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https://doi.org/10.5109/18834

出版情報：九州大学大学院農学研究院紀要. 55 (2), pp.231-232, 2010-10-29. Faculty of Agriculture, Kyushu University

J. Fac. Agr., Kyushu Univ., **55** (2), 231–232 (2010)

Ethylene is not Involved in Rhizome Transition to Storage Organ in Lotus (*Nelumbo nucifera*)

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(*Received June 15, 2010 and accepted July 9, 2010*)

We examined the effects of exogenous ethylene on rhizome transition to storage organ in lotus (*Nelumbo nucifera*) by using seed–derived plants. The rhizomes elongated in long days and enlarged in short days with ethephon treatments as well as those in the control. It is suggested that ethylene is not involved in rhizome swelling in lotus plants.

INTRODUCTION

We previously reported that the rhizome enlargement in lotus (*Nelumbo nucifera*) is induced by short photoperiod (Masuda *et al.*, 2006), and phytochrome plays an important role in the photoperiodic response of the rhizomes (Masuda *et al.*, 2007). There are reports that ethylene induced storage organ formation in some geophyte species such as potato (*Solanum tuberosum*) (Catchpole and Hillman, 1969), *Dahlia* (Biran *et al.*, 1972) and onion (*Allium cepa*) (Levy and Kedar, 1970; Levy *et al.*, 1973). In this paper, we report the effects of exogenous ethylene on induction of rhizome enlargement in lotus plants.

MATERIALS AND METHODS

Plant materials and culture

Open pollinated seeds of *N. nucifera* 'Chugoku' were used in this study. The seeds were prepared for germination by soaking in conc. H_2SO_4 for 3 hrs and rinsed with distilled water. They were then soaked in distilled water for one day at 25 ºC. After removing softened seed coats, the seeds were incubated in distilled water at 25 ºC under continuous fluorescent light (approximately 40 $\mu mol m^{-2} s^{-1}$) until germination (nine days). Five seedlings for one treatment were transplanted into lowland soil containing 5 g slow–release fertilizer (N:P:K=16:5:10%) per plastic container (45×32×23.5 cm). The seedlings were grown at 30 ºC for four weeks in the phytotron glass rooms of the Biotron Institute, Kyushu University.

Effects of ethephon in long days

The seedlings were grown in 14 hr photoperiod (8 hr natural light (9:00–17:00) supplemented with 6 hr light for plant growth (6:00–9:00 and 17:00–20:00) on 20 May 2008. Fluorescent tubes (Toshiba FL20SSBRN/18; Toshiba Lighting and Technology Co., Tokyo, Japan) were used for supplemental lighting. Each container was filled up to 5 cm above soil level with water for the first two weeks and then with water or ethephon (2–chloroethyltrimethyl phosphonic acid), an ethylene–releasing compound, solution of different concentrations. The solutions were replaced twice a week. Measurement of leaf number and rhizome enlargement index (REI) (=maximum internode diameter / internode length in each internode) of the first and second internodes, counted from the apical end, took place after the culture.

Effects of ethephon in short days

The seedlings were grown in each container filled up to 5 cm above soil level with water or ethephon solution of different concentrations in 8 hr photoperiod (9:00–17:00 of natural light) on 15 June 2009. The solutions were replaced twice a week.

RESULTS AND DISCUSSION

Number of leaves in each treatment, that indicates whether the rhizome growth continued without swelling or ceased to transit to storage organs, decreased by increasing concentrations of ethephon (Table 1). We use 0.2 of REI as a value to judge whether an internode of rhizomes elongated or swelled (Masuda *et al.*, 2006, 2007); >0.2 indicates rhizome enlargement, while <0.2 indicates rhizome elongation. REIs in the 1st and 2nd internodes were lower than 0.2 in the treatments with <10 mgl^{-1} ethephon and control, whereas those treated with 100 mgl^{-1} ethephon showed high (>0.2) value in the 1st internode. It is due to probably toxically shortened internode length, 1.24 cm against >6.8 cm in other treatments, and not due to enlarged internode diameter. There were no significant differences in number of leaves, internode length and diameter in short days (Table 2). REIs in the 1st and 2nd internodes were >0.2 in all the treatments except for those with 1 mgl^{-1} in the 2nd internode. Ethephon treatment seems to have neither inductive nor inhibiting effects on rhizome enlargement in lotus plants.

There is controversy in regard to the role of ethylene in induction of storage organ formation in geophytes. Exogenous application of ethylene caused tuberization in

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Table 1. Effect of ethephon on number of leaves and rhizome growth in long days

Concentration (mg l^{-1})	Number of leaves	Internode[2]					
		First internode			Second internode		
		D (cm)	L (cm)	REI	D (cm)	L (cm)	REI
0	6.2 b[1]	0.38 ab[1]	11.26 bc[1]	0.03	0.38 a[1]	8.50 a[1]	0.04
0.01	5.8 b	0.38 ab	13.70 c	0.03	0.36 a	12.04 a	0.03
0.1	5.6 ab	0.40 b	9.74 bc	0.04	0.40 a	11.40 a	0.04
1	5.4 ab	0.42 b	8.12 b	0.05	0.36 a	8.28 a	0.04
10	5.6 ab	0.36 ab	6.80 b	0.05	0.34 a	8.30 a	0.04
100	4.8 a	0.28 a	1.24 a	0.23	0.30 a	11.86 a	0.03

[1] Statistically significant differences at $P<0.05$ (Tukey's HSD test) are indicated by different lower–case letters.
[2] The order was counted from the youngest internode.
D: Maximum internode diameter
L: Internode length
REI: Rhizome enlargement index

Table 2. Effect of ethephon on number of leaves and rhizome growth in short days

Concentration (mg l^{-1})	Number of leaves	Internode[2]					
		First internode			Second internode		
		D (cm)	L (cm)	REI	D (cm)	L (cm)	REI
0	4.8 a[1]	1.07 a[1]	4.26 a[1]	0.25	1.16 a[1]	4.48 a[1]	0.26
0.1	4.5 a	0.89 a	1.35 a	0.66	1.12 a	2.28 a	0.49
1	5.6 a	0.89 a	4.56 a	0.20	0.81 a	4.40 a	0.18
10	5.8 a	0.92 a	1.84 a	0.50	0.83 a	2.60 a	0.32
100	5.0 a	0.96 a	2.78 a	0.35	0.91 a	3.84 a	0.24

[1] Statistically significant differences at $P<0.05$ (Tukey's HSD test) are indicated by different lower–case letters.
[2] The order was counted from the youngest internode.
D: Maximum internode diameter
L: Internode length
REI: Rhizome enlargement index

potato plants, but starch accumulation was not observed in the tubers (Catchpole and Hillman, 1969). Palmer and Barker (1973), in opposite, observed that ethephon did not promote *in vitro* tuber formation of the plant. Treatments with ethephon enhanced tuberous root formation of growing plants of dahlia in non–inductive conditions of long days, but did not in budless cuttings of the plants (Biran *et al.*, 1972). Endogenous ethylene increased in inducing short days in dahlia plants 2 to 3 weeks after the treatment, suggesting the role of ethylene in tuberous root formation (Biran *et al.*, 1972). Treatments with ethephon caused bulb initiation in onion (*A. cepa*) in non–inducing short day conditions (Levy and Kedar, 1970). Lercari (1983) confirmed the effectiveness of ethephon on onion bulb initiation in short days, but he also found that silverthiosulphate (antiethylene complex) and aminoethoxyvinylglycine (inhibitor of ethylene biosynthesis) did not affect photoperiod–induced bulb formation, suggesting that ethephon–induced bulb formation does not appear to be related to endogenous control of bulbing.

Our results suggest that ethylene is not involved in rhizome swelling in lotus plants.

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