Growing and Flowering of in vitro Propagated *Lilium japonicum* Thunb.

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Lilium japonicum **Thunb., sasa-yuri, proliferated by scale culture and enlarged in vitro, were investigated with regard to growth and flowering in the field. When the annual growth of bulbs in the field was observed, leaf emergence of the first year bulbs was seen to be low, but the second year bulbs showed a high leaf emergence rate regardless of bulb weight. Bulbs weighing 1.5 g and more at transplanting had flowers in the second year. A bulb's weight at harvest time was clearly related to stem leaf emergence and length of shoot. The lilies with long shoots and many leaves had larger bulbs. The flowering rate was higher on the larger bulbs, and 80% of the bulbs, which weighed more than 6 g, had flowers. It is, therefore, possible to produce flowers in the field during the second year of cultivation, provided the bulbs are grown up to 6 g or more during the first year.**

INTRODUCTION

Lilium japonicum occurs naturally in various parts of Japan, it has a nice fragrance and light pink flowers. So, it is one of the lilies which Japanese people have loved very much for a long time. However, nowadays its natural habitats are decreasing due to environmental pollution and plant numbers are decreasing due to excessive gathering. Commercial cultivation is not undertaken as it is very slow to grow and difficult to propagate. *Lilium japonicum* seen for sale are mostly removed from the wild. Therefore, propagation and cultivation techniques need to be established in order to protect and increase the populations of the plant in the wild.

In recent years, micropropagation methods for *L. japonicum* have been established (Fukui et al., 1989; Tanaka et al., 1991; Kawarabayashi, 1993). Also, Niimi (1995) and Mizuguchi and Ohkawa (1995) have reported on the growth of micropropagated bulbs of *L. japonicum* in the field. However, the detailed flowering characteristics of the bulbs are not yet clear. So in this research we investigated the growth and flowering of *L. japonicum*, micropropagated and enlarged in vitro, in the field.

MATERIALS AND METHODS

The original *L. japonicum* bulbs were gathered in Miyama, Gifu Pref., and were cultured in accordance with the methods of Fukui et al. (1989) and Nagase et al. (1990) by shoot tip culture. Propagation of the bulblet was accomplished by scale culture on MS medium supplemented with 3% sucrose and 0.7% agar. Formed

Table 1. Annual growing of cultured bulbs.

First Year					Second year						
		Weight of the bulbs at digging (g)							Weight of the bulbs at digging (g)		
Weight of bulbs at transplanting (g)	Rate of leaf emergence (%)	Leaf emerging bulbs	Leaf non-emerging bulbs	Ave.	Rate of leaf emergence (%)	Length of shoot (mm)	Number of leaf	Rate of flowering (%)	Stl[X]	Sl[W]	Ave.
2 - 2.5	14.3 (2.8)[Z]	4.53	2.12	2.6	100 (5.5)	220.0	6.4	11.1 (11.1)[Y]	4.87	2.13	4.5
1.5 - 2	8.6 (2.8)	4.45	1.82	2.02	97.1 (2.8)	205.0	6.1	5.5 (5.5)	4.64	2.96	4.5
1 - 1.5	2.9 (0)	3.23	1.23	1.29	88.9 (25)	168.9	5.3	0	3.53	2.09	3.2
0.5 - 1	22.9 (20)	1.01	0.81	0.86	91.7 (66.7)	183.2	5.7	0	3.17	2.10	2.3

Z Figure in parentheses are rate of scale leaf emergence
Y Figure in parentheses are rate of having flower bud
X Stem leaf : stem leaf emergence
W Scale leaf : scale leaf emergence

bulblets were placed on MS medium supplemented with 10% sucrose, 0.7% agar, and NAA (α-naphthaleneacetic acid) 10^{-7} M, and subcultured every 2 months under dark conditions for 10 months. After culturing, the bulbs were stored at 5 C for 8 weeks. Following cold treatment, all bulbs were cultivated in a vinyl glasshouse with 70% shading. Liquid fertilizer (10N-10P-10K) was applied once or twice every 2 weeks and water was applied by sprinkler once or twice per week.

Experiment 1. Annual Growth Rates. Bulbs were selected by weight from 0.5 g to 2.5 g and transplanted on 25 March 1993. After confirming that the above-ground parts of the plants had withered, bulbs were dug and weighed on 24 Nov. 1993. The bulbs were planted again on 8 Dec. 1993 and length of shoot, number of leaves, number of flowers, and flowering time were investigated the next spring. Then, bulbs were dug and weighed on 22 Nov. 1994.

Experiment 2. Effect of Bulb Weight on Flowering and Growth. After low temperature treatment, the bulbs were grown in the field for one season from March to November 1993, and then were selected by weight from 0.5 g to 9 g and transplanted on 8 Dec. 1993. The length of shoot, number of leaves, number of flowers, and flowering time were investigated and bulbs were dug and weighed on 29 November 1994.

RESULTS

Experiment 1: Annual Growth Rates. Table 1 shows the annual growth rates achieved. Leaf emergence of the first year's bulbs was low, but the second year's bulbs had high leaf emergence rates of about 90%, regardless of the weight of the bulbs. Moreover, it was seen that a lot of scale leaves appeared on the smaller bulbs. The leaves of the bulbs weighing between 0.5 and 1 g at the end of the second year were 66.7% scale leaves.

Bulb weight at harvest time is related to leaf emergence. The lilies with plenty of leaves had larger bulbs, but those with little leaf growth did not produce large bulbs in the first year. The lilies which produced stem leaves had much larger bulbs at the end of the second year compared with those producing only scale leaves. In the second year of cultivation, bulbs weighing less than 1.5 g at transplanting did not flower. The flowering rate on 2.0 to 2.5 g bulbs was 11.1% (Table 1).

Experiment 2: Effect of Bulb Weight on Flowering and Growth. Table 2 shows the effect of bulb weight on flowering and growth in the second year. The leaf emergence rate was about 100% on all bulbs, although the rate of scale leaves was 62.5% on 0.5- to 1-g bulbs. The shoot length was higher in proportion to the bulb weight at transplanting, reaching 300 mm on bulbs weighing 5 g and more. Also, the number of leaves increased proportionately. Shoot length is shown in Figure 1. Leaves had emerged by 10 April and extended rapidly by 20 April, regardless of the weight of the bulbs. However, the degree of extension was different in relation to bulb weight, being larger on large bulbs. Leaf extension was not observed after the beginning of May. The flowering rate was higher on the larger bulbs, and 80% of the bulbs weighing more than 6 g had flowers (Table 2). From this it was clear that bulb weight and flowering are related. Each flower was single white-pink in colour, no mutation occurred (Fig. 2). All bulbs began flowering within a few days of each other.

Table 2. Effect of bulbs weight on flowering and growing for the second year.

Weight of bulbs at transplanting (g)	Rate of leaf emergence (%)	Length of shoot (mm)	Number of leaf	Rate of flowering (%)	Flowering day (1994)	Weight of bulbs at digging(g)
9 - 10	100	445.0	12.0	100 (100)[Y]	5/30	8.2
8 - 9	100	392.0	10.8	80 (80)	5/30	9.6
7 - 8	100	357.8	9.6	55.5 (77.8)	5/31	5.4
6 - 7	100	397.2	9.1	81.3 (81.3)	5/31	7.6
5 - 6	100	320.2	9.0	33.3 (47.6)	5/31	5.6
4 - 5	100	287.0	7.7	20.8 (20.8)	6/1	6.7
3 - 4	95.2	253.5	7.5	0	-	5.8
3 - 2.5	100 (5.2)[Z]	220.8	7.1	0	-	5.7
2.5 - 2	91.7	181.4	5.8	0	-	3.9
2 - 1.5	100	162.1	5.4	0	-	3.6
1.5 - 1	100 (12.5)	144.5	5.1	0	-	3.7
1 - 0.5	100 (62.5)	137.2	4.9	0	-	2.3

[Z] Figure in parentheses are rate of scale leaf emergence
[Y] Figure in parentheses are rate of having flower bud.

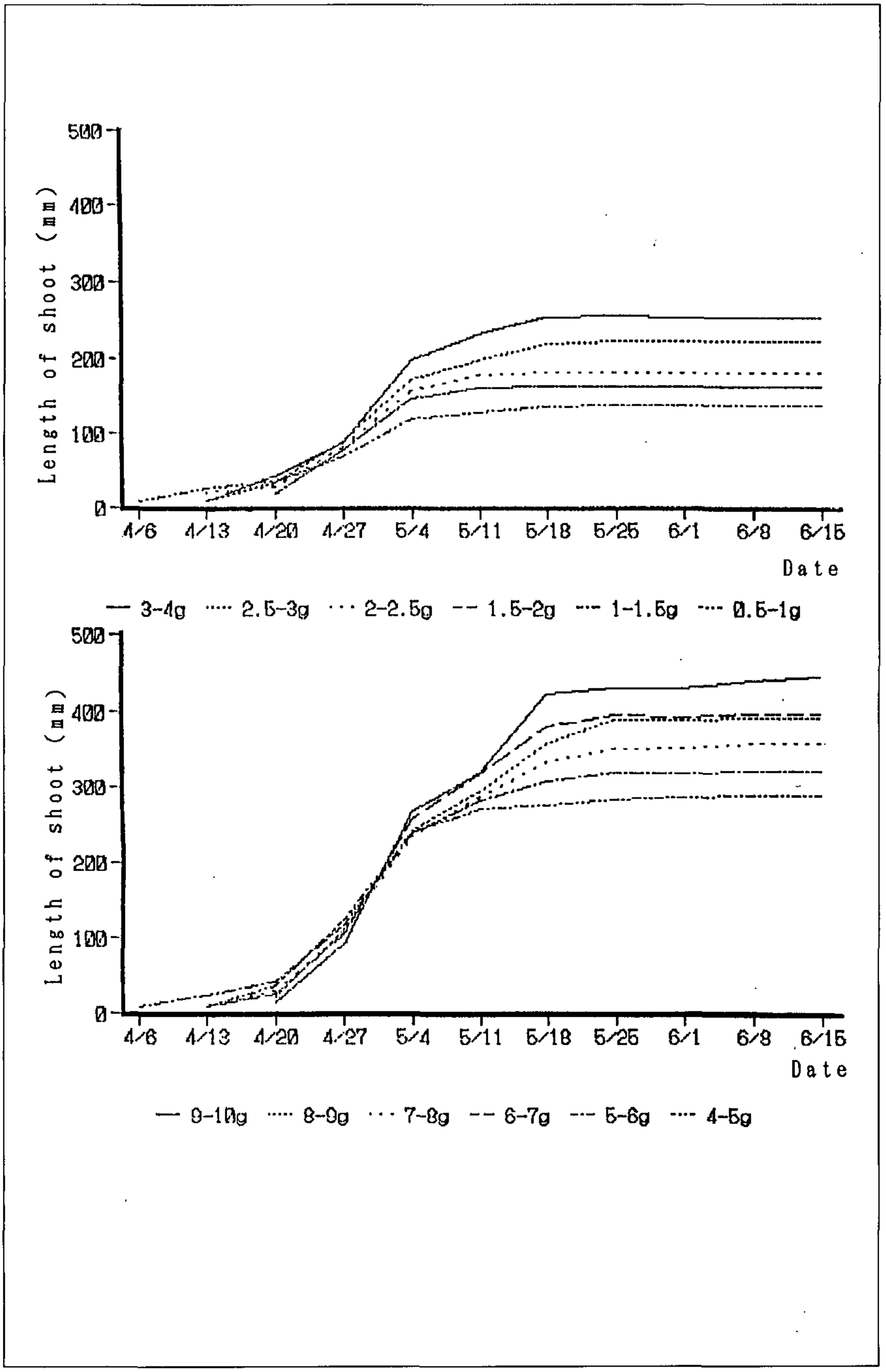

Figure 1. Shoot length of bulbs after transplanting in soil.

Bulb weight at harvest did not increase much on bulbs weighing more than 5 g, at transplanting, but a threefold increase occurred in bulbs weighing 0.5 to 1 g. Figure 3 shows the relation between the weight of bulbs at harvesting, the length of shoot, and the presence of the flower bud. The weight of bulbs at harvesting correlated with the length of shoot, regardless of the weight of the bulb at transplanting. It was clear that bulbs were larger in proportion to the length of the shoot. On the other hand, the bulbs which had no flower bud increased in weight to 8 g, but the bulbs which had a flower bud only increased to 4 g. So it is seen that the presence of a flower bud had an influence on the enlargement of the bulbs. A lot of bulbs above 5 g had a flower bud. Therefore, it was thought that flower bud formation caused a decrease in the weight of the bulbs above 5 g.

DISCUSSION

Takayama (1982) reports that a high sucrose concentration in the medium brings about deep dormancy of *L. auratum* bulbs. Bulblets cultured on a medium which contains 90 g liter^{-1} sucrose require low temperature treatment for a longer period than bulblets cultured on medium which contains 30 g liter^{-1} sucrose. Niimi (1995) reports that bulblets of *L. japonicum* cultured on medium which contains 5% sucrose require low temperature treatment at 4C for 12 weeks, and that short-term treatment produces less leaves. In the current experiment, the low rate of leaf emergence might be caused by insufficient broken dormancy, due to the 8-week period of low temperature treatment being too short.

The enlargement of the bulb in the field differs with the form of leaf which emerges, the lilies which produced stem leaves had the biggest bulbs in the field. Mizuguchi et al. (1995) also reported that bulbs producing stem leaves grew larger than those producing only scale leaves. It has also been reported that the type of leaf produced is affected by the size of the bulb and the light condition under culture in vitro and that bulblets which grew up to 400 mg and more under dark conditions produced stem leaves (Niimi, 1995). From this experiment it can be seen that the length of shoot and presence of flower buds affected the enlargement of the bulbs. It would appear also that the length of shoot is closely related to the number of leaves. The rate at which the shoots lengthened increased as the number of leaves and the consequent rate of photosynthesis increased. Also, it was thought that flower bud growth stimulated an increased consumption of carbohydrates.

As for *L. japonicum*, it takes 4 to 5 years to flower from seed (Shimizu, 1987). A 45-mg bulblet of *L. japonicum* produced by scale culture had a flower after 3 years and 3 months (Mizuguchi et al., 1995). In this experiment, *L. japonicum* flowered after 1 year and 3 months in comparison with the 3 to 4 years required when cultivated from seed. It would appear that the bulbs are enlarged enough during the 10 months of subculture and this equates to 3 years cultivation in the field. Takayama et al., (1990) also reported that the flowering of in vitro-propagated *L. auratum* bulbs is about 2 to 3 years faster than conventional methods of growing from seed. Micropropagation through tissue culture may be an effective propagation method for Oriental lilies that grow slowly and take a long time to germinate from seed. It is thought that *L. japonicum* differentiates flower buds in late autumn during dormancy (Ohkawa, 1989). We would like to try flower bud differentiation in vitro in order to shorten the cultivation period required to flowering.

Figure 2. *Lilium japonicum* flowering in the second spring after transplanting.

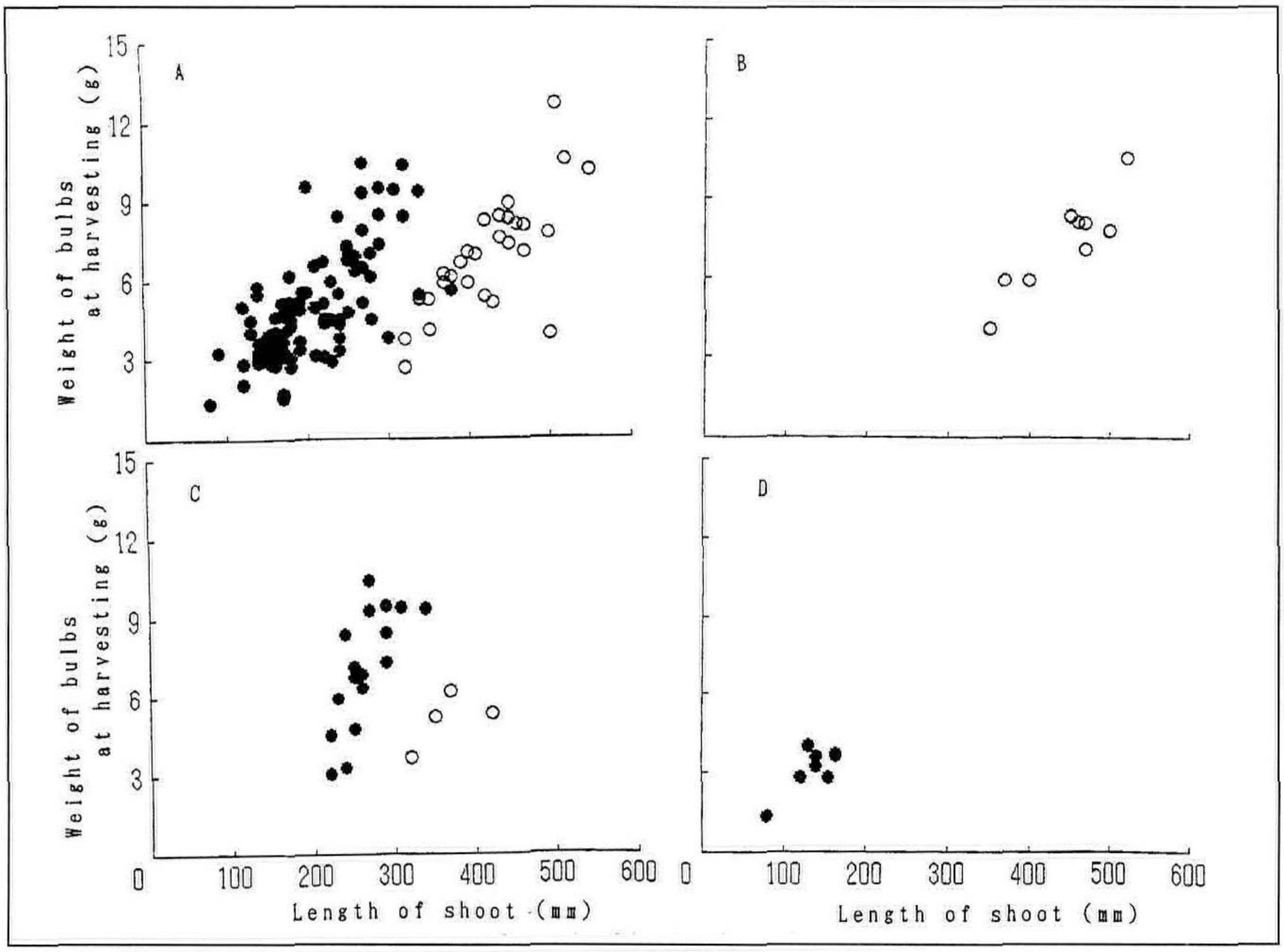

Figure 3. Effect of shoot length and the presence of the flower bud on bulb weight (○, having flower bud bulbs; •, non flower bud bulbs). The weight of bulbs at transplanting were as follows: A, 0.5-10 g; B, 6-7 g; C, 4-5 g; and D, 0.5-1 g.

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