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Induction of Adventitious Buds from Unopened Flower Buds and Expanded Petals of African Violet

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Summary

Streptocarpus ionanthus (H.Wendl.) Christenh. (common name: African violet, saintpaulia), a member of the Gesneriaceae family, is native to the mountainous regions of tropical Africa and is an ornamental plant that is popular as an interior plant because it grows well even in low light interior conditions. Plant regeneration has been reported from petal culture of carnations, and chrysanthemums, but there have been no reports of petal culture of African violet, so this was attempted in this study. Two flowering stages, fully expanded petals and unopened flower buds were set up, and four plots were set up with a combination of α-naphthaleneacetic acid (NAA) at 1.0 or 2.0 mg/L and thidiazuron (TDZ) at 0.25 or 0.5 mg/L as PGRs. It was possible to induce adventitious buds from both of unopened flower bud and fully expanded petal explants. When adventitious buds that had grown to a length of about 5 mm were removed from the test tube and subcultured on a PGR-free medium, rooting was confirmed after 3 weeks and after 8 weeks they had become plantlets that could be acclimatized.

INTRODUCTION

Streptocarpus ionanthus (H.Wendl.) Christenh. (common name: African violet, saintpaulia), a member of the Gesneriaceae family, is native to the mountainous regions of tropical Africa and is an ornamental plant that is popular as an interior plant because it grows well even in low light interior conditions (Tamai, 1989). It can be propagated by leaf cuttings, and various forms of propagation by tissue culture have been attempted, with regeneration of plantlets reported from leaf fragments (Cooke, 1977; Mii and Ohashi, 1988), leaf petioles (Bilkey and Cocking, 1981), and protoplasts derived from leaf blades (Hoshino et al., 1995; Winkelmann and Grunewaldt, 1995).

There are many varieties of African violet, but the bicolored varieties are chimeras, so leaf cutting propagation results in single-colored flowers. In order to propagate the plant while maintaining the chimeric structure, division using apical or axillary buds is performed, but the propagation efficiency is not high. A method of inducing shoots from the axillary buds of the involucre just below the inflorescence has been attempted to maintain the chimeric structure, but there are few research examples. On the other hand, there have been attempts to fix the flower color by tissue culture of the chimeric multicolored parts by color. Plant regeneration has been reported from petal culture of carnations (Nugent et al., 1991), *Hypericum perforatum* (Don Palmer and Kellar, 2011) and chrysanthemums (Takahashi et al., 2024), but there have been no reports of petal culture of African violet, so this was attempted in this study.

MATERIALS AND METHODS

A commercially available potted African violet 'Wood Trail' (blue mono-color flowers) was purchased and used for the experiment. The culture vessel used was a φ20×120 mm glass test tube, into which 10 mL of medium was dispensed and closed with double layer aluminum foil, and then sterilized in an autoclave at 121℃ for 15 minutes. The basal medium was MS (Murashige and Skoog, 1962) composition added with 30 g/L sucrose and 8 g/L agar, and the pH was adjusted to 5.8 before autoclaving.

Confirmation of sterilization method

Fully expanded petals (corolla) were collected with the calyx attached and sterilized in 70 % ethanol for 30 seconds and in 1 % aqueous solution of sodium hypochlorite containing one drop of Tween 20 for 5 minutes, then washed three times with sterilized pure water. The petals were cut into 1 cm square pieces and placed on the basal medium. As a result, there was almost no contamination, so subsequent unopened flower buds and petals were sterilized in the same procedure.

Effects of flowering stage and plant growth regulators (PGRs) on callus and adventitious bud formation

With reference to previous studies (Nugent et al., 1991; Nakano et al., 1994; Don Palmer and Keller, 2011), two flowering stages [fully expanded petals; 1 cm square segments placed adaxial side up and unopened flower buds (bud length approx. 4 mm; cut in half longitudinally and placed cut surface down)] were set up, and four plots were set up with a combination of α-

naphthaleneacetic acid (NAA) at 1.0 or 2.0 mg/L and thidiazuron (TDZ) at 0.25 or 0.5 mg/L as PGRs.

Rooting of adventitious buds and subsequent plantlet cultivation

Adventitious buds formed on the explants that reached about 5 mm in size were subcultured on PGR-free medium. After that, the two adventitious buds that were confirmed to have sufficient roots were removed from the test tubes, washed carefully with tap water so as not to damage the roots, and transplanted into 3 cm pots filled with vermiculite (fine grains). After acclimatization under maintaining high humidity for 2 weeks, they were continued to be cultivated and potted up into 6 cm and then 9 cm pots according to their growth.

The test tubes after the explants were placed on the medium, the acclimation period for the young plantlets removed from the test tubes, and the subsequent pot cultivation were all carried out under the same conditions: 23 ± 1 °C, 16 hours of light (20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) under white fluorescent lamps (FLR40S･EX-N/M-H, Toshiba Lighting and Technology Co., Ltd.) and 8 hours of darkness.

RESULTS AND DISCUSSION

Although omitted in this report, in a similar experiment using the PGR-free medium, the petals of unopened flower buds were expanded but after that no other changes were observed. The expanded petal explants browned and died within 1-2 weeks without any morphological changes. The addition of PGRs was essential for inducing morphological formation in petal culture of African violet.

Although the fully expanded petal explants formed callus, it took longer time for the callus to form than the unopened flower bud explants, and callus formation began 5 to 7 weeks after placement on the medium. When the culture was continued thereafter, adventitious buds formed on the surface of the callus, although not in large numbers. On the other hand, using the unopened flower bud explants, callus formation was confirmed after 3 weeks, and adventitious bud formation was confirmed after 8 weeks from the start of culture (**Fig. 1**). Although there was no significant difference in callus formation between the different types and concentrations of PGRs used in this study, adventitious bud formation was the fastest in the 1.0 mg/L NAA + 0.25 mg/L TDZ.

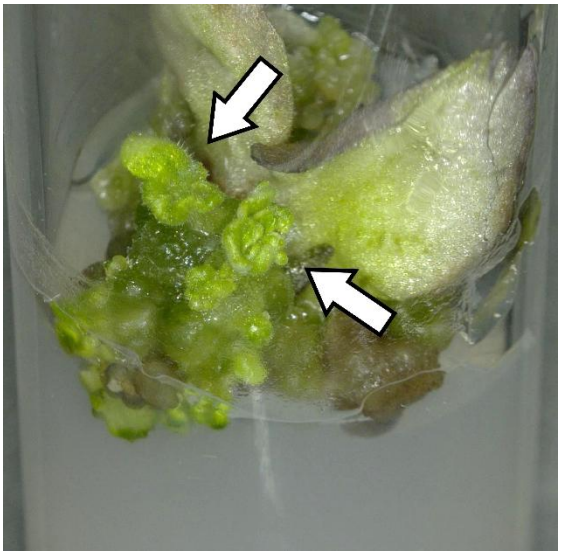

Figure 1. Adventitious bud formation via callus in unflowered flower bud culture (Positions are indicated by two arrows). Eight weeks after culturing in medium containing 1.0 mg/L NAA + 0.25 mg/L TDZ.

When adventitious buds that had grown to a length of about 5 mm were removed from the test tube and subcultured on a PGR-free

medium, rooting was confirmed after 3 weeks and after 8 weeks they had become plantlets that could be acclimatized (**Fig. 2**).

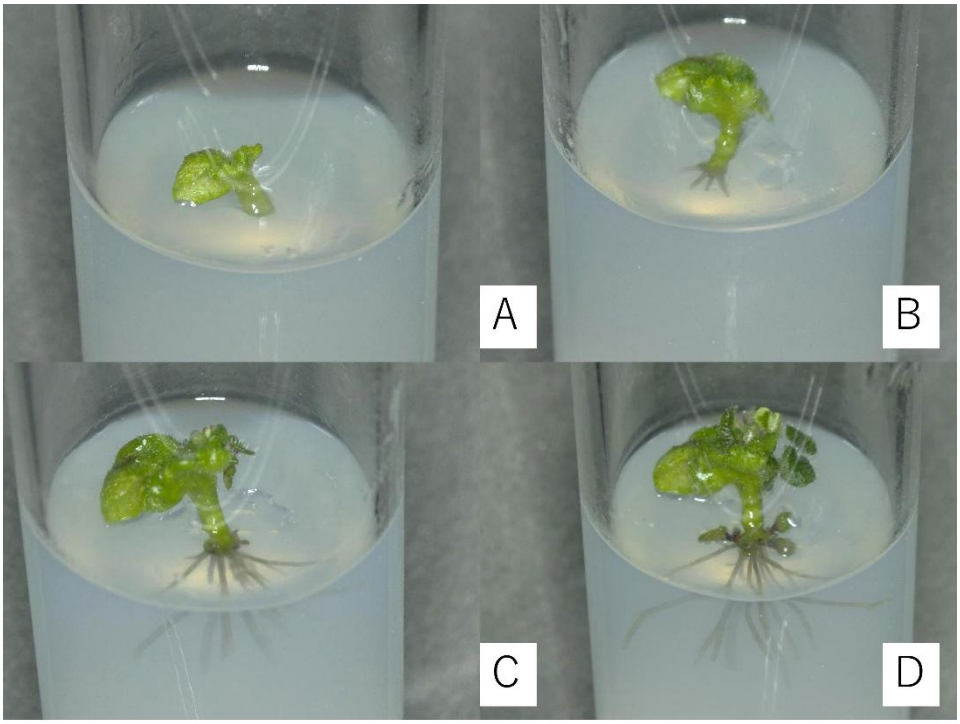

Figure 2. The formed adventitious bud was subcultured to PGR-free medium to induce rooting. A) Adventitious bud when inoculated on the medium, B: 3 weeks after, C) 6 weeks after, D) 9 weeks after subculture.

The plantlets were removed from the test tube, acclimatized and potted to 3 cm pots in the usual way, and grew well. They were re-potted into 6 cm pots 2 months after the start of acclimatization, and 9 cm pots 3.5 months later, and continued to grow vigorously (**Fig. 3**).

Figure 3. Growth status of the plants 5 months after the start of acclimation.

Unfortunately, they had not yet bloomed at this point, so we were unable to check for changes in flower color, etc. In this experiment, unopened flower buds were used that had been cut longitudinally, so the explants contained multiple organs (e.g., petals, sepals, and ovary), and callus was induced from all of them. Although the organ from which the plantlets derived from adventitious buds shown in **Figure 2** originated can be determined only by visual confirmation of the position of the callus at the time of development, it is determined that they are most likely derived from petals. In the future, when using unopened flower buds, it will be necessary to separate them by organ and use them as test materials.

As described above, it was possible to induce adventitious buds from both of unopened flower buds and fully expanded petals in African violets. It was shown that by confirming the flower color from the petals of a chimeric bicolor flower and then taking explants, it may be possible to obtain a strain exhibiting new traits.

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