Micropropagation of *Haworthia cymbiformis* Through Thin-Cell-Layer Tissue Culture©

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Window haworthia [*Haworthia cymbiformis* (Haw.) Duval] was propagated through thin-cell-layer (TCL) tissue culture in vitro. Thin-cell-layer explants were prepared from leaves and stem of offsets. Leaves and stem segments were immersed in 70% ethanol for 10 sec, and then 1% sodium hypochlorite for 13 min. After washing in sterilized distilled water three times, TCL and transverse-TCL (t-TCL) explants were prepared from leaves into 1 mm and 3 mm thickness, respectively. Stem t-TCL explants were dissected to disk explants of 1 mm thickness. Those explants were cultured on the Murashige-Skoog (MS) medium [30 g·L^{-1} sucrose, 8 g·L^{-1} agar (pH 5.6)] supplemented with 0.1 mg·L^{-1} benzyladenine (BA), 0.5 or 1.0 mg·L^{-1} indole-3-acetic acid (IAA) under 24±2 °C and 16-h light with cool white fluorescent lamps (40 µmol·m^{-2}·s^{-1} PPFD) / 8-h dark condition. Only stem t-TCL explants produced adventitious shoots on all media. The maximum number of regenerated shoots was 24.0 per explant on the medium supplemented with 0.1 mg·L^{-1} BA. The respective regenerated shoots produced additional numbers of secondary shoots (7.5 per a divided shoot) and roots after subculture on the growth-regulator-free medium.

INTRODUCTION

Window haworthia [*Haworthia cymbiformis* (Haw.) Duval] produces offsets and is stoloniferous; and its leaves are club-shaped with a separate flattened end area windowed with translucent patches. Although the window haworthia is able to propagate by division of offsets and leaf cuttings, the multiplying efficiency is not that high. In vitro cultures of *Haworthia* plants using inflorescence (Majumdar and Sabharwal, 1968; Kaul and Sabharwal, 1972; Ogihara and Tunewaki, 1978), perianth (Konishi et al., 1982), ovary (Majumdar, 1970), and leaf (Wessles et al., 1976; Beyl and Sharma, 1983) have been reported. However, the main aims of these reports were not the propagation of the true-to-type, and studies were directed at morphogenetic and physiological analysis of *Haworthia* plants. Thin cell layer (TCL) system established and developed by Tran Thanh Van and co-workers (Tran Thanh Van, 1999) has a characteristic point which the accelerative effect of organ formation from various organ and tissue explants prepared as TCL, and the effects have been confirmed in many plant species. It seems that the accelerative effects will effectively act in favor of the micropropagation for the difficult plant species of the increase. In this report, we tried to apply the TCL system for micropropagation of *Haworthia cymbiformis* plants.

MATERIALS AND METHODS

Preparation of TCL Explants from Leaf and Stoloniferous Stem and Their Initial Culture. Thin cell layer explants were prepared from leaves and stoloniferous stems of offsets. Leaves and stem segments (about 1 cm long) were sterilized in 70% ethanol for 10 sec and then 1% sodium hypochlorite for 13 min. After washing in sterilized distilled water three times, TCL and transverse-TCL (t-TCL) explants were prepared from the leaves into 1 mm and 3 mm thickness explants, respectively. The stoloniferous stem were dissected into t-TCL disk explants of 1 mm thickness. Those explants were cultured on the Murashige and Skoog (1962) medium [30 $g \cdot L^{-1}$ sucrose, 8 $g \cdot L^{-1}$ agar (pH 5.6)] supplemented with 0.1 $mg \cdot L^{-1}$ benzyladenine (BA), and 0.5 or 1.0 $mg \cdot L^{-1}$ indole-3-acetic acid (IAA). Ten milliliters of each medium was poured into a 20 × 120-mm glass test tube and autoclaved at 120 °C for 15 min before explant inoculation. All cultures were incubated under 24±2 °C and 16-h light with cool white fluorescent lamps (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) / 8-h dark condition.

Subculture of Regenerated Respective Shoots. The regenerated shoots on each explant in the initial culture were divided into single shoots, and each of them was subcultured on the growth-regulator-free MS medium. Twenty milliliters of the medium was poured into a 40 × 150-mm glass test tube and autoclaved at 120 °C for 15 min before the shoot inoculation. All cultures were incubated at 24±2 °C and 16-h light with cool white fluorescent lamps (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) / 8-h dark condition.

RESULTS AND DISCUSSION

Only stem t-TCL explants produced adventitious shoots on all media. Explants of TCL and t-TCL from leaves showed no response. The earliest shoot formation from the stem t-TCL explant was observed on 11th day from the inoculation of the explants and the first root formation from regenerated shoots was observed on the 17th day from explant inoculation. The maximum number of regenerated shoots was 24.0 per explant on the medium supplemented with 0.1 $mg \cdot L^{-1}$ BA (Table 1). The regenerated shoots produced a considerable number of secondary shoots (7.5 per divided shoot) and roots after subculture on the growth-regulator-free medium (Table 2, Fig. 1). Within 6 months, about 160 plantlets could be obtained through the offset-stem t-TCL explant culture from one offset stem segment when those explants were cultured on the MS medium supplemented with 0.1 $mg \cdot L^{-1}$ BA.

The secondary shoot still showed a vigorous multiplication capacity after the second subculture on the growth-regulator-free medium. The capacity was higher in

Table 1. Effects of BA and IAA on the shoot regeneration from stem t-TCL explants.

Growth regulator					Shoot formation	
Chemical name	($mg \cdot L^{-1}$)	Number of explants	Contamination (%)	Callus formation (%)	(%)	No. per explant
BA	0.1	7	14.3	57.1	28.6	24
IAA	0.5	7	14.3	42.9	28.6	7
IAA	1.0	6	33.3	33.3	33.3	11

Figure 1. Shoot multiplication from a regenerated shoot after subculture on the growth regulator-free MS medium in *Haworthia cymbiformis*.

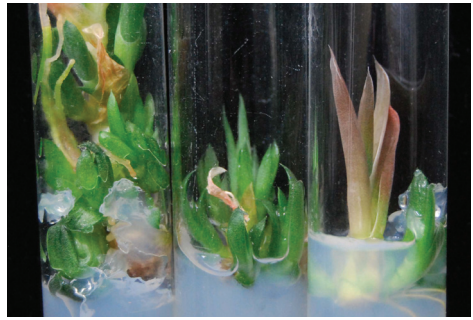

Figure 2. Axillary and adventitious shoot development during the second subculture in *Haworthia cymbiformis*. Left: Axillary shoot development on upper part and adventitious shoot formation on basal part of a whole shoot. Center: Vigorous adventitious shoot formation from the lower half segment. Right: Shoot formation from the upper half segment.

the basal half of the secondary shoots than the upper half. Almost all axially buds of secondary shoots subcultured on the growth-regulator-free medium grew and developed into shoots (Fig. 2 left). The same carrying-over effect of BA was reported by in the inflorescence culture of *H. cymbiformis* (Suzuki and Ijiro, 2011) and shoot culture of *Spathiphyllum wallissii* (Amaki et al., 1996).

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Table 2. Carrying-over effect of BA and IAA on the subcultured shoot multiplication.

Past culture media							
Growth regulator					Regenerated shoot		
Chemical name	($mg \cdot L^{-1}$)	No. of inoculated shoots	Contamination (%)	Callus formation (%)	2nd shoot formation (%)	No. of 2nd shoots	Shoot length (cm)
BA	0.1	7	0	57.1	100	7.5 a	4.5 a
IAA	0.5	7	0	42.9	100	3.1 b	3.3 b
IAA	1.0	6	0	33.3	100	7.5 a	3.0 b