Combined Proceedings IPPS 73:26-31. 2023.

In Vitro Flowering Response in *Iberis umbellata*

Syunya Shimazaki*, Hiroko Nakatsuka, Arisa Noguchi and Wakanori Amaki

Department of Agriculture, Tokyo University of Agriculture, 1737 Funako, Atsugi, Kanagawa 243-0034, Japan; *Currently affiliated with ZEN-NOH PEARL RICE Co., Ltd

amaki@nodai.ac.jp

Keywords: tissue culture, micropropagation, bedding plant

Summary

Iberis species are perennial or annual herbs of the genus *Iberis* in the Brassicaceae family, and is native to the Mediterranean coast, North Africa, and southwestern Asia. In vitro flowering is considered a convenient system to study the entire mechanism of reproductive processes. There have been no reports of in vitro flowering in *Iberis*, although there have been several reports on tissue culture for other species, *I. amara* and *I. semperflorens*. *I. umbellata* used in this research has never been used in in vitro research. Therefore, we investigated the effects of sucrose concentration and explant collected position on the in vitro flowering response. As a result, the addition of sucrose to the medium was essential for flowering, and judging from the flowering reaction, 30 g/L seemed to be optimal concentration. In addition, as a result of examining the explants from different positions, it became clear that the explants containing the apical bud were most likely to flowering.

INTRODUCTION

Iberis species are perennial or annual herbs of the genus *Iberis* in the Brassicaceae family, and is native to the Mediterranean coast, North Africa, and southwestern Asia. The flowering period in Japan is from April to June, and it has the characteristics of long-day plant. It is called “Candytuft” in English because the flowers look like swollen sugar candy (The Royal Horticultural Society, 1992). In Japan, it is called "Magaribana" (meaning a flower that looks crooked) because of its unbalanced flower shape, of which the inner two petals are small and the outer two petals are large. *I. umbellata* L., which is the test material for this research, has small flowers but a wide variety of flower colors, ranging mainly from rose to pink, but also including red, purple, lavender, and white. It has been improved for use in flower beds and is highly branched, reaching a height of about 25 cm when grown outdoors (Tsuda, 1988).

To date, it has been shown that flowering can be induced in various species in vitro (Scorza, 1982; Van Staden and Dicken, 1991; Murthy, 2012). There are various purposes for in vitro flowering. For example, one purpose is to analyze flowering physiology. In vitro flowering is considered a convenient system to study the entire mechanism of reproductive processes, including flower development, flower organ formation, and flower maturation. Another purpose, plants that bloom in vitro are called "in vitro flowers," and they can create new value that is different from conventional flower usage. To date, there have been no reports of in vitro flowering in this genus, *Iberis*, although there have been several reports on tissue culture for other species, *Iberis amara* L. and *Iberis semperflorens* L. (Mudgal et al., 1981; Iapichino and Bertolino, 2009). *Iberis umbellata* used in this research has never been used in in vitro research. Therefore, we investigated the in vitro flowering response of *Iberis umbellata*.

MATERIALS AND METHODS

Cultivation of Plant Material

Seeds of *Iberis umbellata* Candytuft (TAKII & Co., Ltd.) were sterilized in 70 % ethanol for 1 minute and in a 1 % sodium hypochlorite aqueous solution containing 1 drop of Tween 20 for 15 minutes. It was then washed three times with sterile water. Two to three seeds were sown in each test tube (φ40 x 30 mm). A double layer of aluminium foil was used to close the test tube. At the stage when the cotyledons were unfolded, the seedlings were replaced with aluminium plugs with holes of 8 mm in diameter and an air ventilation membrane (MilliSeal, Millipore Co., Ltd.) attached to the hole to improve the ventilation rate, and one good seedling was left and the others were thinned out to serve as test material.

The basal medium used was 1/3 strength of Murashige and Skoog (1962) medium supplemented with 30 g/L sucrose and 8 g/L agar. The pH of the medium was adjusted to 5.8. Twenty mL of the medium was dispensed into each of test tubes, and the tubes were capped with aluminium foil and were autoclaved at 121°C for 15 minutes.

A culture incubator (CL-301, Tomy Seiko Co., Ltd.) was used, and the culture conditions were 20°C, white fluorescence light (FLR40S･EX-N/M-H, Toshiba Lighting and Technology Co., Ltd.) 24-hour

lighting, and photosynthetic photon flux density (PPFD) was 120 μmol · m^{-2} · s^{-1} (When seeds of Brassicaceae plants are sown under 8 or 16 hours of light per day, the hypocotyls tend to elongate unnecessarily. Under 24-hour lighting, this elongation phenomenon is suppressed, so in this experiment, 24-hour continuous lighting was used).

Effects of sucrose concentration on the flowering (Exp.1)

Approximately 1 cm long apical segments cut out from in vitro seedlings (29 days after sowing) were used as explants. Sucrose concentration of the basal medium was changed to 0, 15, 30 and 60 g/L, and the flowering response was observed after culturing for 26 days. Other culture procedures were performed in accordance with the in vitro seedling growth described above.

Effect of stem nodal position on flowering response (Exp.2)

Seedlings (30 days after sowing) were divided into four explants from the top (apical segment 1 cm long) to the base (3 of one-node stem segments, 15 to 20 mm long) and used as explants.Other culture procedures were performed in accordance with the in vitro seedling growth described above. Observations were made on the 45th day after the start of culture.

RESULTS AND DISCUSSION

Effects of sucrose concentration on the flowering

When the sucrose concentration was 0 g/L, the flowering rate was 0 %. In addition, the shoot length was significantly shorter than in other treatments, and no callus was formed. Furthermore, most of the plants were malformed. On the other hand, Flowering was observed in all media containing sucrose, regardless of its concentration (**Table 1**).

Table 1. Effects of sucrose concentration on the in vitro growth and flowering of apical stem explants in *Iberis umbellata*.

Sucrose concentration (g/L)	Number of alive explants	Flowering rate (%)	Shoot length (mm)	Callus formation (%)
0	7	0	15.3b*	0
15	6	33.3	55.5ab	100
30	7	71.4	59.8a	100
60	7	71.4	34.0ab	100

* There are significant differences (5% level) between different letters by Tukey's multiple range test.

These results suggest that sucrose plays an important role in shoot growth and flowering reactions. In addition, although the number of florets was greater at 60 g/L sucrose concentration than at other concentrations, the plant height was small and the

stems turned red (**Table 1** and **Fig. 1**), suggesting that growth inhibition was caused by osmotic stress. At 30g/L, flowering started earlier than at other concentrations and the final flowering rate was the highest. From the above, it is considered that adding 30 g/L of sucrose is suitable for growth and flowering in *Iberis umbellata*.

Figure 1. Growth and flowering of apical explants at different sucrose concentrations in Iberis umbellate (Photographed on the 26th day after the start of culture). From the left: 0, 15, 30, 60g/L sucrose.

Effects of stem nodal position on flowering response

Flowering was observed only in one of the apical explants (**Table 2** and **Fig. 2**). There was no significant difference in shoot length depending on the location of explant collection. Although there was no statistically significant difference in the number of lateral buds formed, there was a tendency for the number of lateral buds to increase in lower explants. The existence of such a gradient of physiological responses from the top to the base of the stem has been reported in tobacco, torenia, perilla, gentian, etc. (Tran Than Van et al., 1974; Tanimoto and Harada, 1979; Tanimoto and Harada, 1980; Zhang and Leung, 2002). In gentian, the shoot formation rate and flower bud formation rate tend to gradually decrease as the node position decreases from the top to the base (Zhang and Leung, 2002). As mentioned above, in tissue culture, when the explant collected position is changed from the top to the base of the stem, there are differences in reactions such as lateral bud formation, flower bud formation and adventitious bud formation depending on the collected position. This suggests that a physiological gradient clearly exists in stem. However, the trends are different between *Iberis* and gentian.

At least, when trying to induce in vitro flowering in *Iberis*, it is better to use the explants contained the apical bud.

Table 2. Effects of stem node position collected explant on the in vitro growth and flowering in *Iberis umbellata* (n = 6).

Stem node position of explant (from top to base)	Flowering rate (%)	Longest shoot length (mm)	Callus formation (%)	Callus formation (%)
Apical	16.7	128a*	15.3b*	0
Second	0	97a	55.5ab	100
Third	0	107a	59.8a	100
Fourth	0	97a	34.0ab	100

* There are significant differences (5% level) between different letters by Tukey's multiple range test.

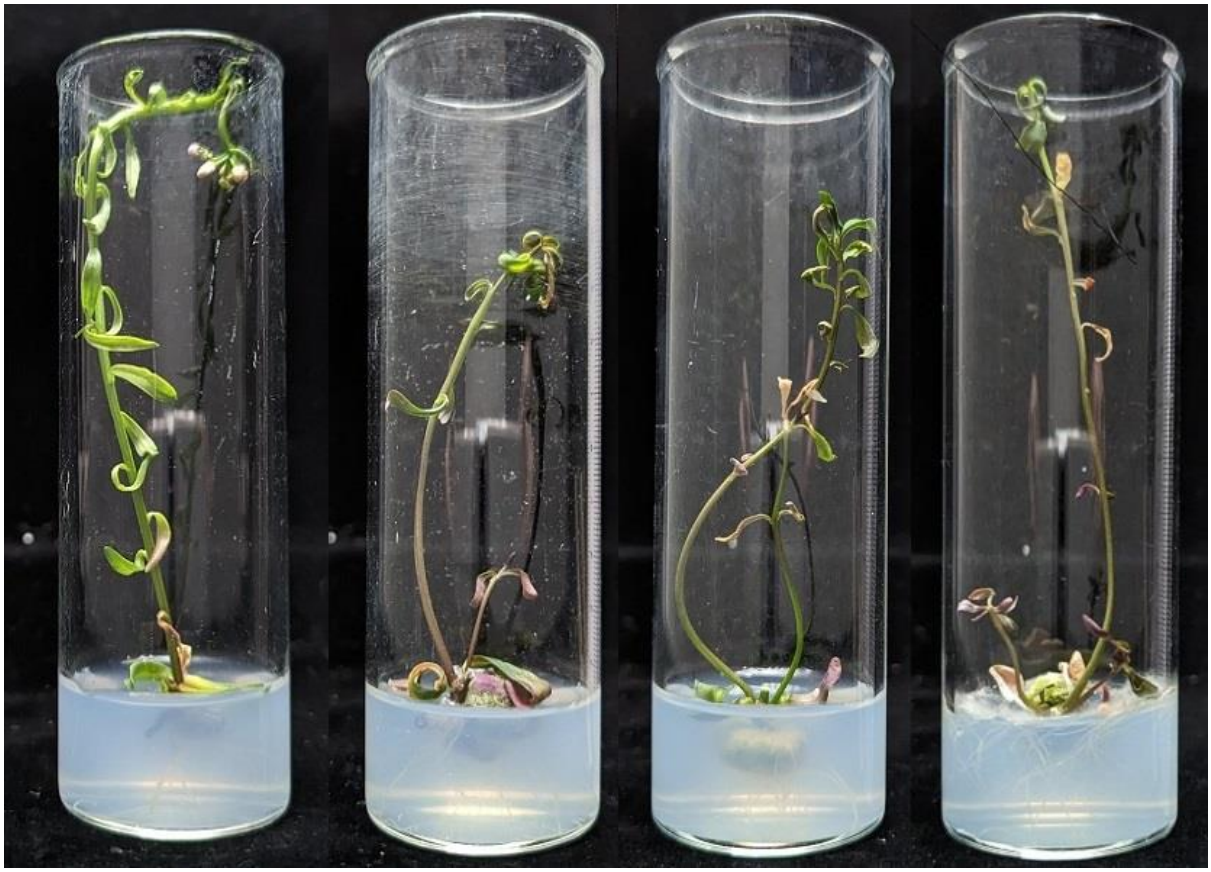

Figure 2. Growth and flowering of explants collected apical explants at different stem position (Photographed on the 26th day after the start of culture). From the left: Apical explant, Second node, Third node, Fourth node.

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