Rapid In Vitro Production of Plants From Immature Seeds in *Lilium japonicum*©

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INTRODUCTION

To control the period from sowing to flowering is important in the cultivation of seed-propagated ornamental plants. However, commercial cultivations of some plants are difficult because of their long periods from sowing to flowering. *Lilium japonicum* Thunb. is a wild lily native to Japan and of great potential as an ornamental plant. Although seed propagation is generally used for its mass propagation, it takes a very long time from sowing to flowering (about 6 to 7 years). Therefore, its commercial cultivation has not been established.

In this report, we investigated characteristics of seed germination and developed a new method with immature seed culture to promote seed germination and to rapidly produce plants, which allows shortening the period to flowering.

MATERIALS AND METHODS

Germinability of Immature Seeds. Immature seeds of *L. japonicum* were collected from six capsules at various maturities. Lengths of seeds and embryos were measured monthly after pollination. Twenty seeds were placed in a 90-mm-diameter petri dish containing 20 ml of the Murashige and Skoog (MS) solid medium (Murashige and Skoog, 1962) or water agar medium without MS salts. Twelve dishes were kept at 20 °C under a long-day photoperiodic condition (74 $\mu mol \cdot m^{-2} \cdot sec^{-1}$, 16 h). The number of germinated seeds was recorded every 40 days for 200 days after the start of culture.

Influence of High Temperature Treatment on Germination of Immature Seeds. Immature seeds 3 or 4 months after pollination were prepared. Sixteen seeds were placed on MS solid medium in a plastic petri dish. For high temperature treatment, 12 dishes were kept at 30 °C for 50 days and then kept at 20 °C. For control, 12 dishes were kept at a constant 20 °C. Both cultures were carried out under a long-day photoperiod condition (74 $\mu mol \cdot m^{-2} \cdot sec^{-1}$, 16 h). The number of germinated seeds was recorded at 40-days intervals during 120-days culture.

Influence of Pre-Treatments on Germination of Immature Seeds. Immature seeds 3 months after pollination were pre-treated with the following methods: (1) scarification (making a small cut in the seed coat); (2) soaking in 0.5% NaClO for 20 min; (3) no treatment (control). Sixteen seeds were placed on MS solid medium in a plastic petri dish. Twelve dishes were used for treatment and kept at 20 °C under a long-day photoperiod condition (74 $\mu mol \cdot m^{-2} \cdot sec^{-1}$, 16 h). The number of germinated seeds was recorded every 40 days for 200 days after the start of culture.

RESULTS AND DISCUSSION

Germinability of Immature Seeds. Seeds and embryos reached their final sizes in 2 months after pollination (Fig.1). Though immature seeds 2 months after pollination did not germinate on water agar medium, they germinated on MS medium. In contrast, there was little difference in germination of seeds collected 3 to 4 months after pollination between water agar medium and MS medium. Culture on MS me-

dium promoted germination of immature seeds. Germination rate of mature seeds 5 months after pollination declined on water-agar medium, while it increased on MS medium. Furuya (1999) suggested that mature seeds of this lily species have dormancy. Our data support that seeds became dormant when mature.

It is unknown why mature seeds did not demonstrate apparent dormancy on MS medium. The possibility that some MS salts may have a breaking effect on seed dormancy remains to be studied in the future.

Influence of High Temperature Treatment on Germination of Immature Seeds. In the previous experiment, immature seeds were also germinable (Fig. 2). However, their germination rate was not high. Therefore, we investigated effect of high temperature treatment on germination of immature seeds to improve the germination rate. High temperature treatment improved germination rate of immature seeds 4 months after pollination (Fig. 3). This suggests that the cause of low germination of immature seeds 4 months after pollination is seed dormancy and that dormancy can be broken by a high temperature treatment. In contrast, germination of immature seeds 3 months after pollination was not affected by high temperature treatment (Fig. 3). The sensitivity to high temperature may develop later than 3 months after pollination. High temperature treatment is effective to promote germination of immature seeds 4 months after pollination. However, it is desirable to use more immature seeds for obtaining plants rapidly. Therefore, we conclude that high temperature treatment is not useful for this purpose.

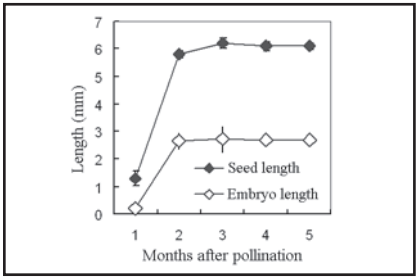

Figure 1. Growth curves of seeds and embryos after pollination in Lilium japonicum. The bars represent SD (n = 6).

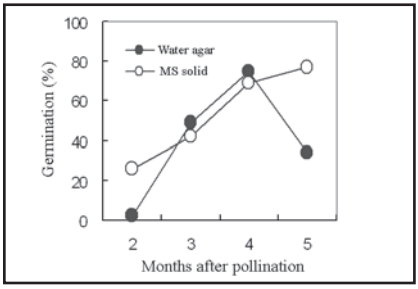

Figure 2. Germination rate of immature seeds at various maturities after pollination on water agar or MS medium. Data were taken after 200 days of culture.

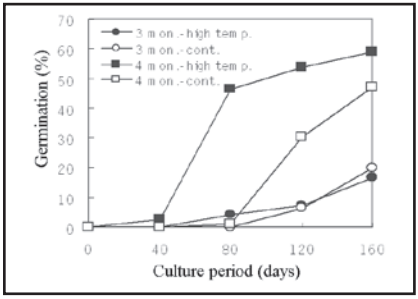

Figure 3 Effect of high temperature treatment on germination of immature seeds three (○, ●) or four months (□, ■) after pollination. Open symbols (○, □), control; closed symbols (●, ■), high temperature treatment.

Influence of Pre-Treatments on Germination of Immature Seeds. In our experiment, we investigated pre-treatment methods to promote germination of immature seeds collected 3 months after pollination. As a result, scarification treatment significantly improved the germination rate of immature seeds 3 months after pollination (Fig. 4). In addition, germination was promoted after a soaking treatment in NaClO (Fig. 4). A soaking treatment is easy for mass propagation compared with scarification. Hence, we consider that soaking treatment in NaClO is effective for pretreatment of immature seeds.

Method of Immature Seed Culture to Promote Germination. Figure 5 shows a comparison of growth among three methods. In the case of existing methods, bulblets weighing 0.1 g were obtained 22 months after pollination (April of 3rd year) when grown in the soil method, or obtained 10 months after pollination (March of 2nd year) using the temperature treatment method (Kamata, 1987). On the other hand, in immature seed culture developed as a new method in our study can provide bulblets with an average weight of 0.5 g 7 months after pollination (January of 2nd year). In vitro cultures to promote bulblet growth have already been investigated extensively (Fukui et al., 1989; Kawarabayashi, 1993; Haruki et al., 1996, 1998; Inagaki et al., 2003). Thus, bulblets weighing over 3.0 g were obtained in April of 2nd year using a liquid culture for 90 days following immature seed culture. When bulbs were planted in containers after experiments, we observed the first flowering 2 years after planting.

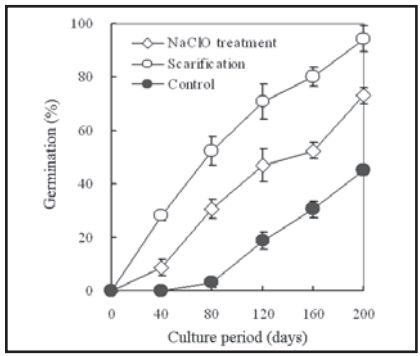

Figure 4. Effect of pre-treatments on the germination rate of immature seeds (3 months after pollination). The bars represent SD (n = 12).

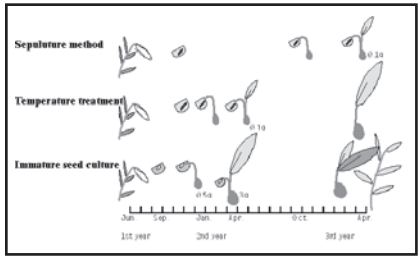

Figure 5. Comparison of growth season among immature seed culture and other two existing methods.

From these results, it is concluded that the culture of immature seeds is an effective method to promote germination. Furthermore, the time to flowering could also be shortened considerably using immature seed culture.

Acknowledgment. The authors are grateful to Prof. H. Ohno of Shizuoka University for careful reading of the manuscript.

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