Bulblets Induction of *Lilium longiflorum* Hybrid var. Formolongo *In Vitro* Culture

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Abstract

The multiplication of the economic flower, *Lilium longiflorum* Hybrid var. Formolongo by *in vitro* culture induction in suitable medium was investigated. The bulb scales of *L. longiflorum* Hybrid var. Formolongo were sterilized with Clorox on various concentration 10%, 20%, 30% and 40% for 20 minutes, then cultured on hormone free MS medium for 1 week. The result showed that Clorox 30% could sterile bulb scales about 62%. The explants were transferred on MS medium with BA and NAA (0, 0.5, 0.75, 1.0, 1.5 and 2.0 mg/l respectively) for 8 weeks. The MS medium with 0.75 mg/l BA and 1.0 mg/l NAA could highly induced multiple shoot formation. After that cutting shoots into small segments were cultured on hormone free MS medium for 6 weeks and got small single shoots (6 mm.). The small single shoots were transferred onto MS medium supplemented with 30, 60, 90 and 120 g/l sucrose respectively for 8 weeks. Bulblets had the biggest diameter (14 mm.) on MS medium with 60 g/l sucrose. Therefore, in this study MS medium with 60g/l sucrose was suitable medium for bulblets induction of *L. longiflorum* Hybrid var. Formolong in culture.

Keywords: *Lilium longiflorum* Hybrid var. Formolongo bulblets induction *in vitro* culture

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การชักนำให้เกิดหัวย่อยของลิลลี่ (*Lilium longiflorum*) ลูกผสมสายพันธุ์ Formolongo ในสภาพปลอดเชื้อ

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บทคัดย่อ

การเพิ่มปริมาณของดอกลิลลี่ Longiflorum สายพันธุ์ Formolongo ซึ่งมีความสำคัญทางเศรษฐกิจ โดยการเพาะเลี้ยงในสภาพปลอดเชื้อเป็นวัตถุประสงค์ของการทำวิจัย เพื่อหาสูตรอาหารที่เหมาะสมในการผลิตหัวย่อยของลิลลี่ในสภาพปลอดเชื้อ โดยการนำกลีบหัวของลิลลี่มาฟอกฆ่าเชื้อที่ผิวด้วยคลอรอกซ์ความเข้มข้นต่างๆ เป็นเวลา 20 นาที แล้วนำมาเพาะเลี้ยงบนอาหารพื้นฐานสูตร MS ที่ไม่มีสารควบคุมการเจริญเติบโตเป็นเวลา 1 สัปดาห์ พบว่าคลอรอกซ์ 30% สามารถฟอกฆ่าเชื้อกลีบหัวได้ดีที่สุด มีเปอร์เซ็นต์การปลอดเชื้อเท่ากับ 62% ย้ายชิ้นเนื้อเยื่อมาเพาะเลี้ยงบนอาหารพื้นฐานสูตร MS ที่เติมสารควบคุมการเจริญ BA ร่วมกับ NAA ที่ความเข้มข้น 0, 0.5, 0.75, 1.0, 1.5 และ 2.0 มิลลิกรัม/ลิตร ตามลำดับ เป็นเวลา 8 สัปดาห์ พบว่าสูตรอาหาร MS ที่เติม BA 0.75 มิลลิกรัม/ลิตร ร่วมกับ NAA 1.0 มิลลิกรัม/ลิตร สามารถชักนำให้เกิดกลุ่มยอดได้มากที่สุด จากนั้นแยกกลุ่มยอดไปเลี้ยงบนอาหาร MS ที่ไม่มีสารควบคุมการเจริญเป็นเวลา 6 สัปดาห์ จนได้ยอดที่มีเส้นผ่านศูนย์กลางของหัวประมาณ 6 มิลลิเมตร แล้วนำไปเลี้ยงบนอาหารพื้นฐานสูตร MS ที่มีปริมาณน้ำตาล 30, 60, 90 และ 120 กรัม/ลิตร เป็นเวลา 8 สัปดาห์ พบว่าสูตรอาหาร MS ที่เติมน้ำตาล 60 กรัม/ลิตร จะได้หัวที่มีขนาดใหญ่ที่สุด (14 มิลลิเมตร) ดังนั้นจากการศึกษาครั้งนี้สูตรอาหาร MS ที่เติมน้ำตาล 60 กรัม/ลิตรจึงเหมาะสมสำหรับการชักนำให้เกิดหัวย่อยของลิลลี่ในสภาพปลอดเชื้อ

คำสำคัญ : ลิลลี่ลูกผสม *Lilium longiflorum* Hybrid var. Formolongo การชักนำให้เกิดหัวย่อย สภาพปลอดเชื้อ

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Introduction

Lilly, a member of the *Lilium* family, is an important ornamental plants for many centuries. Although the very first hybrids originate from the 19th century (1, 2), the systematic breeding of lily cultivars, the number of which exceeds several thousands nowadays, started only in the 1950's by Jan de Graaff. Tissue culture has become an important method in research and mass production of various plants during the last decades. Combining the benefits of mass production and fast regeneration of uniform plant material in tissue culture is a necessity for the future breeding and culture of lilies. However, to make tissue culture a commercially relevant production system, production protocols need to be developed separately for each plant crop and cultivar. Bulbous plants, like lilies, have proved to be ideal for tissue culture, as their regeneration potential is usually high. Furthermore, the compact structure of the shoot makes them easy to handle both in solid cultures. Nowadays, lilies are one of the most important bulbous crops produced in tissue culture also in an industrial scale (3). The aim of the work reported here was to determine the condition for bulblets induction.

Methods

Bulb scales of *Lilium longiflorum* Hybrid var. Formolongo. were used as the starting material. The explants were washed with a detergent and water, rinsed, sterilized in various concentration of Clorox, 10%, 20%, 30% and 40% for 20 minutes, and finally rinsed 3 times with sterile distilled water. The explants were cultured on hormone free MS medium (4) for 1 week. The regenerate, bulblets were transferred on a propagation medium, MS macro and micro nutrients, vitamins and sucrose 30 g/l. For bulblets formation, the combination of BA and NAA were added, 0.5, 0.75, 1.0, 1.5 and 2.0 mg/l, respectively. All the explants were cultured at 25 $\pm$ 2 $^{\circ}$C and 16 hrs. photoperiod (white fluorescent light 100 e mol $m^{-2}s^{-1}$). After that cutting multiple shoots into small segments were cultured on hormone free MS medium for 6 weeks and got small single shoots (6mm.). The small single shoots were transferred on MS medium supplemented with 30, 60, 90 and 120 g/l sucrose, respectively for 8 weeks.

Results

For the culture medium in the present work, MS medium (4) was selected. Bulb scales were sterilized with Clorox on various concentration 10%, 20%, 30% and 40% for 20 minutes. The result showed that Clorox 30% could sterile bulb scales about 62% (Table 1). The strengths of the media have been altered. Increasing the medium strength has been investigated on bulblet formation. Root and shoot induction of lily on MS medium with different phytohormone (BA and NAA) were shown in Table 2. Effect of

sucrose on Growth of bulblets on MS medium was measured the diameter. The result showed that the bulblets had the biggest diameter of bulblets about 14 mm on MS medium with 60 g/l sucrose (Table 3). The result coinside with this study observed in callus induction, somatic embryogenesis and protoplast cultures. The sugar content has a clear effect on morphogenesis because in higher concentrations the sprouting or formation of leaves decreases and the size of the bulb scales increases. (Figure 1).

Table 1 Sterilized explants with different concentration of Clorox.

Concentration of Clorox (%)	Sterilized explants (%)
15	15.00
20	33.33
30	62.00
40	10.00

Table 2 Root and shoot induction of lily on MS medium with different phytohormone (BA and NAA)

BA (mg/l)	NAA (mg/l)				
	0.00	0.50	0.75	1.00	1.50
0.00	R= 0 , B = 1.4	R = +1, B = 2.5	R = +2, B = 4.6	R = +2, B = 4.7	R = +1, B = 4.7
0.50	R= 0, B = 4.6	R = 0, B = +2	R = +1, B = +2	R = +1, B = +3	R = +1, B = +2
0.75	R= 0, B = +3	R = 0, B = +2	R = 0, B = +3	R =0, B = +4	R = 0, B = +3
1.00	R = 0, B = +1	R = 0, B = +2	R = 0, B = +2	R = 0, B = +2	R = 0, B = +2
1.50	R = 0, B = +2	R = 0, B = +1	R = 0, B = +3	R = 0, B =+3	R = 0, B = +2
2.00	R = 0, B = +1	R = 0, B = +2	R = 0, B = +2	R = 0, B = +2	R = 0, B = +1

R Root induction (0 no root, +1 incomplete root, +2 complete root)
B Bulblet induction (+1 bulblet height 1-2 cm less amount, +2 bulblet height 1-2 cm more amount, +3 bulblet height 3-5 cm less amount, +4 bulblet height 3-5 cm more amount)

Table 3 Effect of sucrose on Growth of Bulblet on MS medium.

Sucrose (g/l)	Average Bulblet diameter (mm)
30	8.2
60	14.0
90	11.1
120	9.4

Figure 1 Bulblet cultured on MS medium with various concentration of sucrose for 8 weeks

Discussion

For the culture medium in the present work, MS medium (4) was selected. Bulb scales were sterilized with Clorox on various concentration. The result showed that Clorox 30% could sterile bulb scales about 62%. Although, according to the published data, the different tissue culture media have not been compared in terms of growth of lilies, the MS medium is mostly used. In some cases, Nitsch's (5), White's (6) and Kao's (7) media have been used for germination, embryo and protoplast cultures due to their low ammonium concentration, which may be harmful for susceptible tissues or cells. Also the strengths of the media have been altered for these purposes. Increasing the medium strength has been proved to have an enhancing effect on bulblet formation (8). Growth regulators have a very important role in both induction of differentiation and in growth.

The role of different plant hormones and their concentrations on the morphogenesis of differentiating plants has been studied in various respects. Takayama and Misawa (8) showed clearly the interaction of auxin, α-naphtalene acetic acid (NAA) and cytokinin (kinetin) in the formation of bulblets and roots; higher auxin/cytokinin ratio increased root formation whereas lower ratio increased bulb formation. In this studies on the effect of NAA and BA combination on the number and shoots it has been noticed. The MS medium with 0.75 mg/l BA and 1.0 mg/l NAA could highly induce multiple shoots formation. Sucrose concentration usually varies between 30-120 g/l depending on the tissue were used. The result showed that the bulblets had the biggest diameter of bulblets about 14 mm. on MS medium with 60 g/l sucrose. The result coincide with those observed by other research where the higher concentrations were used in callus induction, somatic embryogenesis and protoplast cultures whereas lower concentrations were used in the mass production of differentiated plants. The sugar content has a clear effect on morphogenesis because in higher concentrations the sprouting or

formation of leaves decreases and the size of the bulb scales increases. The combination of sucrose and mannose has also been proved to promote the bulb growth (9). This tendency may be due to dormancy induction in the shoots caused by elevated osmolality of the medium, which, in turn, imposes a water stress on the tissue (10, 11).

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