園学雑. (J. Japan. Soc. Hort. Sci.) 68 (3) : 513-518. 1999.

Effects of Anti-auxins and Basal Plate on Bulblet Formation in Scale Propagation of Amaryllis (*Hippeastrum* × *hybridum* hort.)

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Summary

Effects of anti-auxins and various morphological types of twin and single scales on bulblet formation in amaryllis (*Hippeastrum* × *hybridum* hort.) were investigated. Auxin inhibited bulblet production on single scales, whereas anti-auxins promoted it. Twin scales without the lower half of the basal plate did not lose their ability to produce bulblets. The rate of bulblet formation in two scales obtained from four adjacent scales by removing inner two scales was very low when the two distant scales were connected by a lower half of the basal plate; it was higher when they were connected by an upper half of the basal plate. Two independent scales without a basal plate but connected by plain agar blocks or agar blocks containing activated charcoal formed bulblets faster than did single scales without a basal plate. A single scale having a thicker basal plate induced bulblet formation faster than did the normal single scales. Thus, auxin has a negative role in bulblet production, whereas the basal plate has a significant role as has the outer scales in twin scale propagation of a new plant. We recommend the use of anti-auxins and scales attached to a portion of the basal plate as efficient means of in vitro bulblet propagation.

Key Words: amaryllis, anti-auxins, *Hippeastrum* × *hybridum*, scaling.

Introduction

Since the development and improvement of twin scaling as an artificial vegetative multiplication method for amaryllis (*Hippeastrum* × *hybridum*) (Luyten, 1926; Traub, 1933; Heaton, 1934), it has become the standard manner of cloning the species. Huang et al. (1990a) found that in twin scaling propagation of the plant, the thickness and length of the outer scale affect the rate of bulblet formation and subsequent leaf development; whereas those of the inner scale do not, and that vascular bundles of the bulblets which are initiated on the abaxial surface of the inner scale eventually connect with the vascular system of the outer scale, but not with that of the inner scale. This indicates an important role of outer scale and why two scales and a portion of basal plate that connects the two are essential in scaling propagation. It is, however, still unclear whether the basal plate portion in twin scaling serves only as a connecting tissue or has some important role on bulblet formation.

Single scaling is inferior to twin scaling in *Hippeastrum* and *Narcissus* (Broertjes and Alkema, 1971; Alkema, 1975; Hanks and Rees, 1979). Regeneration of protocorm like bodies (PLB's), which have morphologically and physiologically similar characteristics to PLB's of orchid plants, was reported to develop in vitro on single scales followed by bulblet formation instead of direct bulblet formation (Huang et al., 1990b). Huang et al. (1985) reported that in twin scaling of *H.* × *hybridum* naphthyl-1-acetic acid (NAA) strongly inhibited bulblet formation, and Tombolato et al. (1994) confirmed the inhibitory effect of auxins on bulblet formation in scaling of the plant. In single scaling, auxin inhibited PLB formation (Huang et al., 1990b). In this study, the roles of auxin, anti-auxins, basal plate and scale in bulblet formation during vegetative propagation were investigated.

Received; March 2, 1998. Accepted; June 19, 1998.

Materials and Methods

Culture

Bulbs of *Hippeastrum* × *hybridum* hort. cv. Apple Blossom, 25 cm in circumference, grown in a plastic-film greenhouse at Kyushu University were used throughout the experiments. All experiments were conducted in August and September 1994.

Bulbs of *Hippeastrum* are composed of enlarged leaf bases only, and there are no true bulb scales. The term "scale(s)" in this paper, therefore, refers to leaf base(s).

Experiment 1. Effects of growth regulators on bulblet formation in single scaling

After the bulbs were lifted, their leaves, roots, outermost two or three scales and innermost undeveloped scales were removed. Twin scales (2.5 cm in height x 1

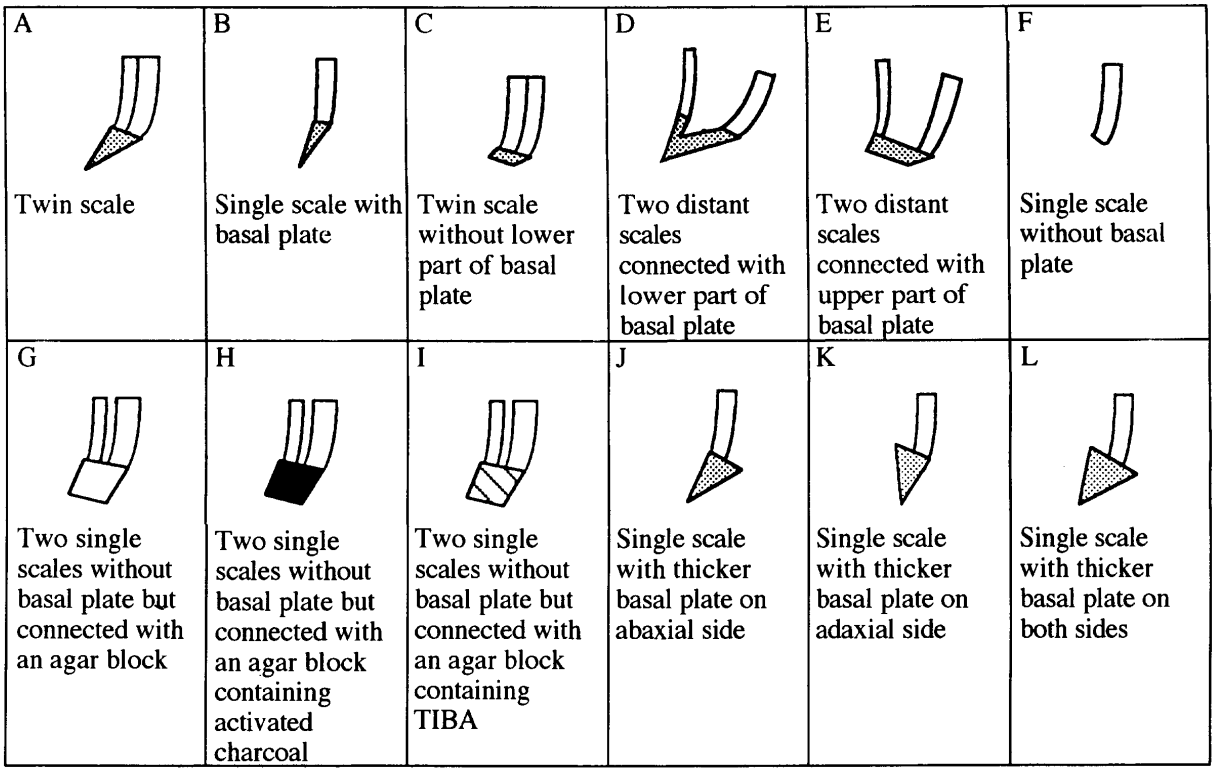

Fig. 1. Scale types prepared for scaling experiments.

cm in width) composed of a thick outer scale and a thin inner scale with a portion of basal plate (Type A scales)(Fig. 1) and single scales of the same size (Type B scales) were prepared from the bulbs by the same procedure as described (Huang et al., 1990a). Single scales were as thick as the outer scale of the Type A scales. The explants were sterilized in 10 % sodium hypochloride (10-13 % available chloride) for 10 min, and then rinsed with sterile distilled water three times. The single scales were then placed on a paper wick in liquid MS medium (Murashige and Skoog, 1962) with 30 g·liter^{-1} sucrose containing 0, 0.1, 1.0 or 10.0 mg·liter^{-1} NAA, naphthylphthalamic acid (NPA; an inhibitor of auxin transport (Morgan and Söding, 1958)), 2, 3, 5-triiodobenzoic acid (TIBA; an inhibitor of auxin transport (Niedergang-Kamien and Leopold, 1957)), 4-chlorophenoxy-isobutyric acid (PCIB; a chemical that interferes with IAA action (Wright and Rayle, 1982)) or 9-hydroxyfluorene-9-carboxylic acid (morphactin; an inhibitor of auxin transport (Krelle and Libbert, 1968)) in a 24 x 100 mm glass tube sealed with double layers of aluminum foils. The twin scales were placed in the same manner as the single scales but with no plant growth regulators in the medium. The pH of the media was adjusted to 5.7 before autoclaving at 120 ℃ for 15 min.

The explants were incubated at 25 ℃ under continuous light (15 μE·m^{-2}·sec^{-1}) for 70 days. Fifty-five explants were used for each treatment.

Experiment 2. Effects of basal plate on bulblet formation

Four adjacent scales joined by a portion of the basal plate were prepared from the bulbs in the same manner as described above. The outermost scale and the third scale from it were thicker than the intermediate ones, the second scale and the innermost scale. The second and third scales were removed, leaving two distant scales connected with the lower part of the basal plate (upper half of the basal plate was removed) (Type D scales). One set of scales was connected with the upper part of the basal plate (lower half of the basal plate was removed) (Type E scales). These explants were incubated as in Experiment 1. No plant growth regulators were added in the medium in Experiment 2 to 4. Twin scales (Type A scales), twin scales without the lower part of basal plate (Type C scales) and single scales (Type B scales) were also prepared and cultured.

Thirty explants for each scale type were used. Measurement were made 120 days after culture.

Experiment 3. Effects of connecting media of two single scales on bulblet formation

Twin scales (Type A scales) were separated into two single scales by removing the basal plate (Type F scales). The two separated scales were implanted closely and vertically into 5 % agar blocks (1.5 x 1.5 x 1.5 cm) (Type G scales) or those containing 5 g·liter^{-1} activated charcoal (Type H scales) or 10 mg·liter^{-1} TIBA (Type I scales) just before the agar was solidified so that they looked like twin scales. The combination and position of two scales, which were the same as the original twin scales, were incubated for 120 days as described above.

Experiment 4. Effects of thickness of basal plate on bulblet formation

Single scales with various shapes of basal plate were prepared as follows: 1) single scales with a basal plate of the same thickness of the scale (Type B scales); 2) single scales without a basal plate (Type F scales); 3) those with a thicker basal plate on abaxial side than the adaxial side (Type J scales); 4) those with a thicker basal plate on the adaxial than on the abaxial side (Type K scales); and 5) those with equally thick basal plates on both sides (Type L scales).

Thirty explants for each treatment were cultured for 120 days as above.

Results

Experiment 1. Effects of growth regulators on bulblet formation in single scaling

All twin scales formed 2.5 bulblets per twin scale, whereas a third of single scales produced 0.9 (Table 1). The former produced no PLB's, whereas the latter yielded three PLB's per scale.

Treatments with NAA decreased the percentage of bulblet formation and number of bulblets and PLB's. PCIB and morphactin of all concentrations examined and 10 mg·liter^{-1} TIBA increased the percentage of single scales that formed bulblets; NPA had a small effect. The bulblets were mainly initiated on the abaxial side of the scale.

Experiment 2. Effects of basal plate on bulblet formation

All twin scales which lacked a lower part of basal plate (Type C scales) formed bulblets as did the normal twin scales (Type A scales) although the number of bulblets was fewer in Type C scales than in Type A scales (Table 2). No PLB's were produced on these scales. The type D scales formed only 33.9 % bulblets, equaling that of Type B scales. In Type E scales, which were connected with the upper half of the basal plates, formed more bulblets than did the Type D scales.

The numbers of bulblets in Type D and E scales were significantly less than that in Type C scales, whereas more PLB's were produced from Type D and E scales than from Type B single scales.

Experiment 3. Effects of connecting media of two single scales on bulblet formation

Type F scales formed a few bulblets, whereas Type G scales formed just one bulblet per scale (Table 3). In Type H scales in agar with activated charcoal, the percentage of bulblet formed was higher than that attained by Type G scales. Two scales connected with agar blocks containing TIBA (Type I scales) decreased the rate of bulblet formation.

Type G, H and I scales produced one or more bulblet but there were no significant differences among the treatments. Likewise the number of PLB's on Type F

Table 1. Effects of growth regulators on bulblet formation in single scaling.

Treatment	Conc. (mg·liter^{-1})	% of bulblet formation[z]			No. of bulblets per twin or single scale[y]	No. of PLB's per twin or single scale[y]
		Total	on adaxial scale surface	on abaxial scale surface		
Twin scaling[x]		100[w]			2.5e	0
Single scaling[x]		31.7	24.1 (76.8)	7.6 (23.2)	0.9bcd	3.1d
NAA	0.1	25.7	10.3 (40.1)	15.4 (59.9)	0.3ab	1.4ab
	1.0	12.8	7.7 (60.2)	5.1 (39.8)	0.1a	1.6abc
	10.0	18.1	13.6 (75.1)	4.5 (24.9)	0.1a	1.2a
NPA	0.1	35.4	20.8 (58.8)	14.6 (41.2)	0.5abc	1.5abc
	1.0	36.0	18.0 (50.0)	18.0 (50.0)	0.2a	2.4abcd
	10.0	34.0	18.0 (52.9)	16.0 (47.1)	0.5abc	1.8abcd
TIBA	0.1	30.9	11.9 (38.5)	19.0 (61.5)	0.9bcd	2.4abcd
	1.0	19.6	17.4 (88.8)	2.2 (11.2)	0.7abc	1.3a
	10.0	47.0	8.8 (18.7)	38.2 (81.3)	0.7abc	2.7bcd
PCIB	0.1	44.5	6.2 (13.9)	38.3 (86.1)	0.4ab	1.3a
	1.0	40.7	14.1 (34.6)	26.6 (65.4)	0.5abc	1.5abc
	10.0	36.5	12.2 (33.4)	24.3 (66.6)	0.6abc	1.9abcd
Morphactin	0.1	50.0	0 (0)	50.0 (100)	0.6abc	2.9bcd
	1.0	57.5	6.4 (11.1)	51.1 (88.9)	1.3d	1.6abc
	10.0	50.0	17.0 (34.0)	33.0 (66.0)	1.1cd	1.9abcd

[z] Numerals in parentheses are the ratio of bulblet formation on adaxial to abaxial scale surfaces.

[y] Mean separation within columns by Duncan's multiple range test, 5%.

[x] Twin and single scales were cultured without plant growth regulators.

[w] All the bulblets were formed on abaxial surface of inner scale.

Table 2. Effects of basal plate on bulblet formation in twin scaling.

Scale type[z]		% of bulblet formation	No. of bulblets[y]	No. of PLB's[y]
A	Twin scale	100	3.2c	0
B	Single scale	30.5	0.7a	3.1a
C	Twin scale without lower part of basal plate	100	2.5b	0
D	Two distant scales connected with lower part of basal plate	33.9	0.8a	6.1b
E	Two distant scales connected with upper part of basal plate	50.0	1.2a	5.0b

[z] A to E; see Fig. 1.
[y] Mean separation within columns by Duncan's multiple range test, 5%.

Table 3. Effects of media on bulblet formation.

Scale type[z]		% of bulblet formation	No. of bulblets[y]	No. of PLB's[y]
F	Single scale without basal plate	3.4	0.1a	4.1a
G	Two single scales without basal plate but connected with an agar block	24.6	1.0b	8.8b
H	Two single scales without basal plate but connected with an agar block containing activated charcoal	37.0	1.3b	5.1a
I	Two single scales without basal plate but connected with an agar block containing TIBA	17.2	1.2b	10.7b

[z] F to I; see Fig. 1.
[y] Mean separation within columns by Duncan's multiple range test, 5 %.

and H scales was not significantly different, but it was about twice that attained from Type G and I scales.

Experiment 4. Effects of thickness of basal plate on bulblet formation

About thirty percent of single scales with thicker basal plates in the abaxial side of the scales (Type J scales) formed bulblets equally well as did the normal single scales (Type B scales) (Table 4). However, the percentage of bulblet formation on abaxial scale surface in the former was about twice that of the latter. Type K and L scales had a high bulblet forming ability, particularly the Type K scales, which had the highest percentage of bulblet formation. Bulblet formation on the abaxial scale surface in Type K and L scales was inferior to that in Type J scales. Type J scales tended to form bulblets on abaxial scale surface, whereas Type K did so on the adaxial scale surface; Type L produced bulblets on both sides of the scales. Type L scales produced the highest yield of bulblets. The frequency of PLB decreased as the percentage of scales that produced bulblets increased.

Bulblets formed on abaxial and adaxial scale surfaces

Table 4. Effects of basal plate on bulblet formation in single scaling.

Scale type[z]		% of bulblet formation		No. of bulblets[y]	Diameter of bulblets[y] (mm)		No. of PLB's[y]
		Total	on abaxial scale surface		on abaxial scale surface	on adaxial scale surface	
B	Single scale with basal plate	30.5	11.9	0.7b	6.4b	6.0b	3.1bc
F	Single scale without basal plate	3.4	3.4	0.1a	4.1b	4.5a	4.1c
J	Single scale with thicker basal plate on abaxial side	29.5	26.7	0.7b	5.1a	6.0b	3.6c
K	Single scale with thicker basal plate on adaxial side	73.6	18.9	0.7b	4.3a	6.3b	2.0ab
L	Single scale with thicker basal plate on both sides	44.1	17.6	1.6c	5.5a	6.8b	1.2a

[z] B, F, J, K and L; see Fig. 1.
[y] Mean separation within columns by Duncan's multiple range test, 5%.

were similar in their diameter in Type B scales, whereas those formed on adaxial scale surface were larger than those on abaxial scale surface in Type J, K and L scales.

Discussion

In single scaling of the plant in vitro, treatment with IAA and NAA produced only PLB's, and the bulblets were formed on the PLB's (Huang et al., 1990b). It is evident from our results that anti-auxin application causes direct bulblet formation in single scales instead of PLB's, and confirms the inhibitory role of auxins in scale propagation of *Hippeastrum*.

In Experiment 2, the upper half of the basal plate played an important role on bulblet formation, but an increase in number of PLB's from Type D and E scales suggests that two distant scales respond as if they were two independent single scales. In Experiment 3 a single scale adjacent to another produced bulblets when they were connected by an agar block which confirms the importance of the second scale as suggested by Huang et al. (1990a). It seems that a portion of basal plate in twin scaling only serves as a connective tissue. The increasing effect of activated charcoal in agar blocks on bulblet formation may indicate some physiological role of basal plate tissue on bulblet formation because the charcoal can be partly substituted for the basal plate. Activated charcoal is known to absorb various compounds including auxins (Reinert and Bajaj, 1977). As anti-auxins can promote direct bulblet formation in single scaling (Experiment 1), they perhaps control bulblet formation by absorbing or inactivating auxins or inhibiting auxin transport. That activated charcoal in agar blocks also decreased the number of PLB's supports the role of the basal plate. It is difficult to explain why the addition of TIBA to agar blocks inhibited bulblet formation.

That bulblets are more easily induced on one side of the scale surface having thicker basal plate than on the opposite side (Experiment 4) indicates the importance of the basal plate. Therefore, the induction of bulblets or PLB's is mainly controlled by a basal plate. Hence its role is that of absorption or inactivation of auxin, inhibition of auxin transport or supply of anti-auxin-like substances.

That large twin-scales gave higher bulblet yields in twin-scaling of *Narcissus* (Hanks and Rees, 1978) and that the number of bulblets formed was proportional to the length of explants of *Hyacinthus orientalis* L. cultured in vitro (Pierik and Ruibing, 1973; Pierik and Post, 1975) indicate that organ regeneration depends on the food reserves in the scales. The differences in bulblet sizes on abaxial and adaxial scale surfaces in single scales having basal plates with different thickness and the location may be attributed to positional effect because the single scales are the outer scales to the bulblets on adaxial side in conventional twin-scaling. Furthermore, the basal plate also contains reserve food.

We conclude that a piece of the basal plate tissue in twin scale propagation of *Hippeastrum* acts physiologically as well as mechanically as a connecting tissue in bulblet formation. Because it controls bulblet or PLB induction, it may be more important than the outer scale.

Our results demonstrate that the use of anti-auxin in in vitro propagation of *Hippeastrum* plants for efficient promotion of bulblet initiation is practical, especially if the scales are cultured attached to the basal plate.

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アマリリス (*Hippeastrum* × *hybridum* hort.) の仔球形成に及ぼす抗オーキシンおよび底盤部の影響

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摘　要

アマリリスのりん片挿しにおける仔球形成に及ぼすオーキシン，抗オーキシンおよび球根底盤部の影響を調査した．オーキシンは2りん片挿しにおける仔球形成を抑制した．1りん片挿しでは仔球形成に先立ちラン科植物に見られるようなPLBが形成されるが，オーキシンはPLBの形成をも抑制した．一方，抗オーキシンはPLB形成を抑制し，直接，仔球形成を促進した．2りん片の底盤部の下半分を切除した場合においても仔球形成能は低下しなかった．底盤部をつけた4りん片を作成し，その内部の2りん片を取り除いた2りん片の仔球形成率は下半分の底盤部を切除した場合に比べて上半分を切除した場合に低かった．1りん片2個を，2りん片であるかのように寒天もしくは活性炭を含む寒天に挿して培養すると，底盤部はなくても高い仔球形成率を示し，活性炭を含む寒天を底盤部の代用とするとより高い仔球形成率を示した．底盤部のみを厚くした1りん片はりん片の厚さと同じ厚さの底盤部をもつ通常の1りん片よりも高い仔球形成率を示した．以上のことから，1りん片挿しにおいては抗オーキシンが仔球形成を促進すること，ならびに仔球形成には底盤部が重要な働きをしていることが明らかになった．