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Studies on Bulblet Differentiation in Bulb-scale Segments of *Lilium longiflorum* II. Effects of anaerobic treatment

Nahoko ISHIOKA and Shizufumi TANIMOTO
(Laboratory of Genetic Engneering)
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Summary

Anaerobic treatment applied to bulb-scale segments of *Lilium longiflorum* promoted adventitious bulblet initiation. A short term treatment (15 to 90 min) with 100 % N_2 stream caused an increment in the number of bulblet formed on the explants. Similar treatment with air stream showed no effects. The treatment with N_2 was effective only when it was given to the explants just after the excision from mother bulb-scales. The treatment applied to the explants which were in culture for more than 60 min could not cause any effect. Addition of anti-auxin or anti-cytokinin to the medium did not inhibit or stimulate the promotive effects of anaerobic treatment.

Key words: anaerobic treatment, bulblet differentiation, *Lilium longiflorum,* wounding

Introduction

In the previous experiments[3], we reported that wounding treatment and addition of traumatic acid were promoted adventitious bulblet initiation in bulb-scale explants of *Lilium longiflorum,* and effects of traumatic acid were closely correlated with wounding. We also demonstrated same promotive effects of wounding and traumatic acid on adventitious bud initiation in *Torenia* stem segments[8]. In this material, anaerobic treatment was also effective and correlated with wounding[6, 7]. The anaerobic treatments promoted somatic embryogenesis in carrot suspension cultures[4] and N_2 treatment given to cultured tobacco anthers showed same promotive effects on pollen embryogenesis[1, 2]. The effectiveness of anaerobic treatment may provide some clue to elucidate intriguing questions of plant organogenesis although its action mechanism is presently unknown. Therefore, we tried to examine the effects of anaerobic treatment on bulblet differentiation in bulb-scale explants of *L. longiflorum.*

Materials and Methods

The material bulb-scales (12 mm in length, 8 mm in width and 6 mm in depth) were prepared from cultured plantlets of *Lilium longiflorum* Thunb., the bulb-scales were

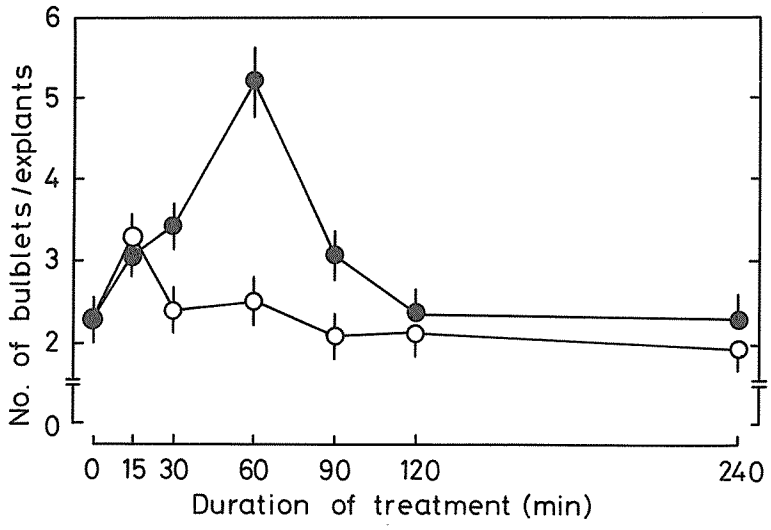

Fig. 1. Effects of N_2 or air treatment on bulblet initiation in bulb-scale explants. Explants were arranged in Petri dishes with basal culture medium, and treated with air (○) or N_2 (●) stream for various period. For each treatment, 200 explants were used, the experiments were repeated at least 3 times, and the standard errors were then calculated.

transversally cut to 6 pieces[3], and the each peace was used as explant. The explants were cultured on the basal medium contained Murashige and Skoog's mineral salts[5], 4 % sucrose, 0.25 % Gelrite, 0.1 μM of naphthaleneacetic acid (NAA) and 1 μM of benzyladenine (BA). The explants were arranged in open Petri dishes (9×6 cm). The Petri dishes with explants were placed in a glass desicator and given air or N_2 treatment for various periods. Filter-sterilized air or N_2 at 1 bar was flowed (300 ml/min) through the desicator. To examine the correlative effects of anti-cytokinin and anti-auxin, 4-cyclobutylamino-2-methylpyrrolo-[2,3-d]pyrimidine (CB-P; one of anti-cytokinins kindly supplied to Dr. H. Iwamura, Kyoto University) and triiodobenzoic acid (TIBA) were added to the culture medium. The cultures were maintained under 16 hr long day photoperiod (6,000 lux) at constant temperature of 25±2 °C. After 3 weeks of culture, bulblet initiation and number of bulblet formed were observed.

Table 1. Effects of dryness and wetness on bulblet initiation in bulb-scale explants.

Treatment	No. of bulblet per explant
No treatment	2.3±0.4
Incubation with liquid medium (15 min)	1.0±0.2
Air treatment (15 min)	3.1±0.6

The explants were pre-incubated with liquid culture medium for 15 min, folowed by air for 15 min, or no treated, and then cultured on the basal solid culture medium. For each treatment, 200 explants were used, the experiments were repeated at least 3 times, and the standard errors were then calculated.

Results and Discussion

Effects of anaerobic treatment

When the explants were cultured on the basal culture medium without anaerobic treatment, the number of bulblets formed in the explant ranged from 2.0 to 2.4[3]. On the contrary, as shown in Fig. 1, a short term treatment (15 to 90 min) with 100 % N_2 stream caused an increment of bulblet number, and the best result was obtained by N_2 treatment for 60 min (5.4 bulblets were formed in the explant). Relative long treatment (120 to 240 min) with N_2 stream showed no effect of bulblet initiation, though the explants did not exhibit any damage. The short term treatment (15 min) with air slightly stimulated the bulblet initiation, however, only 3 bulblets were formed in the explant and long term treatment with air showed no effects.

Table 2. Effects of N_2 treatment during various periods of cultures on bulblet initiation in bulb-scale explants.

Period of N_2 treatment	No. of bulblet per explant
No treatment	2.3±0.2
0-1 hr	5.6±0.6
1-2 hr	2.6±0.4
2-3 hr	2.4±0.2
4-5 hr	2.2±0.2
24-25 hr	2.2±0.2

Firstly, explants were cultured on the basal medium for 0, 1, 2, 4 or 24 hr, then treated with N_2 stream for 1 hr. For each treatment, 200 explants were used, the experiments were repeated at least 3 times and the standard errors were then calculated.

Although short term treatment with air showed slight promotion, this effects were thought to be due to the dryness of explants. Thus, we examined the effects of dryness on bulblet initiation. The explants were incubated on the liquid culture medium for 15 min, and then cultured on the solid medium. As shown in Table 1, incubation with liquid medium showed suppressing effects on bulblet initiation.

To find out the most effective period of anaerobic treatment, the explants were cultured first on the basal culture medium for various durations (0 to 24 hr), then treated with N_2 stream for 60 min. Promotive effects of N_2 treatment on bulblet initiation were obtained only when the treatment was applied just after the excision of explants (Table 2). The treatment applied to the explants which were pre-cultured for more than 1 hr, could not cause any stimulative effect.

Table 3. Correlative effects of anti-cytokinin (CB-P), anti-auxin (TIBA) and anaerobic treatment on bulblet initiation in bulb-scale explants.

Treatment	No. of bulblet per explants		
	− CB-P − TIBA	+ CB-P (10 μM)	+ TIBA (10 μM)
Air treatment (60 min)	2.3±0.2	2.1±0.2	3.4±0.4
N_2 treatment (30 min)	3.3±0.4	3.0±0.4	3.8±0.6
N_2 treatment (60 min)	5.2±0.6	5.0±0.4	5.6±0.8

Explants were treated with air for 60 min, or N_2 stream for 30 or 60 min immediately after the excision, and cultured on the basal medium with or without CB-P or TIBA. For each treatment, 200 explants were used, the experiments were repeated at least 3 times, and the standard errors were then calculated.

These results showed that anaerobic treatment may affect the initial process of bulblet initiation, and the initial stage is controlled by excision (wounding) itself. The stimulatory effects of wounding[3, 6, 7] can be amplified by the anaerobic treatment.

Correlative effects of anti-auxin, anti-cytokinin and N_2 treatment.

The treatment with N_2 stream caused stimulative effects on bulblet initiation. This effectiveness seemed to be due to the increment of endogenous phytohormones. To examine the possibility, we tried to investigate the correlative effects of CB-P, TIBA and N_2 treatment on bulblet initiation. The results (Table 3) clearly showed that N_2 treatment did not correlate with endogenous phytohormone levels.

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鉄砲ユリの鱗片切片培養における球根分化に関する研究

II. 嫌気処理の影響

石岡奈穂子・谷本　靜史

(生物工学講座・遺伝子工学研究室)

摘　　要

鉄砲ユリの鱗片切片培養において，切片を窒素ガスを用いて短時間（15から90分間）嫌気処理した後に培養することにより，球根分化が促進される．同様の処理を空気で行った場合には全く効果はみられない．窒素ガスによる分化促進効果は切片を採取直後に嫌気処理した時にのみみられ，採取後1時間以上経過した切片では効果はない．抗オーキシンや抗サイトカイニンが嫌気処理の効果に影響しないことから，内生ホルモンレベルの関与は否定される．嫌気処理は切片の採取，すなわち切片に与えた傷害の効果を増幅することにより球根分化を促進するものと考えられる．