Effects of Rooting Substrates on *In Vitro* Rooting of *Anthurium andraeanum* L. cv. Avanti

Wararat KEATMETHA and Padungsak SUKSA-ARD

School of Agricultural Technology, Walailak University, Thasala, Nakhon Si Thammarat 80160, Thailand.

ABSTRACT

A study was made of the effects of rooting substrates on *in vitro* rooting of *Anthurium andraeanum* L. cv. Avanti, orange flower. Initiation of root was attempted in several rooting substrates with modified ½ MS medium supplemented with 30 g/l sucrose. The cut end of the shoot was dipped in 2.5 g/l indole-3-butyric acid (IBA) before insertion in substrates. After 4 weeks of culture, it was found that roots were markedly induced in 8 and 12 g/l agar with 86.67 and 73.33% in root induction rate, 14.62 and 12.41 mm in root length, with 3.54 and 3.91 roots in root number respectively. However, 93.33% of root induction rate with 3.00 roots, and 11.66 mm in root length were produced on medium containing 1.5 g/l phytagel while peat moss and vermiculite could induce rooting at 46.67% with 7.86 and 6.66 mm in length, with 1.00 and 1.86 roots respectively. Root could not be induced in sphagnum moss. Anatomical study of root showed no abnormality in all rooting substrates. The roots formed in high concentrations of agar at 12 and 16 g/l, 2.0 and 2.5 g/l phytagel, and peat moss were thicker than those formed in other rooting substrates. Especially, root formed in peat moss had the largest vascular diameter. However, roots formed in phytagel, peat moss, and vermiculite had more root hairs than those formed in agar substrate. Plantlets, rooted in peat moss and vermiculite, survived at 100% after acclimation in a mixture of soil and coconut husk.

Key words: *Anthurium andraeanum* L. - Rooting substrates - Agar - Phytagel - Peat moss - Sphagnum moss - Vermiculite

INTRODUCTION

Anthurium is one kind of major cut flower species in the tropical and sub-tropical countries. Although mass propagation of *Anthurium* spp. can be successfully performed by the use of *in vitro* technique (1,2,3), it is very important to improve plantlet survival rate during acclimation as well as to enhance shoot proliferation and rooting *in vitro*. Besides environmental conditions such as humidity and light intensity, the quality of roots, which has been determined *in vitro* before transplanting, can markedly affect the survival rate (4). Difficulty in acclimation of micropropagated plantlets often becomes a practical problem. Agar is normally used as a solidifying agent in *in vitro* cultures. Many authors have mentioned problems relating to the root quality when developed in agar medium (4,5). Plantlets that develop roots in anaerobic conditions in agar substrate have no root hair and are very inefficient when placed in

aerobic substrate, leading to loss of vigor of the plantlets when transferred to greenhouse (6).

The purpose of this work was to evaluate the influences of several rooting substrates on root induction rate and anatomical characteristics of root of *Anthurium andraeanum* L. cv. Avanti under *in vitro* conditions.

MATERIALS AND METHODS

Establishment of Proliferation Culture and Preparation of Shoots

Shoot tip culture of *Anthurium andraeanum* L. cv. Avanti, orange flower, was established on Murashige and Skoog (7) (MS) basal medium supplemented with 0.5 mg/l 6-benzylamino purine (BA), 0.1 g/l adenin sulfate, 30 g/l sucrose and 8 g/l agar, pH 5.6. The cultures were incubated at 25±1°C under a photoperiod of 16 h at 40 μmol/m^2/s with cool white fluorescent lamps. The proliferation culture was maintained by dividing shoot clump, as a single shoot with 10 mm long, and subculturing every 4 weeks.

Two actively growing shoot tips about 10 mm in length were placed in each 200 ml bottle, capped with clear plastic cap. Each bottle contained 20 ml of MS basal medium supplemented with 0.5 mg/l 6-(γ,γ-dimethylallylamino)-purine (2iP), 30 g/l sucrose, and 10 g/l agar, pH 5.6. After 4 weeks of culture under the same conditions as previously described, uniformly elongated shoots about 15 mm long were collected and prepared for rooting. The basal leaves were removed and three terminal leaves were left.

Effect of Rooting Substrates on Root Induction Rate and Root Anatomy

Various rooting substrates, agar, phytagel, sphagnum moss, peat moss, and vermiculite were tested. A half-strength MS basal medium supplemented with 30 g/l sucrose and 8 g/l activated charcoal was solidified with 8, 12 and 16 g/l agar or with 1.5, 2.0 and 2.5 g/l phytagel (Sigma) individually, then 20 ml of medium was dispensed per test tube (25×150 mm) while twenty milliliters of sphagnum moss, peat moss, and vermiculite were placed in test tube and moistened with 10 ml of the half-strength MS liquid medium containing 30 g/l sucrose. All test tubes were capped with aluminum foil before sterilization at 121°C with pressure of 1.2 kg/cm^2 for 15 min.

The cut end of the shoot was dipped in 2.5 g/l IBA (dissolved in 50% ethanol), which had been sterilized by filtering through a Millipore membrane with pore size of 0.45 μm, for 5 s before being inserted in rooting media, one shoot per tube. Thirty-five shoots were used per treatment. After 4 weeks of culture under the same conditions as proliferation, the percentage of rooting, root length, number of roots longer than 3 mm, root diameter, and vascular diameter were evaluated. Five complete plantlets from each treatment were randomly selected for root anatomical observation. Transverse sections of the roots at a distance of 3 mm from the root tips were stained with 5 g/l safranine and then observed under a light microscope. The rest of the complete plantlets were removed from culture vessel and carefully washed in distilled water in order to remove completely rooting substrate and culture medium before being transferred to small black plastic bags (4×6 in) containing the mixture of soil and coconut husk at a ratio of 1:1. All plants were maintained under mist conditions for first 2 days, then moved to 75% shading lathhouse for the next 7 days, and finally left to grow in 50% shaded lathhouse. The plants were watered twice a day.

Statistical Analysis

The data expressed were analyzed using the one-way ANOVA. When significant differences occurred ($P<0.05$), the interaction of various factors prior to Duncan's multiple range were tested for pairwise comparisons.

RESULTS AND DISCUSSION

Root induction of the shoots started after the swelling of the basal ends about 10 days after the initiation of culture. Roots developed well in medium solidified with agar and phytagel at the range from 60.00 to 93.33%. Equal rooting percentage at 46.67 was obtained in peat moss and vermiculite. Roots formed in agar and phytagel solidified medium were longer than those obtained in peat moss and vermiculite. No root was observed in sphagnum moss **(Table 1)**. Phytagel at concentration of 1.5 and 2.0 g/l was more effective in enhancing root induction rate than other substrates tested. The increasing of agar and phytagel concentrations up to 16 g/l and 2.5 g/l respectively caused the reduction in root induction rate of shoot. Although shoot could be rooted at 93.33% in phytagel medium, large amounts of root at 3.54, 3.91 and 3.42 roots with 14.62, 12.41 and 14.23 mm in length were produced in medium solidified with 8, 12 and 16 g/l agar respectively. The phytagel at concentrations higher than 1.5 g/l markedly reduced root number, but increased the root length of shoot **(Table 1)**. The visual observation of shoot growth showed that large amounts of calli were produced at the basal end of the shoot cultured in agar and phytagel containing medium, whereas a small amount of calli was formed in the sphagnum moss, peat moss, and vermiculite containing one.

Table 1. Effects of rooting substrates on *in vitro* rooting of *Anthurium andraeanum* L. cv. Avanti.[z]

Rooting substrates	Percentage of rooting (%)	Root number (root) [y]	Root length (mm) [y]	Root diameter (mm) [y]	Vascular diameter (mm) [y]
8 g/l agar	86.67	3.54 d [x]	14.62 c	0.87a	0.13 a
12 g/l agar	73.33	3.91 d	12.41 c	1.06 c	0.14 bc
16 g/l agar	60.00	3.42 cd	14.23 c	1.08 cd	0.15 c
1.5 g/l phytagel	93.33	3.00 bc	11.66 bc	0.97 b	0.13 ab
2.0 g/l phytagel	93.33	1.64 a	13.14 c	1.07 cd	0.15 c
2.5 g/l phytagel	80.00	1.33 a	13.86 c	1.11 d	0.19 d
sphagnum moss	0	N/A [w]	N/A	N/A	N/A
peat moss	46.67	1.00 a	7.86 ab	1.05 c	0.21 e
vermiculite	46.67	1.86 ab	6.66 a	0.99 b	0.17 d

[z] The cultures were incubated at 25±1°C under a photoperiod of 16 h at µmol/m^2/s with cool white fluorescent lamps.
[y] Data were collected from the roots longer than 3 mm after 4 weeks of culture.
[x] Mean separation within column by Duncan's multiple range test at 5% level.
[w] Root could not be induced in sphagnum moss.

Anatomical observation revealed that roots formed in higher concentrations of agar and phytagel, and in peat moss were significantly thicker than those cultured in the rest of rooting media tested **(Table 1)**. The roots of shoots cultured in phytagel, peat moss, and vermiculite had longer root hairs than those cultured in agar medium, especially roots formed in peat moss and vermiculite had abundant root hairs when observed under light microscope **(Figure 1)**. Although abnormality of inner structure of root was not found in all rooting media, the largest vascular diameter was found in root formed in peat moss **(Table 1)**. The plantlets were successfully established in a mixture of soil and coconut husk in black plastic bags after acclimation (data not shown).

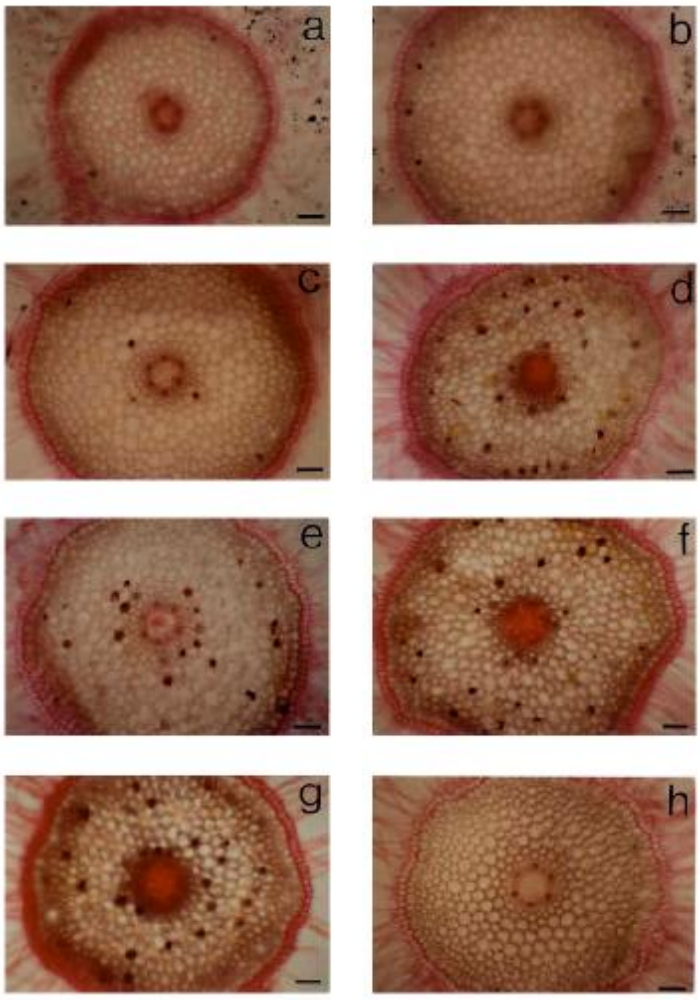

Figure 1. Transverse sections of *Anthurium andraeanum* L. cv. Avanti roots formed in different rooting substrates. Data were collected after 4 weeks of culture. (a) 8 g/l agar, (b) 12 g/l agar, (c) 16 g/l agar, (d) 1.5 g/l phytagel, (e) 2.0 g/l phytagel, (f) 2.5 g/l phytagel, (g) peat moss, (h) vermiculite. Bar : 0.1 mm. (×100).

The lack of development of root hairs in roots formed in agar medium generally made it difficult to get a good functional root system *in vitro*. Anaerobic root development without root hair in agar medium induced a lack of vigor of the plantlet after transplanting (8). Furthermore, it is possible that growth-inhibiting substances may be present in agar (9) which results in growth suppression of *Anthurium* root hairs in agar solidified medium in this study. Although the root induction rate could be improved by increasing the concentration of agar in the medium (10), it was found in this study that increased concentration of agar and phytagel suppressed the root induction rate of shoot. Therefore, it is possible that poor aeration of agar substrate might be the major cause of root reduction. In order to optimize root induction *in vitro*, the replacement of the agar medium by another substrate such as vermiculite and rockwool block is recommended (4,6). Aeration of the rooting medium was an important factor in the formation of adventitious root. It is assumed that insufficient aeration could be the reason for low root quality and loss of root functionality (11). Shoots of *Pyrus* 'Bartlett' and 'OH×F97' clones did not produce secondary roots in the agar substance while both clones could be rooted in vermiculite at the frequency of 31.07 and 53.61% respectively (6). Zimmerman and Broome (12) reported that better rooting results for various apple varieties could be better enhanced by using vermiculite or a mixture of vermiculite and perlite than agar substrate. Hutchinson (13) also obtained significantly higher rooting percentage of apple cv. Northern Spy growing in coarse sand and perlite.

The study demonstrated that different responses of rooting *in vitro* were the result of rooting substrate. The lack of aeration is frequently referred to as the main problem for the roots grown in the medium solidified with agar (5). The fine particles of rooting medium had the tendency for aerobic capacity reduction with negative effects on rhizogenesis (4). To enhance root induction of shoot *in vitro*, more aeration of rooting substrate is recommended since it has higher dissolved oxygen rate and contains more water in comparison with the others. The higher water potential in the leaves of shoot/plantlets cultured in vermiculite medium compared to those cultured in agar and gelrite results in a greater number of primary roots in *Eucalyptus* (14,15). The findings of earlier studies as well as the results of this study suggest that selection of rooting substrate with better air permeability as well as appropriate water retention is essential for the normal root development of *Anthurium andraeanum* L.

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บทคัดย่อ

วรารัตน์ เกียรติเมธา และ ผดุงศักดิ์ สุขสอาด

อิทธิพลของวัสดุออกรากที่มีต่อการออกรากของหน้าวัวพันธุ์ Avanti ภายใต้สภาพปลอดเชื้อ

การชักนำรากของหน้าวัว (*Anthurium andraeanum* L.) พันธุ์ Avanti ดอกสีส้มในวัสดุออกรากชนิดต่างๆ ภายใต้สภาพปลอดเชื้อ ด้วยการใช้อาหารดัดแปลงสูตร ½ MS ที่เติมน้ำตาลซูโครสเข้มข้น 30 กรัมต่อลิตรร่วมกับการจุ่มส่วนโคนยอดลงในสารละลาย IBA ความเข้มข้น 2.5 กรัมต่อลิตรก่อนนำไปเพาะเลี้ยงในวัสดุออกรากเป็นเวลา 4 สัปดาห์ พบว่าหน้าวัวเกิดรากได้ดีในวุ้นที่ระดับความเข้มข้น 8 และ12 กรัมต่อลิตร โดยมีอัตราการเกิดรากเป็น 86.67 และ 73.33 เปอร์เซ็นต์ ความยาวเฉลี่ย 14.62 และ 12.41 มิลลิเมตรและมีจำนวนรากเฉลี่ย 3.54 และ 3.91 ราก ตามลำดับ ส่วนไฟตาเจลที่ความเข้มข้น 1.5 กรัมต่อลิตรชักนำให้ยอดออกรากได้ 93.33 เปอร์เซ็นต์ จำนวนรากเฉลี่ยเพียง 3 ราก ที่ความยาว 11.66 มิลลิเมตร ในขณะที่พีทมอสและเวอร์มิคูไลท์ชักนำรากได้ 46.67 เปอร์เซ็นต์เท่ากัน จำนวนรากเฉลี่ย 1 และ 1.86 ราก ความยาว 7.86 และ 6.66 มิลลิเมตรตามลำดับ นอกจากนี้ไม่สามารถชักนำให้ยอดหน้าวัวออกรากได้ในสแฟกนั่มมอส จากการตรวจสอบโครงสร้างภายในรากไม่พบลักษณะผิดปกติของรากที่เกิดในวัสดุออกรากทุกชนิดที่ทดสอบ แต่พบว่ารากที่เกิดในวุ้นและไฟตาเจลความเข้มข้นสูง รวมทั้งพีทมอสมีขนาดใหญ่กว่ารากที่เกิดในวัสดุออกรากอื่น โดยเฉพาะรากที่เกิดในพีทมอสจะมีขนาดเส้นผ่าศูนย์กลางของระบบท่อลำเลียงมากที่สุด ยอดผลิตรากขนอ่อนได้ดีเมื่อใช้ไฟตาเจล พีทมอสและเวอร์มิคูไลท์เป็นวัสดุออกรากเทียบกับการใช้วุ้น ต้นหน้าวัวที่ออกรากในพีทมอสและเวอร์มิคูไลท์มีอัตรารอดตาย 100 เปอร์เซ็นต์หลังย้ายปลูกลงวัสดุปลูกผสมระหว่างดินและขุยมะพร้าว

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