Inducing Callus and Regeneration of *Haworthia*

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***Haworthia* is a genus of succulent plants which belongs to the family *Liliaceae*. There are about 500 species, mainly located in South Africa. In the present study, leaf and flower stem segments were cultured in order to induce callus in four different species of *Haworthia*. It is considered difficult to propagate *Haworthia* via leaf cuttings, root cuttings, division, or seed. In this study, the propagation of *Haworthia* by tissue culture was investigated.**

MATERIALS AND METHODS

Four different species of *Haworthia*, *H. truncata, H. arachnoidea* (syn. *setata*), *H. emelyae* (syn. *picta*), and *H. turgida*, were used. Leaf segments and flower stems were washed for 24 h with water and sterilized using ultrasound for 15 min, 70% alcohol for 2 min, and 50% sodium hypochlorite solution with a few drops of Tween 20 for 15 min, followed by three rinses in sterile distilled water. Each leaf was cut into two parts and the flower stems were cut about 1 to 2 cm in length.

Naphthalene acetic acid or 2,4-D plus kinetin or BA were added to Murashige and Skoog (MS) basal medium to induce callus. In total, 21 different media containing either NAA or 2,4-D combined with kinetin or BA were used in order to investigate the effects of plant growth regulators on callus proliferation. The leaf and flower stem segments were cultured at 23±2C and 300 lux (16-h photoperiod) or in the dark. During callus culture, shoot and root numbers were recorded. MS medium without any growth regulator and Hyponex medium were also used to examine their effect on induction of root formation.

RESULTS AND DISCUSSION

Inducing Callus. Calli were induced from leaf segment of all four species of *Haworthia*. Although there are reports of callus being induced from flower stems in *Haworthia*, this is the first report of callus being induced from leaf segments.

Proliferation of Callus. The most effective callus-proliferation medium was the MS medium supplemented with 0.2 mg liter^{-1} NAA plus 0.2 mg liter^{-1} BA under dark rather than light conditions. After a month of culture, *H. arachnoidea* showed the highest proliferation rate, 316.0%; followed by *H. turgida*, 202.7%; *H. emelyae*, 200.0%; and *H. truncata*, 50.6% F.W.

Shoot Formation. The most effective shoot induction medium was the MS medium with 0.2 mg liter^{-1} NAA plus 0.2 mg liter^{-1} kinetin under light conditions. The number of shoots formed during 4 months of culture were: *H. arachnoidea*, 53.8±14.8; *H. emelyae*, 39.9±24.2; *H. truncata*, 22.1±13.9; and *H. turgida*, 10.3±5.3.

Root Formation from Shoots. The most roots, 7.8 was the average, were formed from shoots of *truncata* during the 1 month callus culture on the MS medium

containing 0.2 mg liter^{-1} kinetin. The Hyponex medium without growth regulators was not as effective in inducing roots on shoots, with 2.3 roots on average formed. The numbers of roots were: *H. truncata*, 4.1±2.0; *H. emelyae*, 3.3±2.1; *H. turgida*, 1.3±0.9; and *H. arachnoidea,* 0.8±1.0. The regeneration rate of plantlets from callus was similar to the rate of root formation from shoots. The difficulty of regenerating plantlets from callus still remains.

Induction of Axillary Buds by Nodal Segment Culture and Rooting of Axillary Shoots of *Epipremnum aureum*

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Nodal segments (5 mm in length) were excised from the shoots of potted *Epipremnum aureum* Bunt. which had been obtained from a market. After sterilization with 1% sodium hypochlorite solution, nodal segments were placed on Murashige and Skoog (MS) media with differing concentrations of benzyladenine (BA 0, 5, 10, and 15 mg liter^{-1}). The highest value of axillary bud induction (%) was observed in the MS medium supplemented with 10 mg liter^{-1} BA. When young nodes were used as explants, a number of adventitious shoots were formed on the explants. In this case, two types of shoots were observed, one with spotted leaves similar to the donor plant and another with unspotted green leaves.

It was found in both the media supplemented with 10 mg liter^{-1} BA or 10 mg liter^{-1} kinetin that the axillary bud break (%) of the plants derived from micropropagation was higher than that of the plants grown from soft cuttings.

When the axillary shoots reached about 2 cm in length, the shoots were excised from the explants and transferred to a vermiculite medium in vivo. As shown in Table 1, the rooting (%) of the shoots induced on the medium with 10 mg liter^{-1} kinetin was higher than those on the media with 10 mg liter^{-1} BA and control. Benzyladenine in the shoot-induction medium suppressed the rooting in the vermiculite medium. The shoot induction medium with 10 mg liter^{-1} kinetin had a promotive effect on the number of roots and on the maximum root length. Using this method the period necessary for obtaining good transplants was shortened, and the regenerated plants are now growing normally in a greenhouse.

Table 1. Rooting of axillary shoots in a vermiculite medium.

Hormones in axillary bud induction medium	Rooting (%)			Number of roots**	Length of maximum root (cm)
	5	10	20*		
Free	20	30	75	1.7±0.2	3.0±1.0
Kinetin (10 mg liter^{-1})	50	83	83	2.6±0.3	7.7±2.6
BA (10 mg liter^{-1})	5	10	70	1.8±0.3	3.6±0.7

* Days after transplantation
** Values 25 days after transplantation