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APPLICATION OF LEAF-SEGMENT CULTURES TO IN VITRO BULBLET PRODUCTION OF SIX LILIUM SPECIES

Y. NIIMI

Faculty of Agriculture, Laboratory of Horticultural Science, Niigata University, Niigata 950–21, Japan.

SUMMARY

Three 5 mm strips excised from the basal region of young leaves of 6 *Lilium* species were cultured in vitro, and the rates of regeneration and the number of bulblets per explant were investigated. The bulblet productivity depended both on the kind of *Lilium* species and the origins of each explant. Growth regulators also affected the bulblet productivity; α-naphthaleneacetic acid (NAA) was absolutely required for the induction and growth of bulblets whereas addition of 6-benzylaminopurine (BA) was ineffective.

1. INTRODUCTION

Leaf-segments excised from growing leaves of *Lilium rubellum*, *L. japonicum*, *L. auratum*, and *L. speciosum* × *L. auratum* produced bulblets (NIIMI & ONOZAWA 1979). Furthermore, the productivity of *L. rubellum* leaf segments proved to be superior to that of scale-, stem- and tepal-segments (NIIMI 1984). On the other hand, TAKAYAMA & MISAWA (1979) reported that in *L. speciosum*, segments of leaves excised just before anthesis were a poor source for the in vitro production of bulblets.

The present study was carried out in order to investigate whether leaf-segments excised from 6 *Lilium* species, including *L. speciosum* and *L. rubellum*, can form bulblets and was attempted to explain the inconsistent results in bulblet productivity for *L. rubellum* and *L. speciosum*.

2. MATERIALS AND METHODS

The plants of 6 *Lilium* species were cultivated in an experimental garden under the same conditions. Young leaves of each *Lilium* species were excised and cultured on the following dates: *Lilium maculatum* Thunb., May 1; *L. rubellum* Baker, May 3; *L. auratum* Lindl. and *L. speciosum* Thunb., May 7; *L. platyphyllum* Makino, May 9; and *L. formosanum* Wallence, May 12.

Preceding culturing in vitro, young growing leaves excised from each parent plant were surface-sterilized by swabbing them with cotton wool containing 70% ethanol, and then by immersing them for 5 min. in a 10% solution of a commercial bleach containing 6% sodium hypochloride in water. Next, the leaves were

rinsed 3 times in sterilized distilled water. After removal of a 3 to 7 mm long basal region including the petiole, three 5-mm transverse strips were dissected from the basal region of each leaf and designated as LS-I, LS-II and LS-III in the order of their location in the basal region.

The basal medium consisted of MURASHIGE & SKOOG's medium (1962) supplemented with 2 mg/l glycine, 100 mg/l myo-inositol, 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 the growth regulators NAA and BA were added to the basal medium in each experiment (see *table 1* and *2*). 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, dissolved in a 1000 ml Erlenmeyer flask, was autoclaved for 10 minutes at 121 °C. About 40 ml of the autoclaved medium was poured into sterilized plastic petri dishes (94 × 21 mm) (Corning Co.) under sterilized conditions.

The 5-mm transverse strips (6 per petri dish), dissected from each of two leaves, were placed with the abaxial side down on each medium. Each treatment consisted of 4 to 6 petri dishes. The cultures were kept in the dark at 24 ± 1 °C. The number of leaf-segments forming bulblets (% of regeneration) and the number of bulblets per explant were determined 90 days after culture. The significance of these findings was tested at 5-percent level.

3. RESULTS AND DISCUSSION

Table 1 shows that the regenerative ability of strips excised from the different regions of leaves depended on *Lilium* species.

In *L. rubellum*, the regenerative ability of LS-I was superior to that of LS-II and LS-III, and the highest rates of regeneration and bulblet production were observed in the explants cultured in the medium containing 1.0 mg/l NAA and 0.1 mg/l BA. These results were in accordance with those in a previous report (NIIMI & ONOZAWA 1979).

In *L. maculatum* and *L. formosanum*, all of LS-I regenerated bulblets when cultured in media supplemented with growth regulators, and several of LS-II also formed bulblets in media containing more than 0.5 mg/l NAA irrespective of addition of BA, whereas none of LS-III formed bulblets even when cultured in any kind of media (*table 1*). LS-I, LS-II and LS-III of *L. speciosum*, irrespective of the kind of culture media, showed high regenerative rates as compared with the strips of other *Lilium* species: when the leaf-segments were cultured in the medium supplemented with NAA 0.5 mg/l and BA 0.01 mg/l, all of them formed bulblets; and several of the leaf-segments formed bulblets even when cultured in the basal medium (*table 1*), indicating that *L. speciosum* leaf segments excised from young growing leaves have high regenerative ability.

The leaf-segments of *L. platyphyllum* and *L. auratum* as well as those of *L. speciosum* formed bulblets at relatively high rates, except in a few cases where the strips of LS-II and LS-III cultured in the basal medium or in media containing growth regulators in some combinations of their concentrations had no response (*table 1*).

Table 1. Percentage of leaf-segments forming bulblets in *6 Lilium* species. Leaf-segments were excised from different regions of each individual leaf and cultured in a Murashige and Skoog's medium supplemented with NAA and BA at different concentrations.

Growth regulators (mg/l)		Percentage of explants forming bulblets in:																							
		L. rubellum Baker				*L. maculatum* Thunb.				*L. platyphyllum* Makino				*L. auratum* Lindl.				*L. speciosum* Thunb.				*L. formosanum* Wallance			
NAA	BA	I	II	III	M*	I	II	III	M	I	II	III	M	I	II	III	M	I	II	III	M	I	II	III	M
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	17	8	17	7	0	0	2
0.05	0	0	0	0	0	67	0	0	22	50	44	13	33	89	22	22	44	100	83	50	78	100	0	0	33
0.05	0.01	0	0	0	0	50	0	0	17	72	72	13	54	89	0	0	30	100	75	33	69	33	0	0	11
0.1	0	8	0	0	3	44	0	0	15	61	44	38	50	67	22	0	30	100	83	58	81	83	0	0	28
0.1	0.01	25	0	0	8	89	0	0	30	72	77	50	67	56	11	11	26	100	100	67	89	100	0	0	33
0.5	0	25	0	0	8	100	11	0	37	78	44	50	58	100	56	11	56	100	100	75	92	83	0	0	28
0.5	0.01	17	0	0	6	100	17	0	39	72	83	75	79	100	67	44	70	100	100	100	100	17	17	0	11
1.0	0	33	17	0	17	78	0	0	26	63	61	75	67	78	33	0	37	100	100	92	97	83	0	0	28
1.0	0.01	33	8	0	14	100	11	0	37	83	72	88	83	100	67	22	63	100	100	92	97	83	17	0	33
1.0	0.1	83	50	17	50	67	100	0	56	44	44	88	63	76	44	44	56	92	100	100	97	67	17	0	28
LSD	5%	25	14	9		38	21			NS	NS	29		56	50	29		16	17	29		38	NS		

*I, II and III represent three 5-mm transverse strips dissected in the order of their location from the proximal regions of each individual leaf. M represents the average of I, II and III.

These results indicate that *Lilium* species investigated in the present study could be divided into two groups: the formation of bulblets was nearly restricted to the strip LS-I as seen in *L. rubellum*, *L. maculatum* and *L. formosanum* seem to belong to this group; *L. speciosum*, *L. auratum* and *L. platyphyllum* were classified into another group, in which the strips excised from the basal regions easily formed bulblets when they were cultured in media containing NAA. It is difficult to explain the difference in their regenerative ability between these two groups. This may be attributable to the cellular activity and totipotency of the excised strips, suggesting not only that the rates of regeneration greatly depends on the physiological state of cultured explants but also that the reversion from adult status to juvenile one is relatively difficult in *Lilium* leaf-segments even when *Lilium* leaf-segments are cultured in media containing NAA and BA in different combinations of their concentrations. The difficulty in the reversion observed in the present study was also recognized in the tissue cultures of other plants (BHOJWANI & RAZDAN 1983).

The number of bulblets per explant depended on the leaf-segments dissected from the location in the basal region of leaves and was also affected by the addition of growth regulators (*table 2*). In the leaf-segment cultures of *L. speciosum* the numbers of bulblets formed in LS-II and LS-III were greater than those in LS-I although the regenerative rates of LS-I were higher than those of LS-II and LS-III. Similar tendency was observed in the leaf-segment cultures of *L. platyphyllum* and *L. auratum*. These results are different with those of *L. rubellum*, in which the leaf-segments of LS-I were effective explants for the production of bulblets, as compared with the strips LS-II and LS-III (*table 2*; NIIMI & ONOZAWA 1979). This inconsistency can be explained as follows: the strips LS-II and LS-III of *L. speciosum*, *L. platyphyllum* and *L. auratum* were as regenerable as those of LS-I (*table 1*); the whole areas of the strips LS-II and LS-III became larger than those of LS-I only because the width of each strip was not trimmed in the present study; as a result, the numbers of bulblets formed in LS-II and LS-III were greater than those in LS-I.

The optimum concentrations of growth regulators for increasing the number of bulblets depended on the strips from the location in the basal region of leaves in each *Lilium* species. The addition of NAA at 0.1 mg/l and above to the basal medium generally increased the number of bulblets, whereas BA was a less important additive. Similar phenomena have been reported in *Lilium* scale cultures, in which NAA was absolutely required for the formation and growth of bulblets, whereas the addition of BA was ineffective (STIMART & ASCHER 1978, NIIMI 1985). Hence it can be concluded that cytokinin is a less important growth regulator in *Lilium* bulblet production in vitro than auxin is.

The present study showed not only that the strips excised from growing leaves were appropriate for the in vitro production of bulblets of *Lilium* species, but also that *L. speciosum* leaf-segments were an excellent explant to produce bulblets, although TAKAYAMA & MISAWA (1979) reported that no bulblets developed when the segments of the mature leaves excised just before anthesis were cultured in MURASHIGE & SKOOG's medium (1962) containing 1 mg/l NAA and 1 mg/l

Table 2. Number of bulblets formed in the leaf-segments of *6 Lilium* species shown in table 1.

Growth regulators (mg/l)		Number of bulblets per cultured leaf-segment in:																	
		L. rubellum Baker			*L. maculatum* Thunb.			*L. platyphyllum* Makino			*L. auratum* Lindl.			*L. speciosum* Thunb.			*L. formosanum* Wallance		
NAA	BA	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7	0.6	0.1	0.1	0	0
0.05	0	0	0	0	1.5	0	0	0.6	1.9	0.8	3.7	2.6	0.8	2.4	6.5	1.5	3.0	0	0
0.05	0.01	0	0	0	2.2	0	0	1.1	2.7	0.3	2.2	0	0	2.6	3.3	2.0	1.2	0	0
0.1	0	0	0	0	1.7	0	0	0.7	1.8	0.3	1.7	1.9	0	2.3	5.3	2.8	2.7	0	0
0.1	0.01	2.4	0	0	2.8	0	0	1.0	2.1	2.4	1.8	0.2	0.2	2.6	5.8	4.0	5.3	0	0
0.5	0	1.3	0	0	2.6	0.1	0	1.0	0.7	2.4	2.8	2.9	0.9	2.2	6.0	5.1	2.7	0	0
0.5	0.01	0.6	0	0	3.7	0.5	0	1.6	2.0	2.4	4.1	2.9	4.7	2.4	4.9	6.5	0.5	0.5	0
1.0	0	1.6	0.4	0	1.6	0	0	1.0	1.4	2.3	2.8	0.7	0	2.8	5.7	7.2	1.8	0	0
1.0	0.01	1.3	0.3	0	3.7	0.3	0	0.9	2.2	2.1	2.0	3.3	0.3	1.8	4.3	5.4	2.0	0.3	0
1.0	0.1	2.2	1.3	0.2	2.4	3.3	0	0.4	1.2	1.6	1.7	0.7	0.9	1.4	3.4	4.5	2.0	1.2	0
LSD	5%	1.3	0.5	0.1	1.6	1.4		0.8	NS	NS	NS	NS	1.8	0.9	2.0	2.3	1.8	NS	

BA. This inconsistency of the bulblet productivity of *L. speciosum* leaf-segments cultured in vitro may be attributable to the difference in the developmental stages of the excised strips and in the concentration of the added growth regulators NAA and BA, suggesting that the induction of cellular division and totipotency in the mature leaves of *L. speciosum* is difficult. More systematic work on the whole area of plant physiology and biochemistry in special reference to plant regeneration is required to discover the culture conditions necessary to elicit cellular totipotency.

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