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Okubo, Hiroshi
Laboratory of Horticultural Science, Faculty of Agriculture, Kyushu University

Yoshida, Satoshi
Laboratory of Horticultural Science, Faculty of Agriculture, Kyushu University

Uemoto, Shunpei
Laboratory of Horticultural Science, Faculty of Agriculture, Kyushu University

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Effect of Scaling Date on Leaf Emergence and Endogenous Plant Hormone Levels in Hyacinth *(Hyacinths orientalis* L.)

Hiroshi Okubo, Satoshi Yoshida* and Shunpei Uemoto

Laboratory of Horticultural Science, Faculty of Agriculture, Kyushu University 46-01, Fukuoka 812, Japan.

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Leaf emergence from bulblets during scaling in hyacinth (*Hyacinthus orientalis* L.) was affected by stage of dormancy. It occurred in the post-dormancy stage, at the end of the period of high summer temperature, but not in the pre- (during winter) and true-dormancy (flowering and bulb maturing periods) stages. Changes in plant hormone levels in the scales and basal plates of mother bulbs were synchronized with these phenomena, i. e. auxin activity was high in the post-dormancy stage and then decreased while abscisic acid activity was detected during the period from pre- to true-dormancy. It was recognized that the induction of dormancy in hyacinth is caused by low temperature. Close relationship between the phenomenon of the induction of dormancy in autumn planting bulbous plants and the induction of bulb formatin is suggested.

INTRODUCTION

Many autumn planting bulbous plants such as *Tulipa, Iris, Hyacinthus* and *Narcissus* originated mainly in the Mediterranean climate areas. They have features of dormancy (summer dormancy) and a cold requirement adapting them to a hot dry summer and a winter, respectively. These features are also seen in *Lilium longiflorum* which originated in the Ryukyu Islands of Japan.

Effects of various factors to overcome bulb dormancy have been well studied for the purpose for forcing cultivation and extension of flowering period, but little is known about factors inducing dormancy in bulbous plants. It is recognized that dormancy in plants is a survival mechanism which has evolved as an adaptation to unsuitable environments for normal growth. It is, therefore, considered that the dormancy in these bulbous plants is induced by the rising temperature or increasing daylength after the occurrence of bulb formation which is induced by low winter temperature. As induction of dormancy in these bulbs, however, is always accompanied by a process of bulb swelling, the relationship between the induction of bulb formation and the induction of bulb dormancy is still unclear. Our objective is to clarify how different the induction of bulb formation is from the induction of dormancy in bulbous plants. We have already obtained some results suggesting closeness of these two phenomena in Iris (Okubo and Uemoto, 1981) and *Lilium* (Uemoto *et al.,* 1983). In this study, leaf emergence during scaling at various physiological stages of mother

*Present address : Biotron Institute, Kyushu University, Fukuoka 812

bulbs of hyacinth was investigated with respect to changes in endogenous plant hormone levels to clarify the relationship between these two phenomena.

MATERIALS AND METHODS

Bulbs of hyacinth cv. Delft Blue, 15 cm in circumference, were obtained from a commercial grower in Japan on August 24, 1984 and kept at room temperature until used.

A fifth of the bulbs without a developed root system was used for initial scaling and extraction of endogenous plant hormones on October 6. The remaining four fifths were planted on October 7 in sand in pots and grown outdoors. The growing bulbs were lifted on December 4, February 8, April 3 and June 14 and used for scaling and extraction. Bulbs used for scaling were divided vertically into eight parts and twin-scales, two scales attached to the basal plate, 15 mm wide and 25 mm long, were prepared. After curing treatment at 25°C with 100% relative humidity for 5 days, they were placed in sand in plastic flats (16 *x* 10 *x* 5 cm) to a depth of about half their lengths, and incubated for 180 days at 15, 20, 25 and 30°C constant temperatures under natural light in the phytotron of the Biotron Institute, Kyushu University. The growth cabinets in which the temperature and the daylength were controlled to keep them similar to those in the phytotron were used for scaling from October 1 to 31 and from April 1 to 30, because the phytotron was out of service during these periods for technical maintenance. Twenty scale sets in two flats were used in each treatment. Observations of growth of scale bulblets and leaf emergence from them were made at one-week intervals.

Fifty grams fresh weight of the scales and basal plates of mother bulbs were used for each extraction of plant hormones. Each lyophilized sample was homogenized and extracted with 200 ml of 80% cold methanol for 24 h at 5°C in the dark by shaking. After filtration, the extract was concentrated *in vacuo* at 37°C and distilled water was added to make the volume to 200 ml. The solution was then acidified to pH 3.0 with 6 M HCI and extracted with an equal volume of ethyl acetate. The ethyl acetate phase was extracted with an equal volume of 2% sodium bicarbonate. After reacidification of the sodium bicarbonate phase to pH 3.0 with 6 M HCl, it was extracted with an equal volume of ethyl acetate. The ethyl acetate acidic fraction was concentrated to dryness, dissolved in a small amount of ethanol, line-loaded on 20 *x* 20 cm Toyo No. 51 paper and developed by ascending chromatography with isopropanol : ammonia : water = 10 : 1 : 1 (v/v/v). The chromatogram was divided into 11 equal areas and the biological activities in each area were tested using *Avena* coleoptile straight growth test.

RESULTS

In preliminary experiments, twin-scales were prepared from 5 different positions of mother bulbs, the outermost, outer, middle, inner and innermost. Number and diameter of bulblets produced, percentage of leaf emergence from bulblets and number of leaves were significantly less in those from the outermost and the innermost regions of mother bulbs after incubation, No differences in these characters were found

between 3 inner positions, outer, middle and inner. Experiments thereafter were carried out with twin-scales prepared from outer, middle and inner regions of mother bulbs mixed.

As shown in Fig. 1, growth of leaves and inflorescence stalk was slow during winter. Their growth rate increased as temperature rose and flowering began on April 1. Growth of daughter bulbs was inhibited until flowering, then accelerated growth occurred from April to June.

As shown in Table 1, number of bulblets produced per scale set ranged from 1 to

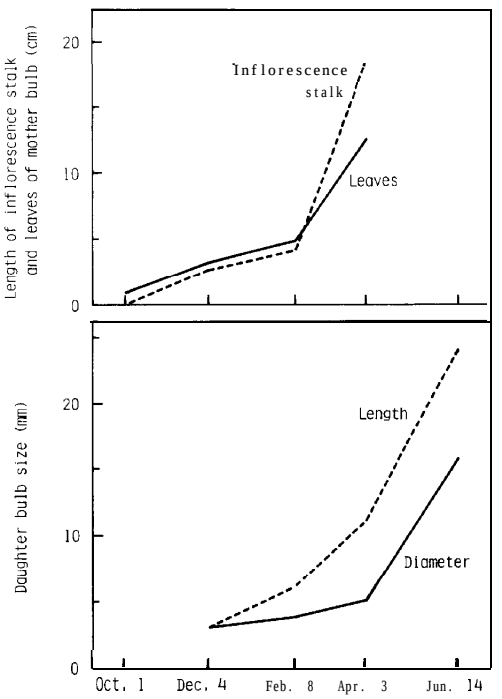

Fig. 1. Growth of inflorescence stalk, leaves and daughter bulbs under outdoor conditions.

Table 1. Effects of time of scaling and scaling temperature on number of bulblets produced. Values within a column followed by different letters are significantly different at 5% level according to Duncan's new multiple range test.

	Scaling temperature			
	15°C	*20°C*	*25°C*	*30°C*
October	1.7* bc	1.3 ac	2.2 bc	1.5 a
December	1.2 ab	1.2 ac	1.1 a	1.1 a
February	1.0 a	1.2 ac	1.4 ac	1.1 a
April	1.0 a	1.0 a	0	0
June	1.9 cd	1.5 bc	2.1 bc	1.2 a

. Number of bulblets per scale.

Table 2. Effects of time of scaling and scaling temperature on diameter of bulblets produced. Values within a column followed by different letters are significantly different at 5% level according to Duncan's new multiple range test.

	Scaling temperature			
	15°C	20°C	25°C	30°C
	mm			
October	6.9 b	6.5 c	4.8 b	6.4 b
December	7.5 b	7.5 e	8.6 c	7.6 b
February	6.5 b	7.3 d	6.1 b	6.8 b
April	6.2 b	5.3 b		
June	2.4 a	3.7 a	1.5 a	1.0 a

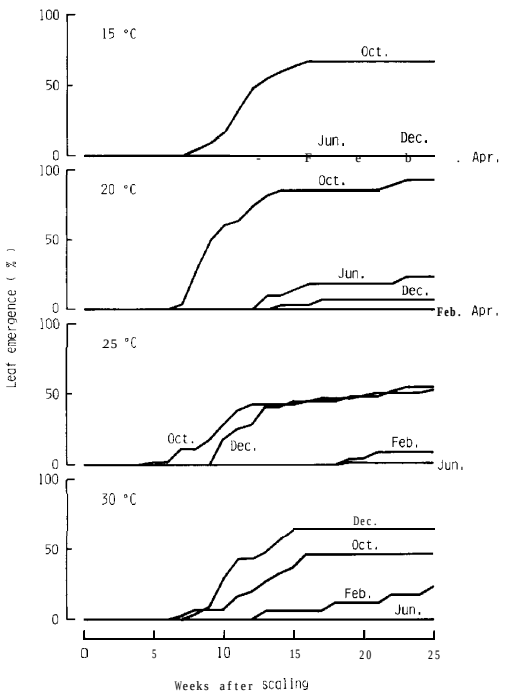

Fig. 2. Effects of time of scaling and scaling temperature on leaf emergence from bulblets.

2 in all treatments, and fewer bulblets resulted when scalings were done during the period from December to April than in October and June. No bulblet formation was observed at incubation temperature of 25 and 30°C when scaling was started in April. The largest bulblets were obtained when scaling was done in December at any incubation temperature, then the diameter declined towards June (Table 2).

Changes in percentage of leaf emergence from newly developed bulblets are shown in Fig. 2. At incubation temperature of 15 and 20°C, leaf emergence was earliest and percentage was highest when scaling was done in October. It was delayed and percentage remained low when scaling was started in December, and there was no leaf

Table 3. Effects of time of scaling and scaling temperature on number of leaves sprouted per bulblet. Values within a column followed by different letters are significantly different at 5% level according to Duncan's new multiple range test.

	Scaling temperature			
	15°C	20°C	25°C	30°C
October	1.3 a	2.1 a	1.8 b	1.2 a
December	1.0 a	1.5 a	1.2 a	1.1 a
February	0	0	1.0 a	1.0 a
April	0	0		
June	1.0 a	1.5 a	1.0 a	0

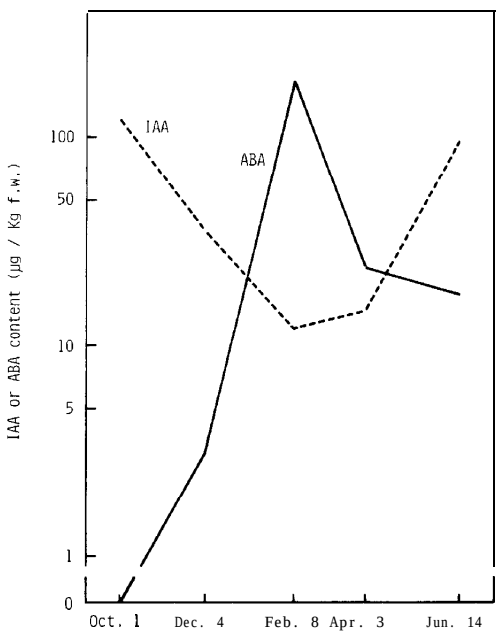

Fig. 3. Changes in auxin and abscisic acid activities in scales and basal plates of mother bulbs.

emergence observed when scalings were started in February and April. Leaf emergence was observed again when scaling was done in June, though its percentage was low. At higher incubation temperatures, 25 and 30°C, the earliest leaf emergence was also observed when scaling was done in October. Percentage of leaf emergence in October-scaling was almost the same as that in December-scaling at 25°C, and the latter was higher than the former at 30°C. Delayed and low percentage of leaf emergence was observed when scaling was done in February, and leaf emergence was almost inhibited when scaling was done in June. Number of leaves sprouted per bulblet 21 weeks after scaling is presented in Table 3. The results obtained here showed a similar tendency to those seen in Table 1. Growth of leaves was most vigorous when scaling was started in October, with that from June-scaling in the second place.

As shown in Fig. 3, auxin activity at Rf zones 0.2 to 0.4 on paper chromatograms, the region to which authentic IAA migrated, was higher in October, and it dramatically decreased during the period from December to April. In contrast, inhibitory activity at higher Rf zones, where authentic ABA is detected, increased after December in proportion to the decrease in auxin activity, and was highest in February. Auxin activity increased again in June, but inhibitory activity still remained high.

DISCUSSION

Changes in rate of leaf emergence from newly developed bulblets coincided with changes in the physiological state of mother bulbs. Leaf emergence was vigorous when twin-scales were prepared from mother bulbs which had already received high summer temperatures. When scalings were done during the winter period, leaf emergence was inhibited at lower incubation temperatures, but not at higher temperatures. There was almost no leaf emergence and bulblets continued swelling when scalings were done at and after flowering times. It is, therefore, considered from leaf emergence behavior that dormancy of hyacinth bulbs is released by high summer temperatures, elongation of leaves and inflorescence stalk and flowering occur after receiving cold winter temperatures, and that dormancy is again induced in the meanwhile.

Bulbs receiving low winter temperatures are thought to be in a state of pre-dormancy as noted in deciduous tree buds by Wareing and Phillips (1981). Then pre-dormancy may subsequently become true-dormancy as temperature rises. These seasonal changes in scaling were also recognized in *Lilium longiflorum* (Uemoto *et al.*, 1983). Matsuo and Arisumi (1979) also found that in *L. longiflorum* the longer the period of cold treatment during the 25°C storage of mother bulbs, the lower the percentage of leaf emergence from newly formed bulblets in scaling outdoors. Matsuo and van Tuyl (1984) reported that a higher storage temperature of mother bulbs of the same plants cv. White American promoted leaf emergence from scale bulblets, whereas a lower temperature delayed it.

At a high incubation temperature of 30°C, percentage of leaf emergence was higher when scaling was done in December than in October, and it decreased in February. It was shown that in *Oxalis* (Aoba, 1972) and in *Iris* (Aoba, 1974), induction of bulb formation was caused by low temperature but its effect was negated by high temperature if low temperature was not given long enough. He concluded that these phenomena resemble vernalization, devernalization and revernalization in flower formation. In hyacinth, it is considered that low temperature was not yet sufficient in December and its inhibition of leaf emergence might be negated by a high incubation temperature, but not in February.

Comparison of chromatographic behaviors and biological activities of substances which migrated to lower and higher Rf zones with authentic indoleacetic acid (IAA) and abscisic acid (ABA) showed the former to be IAA and the latter to be ABA. ABA has been identified in hyacinth bulbs (Nowak et al., 1973).

In accordance with the decrease in percentage of leaf emergence from bulblets during the period from December to April, abscisic acid increased and auxin decreased in the scales and basal plates of mother bulbs, and high activity of abscisic acid continued until June. The fact that breaking of the dormancy of iris bulbs is due to a

decrease of abscisic acid at high temperature (Tsukamoto and Ando, 1973) is evidence that abscisic acid is involved in controlling dormancy in bulbous plants. Our results, therefore, suggest that bulbs of hyacinth are induced into dormancy by low temperature. Increase in auxin activity in June may have a relation only to the swelling growth of daughter bulbs, because abscisic acid activity was still high and percentage of leaf emergence remained low.

Results obtained here are in disagreement with those by Rudnicki and Nowak (1976). In their investigations, cold storage of hyacinth bulbs resulted in a gradual decrease in abscisic acid and an increase in IAA in the bulbs. They analyzed these hormones from whole bulbs including flower buds while our analysis was only of scales and basal plates. We agree that their results might be obtained mainly from flower buds. Saniewski *et al.* (1978) analyzed the hormones in different organs of hyacinth. Abscisic acid was at a high concentration before bulb lifting time. It was not detected in summer, and it was again present in winter in the basal plates and scales in accordance with our results.

In consideration of the facts that abscisic acid increased during growth of bulbous iris under bulb-forming conditions (low temperature) whereas auxin activity increased under non bulb-forming conditions (Okubo and Uemoto, 1981), that induction of dormancy or an increase in abscisic acid in hyacinth is triggered by low temperature (results obtained here), and that low temperature is the main factor for the induction of bulb formation in bulbous plants (Aoba, 1971, 1972, 1974, 1976; Okubo and Uemoto, 1981), it seems to be reasonable for us to conclude that there is a close relationship between the induction of bulb dormancy and the induction of bulb formation in bulbous plants.

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