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Factors Affecting the Regeneration and Growth of Bulblets in Bulb-scale Cultures of *Lilium rubellum* Baker[1]

Yoshiji NIIMI

Faculty of Agriculture, Laboratory of Horticultural Science, Niigata University, Niigata 950-21

Summary

Lilium rubellum Baker bulb-scales excised from bulbs growing *in vitro* were cultured at different temperatures, in various lengths of light period, or in a medium containing different concentrations of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA).

Temperature affected the percentage of regenerating bulb-scales, the number of bulblets per scale, and the growth of bulblets. The optimum temperature for each of the parameters was 25°C.

The percentage of regenerating bulb-scales and the number of bulblets per scale were stimulated in a lighted environment, but the fresh weight of bulblets formed in this environment was lower than the weight of those formed in darkness.

NAA at concentrations of 0.05 and 0.1 mg/*l* gave a stimulatory effect on each of the parameters, but the higher concentrations suppressed bulblet formation. BA scarcely affected any of the parameters, except in the combination of 0.1 mg/*l* BA with 1.0 mg/*l* NAA, which enhanced the fresh weight of the bulblets.

Introduction

In vitro asexual propagation has been applied to *Lilium rubellum* Baker for the proliferation of plantlets which were genetically identical to the parent plants (10), because the extent of rose color, the flowering time, the length of stem, the number and shape of leaves depend on the parent plants (6). As shown in a previous paper (11), however, the productivity of the explants excised from each organ of *L. rubellum* parent plants was found to be very low as compared with *L. longiflorum* (19). To increase the number of bulblets for a short period, the bulb-scale culture, in which bulb-scales are excised from bulbs formed *in vitro*, seemed to be one of several useful methods. However, it has been reported that in axenic bulb-scale cultures concentrations of growth regulators (1, 13, 19) and culture conditions (2, 7, 19) suitable for the development of bulblets depend on *Lilium*. The present study was made to clarify effect of temperature, of length of light period and of concentration of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA) on the regeneration and growth of bulblets in *L. rubellum* bulb-scale cultures.

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Materials and Methods

Medium. ——The basal medium consisted of Murashige and Skoog's salt (8) together with 2 mg/*l* glycine, 100 mg/*l* myo-inositol, 0.5 mg/*l* nicotinic acid, 0.5 mg/*l* pyridoxine-HCl, 0.1 mg/*l* thiamine-HCl, 50 g/*l* sucrose, and 7 g/*l* Difco Bacto agar. Different concentrations of growth regulators NAA and BA were added to the basal medium in each experiment, and the concentrations are shown in results. All media were adjusted to pH 5.6-5.7 with 0.1 N NaOH and 0.1 N HCl before the addition of sucrose and agar. The medium was dissolved and about 20 ml of it was then poured into each 50 ml Erlenmeyer flask, all of which were then capped with aluminium foil and autoclaved for 10 minutes at

121°C under a pressure of 1.2 kg/cm².

Bulb-scale culture. ——Unexpanded leaves were dissected from mother plants and cultured for 120 days in darkness in a basal medium supplemented with 1.0 mg/*l* NAA and 0.1 mg/*l* BA, according to the procedures described previously (10, 11). Developing bulblets were dissected and subcultured in the basal medium at 24±1°C in darkness for a few months before experiments on bulb-scale cultures began.

All bulb-scales, excluding the innermost scales near the growing point, were dissected from the subculturing bulbs. The bulb-scales, weighing more than 10 mg in fresh weight, were chosen, and 3 to 5 bulb-scales were placed in each of flasks with the abaxial side down on the basal medium or on the basal medium supplemented with growth regulators at various concentrations.

Bulb-scales were cultured for 60 days in darkness or in a lighted environment consisting of 20 W, white fluorescent tubes, giving about 1000–1200 lx at the culture surface. The number of bulb-scales cultured in each of the experiments is shown in results.

Determining number and weight of developed bulblets. ——At the end of the incubation period, bulb-scales were removed from each of the flasks for evaluation. After the number of bulb-scales forming bulblets were determined, the average number and fresh weight of the bulblets formed were evaluated in each of the flasks. Fresh weight of rooted bulblets was determined after removal of the roots. The results were subjected to statistical analysis at the 5% level following Duncan's multiple range test (3).

Results

Effect of temperature. ——Table 1 shows the results obtained at the various temperature, 15, 20, 25 and 30°C. The highest percentage of regenerating bulb-scales was observed at 25°C, and 30°C reduced the regeneration rate with occasional death of bulb-scales. The number of bulblets per scale and the fresh weight of bulblets were greatest at 25°C, and the nearest suboptimum temperature for the formation of bulblets was 30°C, at which their growth, however, was extremely inhibited. In conclusion, 25°C was the optimum temperature for all parameters.

Table 1. Regeneration and bulblet growth in bulb-scales cultured in a basal medium supplemented 1.0mg/*l* NAA and 0.1mg/*l* BA, at different temperatures in darkness for 60 days. Eighteen bulb-scales were cultured in each of the treatments. Means in columns not followed by the same letter are significantly different at the 5% level.

Temperature (°C)	Number of bulb-scales with bulblets (%)	Number of bulblets per scale	Mean fresh weight of bulblet (mg)
15	66^{b}	1.4^{b}	34^{b}
20	83^{ab}	1.4^{b}	23^{b}
25	89^{a}	2.3^{a}	57^{a}
30	66^{b}	1.8^{ab}	14^{b}

Effect of length of light period. ——Table 2 shows the results obtained in various lengths of light period. The percentage of regenerating bulb-scales increased as the length of light period increased, although there were statistically no differences among treatments. This was also true for the number of bulblets per scale, which was greatest in a continuously lighted environment and lowest in darkness. In contrast, the fresh weight of bulblets was greatest in darkness although there was statistically no difference between the fresh weight of the bulblets developed in darkness and those developed in a continuously lighted environment.

Effect of NAA alone or of NAA in combination with BA. ——Table 3 gives the results of growth regulators. As compared with the control, the addition of growth regulators generally reduced the percentage of regenerating bulb-scales, particularly at 0.5 mg/*l*

Table 2. Regeneration and bulblet growth in bulb-scales cultured at various lengths of light period(h) at 24±1°C. Thirty bulb-scales in each of the treatments were cultured in the same kind of medium as in Table 1. Statistical analysis as in Table 1.

Length of light period (h)	Number of bulb-scales with bulblets (%)	Number of bulblets per scale	Mean fresh weight of bulblet (mg)
0	80^{a}	1.6^{c}	60^{a}
8	83^{a}	2.5^{abc}	37^{b}
16	93^{a}	2.8^{ab}	34^{b}
24	100^{a}	3.4^{a}	47^{ab}

Table 3. Regeneration and bulblet growth in bulb-scales cultured in a basal medium supplemented with various concentrations of NAA alone or of NAA together with BA. Forty scales in each of the treatments were cultured at 24±1°C in a continuously lighted environment. Statistical analysis as in Table 1.

Growth regulators (mg/l)		Number of bulb-scales with bulblets (%)	Number of bulblets per scale	Mean fresh weight of bulblet (mg)
NAA	BA			
0	0 (control)	88^{a}	2.0^{bcde}	16^{de}
0.01	0	80^{ab}	2.5^{abcd}	23^{bcde}
0.05	0	88^{a}	3.4^{a}	32^{bc}
0.1	0	83^{ab}	3.0^{ab}	37^{b}
0.5	0	58^{bcde}	1.9^{bcde}	29^{bcde}
1.0	0	30^{fg}	1.0^{ef}	32^{bc}
2.0	0	35^{efg}	0.9^{ef}	14^{e}
3.0	0	15^{g}	0.2^{f}	20^{cde}
0.01	0.001	75^{abc}	2.1^{bcde}	23^{bcde}
0.01	0.01	75^{abc}	1.8^{bcde}	16^{de}
0.01	0.1	73^{abc}	1.6^{cde}	24^{bcde}
0.1	0.001	70^{abcd}	2.7^{abc}	25^{bcde}
0.1	0.01	88^{a}	3.4^{a}	27^{bcde}
0.1	0.1	90^{a}	2.2^{bcd}	30^{bcd}
1.0	0.001	55^{cdef}	1.9^{bcde}	22^{bcde}
1.0	0.01	45^{defg}	1.4^{de}	23^{bcde}
1.0	0.1	65^{abcd}	1.6^{cde}	55^{a}

NAA and more, and at 1.0 mg/l NAA in combination with BA. The number of bulblets per scale increased when only NAA at the concentrations of 0.05 and 0.1 mg/l was added. The addition of BA with NAA scarcely affected the increase in the number of bulblets, except for 0.01 mg/l BA together with 0.1 mg/l NAA, in which 3.4 bulblets developed as shown in 0.05 mg/l NAA. The fresh weight of bulblets was greatest in 1.0 mg/l NAA in combination with 0.1 mg/l BA, in which the number of bulblets per scale was lower than the control. NAA at concentrations from 0.01 up to and including 1.0 mg/l also stimulated the growth of bulblets. From a comprehensive analysis of the combined parameters, NAA at the concentrations of 0.05 and 0.1 mg/l seemed to be most favorable for the production of bulblets.

Discussion

The present study showed that temperatures affected all parameters, i. e., the percentage of regenerating bulb-scales, the number of bulblets per scale, and the growth of bulblets (Table 1). The optimum temperature for the *in vitro* production of bulblets in other bulb plants was as follows : 25°C was favorable for bulblet formation in *L. speciosum* (2) ; 21.6 or 24.8°C for bulblet growth in hyacinths (14) ; and 25°C for all parameters in narcissus (17). Furthermore, it has been reported that a temperature increase from 15 to 22°C increases the active sucrose uptake rate about 50% in the *in vitro* root discs of sugar beets (16). These data show that the optimum temperatures for *in vitro* development of bulblets probably lie from 20 to 25°C. The range of temperatures has been not only applied to the *in vivo* scale propagation of *Lilium* (18), but also has favored scale initiation and filling of *L. longiflorum* daughter bulbs (20). Hence, it could be concluded that 25°C was the favorable temperature for the *in vitro* propagation of *L. rubellum* Baker.

Table 2 showed that illumination, particularly continuous light, strongly promoted bulblet formation. The effect of illumination on bulblet formation depended on *Lilium* : The lighted environment was favorable for *L. longiflorum* 'Osnat' (7) ; but unfavorable both for *L. longiflorum* 'Ace' (19) and *L.* oriental hybrids (13). Darkness was also favorable for the regeneration and growth of bulblets in the leaf segment cultures of *L. rubellum* Baker (10). The discrepancy seems to be due partly to the differences in the physiological states of the explants investigated. Table 2 also showed that the growth of bulblets formed in darkness was superior to those formed in a lighted environment. This seems to be related with the number of bulblets formed on a bulb-scale : Since rooting in developing bulblets began 1 to 1.5 months after bulb-scale culture (data not shown), the developing bulblets seemed to uptake nutrition through each individual bulb-scale nearly throughout the entire period of bulblet growth ; and as a result, the nutrient competition in the bulblets grown in lighted conditions is assumed to be greater than in those formed in darkness.

The present study gave the following growth-regulator results : NAA was clearly required for the formation and growth of *L.*

rubellum bulblets ; and low concentrations were more favorable for them. Growth of isolated bulblets of *L. rubellum* Baker was also stimulated in the presence of 0.1 mg/*l* NAA or of 0.1 mg/*l* NAA in combination with 0.001 mg/*l* BA (12). Similarly, it has been reported elsewhere that the development of bulbets was stimulated at 0.03 mg/*l* NAA in *L. longiflorum* (19) and 0.1 mg/*l* in *L. speciosum* (1). This may be related to the fact that *Lilium* bulb-scales ordinarily form new bulblets in the *in vivo* scale propagation without difficulty. The present study also showed that BA was not a critical addendum to increase the number of bulblets. The same phenomena have been observed in several other bulb plants (4, 5, 15), and furthermore, cytokinin 2 iP suppressed the regeneration and growth of bulblets in *L. longiflorum* (19). Hence, cytokinins seem to be less important addendum in the bulb-scale cultures in *Lilium*, although Murashige indicated that cytokinins were one of critical compounds in increasing the proliferation rate of herbaceous and woody plants (9).

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ヒメサユリのりん片培養における子球の形成及び生長に及ぼす要因

新 美 芳 二
新潟大学農学部 950-21 新潟市五十嵐2の町

摘 要

試験管内でヒメサユリの子球の生産を効果的にすすめるため，試験管内で育成された子球のりん片を外植体として用い，それらを異なる培養温度（15, 20, 25及び30℃）及び日長（0, 8, 16及び24時間），あるいは生長調整物質（NAA 及び BA）を種々の濃度で添加した培地で培養した．そして，各条件下で培養したりん片の子球分化率，りん片当たりの子球数及び子球生体重を調査した．

培養温度はりん片の子球分化率，りん片当たりの子球数及び子球生体重のいずれにも影響し，25℃ が子球の形成及び生長に最適であった．

明条件は子球の分化率及び子球形成を促進し，特に24時間日長の連続光は著しい効果を示した．暗条件では子球の形成は劣ったが，形成された子球の生体重は最大であった．

0.05 及び 0.1 mg/*l* NAA の添加は子球の形成及び生長を促進したが，1.0 mg/*l* 以上の濃度では阻害的に作用した．NAA とともに添加した BA は，0.1mg/*l* BA と1.0mg/*l* NAA を添加した区で形成された子球の生長を促進した以外はりん片の子球分化率及び子球形成数にほとんど効果がないことがわかった．