Growth Regulators Influence Root and Shoot Development of Micropropagated Algerian Ivy

K.H. Al-Juboory¹

Department of Horticulture, College of Agriculture, University of Baghdad, Abu-Ghreib, Baghdad, Iraq

D.J. Williams² and R.M. Skirvin²

Department of Horticulture, University of Illinois, 1005 Plant Science Laboratory, 1201 South Dornier, Urbana, IL 61801

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Abstract. Shoots of greenhouse-grown Algerian ivy (*Hedera canariensis* L.) were surface disinfected and explanted on modified Murashige and Skoog (MS) medium supplemented with BA (10 μ M) and NAA (2.5 μ M). One month later the shoots were transferred to MS proliferation medium supplemented with TDZ (0.1 or 0.5 μ M) and NAA (40 μ M). An average of three microshoots developed on each stem treated with TDZ. Pruned shoots grown on MS medium supplemented with GA₃ (20 μ M) and BA (20 μ M) branched better than unpruned shoots (3.7 vs. 1 per explant, respectively). Rooted shoots grown ex vitro grew and developed a shape suitable for commercial sale in 3 months. Chemical names used: *N*-(phenyl-methyl)-1 *H*-purine-6-amine (BA); gibberellic acid (GA₃); 1-naphthaleneacetic acid (NM); *N*-phenyl-W-1,2,3-thiadiazole-5-yl urea (Thidiazuron, TDZ).

Algerian ivy, a popular woody ornamental species, is used as a hanging basket plant or as a groundcover in warm climates. It displays strong apical dominance and does not readily branch even after pruning. Improved production methods that increase branching would reduce the time required to produce a saleable crop. Anderson (1975) reported that tissue culture-produced rhododendrons have a more compact habit and more branches. One method to accelerate the production of Algerian ivy could be through micropropagation in vitro.

There have been two reports of Algerian ivy being cultured in vitro (Shantz and Steward, 1959; Stoutemyer and Britt, 1965); both involved callus cultures, and shoot formation was not reported. In contrast, a related species, English ivy (*Hedera helix* L.), has been studied extensively in vitro; the majority of this literature examines callus cultures and the role of juvenility and maturity in root development (Geneve et al., 1988, 1990; Hackett, 1988). Sporadic somatic embryogenesis and adventitious bud formation have been reported for English ivy callus (Banks, 1979; Banks et al., 1979). Therefore, this

study was initiated to develop an in vitro technique to propagate Algerian ivy.

Stem cuttings (eight to 10 nodes) of greenhouse-grown juvenile phase Algerian ivy were divided into one-node explants. The explants were washed for 1 min in a 0.1 w/v Alconox solution, rinsed with sterile distilled water (SDW), soaked for 5 sec in 70% ethanol, rinsed with SDW, and soaked for 15 min in a 0.5% NaOCl solution containing 0.1% (v/v) Tween 20. Nodal segments were then rinsed three times (5 min each) with SDW

and aseptically separated into 1.5- to 2.0-cm sections, each containing one bud.

All explants were placed on a modified (MS) medium (Murashige and Skoog, 1962) supplemented with 10 μ M BA, 2.5 μ M NM, and (in mg-liter⁻¹) 0.5 thiamine HCl, 1 pyridoxine, 5 nicotinic acid, 2 glycine, 100 myo-inositol, and 30 g sucrose/liter. Before autoclaving, gentamicin sulfate at 3 mg-liter⁻¹ was added to suppress bacterial growth. The pH was adjusted to 5.6 to 5.7 and agar (7 g-liter⁻¹) was added. The medium was autoclaved at 106 kPa pressure at 120°C for 15 min. All cultures were grown at 22 to 24°C under cool-white fluorescent lights (40 to 60 μ mol m⁻²s⁻¹) with a 16-h photoperiod. All the explants were maintained for 1 month on MS medium.

Media with NAA or IBA and shoot growth (Expt. 1). Single node microshoots were excised from whole explants growing in vitro and moved either to full MS or half-strength MS salts (one-half MS) containing either auxin (NAA) or 1 *H*-indole-3-butyric acid (IBA) at seven concentrations (0, 2.5, 5, 7.5, 10, 20, or 30 μ M). A single microshoot (experiment unit) was placed into a 150 × 25-mm culture tube containing 15 ml of medium. A completely randomized experimental design with 24 single microshoot replicates per treatment was used. Cultures were evaluated for number of shoots produced by the single axillary bud per explant.

Media with NAA and TDZ and growth characteristics (Expt. 2). MS media containing 5 × 3 factorial combinations of NAA (0, 10, 20, 30, or 40 μ M) and TDZ (0, 0.1, or 0.5 μ M) were dispensed 50 ml/vessel into 350-ml propylene GA7 (Magenta Corp., Chicago) vessels randomly arranged in culture racks. Terminal microshoots (1 to 2 cm long) were taken from stock cultures and placed one in each culture vessel. There were

Table 1. Effects of combinations of NAA and TDZ on root and shoot growth characteristics and on callus formation of Algerian ivy microshoots grown in vitro and evaluated 4 weeks after explanting.²

NAA (μ M)	TDZ (μ M)	Roots/explant	Root length (cm)	Rooting (%) ³	Shoot length (cm)	Leaf no.	Shoot no.	Callus rating ⁴
0	0.0	0.0 a	0.0 a	0.0 a	1.5 a	1.0 a	1.0 a	+
	0.1	0.0 a	0.0 a	0.0 a	1.5 a	1.0 a	1.0 a	+
	0.5	0.0 a	0.0 a	0.0 a	1.4 a	1.0 a	1.0 a	+
10	0.0	8.8 de	2.0 c	84.0 e	1.5 a	1.0 a	1.0 a	+
	0.1	2.8 c	1.0 b	10.0 b	1.5 a	3.3 b	1.0 a	+
	0.5	0.0 a	0.0 a	0.0 a	1.5 a	3.4 b	1.0 a	+
20	0.0	8.4 de	4.0 e	78.0 e	1.4 a	3.2 b	1.0 a	+
	0.1	2.1 c	1.1 b	20.0 c	2.1 a	3.5 b	1.6 a	++
	0.5	0.0 a	0.0 a	0.0 a	2.0 a	3.0 b	1.0 a	+
30	0.0	9.2 d	4.0 e	75.0 e	1.5 a	3.1 b	1.0 a	+
	0.1	1.3 b	1.2 b	10.0 b	2.0 a	3.2 b	1.7 a	++
	0.5	0.9 b	0.9 b	10.0 b	2.0 a	4.0 b	1.0 a	+
40	0.0	8.1 d	4.2 e	77.0 e	1.6 a	3.2 b	1.0 a	+
	0.1	10.0 e	3.1 d	80.0 e	5.5 b	5.0 b	3.2 b	+++
	0.5	12.0 f	4.0 e	50.0 d	6.3 b	6.0 b	3.1 b	+++
Significance								
NAA		*	*	*	*	*	*	
TDZ		NS	NS	NS	NS	NS	NS	
NAA × TDZ		**	**	**	**	**	**	

²Means separation in columns by LSD at *P* = 0.05.

³Percent of microshoots that rooted.

⁴Callus rating denoted by + for small, ++ average, and +++ large callus.

NS,*,**Nonsignificant or significant at *P* = 0.05 or 0.01, respectively.

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¹Lecturer, Univ. of Baghdad, Iraq.

²Professor.

Fig. 1. Effects of NAA, IBA, and MS salt strength on number of shoots per explant of Algerian ivy in vitro. Mean separation within MS salt strength by LSD at $P = 0.05$.

24 single microshoot culture replicates used per treatment. Percent rooting, root count and length, leaf count, shoot count, and callus growth rating per explant were determined after 4 weeks. Callus rating was evaluated by assigning + for a small amount of callus, ++ for an average-sized callus, and +++ for a large amount of callus.

GA₃ plus BA and branching (Expt. 3). To determine the effect of GA₃ + BA on the branching of Algerian ivy, single terminal microshoots (1 to 2 cm) were harvested from stock cultures. Shoot apices were removed (pruned) from one-half of the microshoots with a scalpel; the others were left unpruned. Explants were placed on MS medium supplemented with filter-sterilized GA₃ (0, 20, or 40 µM) after being autoclaved in combination with BA (0, 20, or 40 µM). Fifteen single microshoot replicates, one per test tube for each treatment, were used in a 3 × 3 factorial experiment. The cultures were evaluated for shoot count 4 weeks after transfer.

Acclimatization. Rooted explants from Expt. 2 were transferred to a 1 peat : 1 perlite : 1 soil (by volume) mixture and were maintained in plastic bags in a culture room to prepare them for greenhouse conditions. After 1 month, they were transferred to a greenhouse and grown at 18°C night/24°C day. Plants were fertilized weekly with soluble fertilizer water containing 150 ppm N.

Data were subjected to an analysis of variance (ANOVA) and all proportional data were transformed into arcsin values before analysis. Treatment effects were partitioned into their appropriate orthogonal contrasts. For quantitative factors, the ANOVA was followed by the least significant difference mean separation procedure, with significance at $P = 0.05$ (SAS Institute Inc., 1985).

Experiment 1. Up to 3.5 lateral buds grew

from microshoots on full MS medium supplemented with auxin (Fig. 1). In one-half MS medium, not more than two branches per microshoot were produced even with auxin. Without auxins, axillary buds did not elongate. NAA induced shoot development at all concentrations tested. On full MS, IBA induced shoot branching at all concentrations, but on one-half MS, IBA induced it only at the lower concentrations (Fig. 1). Auxin also stimulated root initiation on the microshoots (data not shown). Cytokinins are thought to be synthesized in root tips and then transported through the xylem to the shoot (Davies, 1987). The ability of roots to produce cytokinins as well as to absorb nutrients may account for the increased shoot growth of auxin-treated microshoots. Once the microshoots rooted, they continued to elongate and branch without the formation of calli.

Experiment 2. There was a significant NAA × TDZ interaction for the ability to induce ivy rooting in vitro. NAA was necessary for root development; without NAA, there was no root formation (Table 1). The most roots per microshoot (12.0) were obtained on medium supplemented with 40 µM NAA with 0.5 µM TDZ. There was also a significant NAA × TDZ interaction for shoot count. Multiple shoots did not form without NAA and TDZ in the medium (Table 1). The highest number of shoots that developed from a single microshoot (3.2 and 3.1) grew on media supplemented with 40 µM NAA plus 0.1 µM and 0.5 µM TDZ, respectively. Since neither NAA nor TDZ alone was able to induce shoots, there appears to be a synergistic effect between NAA and TDZ on microshoot development. The highest number of shoots and the longest shoots were observed with NAA at 40 µM and TDZ at 0.1 µM and 0.5 µM (Table 1). TDZ has been used to stim-

ulate in vitro shoot formation in pear (Chevreau et al., 1989) and shoot proliferation of azalea (Fellman et al., 1987). TDZ has also been found to induce adventitious buds on corm explants of *Lilium flexuosum* L. (corm lily) that were more vigorous than those produced with BA treatment (Meyer and van Staden, 1988).

Experiment 3. Pruned microshoots produced 1 to 3.7 shoots per explant; unpruned microshoots did not branch. The most branching, 3.7 shoots per explant, occurred at 20 µM GA₃ and 20 µM BA. This result might be explained by a synergistic effect on branching, since neither GA₃ nor BA alone induced branching (Al-Yuboory and Williams, 1990). Other combination hormone applications have been shown to increase lateral bud inhibition relative to the effect of IAA alone (Davies et al., 1966).

Acclimatization. Sixty rooted plants from these experiments were moved to soil ex vitro. These plants grew well and developed a shape and size suitable for commercial sale after 3 months. This was accomplished in only half the time required to produce rooted plants by standard commercial systems. These results indicate that Algerian ivy produced by micropropagation techniques branch well and can be readily adapted into a commercial production system.

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