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THEOBROXIDE AND DAY-LENGTH EFFECTS ON THE GROWTH OF YAM (*DIOSCOREA* SPP.)

Shuwan Chen, Hironobu Shiwachi, Atsushi Sanada and Hidekazu Toyohara

Graduate School of Agriculture Science, Tokyo University of Agriculture,
1-1-1, Sakuragaoka, Setagaya, Tokyo 156-8502, Japan
*To whom correspondence should be addressed (e-mail: h1shiwac@nodai.ac.jp)

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ABSTRACT

The effects of theobroxide (a novel compound isolated from the fungus *Lasiodiplodia theobromase* culture) and day length on flowers, tubers, and bulbils development at the primary and rapid growth stages of yam tubers were investigated in four varieties of water yam (*Dioscorea alata*) and one variety of Chinese yam, cv. Nagaimo (*D. opposita*) in 2005 and 2007. The yam plants were exposed to 10 h day length (short-day) and theobroxide application, with natural day length serving as control. Flower development was not influenced by the treatments in all the varieties evaluated. The effects of theobroxide and short-day to tubers and bulbils development were varied, depending on the varieties tested and the growth stage of the plant. Theobroxide or short-day treatment did not affect the growth of tubers and bulbils in *D. opposita*. The treatments promoted tubers and bulbils development in *D. alata*, but some varieties were inhibited. The short-day treatment tended to promote tuber growth at the primary tuber growth stage of the plant, and bulbil development at the rapid tuber growth stage in most varieties. The short-day length was the important environmental factor for bulbil development in *D. alata*. The short-day treatment in *D. alata* first induced the thickening growth of the tubers and then the development of bulbils with some time lag. The theobroxide application was most effective during the tuber enlargement period in encouraging the development of bulbils. However, the effectiveness of theobroxide on the development of flowers, tubers, and bulbils was not very clear in this study. Further study is necessary to identify the optimum treatment period for each yam species and variety. This is the first reported use of theobroxide in yam.

Key words: Bulbils, flower development, short-day, tuber growth

INTRODUCTION

Yam (*Dioscorea* spp.) is one of the important starchy tuberous crops in the tropics. Yam propagation in tropical Asia and Africa has utilized traditional techniques and methods. Yam breeding is constrained by some factors especially low levels of flowering that limit the development of new varieties. Several environmental factors affect the growth of yam (*Dioscorea* spp.), in particular, the development of the flowers, tubers and bulbils. Tuber growth in yam is slow immediately after initiation; then it becomes very rapid at full canopy formation and finally slows down during maturation with a loss in dry matter (Onwueme, 1978). However, details of the tuber growth pattern differ, especially the start of rapid growth depending on the species or varieties (Shiwachi *et al*., 1999, 2001). The enlargement of the tuber in yam is known to be affected by photoperiod. Short days stimulate tuberization, while long days inhibit it in water yam (*D. alata*). The translocation of photosynthetic product is encouraged under short-day condition. However, *D. opposita* is more sensitive to the short-day treatment than *D. alata* (Shiwachi *et al*., 1999). The difference in the response is noticeable at the beginning of the rapid growth of the tuber and seems related to the maturity period of tubers and to the geographic distribution of *Dioscorea* species (Shiwachi *et al*., 2000, 2002). Knowledge of the relations between photoperiod and tuber growth would be useful for varietal selection and the breeding program in yam.

Bulbils in *D. opposita* proliferate under short-day conditions (Yoshii, 1949) that encourage their growth (Sato, 1974). However, little is known about bulbil development in tropical and subtropical yams, and the physio-ecological profiles are almost unknown. The environmental factors for the development of inflorescences in yam are also not well defined. The characteristics and eco-physiologies on flowering vary among species and varieties in yam. For example, flowering is frequent in white Guinea yam (*D. rotundata*) and *D. opposita*, but very rare in *D. alata*(Asiedu *et al*., 1998). The effects of photoperiod on the development of inflorescences in yam were complicated by differential responses by the varieties (Shiwachi *et al*., 2005). More knowledge about this could provide options for manipulating flowering periods and thus enhance the efficiency of yam hybridization program.

Yoshihara *et al*. (2000) reported that theobroxide; a novel compound isolated from the fungus *Lasiodiplodia theobromae* culture, induces flowering of potato (*Solanum tuberosum*) under non-induced long-day condition. And more, potato tubers were produced under non-induced long-day condition by the theobroxide treatment (Arimoto *et al*., 2005). Theobroxide is the trigger for jasmonic acid production to induce potato tuberization (Xiquan *et al*., 2005). Several plant hormones affect tuber growth in yam (Chang *et al*., 1995). The enlargement of tubers in *D. alata* was promoted by jasmonic acid (Kikuno *et al*., 2002a, 2002b). Thus, it is expected that theobroxide application would induce the flower and tuber development in yam. But this has not been tested yet. In this study the influences of theobroxide and day-length on the development of flowers, tubers and bulbils at primary and rapid growth stages of the tubers in yam were investigated.

MATERIALS AND METHODS

Two experiments were carried out at the Tokyo University of Agriculture (TUA), Tokyo, Japan, from March to December, 2005 and 2007. Four varieties of *D. alata* and one variety of *D. opposita* were obtained from the germplasm collection at TUA. Yam seed-sets, each weighing about 50g, were planted in plastic pots (30 cm diameter × 30 cm depth) that had been filled with topsoil for the experiments at the end of March, 2005 and 2007. 23.8 g of ammonium sulfate, 26.3 g of calcium super-phosphate, and 25.0 g of potassium silicate were initially applied in each pots. These pots were kept under plastic cover, air temperature controlled at 20 - 22 °C from planting in March to April, and under natural air temperature conditions from May. The plants were staked with a stick 2.0 m long. The pots were kept under natural day-length conditions until specific day-length treatments were imposed.

Effect of theobroxide and day length at the rapid growth stage on tubers

The varieties Obukosumbori, Basmi and Malaysia-A were used in this experiment. Fifteen plants from each variety were randomly selected on 4 th August 2005. Ten plants were kept under 10 hr day length (short-day treatment: ST) imposed by shielding the plants from light from 1800 hr each day using a rectangular plastic canopy. The rest were kept under natural day-length (control) during the experiment. The foliage of five plants from each variety was sprayed with 100 $\mathrm{ml plant^{-1}}$ of 250 $\mathrm{mg l^{-1}}$ of theobroxide (short-day-theobroxide treatment: STT) every 7 days from 16 th August to 13 th: September. The concentration of theobroxide was specified in a previous report (Yoshihara *et al*., 2000). Control and short-day treatment plants of the same varieties were sprayed with water on the same date. Natural day-length during treatment was from 13 hrs 30 min to 12 hrs 30 min. Flower and bulbil development was observed every 7 days from planting.

The plants from each variety were harvested in December. The roots were washed with water and each plant was divided into leaves (petioles included), vines, roots, and tubers. The fresh weights of these portions were recorded and their dry weights were estimated following oven drying at 90°C for 4 days.

Influence of theobroxide and short-day length at the primary growth stage of tubers

The varieties Basmi, Malaysia-A and Taiwan local (*D. alata*), and Nagaimo (*D. opposita*) were used in this experiment. These were obtained from the germplasm collection at TUA (Table 1). Forty plants of each variety were randomly selected on 20 th July 2007. Twenty plants of each variety were exposed to a ten-hr day-length as in experiment 1. The other 20 plants were kept under natural day-length throughout the experiment. Foliage of the 10 plants of each variety from the short-day treatment (ST), short-day-theobroxide treatment (STT) and natural day-length theobroxide treatment (TT) was sprayed with 100 $mlplant^{-1}$ of 250 mgl^{-1} of theobroxide every 7 days from 3 rd to 24 th August. The concentration of theobroxide was used as same as in experiment 1. The rest were kept under natural day-length during the experiment. Natural day-length during treatment was from 13 hrs 54 min. to 13 hrs 17 min. Flower and bulbil development were observed as in experiment 1, and these were sprayed with water on the same date. The yam plants were harvested in December.

Table 1. Yam species, maturity class and origin of the yam varieties used in the study.

Dioscorea **species**	**Variety**	**Maturity class**	**Origin**
D. alata	Obukosumbori	Unknown	Papua New Guinea
	Basmi	Early maturing	Papua New Guinea
	Malaysia-A	Early maturing	Malaysia
	Taiwan Local	Unknown	Taiwan
D. opposita	Nagaimo	Early maturing	Hokkaido, Japan

RESULTS

Air temperature in Tokyo during the experimental period 2005 and 2007 is shown (Table 2).

Table 2. Air temperature in Tokyo during the 2005 and 2007 experiments.

	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
2005										
Max.	12.4	18.9	21.4	26.2	28.5	31.3	27.8	22.2	16.5	9.8
Min.	4.9	10.9	14.0	20.1	22.6	24.8	21.8	16.5	9.1	2.5
Avg.	8.6	14.9	17.7	23.2	25.5	28.0	24.8	19.4	12.8	6.2
2007										
Max.	14.4	25.8	29.0	31.5	32.7	37.5	32.9	26.5	21.2	15.8
Min.	6.8	4.0	13.0	15.7	18.2	23.1	15.7	12.8	4.6	2.7
Avg.	10.6	13.7	19.8	23.2	24.4	29.0	25.2	19.0	13.3	9.0

Effect of theobroxide and day length at the rapid growth stage on tubers

Flower development was not observed in the three tested varieties. The effects of theobroxide and day-length treatments on the growth of yam at the rapid growth stage of the tuber are shown (Table 3). Most vigorous growth of leaves and vines in three varieties was observed in the control. In cv. Obukosumbori, the dry weight of leaves and vines was higher in the control than in ST and STT. The dry weight of ST and STT were not significantly different from one another while the best growth was observed in the control; there was no significant difference between ST and STT.

Theobroxide treatment reduced tuber growth and varietal response was observed. In cv. Obukosumboni, the smallest tuber weight was observed in ST. The difference between the control and STT was not significant. The least tuber growth was observed in cv. Malaysia-A where better tuber growth was observed in the control; there was no significant difference between ST and STT.

Table 3. Effect of daylength and theobroxide on the growth of *D. alata* at rapid tuber growth stage, 2005.

Variety		Control	Short day length (SE)	Short day length• theobroxide
	Leaf and vine (g)	98.7 (5.2)	43.5 (6.8)	46.7 (4.1)
Obukosumbori	Tuber (g)	392.5 (37.3)	276.6 (32.7)	392.5 (30.1)
	Leaf and vine (g)	107.7 (3.9)	53.3 (1.0)	46.7 (2.4)
Basmi	Tuber (g)	397.2 (21.5)	272.9 (44.3)	248.5 (42.5)
	Leaf and vine (g)	89.0 (8.9)	56.0 (2.1)	52.0 (2.1)
Malaysia-A	Tuber (g)	427.3 (15.1)	256.5 (36.7)	238.3 (46.1)

Influence of theobroxide and short-day length at the primary growth stage of tubers

Bulbil development was affected by theobroxide treatment and varietal response was observed in this experiment (Table 4). In cv. Obukosumbori, bulbils did not develop or only a few were observed in the control, ST and STT, however some bulbils were recorded in the other varieties in all treatments. The highest number of bulbils developed was demonstrated in cv. Basmi at short day length while the number of bulbils of cv. Malaysia increased significantly in ST and STT treatments. In cv. Basmi, the weight of bulbils in ST and STT was consistently greater than in the control.

Table 4. Effect of daylength and theobroxide on the development of bulbils in *D. alata* at the rapid tuber growth stage, 2005.

Variety		Control	Short day length (SE)	Short day length• theobroxide
Obukosumbori	Number	0	0.2	0.4
	Weight (g)	0	0.4	0.8
Basmi	Number	12.4 (3.8)	23.2 (5.3)	33.0 (4.2)
	Weight (g)	27.8 (11.6)	69.7 (20.0)	86.2 (15.4)
Malaysia-A	Number	10.6 (3.0)	26.2 (7.3)	25.4 (4.5)
	Weight (g)	43.5 (13.7)	96.7 (21.6)	73.7 (13.6)

Experiments on Taiwan local and cv. Nagaimo

Taiwan local and cv. Nagaimo varieties both have female and male flowers that are varieties setting used for the flowering effects observation in the theobroxide treatment. The variety cv. Obukosumbori was not used in this experiment, because bulbil formation was not observed in any of the treatments. Flowers were not observed on the *D. alata* varieties in experiment 1. Flowering was observed in cv. Nagaimo, however the number of flowers and date of emergence on the flower spike did not differ among treatments and control.

The effects of theobroxide and day-length treatments on the growth at the primary growth stage of tuber are shown on Table 5. In cv. Nagaimo, a low dry weight of leaves and vines was recorded in the control and TT, while ST and STT treatments, were heavier than the control. For Taiwan local, the largest weight was in the TT treatment while ST was the same as the control.

For cv. Basmi the biggest growth of leaves and vines was observed in the TT treatment. The growth in ST and STT was smaller than in the control. For cv. Malaysia-A the TT treatment did not differ from the control; results in ST and STT were smaller than in the control.

For cv. Nagaimo, the dry weight of tubers was smaller in TT and larger in ST and STT treatments compared with the control. Taiwan local had lower tuber weight in TT than in ST and STT, which were heavier than in the control. For cv. Basmi, the biggest tuber growth was in TT and the smallest in control. There was no difference in tuber weight between ST and STT treatments For cv. Malaysia-A, the biggest tubers were observed in STT, followed by ST, and least was control and TT.

Table 5. Effect of daylength and theobroxide on the growth of *D. alata* and *D. opposita* at the primary growth stage of tuber, 2007 (SE).

Variety		Control	Theo-broxide	Short day length	Short day length · theobroxide
Nagaimo	Leaf and vine (g)	7.2 (1.6)	5.5 (0.7)	12.5 (1.2)	12.6 (0.9)
	Tuber (g)	91.4 (19.3)	42.6 (7.2)	132.6 (10.7)	152.7 (16.5)
Taiwan Local	Leaf and vine (g)	25.8 (2.1)	34.4 (2.4)	24.0 (2.3)	19.4 (1.6)
	Tuber (g)	76.8 (5.4)	49.7 (4.3)	113.1 (9.9)	115.6 (12.1)
Basmi	Leaf and vine (g)	30.8 (3.4)	38.4 (2.2)	21.0 (2.0)	21.0 (0.7)
	Tuber (g)	117.6 (9.7)	162.8 (28.3)	149.1 (9.7)	135.5 (11.7)
Malaysia-A	Leaf and vine (g)	46.7 (3.4)	48.4 (4.7)	26.0 (2.4)	21.6 (1.7)
	Tuber (g)	240.4 (15.5)	184.2 (16.1)	104.8 (15.3)	89.0 (7.1)

The effect of theobroxide and day length treatments on the development bulbils at the tuber initiation stage are shown in Table 6. Bulbils developed in all tested varieties. More of bulbil development was observed in cv. Nagaimo compared with *D. alata* varieties. Less bulbils formation and enlargement was observed in cv. Basmi and Malaysia-A compared with results from experiment 1 in 2005. The number and enlargement of bulbils were not significantly different among treatments. Bulbil development in cv. Nagaimo started before theobroxide and day-length treatments in June. The physiology of bulbil formation in cv. Nagaimo was different from that in the *D. alata* varieties.

Table 6. Effect of daylength and theobroxide on the weight of bulbils in *D. alata* and *D. opposita* at the primary growth stage of tubers, 2007 (SE).

Variety		Control	Theo-broxide	Short day length	Short day length - theobroxide
Nagaimo	Number	14.8 (3.8)	12.8 (5.4)	19.8 (5.1)	18.7 (5.9)
	Weight (g)	1.7 (0.6)	2.2 (0.9)	3.6 (1.2)	2.5 (0.8)
Taiwan	Number	2.9 (1.4)	2.7 (1.2)	2.4 (1.2)	1.1 (0.6)
Local	Weight (g)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.1 (0.1)
Basmi	Number	0.8 (0.4)	0.4 (0.3)	1.9 (1.8)	0.2 (0.1)
	Weight (g)	-	-	0.1 (0.1)	-
Malaysia-A	Number	2.1 (1.0)	2.3 (0.9)	1.0 (0.6)	1.5 (0.5)
	Weight (g)	0.1 (0.1)	0.1 (0.1)	-	0.1 (0.1)

-: weight was observed less than 0.1 g.

DISCUSSION

Flower development

Flower development did not occur under natural day-length in Tokyo in all of the *D. alata* varieties. Flowering and non-flowering types of *D. rotundata* and *D. alata* are found, and there are a few flowering varieties of *D. alata* (Asiedu *et al*., 1998). The results shows that the three varieties used in experiment 1 were non-flowering types, because none of them developed flower bud. The flowering variety of *D. opposita* and a Taiwan local variety of *D. alata* which were used in experiment 2 had been observed to develop flowers in Tokyo and Okinawa, Japan. However, Taiwan local, a flowering variety, did not develop flowers in this experiment. *D. rotundata* and *D. alata* have been described as short-day plants for flowering (Bai and Ekanayake, 1998). But Taiwan local, a female flowering variety did not develop flower buds under the short-day treatment. The short day period did not affect the number of flowers and the date of emergence on the flower spike in cv. Nagaimo (experiment 2).

Varieties of *D. rotundata* developed flower spikes under long-day period (Shiwachi *et al*., 2005). Flower development in *D. rotundata* was caused by combination and rate of change of photoperiod (Ile *et al*., 2007). It suggests that *D. alata* and *D. opposita* have similar flowering behavior in the flower bud development. Theobroxide induces the flowering of potato (*Solanum tuberosum*) and morning glory (*Ipomoea purpurea*) under non-induced long-day conditions (Yoshihara *et al*., 2000). Theobroxide had no influence on yam flowering at the rapid and primary growth stages of tubers in these experiments. The usefulness of theobroxide in yam flowering would be tested to determine the appropriate treatment time and concentration.

Plant growth and tuber development

The growth of tested yam plants was smaller in 2007 than in 2005. The experiment was conducted in less sunny conditions with the cooler minimum air temperatures in June and July and this had a negative effect on their growth. The short-day length stimulates tuberization in yam (Shiwachi *et al*., 2002). The short-day treatment inhibited leaves, stems, and tuber growth at the rapid growth stage of tuber (experiment 1). In the primary growth stage of tubers, plant and tuber growth varied among the varieties under the short-day treatment (experiment 2). These results were in line with those of Shiwachi *et al*. (2000). The short-day treatment applied at the primary growth stage of tubers promoted the enlargement of tubers, but when applied after the rapid growth stage of the tubers it tended to inhibit tuber enlargement. When the short-day treatment was applied to plants in experiment 2, it seemed that the tubers of cv. Nagaimo, Taiwan local, and cv. Basmi had already changed to the rapid growth stage. The *D. opposita* variety had a very low sensitivity to short-day length; tubers continued to enlarge slowly, even under long-day length conditions (Yoshida and Kanahama, 1999; Shiwachi *et al*., 2000).

D. opposita is an early maturing plant. Low sensitivity to short-day length was related to the maturity level of plants in *D. rotundata* and *D. alata* (Shiwachi *et al*., 2002). Taiwan local and cv. Basmi could be considered to have an early maturity level compared with cv. Malaysia-A.

Theobroxide treatment did not affect yam tuber growth at the rapid growth stage. Tuber growth was inhibited in cv. Nagaimo, Taiwan local, and cv. Malaysia-A, but promoted in cv. Basmi at the primary growth stage of tubers. Theobroxide treatment influenced potato yield in a field experiment; tuber yield in most of tested varieties increased but was decreased in one variety (Yoshihara and Murai, 2008). To increase yield, theobroxide treatment should be at the stolon formation period and this should be identified in each variety. The usefulness of theobroxide in inceasing yam tuber yield was not clear in this study. The tuber growth characteristics of specific yam varieties would be limited by the need to determine the appropriate treatment time.

Bulbil development

Bulbil formation varies among species and varieties in yam. *D. bulbifela* produces a large number and size of bulbils; it is used for food. Bulbil setting and non-setting types are found in *D. opposita* and *D. alata* although the latter has few bulbil setting species in yams (Coursey, 1967; Inagaki et al., 1985). Bulbil formation was observed in all varieties tested in this study. However, bulbil setting rate (number and weight) varied in varieties and years. Less or no formation of bulbils was observed in cv. Obukosumbori (experiment 1). This variety was considered a non-setting type. Less bulbil formation in cv. Basmi and cv. Malaysia-A was observed in 2007 compared with 2005. It seemed that the cool minimum air temperature affected bulbil formation as well as tuber growth.

Short-day treatment promotes bulbil formation at the rapid growth stage of tubers, but does not affect the bulbils formation at the primary growth stage. Theobroxide promoted bulbils setting in cv. Basmi. When yam seed-setts were planted in April, bulbil development was observed from June in cv. Nagaimo (*D. opposita*), and from September in *D. alata* varieties in Japan. Development of tubers in *D. alata* was earlier than the development of bulbils. Bulbil development would be started after the rapid growth tuber stage. Therefore, the short-day or theobroxide treatment at the primary growth stage of tubers did not affect bulbil development. Bulbil development started under long-day period in *D. opposita*, but under short-day period in *D. alata*. Results showed that short-day stimulated the development of bulbils in *D. alata*.

The usefulness of the theobroxide treatment in bulbil development was not as clear as for tuber development in this study. Theobroxide is the triggers for jasmonic acid production to induce potato *(Solanum tuberosum)* tuberization (Xiquan *et al*., 2005). The enlargement of tubers in *D. alata* was promoted by jasmonic acid (Kikuno *et al*., 2002a, 2002b). There is evidence that bulbils development would be promoted by jasmonic acid. Further study is expected of the theobroxide treatment in yams. Since bulbils are used for seed-setts in *D. opposita* (Inagaki *et al*., 1985) and could be used for seed-setts in *D. alata*, the short-day or theobroxide treatment would be effective for bulbil production. The short-day or theobroxide treatment should be done after the rapid growth stage of tuber, that is the period of bulbil development in *D. alata*. This is first report of the use theobroxide in yams.

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