or flowers of each group were measured up to day 77 after the treatments.

Morning glory (*Pharbitis nil* cv. Akenokumo normal type) seedlings were grown for 17 days in a mixture of peat moss and perlite (2:1, v/v) in 400-mL pots under long day (18-h light–6-h dark) conditions before treatment. The theobroxide treatments were carried out using the same procedure as for other morning glory (*Pharbitis nil* cv. Sun Smile dwarf type).

The seeds of morning glory (*Pharbitis nil* cv. Violet) were obtained from Marutane Seed Co. (Kyoto, Japan). Growth conditions were almost identical to that of morning glory (*Pharbitis nil* cv. Sun Smile







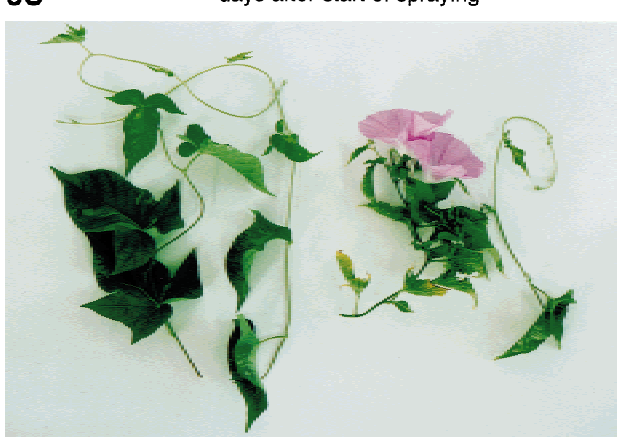


Figure 2. (**A**) Tuber formation and number and weight of the tubers; ■ long day without theobroxide, □ long day with theobroxide, ▨ short day without theobroxide. (**B**) Tuber production after theobroxide treatment on potato plants grown under long days without theobroxide (left), under long days with theobroxide (center), and under short days without theobroxide (right).

Figure 3. Flower bud formation and flowering of dwarf morning glory, *P. nil*, cv. Sun Smile, under different photoperiods with and without theobroxide. These pictures were taken at day 59 after the start of spraying. (**A**) left two: without theobroxide; right two: with theobroxide. (**B**) without leaves, left: without theobroxide; right: with theobroxide. (**C**) ◆ short day without spraying, ● long day with spraying, ■ long day without spraying.

Figure 4. Flowerings of morning glory *P. nil* cv. Akenokumo grown under long days for 74 days; left without theobroxide, right with theobroxide (cut before photography).

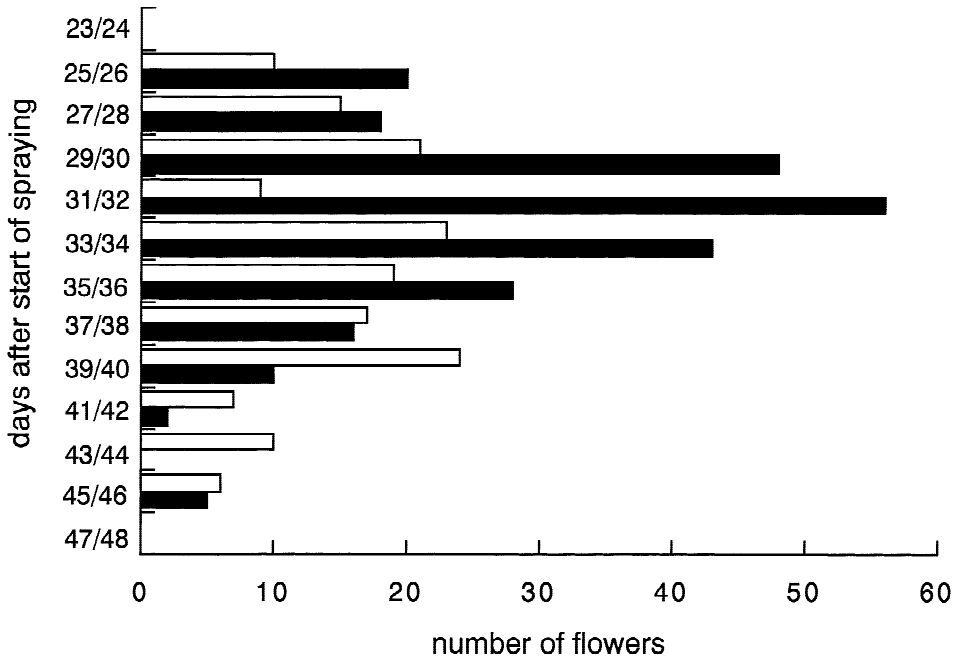


Figure 5. The flowerings of morning glory *P. nil* cv. violet grown under short day conditions (10 hour light–14 hour dark) with theobroxide(■) and without theobroxide(□). Numbers of flowers are totals of 2 days each.

dwarf type). To limit stem growth, the stems were cut between the sixth and seventh node on day 33 after sowing. The seedlings were then transferred to short day (10-h light–14-h dark) conditions and divided into theobroxide treated and nontreated groups.

Results and Discussion

A solution (10^{-3} M in 100 ppm Tween 20 solution) of theobroxide was sprayed over a 2-week period onto the leaf surface of 14 potato plants grown under long days. The plants were harvested 3 weeks after treatment, and the numbers and weights of the tubers were measured (Fig. 2A). Tuber production was observed in 100% (14 of 14) of the controls grown under short days. The total number of tubers was 68 and the total weight was 65.0 g. Under long days, 50% (7 of 14) of the potato plants not receiving theobroxide produced a total number of 9 tubers with a total weight of 1.6 g. On the other hand, 93% (13 of 14) of the potato plants sprayed with theobroxide produced a total number of 48 tubers with a total weight of 26.2 g. The leaves of theobroxide treated plants were slightly chlorotic.

Flowering of potato is also controlled by photoperiod, but, contrary to tuberization, flowering is promoted under long days (Turner and Ewing 1988). Flower bud formation was stimulated on some of the theobroxide-treated plants grown under long days (Fig. 2B, center).

Three batches of 22 dwarf morning glory plants, cv. Sun Smile, grown under long days for 33 days, were subjected to the following treatments. The controls were transferred to inducing conditions (short days). One hundred of the seedlings produced flower buds starting from day 20 after transfer. The first flowers were observed at day 36. Onto plants grown under long days, a solution of theobroxide was sprayed at intervals for 33 days. Flower bud formation was observed in 100% of the seedlings beginning from day 34 after spray initiation. The first flowering was observed at day 37. On the other hand, plants grown under long days, without theobroxide treatment, produced no flower buds even after 77 days (Fig. 3A–C). Theobroxide sprays also appeared to stimulate the growth of stems. The leaves of seedlings sprayed with theobroxide were slightly pale.

Normal type morning glory, cv. Akenokumo, produced flower buds and flowered (Fig. 4) under the same conditions as dwarf cv. Sun Smile. Plants grown under short days began flowering at day 30 and under long days with theobroxide at day 57.

Additional application of theobroxide to cv. Violet under inducing conditions, namely short days, produced about 1.5 times more flowers compared with nonsprayed plants (Fig. 5). The total number of flowers per plant, up to day 48, was 14.3 and 9.3, whereas the number of immature fruits was 7.1 and 4.7, respectively, with and without theobroxide. The growth of plants was not changed by theobroxide, but the leaf color was slightly pale in sprayed plants.

It is not possible to state that theobroxide is in

itself a single trigger for the formation of tubers and flower buds, because in contrast to [2-^{14}C](±) jasmonic acid (Yoshihara and others 1996) neither metabolism nor transportation occurred in an experiment with the application of [3,6-^{3}H] (±) theobroxide to the leaves of potato plants (unpublished data). Theobroxide may stimulate the biosynthesis of a common plant growth regulator. It has been reported that polyamines induced flower buds in *Pharbitis nil* grown under noninductive conditions, that is, under continuous light (Wada and others 1994). Jasmonic acid, or its derivatives, may also be good triggering candidates. Although theobroxide induces both the formation of tubers and flower buds, the inducing endogenous signal substance for tuber formation is known to be tuberonic acid (Yoshihara and others 1989), a jasmonic acid analogue. Theobroxide may be a valuable tool in basic studies of formation of tubers and flowers and may find a practical use in agriculture, seed production, and horticulture.

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REFERENCES

Chailakhyan MKh, Yanina LI, Devedzhyan AG, Lotova GNL. 1977. Regulation of the flowering of plants of long-short day species by gibberellin and continuous darkness. Doklady Akademic Nauk S.S.S.R. 237:1536–1539.

Chapman HW. 1958. Tuberization in the potato plant. Physiol Plant 11:215–224.

Gregory L. 1956. Some factors for tuberization in the potato plant. Am J Bot 43:281–288.

Koda Y, Kikuta Y, Tazaki H, Tsujino Y, Sakamura S, Yoshihara T. 1988. Potato tuber-inducing activities of jasmonic acid and related compounds. Phytochemistry 30:1435–1438.

Koda Y, Okazawa Y. 1988. Detection of potato tuber-inducing activity in potato leaves and old tubers. Plant Cell Physiol 29:969–974.

Koda Y, Omer EA, Yoshihara T, Shibata H, Sakamura S, Okazawa Y. 1988. Isolation of a specific tuber-inducing substance from potato leaves. Plant Cell Physiol 29:1047–1051.

Martin C, Vernoy R, Paynot N. 1982. Photoperiodism, tuberisation, floraison et phenolamides. C. R. Acad Sc Serie III 295:565–568.

Matsuura H, Yoshihara T, Ichihara A, Kikuta Y, Koda Y. 1993. Tuber-forming substances in Jerusalem artichoke (*Helianthus tuberosus* L.). Biosci Biotech Biochem 57:1253–1256.

Nakamori K, Matsuura H, Yoshihara T, Ichihara A, Koda Y. 1994. Potato micro-tuber inducing substances from *Lasiodiplodia theobromae*. Phytochemistry 35:835–839.

Okazawa Y. 1960. Studies on the relation between the tuber formation of potato and its natural gibberellin content. Proc Crop Sci Japan 29:121–124.

Pelacho AM, Mingo-Castel AM. 1991. Jasmonic acid induces tuberization of potato stolons cultured in vitro. Plant Physiol 97:1253–1255.

Perl A, Aviv D, Willmitzer L, Galun E. 1991. *In vitro* tuberization in transgenic potatoes harboring β-glucuronidase linked to a patatin promoter. Plant Sci 73:87–95.

Racca RW, Tizio R. 1968. Study of changes in the content of gibberellin-like substances in the potato plant in relation to the tuberization mechanism. Eur Potato J 11:213–220.

Ravnikar M, Zel J, Plaper I, Spacapan A. 1993. Jasmonic acid stimulates shoot and bulb formation of garlic *in vitro*. J Plant Growth Regul 12:73–77.

Turner AD, Ewing EE. 1988. Effects of photoperiod, night temperature, and irradiance on flower production in the potato. Potato Res 31:257–268.

Vreugdenhil D, Bindels P, Reinhoud P, Klocek J, Hendriks T. 1994. Use of the growth retardant tetcyclacis for potato tuber formation *in vitro*. J Plant Growth Regul 14:257–265.

Wada N, Shinozaki M, Iwamura H. 1994. Flower induction by polyamines and related compounds in seedlings of morning glory (*Pharbitis nil cv. Kidachi*). Plant Cell Physiol 35:469–472.

Yoshihara T, Omer EA, Koshino H, Sakamura S, Kikuta Y, Koda Y. 1989. Structure of a tuber-inducing stimulus from potato leaves (*Solanum tuberosum* L.). Agric Biol Chem 53:2835–2837.

Yoshihara T, Amanuma M, Tsutsumi T, Okumura Y, Matsuura H, Ichihara A. 1996. Metabolism and transport of [2-^{14}C] (±) jasmonic acid in potato plant. Plant Cell Physiol 37:586–590.