Genotypic Variation in the Micropropagation of Sri Lankan *Exacum* Hybrids

Andrew Riseman¹ and Siva Chennareddy

UBC Botanical Garden and Centre for Plant Research, University of British Columbia, 6804 SW Marine Drive, Vancouver, BC, V6T 1Z4 Canada

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ABSTRACT. Protocols for in vitro propagation are reported for interspecific hybrids of *Exacum* L., derived from Sri Lankan taxa. Four genotypes were used to evaluate the effects of MS (Murashige and Skoog) and WP (Woody Plant) media supplemented with 2-iP, BA, or KIN during establishment and multiplication phases. In addition, rhizogenesis and associated root characteristics were evaluated using MS medium supplemented with NAA or IBA. Overall, either 2-iP or BA was significantly more effective than kinetin in establishment and shoot proliferation with significant genotype $\times$ treatment interactions present. Maximum multiplication rates were achieved in the following genotype-hormone combinations: E-6, 2 mg·L⁻¹ BA (4.5 per explant); E-23, E-32, and E-37, 2 mg·L⁻¹ 2-iP (3.5, 2.5, and 3.6 per explant, respectively). In vitro rhizogenesis was greatest in liquid MS medium supplemented with 1 mg·L⁻¹ NAA while significantly reduced rooting was observed with IBA supplements. Our results demonstrate that micropropagation of Sri Lankan *Exacum* hybrids is possible and that adequate multiplication and rooting percentages can be achieved. However, the high level of genetic variation identified requires genotype-specific media modifications. Chemical names used: benzyladenine (BA); 2-isopentenyladenine (2-iP); indole-3-butyric acid (IBA); α -naphthaleneacetic acid (NAA); kinetin (KIN).

Hybrids of *Exacum* (L.), derived from *E. trinervium* (L.) Druce ssp. *macranthum* (Arn.) Cramer, *E. trinervium* ssp. *pallidum* (Trimen) Cramer, *E. trinervium* ssp. *ritigalensis* (Willis) Cramer, and *E. trinervium* ssp. *trinervium* (Cramer, 1981), all of which are native to Sri Lanka, possess many horticulturally desirable traits. These include season-long flowering; production of numerous large flowers (>2.5 cm); a range of flower colors from sky blue to deep violet; attractive dark green foliage; and plant habits suitable for either bedding, pot, or cut flower production (Riseman and Craig, 1995). A current obstacle to the commercialization of this crop is the generation of propagules that are uniform, easily produced, and cost effective.

Development and introduction of new ornamental crops requires adequate propagation protocols that generate high-quality, disease-free, uniform propagules in sufficient quantities to satisfy production demand. In crops where inbred breeding lines are available, these propagules are normally produced in the form of seeds. However, in crops that are not yet to the point of genetic stability, or when heterozygosity or fertility cannot be adequately managed, clonal propagation of specific genotypes is required. This can be accomplished either through traditional vegetative propagation methods or through micropropagation.

Exacum germplasm under development for horticultural use is highly heterozygous and suffers from intense inbreeding depression (Riseman, 1990) that precludes commercial propagation via seed. In addition, attempts to develop traditional vegetative propagation protocols have failed due to ontogenic interactions between rhizogenesis and reproductive status of the stock plants where a reproductive cutting fails to form roots (Riseman, unpublished data). Based on these observations, we suggest that

the most efficient means to generate the required propagules for commercial introduction is through micropropagation.

Micropropagation protocols exist for commercially available *E. affine* (Torres and Ntarella, 1984) but in preliminary trials, they were not effective for our taxa. Thus, we investigated additional factors affecting micropropagation specific for this germplasm with the following objectives: 1) to evaluate the effects of BA, KIN, and 2-iP on the in vitro establishment of four interspecific *Exacum* genotypes; 2) to evaluate the effects of BA and 2-iP on shoot multiplication rate over successive sub-cultures of four *Exacum* genotypes; 3) to evaluate the effects of IBA, NAA, KIN, BA, and 2-iP on rhizogenesis and associated root characteristics of two *Exacum* genotypes; and 4) to evaluate the genotype $\times$ medium interaction for multiplication and rooting responses.

Materials and Methods

PLANT MATERIAL. The four *Exacum* genotypes (E-6, E-23, E-32, and E-37) used in this study were 10th generation hybrids arising from directed hybridizations among four taxa within the *E. trinervium* complex: *E. trinervium* (L.) Druce ssp. *macranthum* (Arn.) Cramer, *E. trinervium* ssp. *pallidum* (Trimen) Cramer, *E. trinervium* ssp. *ritigalensis* (Willis) Cramer, and *E. trinervium* ssp. *trinervium* (Cramer, 1981). Ploidy level has not been established for these taxa, but based on the fertility of the interspecific hybrids, inheritance patterns (Riseman, 1990), and chromosome counts (Sumanashinge, 1986), these hybrids appeared to be diploidized allo- or autohexaploids with possible aneuploid reduction (Riseman, in press). These specific genotypes were selected based on horticultural quality and high level of fertility. Stock plants were maintained in glasshouses at the Univ. of British Columbia, Vancouver, BC, Canada, under 22/18 °C (± 3) day/night temperature and natural photoperiod. All four genotypes were included in the shoot multiplication studies and based on explant availability, only E-37 and E-6 were continued in the rhizogenesis study.

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¹To whom reprint requests should be addressed. E-mail: ariseman@interchange.ubc.ca

TISSUE CULTURE METHODS. Tissue culture production cycle was based on three stages: establishment, multiplication, and rooting. The establishment stage lasted for 4 weeks and allowed for the production of initial aseptic cultures. This was followed by multiplication in which cultures went through repeated cycles of sub-culturing at 4- to 6-week intervals. The rooting stage consisted of four weeks of treatment followed by data collection.

Based on preliminary trials, the following standard procedures were developed for tissue handling, media composition and culture conditions. For establishment, shoot cuttings 5 to 10 cm in length were harvested and transported to the laboratory, cleaned with 70% ethanol, cut into one to two cm pieces with one to two nodes each, and placed in a container with 10 mL liquid detergent then washed under running tap water. Each shoot was transferred to a sterile flow hood then surface sterilized by successive treatments with 70% ethanol for 30 s, 10% commercial bleach solution containing two drops of Tween-20 for 10 min, and three 5-min rinses in sterile distilled water. Each nodal explant was cut aseptically and placed in a 25 × 150-mm culture tube containing 20 mL media supplemented with 30 g·L⁻¹ sucrose and 5.6 g·L⁻¹ high gel strength agar (Sigma Type A). The pH of all media was adjusted to 5.8 before autoclaving for 15 min at 121 °C. During multiplication, shoots were sub-cultured into sterile 100-mL baby food jars for added space per explant. Solid media were used during establishment and multiplication stages while liquid media was used during the rooting stage. The cultures were incubated at 25 °C under 16-h photoperiod provided by cool-white fluorescent lamps (120 μmol·m⁻²·s⁻¹).

TREATMENTS. Based on literature for the relative, *E. affine* (Torres and Natarella, 1984), the following treatments were evaluated. During the establishment stage, both Murashige and Skoog (MS) (Murashige and Skoog, 1962) and Woody Plant (WP) (McCown and Lloyd, 1983) media were tested in conjunction with either KIN, 2-iP, or BA supplements. However, WP-based media were discontinued early in the research due to very poor explant performance. During the multiplication stage, only MS based media were used with hormone combinations between 2-iP and BA (Table 1). During the rooting stage, MS media were supplemented with one of KIN, 2-iP, or BA, plus either NAA or IBA (Table 1). Explants used for subsequent experiments were selected based on morphological uniformity and randomized prior to use.

DATA COLLECTION AND STATISTICS. At least 40 explants per treatment were used in all experiments and statistical analyses. Multiplication rate was calculated as the number of shoots produced per subculture period divided by the number of shoots initially cultured. Data for the rhizogenesis stage were from uniform shoots consisting of two nodes and two fully expanded leaf pairs collected 4 weeks after the start of treatments. Data from shoot included shoot length, number of leaves, fresh mass, and

dry mass. Measured root characteristics were rooting percentage, the number of root initials per shoot, total root length, fresh mass and dry mass. Root length measurements for each root segment were made by alignment to a ruler and summed to calculate total root length. After measurement, root and shoot tissues were weighed and then dried at 50 °C for 2 d and reweighed for dry weight determination.

Replication was concurrent in time. Descriptive statistics and analysis of variance (ANOVA) were computed with Systat for the Macintosh Version 5.2.1 (Systat, Evanston, Ill.). SuperANOVA software (Abacus Concepts, Berkeley, Calif.) was used for mean separation analyses using Fisher's Protected least significant difference (LSD) procedure.

Results

INITIATION STAGE. Culture growth on a WP medium, regardless of hormone supplement, was significantly less productive than on MS medium with vitrification occurring within 2 weeks postestablishment. In addition, KIN supplements had less effect on overall explant growth than either the BA or 2-iP supplements (data not shown). Explant responses to KIN included reduced apical bud expansion and shoot growth while shoot responses to both BA and 2-iP treatments included bud expansion and vigorous shoot production from both apical and axillary meristems. When NAA was withheld from the media, no shoot development was observed, regardless of genotype or kinetin supplement (data not shown). No genotype × medium interactions were observed during the establishment stage. Based on these observations, only base MS media with various BA or 2-iP supplements were used in subsequent experiments. In addition, at least 0.1 mg·L⁻¹ NAA was added to all subsequent treatments.

MULTIPLICATION STAGE. Apical and axillary meristem growth was observed after 2 weeks on MS medium, regardless of hormone supplement. Callus growth was observed in treatments supplemented with BA, while no callus was produced in the 2-iP treatments. Shoots produced in the BA treatments were thinner and weaker than with the 2-iP treatments, regardless of hormone concentration.

In the first sub-culture, a trend was observed for increased multiplication rate with increased concentrations of either 2-iP or BA, regardless of genotype. However, no significant differences were detected. In the second sub-culture, a significant genotype × supplement interaction was identified (Fig. 1). For genotypes E-23, E-32, and E-37, multiplication rate was significantly higher with increased concentrations of 2-iP. However, for genotype E-6, the multiplication rate was significantly lower with increased concentrations of 2-iP. For genotypes E-6, E-23, and E-37, multiplication rate was significantly higher with increased concentrations of BA while for genotype E-32, a significant decrease

Table 1. Hormone supplements (mg·L⁻¹) used in conjunction with base media [Murashige and Skoog (MS) or Woody Plant (WP)] for initiation, multiplication, or rooting phase of *Exacum* tissue explants.

Initiation phase (MS and WP)	Multiplication phase (MS)	Rooting phase (MS)
1.0 mg KIN ^a + 0.01 mg NAA	1.0 mg 2-iP + 0.01 mg NAA	0.1 mg KIN + 1.0 mg NAA
2.0 mg KIN + 0.01 mg NAA	2.0 mg 2-iP + 0.01 mg NAA	0.1 mg KIN + 1.0 mg BA
1.0 mg 2-iP + 0.01 mg NAA	1.0 mg BA + 0.01 mg NAA	0.1 mg 2-iP + 1.0 mg NAA
2.0 mg 2-iP + 0.01 mg NAA	2.0 mg BA + 0.01 mg NAA	0.1 mg 2-iP + 1.0 mg IBA
1.0 mg BA + 0.01 mg NAA		0.1 mg BA + 1.0 mg NAA
2.0 mg BA + 0.01 mg NAA		0.1 mg BA + 1.0 mg IBA

^aKIN = Kinetin; NAA = α-naphthaleneacetic acid; 2-iP = isopentenyladenine; BA = benzyladenine.

in multiplication rate was observed with increased concentrations of BA. Maximum multiplication rates were achieved in the following genotype–hormone combinations: E-6, 2 mg·L⁻¹ BA (4.6 per explant); E-23, E-32, and E-37, 2 mg·L⁻¹ 2-iP (3.5, 2.5, and 3.6 per explant, respectively).

In subsequent sub-cultures, multiplication rates increased in the 2 mg·L⁻¹ 2-iP or the 2 mg·L⁻¹ BA treatments depending on genotype. Maximum multiplication rates of 4.5 and 6.1 per explant were achieved for genotypes E-37 (with 2.0 mg·L⁻¹ 2-iP) and E-6 (with 2.0 mg·L⁻¹ BA), respectively, an average increase of 40% over the initial multiplication sub-culture (data not shown). However, the higher rates achieved with BA continued to produce shoots that were thinner, weaker, and more often vitrified than in the 2-iP treatments.

ROOTING STAGE. Tissue availability after the 3rd sub-culture limited our study on in vitro rhizogenesis to the shoots of only genotypes E-37 and E-6. Initial attempts using solid media with supplements led to weak or no root induction (data not shown). Therefore, all subsequent rooting studies were conducted using media without the solidifying agent.

The NAA-based auxin treatments produced significantly greater absolute rooting percentages after 4 weeks, regardless of cytokinin supplement, than IBA based treatments, with averages of 67% and 98% rooting for genotypes E-6 and E-37, respectively. Rooting percentage was significantly lower in all IBA treatments, regardless of cytokinin, with averages of 16% and 8% rooting for E-6 and E-37, respectively (Fig. 2).

In evaluating specific rooting parameters, there were significant genotype, auxin supplement, and genotype × auxin supplement interactions for number of root initials, total root length, root fresh weight, and root dry weight (Table 2). In comparing overall genotypic averages, genotype E-37 produced about twice the number of root initials as compared to E-6 in most treatments. However, the significant genotype × auxin supplement interaction necessitates further analysis. In evaluating the genotype × auxin interaction, E-37 was significantly and positively affected by NAA supplement while E-6 was affected to a lesser degree. In addition, E-6 was slightly more responsive to IBA supplement than E-37. For E-37, the highest number of root initials (45 per explant) was observed in 0.1 mg·L⁻¹ BA + 1.0 mg·L⁻¹ NAA supplement while for E-6, the highest number of root initials (24 per explant) was observed in 0.1 mg·L⁻¹ 2-iP + 1.0 mg·L⁻¹ NAA. Total root length and root fresh and dry weights were, in general, greatest in treatments where more root initials were produced. However, when average root initial production was <2, average root length was greater than expected. This is

Error bar=SE

Fig. 1. Genotypic variation in multiplication rate of *Exacum* tissue explants during 2nd sub-culture in MS media with hormone supplements.

Error bar = SE

Fig. 2. Genotypic variation for rooting percentage in response to hormone combinations of NAA, IBA, KIN, 2-iP, and BA.

probably a result of resource allocation to fewer root initials, thus allowing for greater growth.

For shoot characteristics after rhizogenesis, statistically significant genotype, kinetin, genotype × kinetin differences were observed for all characteristics measured (Table 3). In addition, a significant genotype × auxin interaction was identified for both shoot fresh and shoot dry weights. However, the relationship between statistical significance and biological significance is neither straightforward nor apparent for these characteristics.

Table 2. Effects of genotype, kinetin, and auxin supplement on root characteristics and significance determined by analysis of variance (ANOVA).

Genotype	Supplement		Root characteristic ^z				
	Kinetin	Auxin	# Initials ^y	Total length ^y	Avg length/Initial ^s	Fresh wt (mg) ^y	Dry wt (mg) ^y
E-6	0.1 mg KIN ^w	1.0 mg NAA	14.4 (2.3) a	82.4 (39.8) b	19.3 (4.2) b	291 (100) b	29 (8) b
	0.1 mg KIN	1.0 mg IBA	2.1 (0.9) b	46.6 (19.2) b	32.5 (27.7) b	286 (113) b	25 (13) b
	0.1 mg 2-iP	1.0 mg NAA	23.9 (12.7) a	802.5 (351.7) a	31.6 (7.1) b	1550 (486) a	101 (22) a
	0.1 mg 2-iP	1.0 mg IBA	1.2 (0.4) b	78.8 (35.3) b	63.5 (8.3) a	518 (135) b	68 (18) b
	0.1 mg BA	1.0 mg NAA	10.5 (6.4) a	147.5 (18.5) b	11.3 (5.7) b	666 (294) b	58 (27) b
	0.1 mg BA	1.0 mg IBA	6.5 (2.8) b	221.3 (75.1) b	32.1 (5.7) b	550 (165) b	59 (21) b
E-37	0.1 mg KIN	1.0 mg NAA	35.4 (12.3) a	998.5 (397.8) a	30.1 (8.3) b	1891 (537) a	109 (23) a
	0.1 mg KIN	1.0 mg IBA	1.4 (0.8) b	58.6 (18.8) b	32.7 (18.1) b	878 (311) b	67 (31) b
	0.1 mg 2-iP	1.0 mg NAA	35.8 (11.4) a	838.4 (201.2) a	23.4 (2.5) b	2056 (721) a	111 (35) a
	0.1 mg 2-iP	1.0 mg IBA	2.2 (1.6) b	53.1 (23.6) b	22.9 (9.0) b	1073 (280) b	69 (54) b
	0.1 mg BA	1.0 mg NAA	45.1 (11.4) a	978.5 (466.4) a	21.1 (5.5) b	1728 (291) a	102 (30) a
	0.1 mg BA	1.0 mg IBA	1.4 (0.6) b	82.2 (34.9) b	62.1 (7.7) a	227 (120) b	20 (9) c
ANOVA							
Genotype			***	***	NS	***	***
Kinetin			NS	NS	NS	NS	NS
Auxin			***	***	**	***	***
Genotype × kinetin			NS	NS	NS	NS	NS
Genotype × auxin			***	***	*	***	***
Kinetin × auxin			NS	NS	NS	NS	NS
Genotype × kinetin × auxin			***	***	**	**	***

^zMean (standard deviation) per explant.^yWithin genotype mean separation by Fisher's Protected LSD at $P \leq 0.05$.^sMean separation by Fisher's Protected LSD at $P \leq 0.05$.^wKIN = kinetin; NAA = α -naphthaleneacetic acid; 2-iP = isopentenyladenine; BA = benzyladenine.NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table 3. Effects of genotype, kinetin, and auxin supplement on shoot characteristics and significance determined by analysis of variance (ANOVA).

Genotype	Supplement		Shoot characteristic ^z			
	Kinetin	Auxin	Length (mm) ^y	Leaf no. ^y	Fresh wt (mg) ^y	Dry wt (mg) ^y
E-6	0.1 mg KIN ^s	1.0 mg NAA	68.3 (5.7) b	12.2 (1.2) a	780 (177) c	76 (17) c
	0.1 mg KIN	1.0 mg IBA	53.3 (4.9) b	10.0 (1.7) a	1025 (312) bc	91 (22) bc
	0.1 mg 2-iP	1.0 mg NAA	80.1 (7.9) a	11.2 (1.6) a	932 (204) bc	82 (13) bc
	0.1 mg 2-iP	1.0 mg IBA	86.5 (7.0) a	12.4 (1.5) a	1251 (336) a	109 (26) a
	0.1 mg BA	1.0 mg NAA	47.2 (11.3) b	8.6 (0.8) b	573 (238) c	57 (19) c
	0.1 mg BA	1.0 mg IBA	76.3 (6.7) a	10.6 (1.4) a	1071 (113) b	96 (11) b
E-37	0.1 mg KIN	1.0 mg NAA	68.5 (9.7) a	9.6 (2.0) a	877 (283) a	73 (25) a
	0.1 mg KIN	1.0 mg IBA	75.4 (16.9) a	10.8 (1.0) a	995 (478) a	88 (34) a
	0.1 mg 2-iP	1.0 mg NAA	75.6 (10.4) a	9.4 (1.2) a	770 (219) a	60 (16) a
	0.1 mg 2-iP	1.0 mg IBA	68.1 (21.6) a	10.2 (3.7) a	657 (326) ab	65 (32) ab
	0.1 mg BA	1.0 mg NAA	49.5 (12.7) b	9.2 (2.3) a	467 (192) b	51 (15) b
	0.1 mg BA	1.0 mg IBA	30.0 (14.3) b	8.0 (0.8) b	462 (149) b	47 (17) b
ANOVA						
Genotype			**	***	***	***
Kinetin			***	***	***	***
Auxin			NS	NS	NS	NS
Genotype × kinetin			***	*	*	*
Genotype × auxin			NS	NS	***	***
Kinetin × auxin			NS	NS	NS	NS
Genotype × kinetin × auxin			***	***	NS	NS

^zMean (standard deviation) per explant^yWithin genotype mean separation by Fisher's Protected LSD at $P \leq 0.05$ ^sKIN = kinetin; NAA = α -naphthaleneacetic acid; 2-iP = isopentenyladenine; BA = benzyladenine.NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively

In general, the 2-iP treatments produced greater responses (e.g., longer shoots, greater weight) while the BA treatments produced lesser response (e.g., shorter shoots, less weight) regardless of genotype.

Discussion

The development of adequate micropropagation protocols is prerequisite to successful introduction and commercialization of plants that are recalcitrant or produce inconsistent results with traditional propagation methods. The *Exacum* hybrids currently under development fit into this category. Propagation difficulties combined with their genetic status (Riseman and Craig, 1995; Sumanasinghe, 1986) indicate that this germplasm is best suited to micropropagation-based production.

INITIATION STAGE. Culture in WP media, regardless of hormone supplements, produced a significant number of vitrified (hyperhydric) shoots. In *Gypsophila paniculata*, vitrification has been associated with either high relative humidity resulting in a lack of transpiration in vitro and the abnormal formation of leaf epidermis, calcium or potassium concentrations outside of adequate ranges (Gribble et al., 1996), or with high cytokinin levels in the media (Gribble, 1999). Since relative humidity and cytokinin concentrations in base media comparisons (i.e., MS vs. WP) were not different, we conclude the increased incidence of vitrification in the WP medium was related to its elemental composition. The WP medium contained significantly less calcium and potassium than MS media and we conclude these concentrations were below the required levels needed by *Exacum*.

MULTIPLICATION STAGE. Various cytokinin supplements were added to basal MS media to evaluate their effects on establishment and multiplication rate. Both explant establishment and multiplication rates were enhanced by the addition of either BA or 2-iP, depending on genotype. The effectiveness of these cytokinins in breaking apical dominance and promoting axillary bud growth in semi-woody and woody species is well documented (Arrillaga et al., 1991, 1992; Gavidia et al., 1996; Tripepi, 1977). In addition, KIN has been reported to promote multiple shoot formation from terminal and lateral buds of *E. affine* (Torres and Natarella, 1984), a close relative to the interspecific *Exacum* hybrids used in this study. However, when KIN was used on our germplasm, shoot growth and bud expansion was reduced and little to no shoot growth was observed.

There were significant differences for multiplication rate across hormone treatments. Highest multiplication rates were achieved when NAA was added to either 2-iP or BA supplements. Low concentrations of auxins can promote the growth of axillary buds by counteracting the inhibitory effects of high cytokinin concentrations on shoot elongation (Nehra and Kartha, 1994). The cytokinin 2-iP has been reported to be the most potent form in this class of plant hormones for shoot multiplication (Lloyd and McCown, 1980; Norton and Boe, 1982; Norton and Norton, 1985) when compared to either BA or KIN. Torres and Natarella (1984) reported that the addition of 0.01 mg NAA to 2.0 mg KIN produced adequate multiplication rates of *E. affine*. However, while *E. affine* responded favorably to KIN treatments, our germplasm did not display this response (data not shown).

The genotypic differences observed in response to BA and 2-iP supplements may relate to differences in the induction and activity of cytokinin oxidase (Motyka and Kaminek, 1994), the binding affinity of cytokinin oxidase to the respective cytokinin substrate (Whitty and Hall, 1974), or to differences in the quantity

or metabolism of endogenous cytokinins (Auer et al., 1999). Mok et al. (1978, 1982) suggested that the different effects of BA and 2-iP in in vitro cultures may be due to preferential degradation of 2-iP over BA. This enzyme degrades cytokinins by cleaving the N6-isopentenyl side chain at the double bond with resultant loss of activity (McGaw and Horgan, 1983; Whitty and Hall, 1974). Exogenously applied 2-iP may be degraded by genotype specific isoforms of this oxidase, and thus required a higher concentration of 2-iP for shoot formation. This could explain the genotypic differences in optimal concentration of either BA or 2-iP and suggests that cytokinin oxidases are important in controlling endogenous hormone levels. In addition to differential oxidase activity, the observed differences may relate to the total amount of endogenously produced cytokinin. Although endogenous cytokinins levels were not measured, greenhouse observations on branching and resistance to leaf chlorosis under stress (data not shown) support genotypic differences in endogenous cytokinin production among these genotypes.

ROOTING STAGE. The use of liquid medium had a positive and significant effect on root promotion in the present study as compared to solid media. In our study, use of solid media produced only weak to no root formation. This phenomenon was reported in other difficult-to-root species (Sriskandarajah and Mullins, 1981). According to George and Sherrington (1984), the favorable effects of liquid medium have been associated with either increased availability of both nutrients and growth regulators, to a more effective removal of toxic/inhibitory metabolites, or to increased diffusion of oxygen. In addition, liquid media are reported to be superior to solid media in maintaining adequate turgor pressure for cell division and subsequent shoot and root growth (Green, 1968). In production trials, rooting of interspecific *Exacum* cuttings was very sensitive to water logging or constantly wet media where oxygen available for root respiration may have been limiting. This would support the conclusion that solid media inhibited rooting through reduced oxygen diffusion to the explant.

Rooting percentages were significantly greater in all NAA treatments than in IBA treatments, regardless of genotype. In contrast, the related species *E. affine*, displayed the opposite response where significantly greater rooting occurred with IBA than with NAA treatments (Torres and Natarella, 1984). These differential responses in adventitious root formation based on exposure to either NAA or IBA has been reported for several crops. In *Malus* (De Klerk et al., 1997) and *Vitis* (Heloir et al., 1997), IBA was significantly better in root promotion than NAA while in *Bambusa* (Sarita and Sharma, 1998), *Santolina* (Casado et al., 2002), and *Cucumis* (Compton et al. 2001), the opposite effect was observed where NAA was significantly better than IBA. No pattern or fundamental biological process has yet been identified to explain these differences, but they appear to be species, not genera, specific.

The observed level of variation is not surprising considering the genetic constitution of both the *Exacum* genus as a whole and specifically this germplasm. The genus is thought to comprise a polyploid series of species with *E. affine* hypothesized to be tetraploid (Rork, 1949). Darlington and Wylie (1955) suggested the basic chromosome number for *Exacum* to be $X = 9$, thereby supporting this hypothesis ($2n = 4X = 36$). However, a haploid number of 10 is also common in the genus (Riseman, in press). For the Sri Lankan taxa used in the primary interspecific hybridizations, *E. trinervium* ssp. *macranthum* ($2n = 6X? = 54$) is hypothesized to be a hexaploid while, *E. trinervium* ssp. *pallidum* ($2n =$

6X-2? = 52) is thought to be hexploid with aneuploid reduction (Sumanasinghe, 1986). In addition, *E. trinervium* ssp. *trinervium* and ssp. *ritigalensis* (both $2n = 6X? = 60$) are hypothesized to be allopolyploids based on chromosome number and morphology (Sumanasinghe, 1986). When we consider the polyploid status of the native taxa combined with the interspecific background of this specific germplasm, the opportunity for gene diversification, in the long term, and recombination, in the short term, is great. With greater gene diversification and recombination, it is expected that a greater level of variation would be expressed. We conclude that the level of variation expressed by these four genotypes is representative of the high level of heterogeneity within this germplasm and that specific hormone regimens need to be developed for individual genotypes in order to achieve optimal performance.

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