

In vitro propagation of 'Echo' cultivars of *Eustoma grandiflorum* (Raf.) Shinn.

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Summary: *Eustoma grandiflorum* (Raf.) Shinn. 'Echo' F1 cultivars ('Echo White', 'Echo Rose', 'Echo Blue', 'Echo Blue Picotee') were used and multiplication of shoots was evaluated on Murashige and Skoog (1962) basal medium with 11 g/l agar-agar and 20 g/l sucrose. To test the effect of BA different concentrations were added: 0.10, 0.25 mg/l and a culture medium without BA. Differentiation of roots was examined on Jámbor-Benczúr and Márta (1990) basal medium with the same concentration of agar-agar and sucrose. To examine the effect on rooting, various concentrations of NAA were used: 0.5, 1.0, 2.0, 3.0 mg/l. The pH was adjusted to 5.6 in every case using KOH. We studied the after-effect of different concentrations of BA during the acclimatisation. During the multiplication, the cultivar 'Echo White' formed the most shoots and the smallest leaves on the medium with 0.10 mg/l BA. Fortunately, in the case of this cultivar, the number of shoots was reduced and the length of leaves was increased successfully on the medium without BA. The other three cultivars developed the longest leaves on the medium containing 0.10 mg/l BA. Sometimes not only shoot regeneration but spontaneous rooting was observed during the multiplication. Examining the rooting, the highest percent of roots was found on the medium with 1.0 mg/l NAA, and the cultivar 'Echo Rose' formed the most roots on this medium. Higher concentration (2.0 and 3.0 mg/l) of NAA already reduced the number of roots in all of the cultivars. During the acclimatisation, the percentage of survival was 76.3% and the tallest plants with the longest leaves were found on the multiplication medium with 0.25 mg/l BA. 'Echo Blue Picotee' gave the best results with the tallest pieces and longest leaves on this medium.

Key words: *Eustoma*, *in vitro* propagation, BA, NAA

Introduction

The *Eustoma grandiflorum* (Raf.) Shinn. belongs to the *Gentianaceae* family and is native in the Southern part of the United States (Texas, Nebraska, Kansas, Colorado, Luisiana), and North Mexico. This plant tolerates drought and requires sunshine. It is an annual or biannual plant with taproot, tender stem, oval-shaped leaves and bold flowers similar to a poppy. (Tjia & Sheehan, 1986; Halevy & Kofranek, 1984). Although seeds can be sown in the nurseries, it is not an easy way of propagation due to the slow growth (about 6–8 months are required from seed to flowering) and seedling populations are fairly variable with respect to flowering time, stem length and flower qualities (maybe micropropagation can be of help). The 'Echo' F1 cultivars are excellent cut-flowers with large, double-lobed flowers and strong, wiry, long stems. The *Eustoma grandiflorum* is a very important and popular cut-flower or pot plant, and numerous new cultivars have been developed, especially in Japan (Ohkawa & Sasaki, 1999).

Griesbach *et al.* (1988) attempted to develop a method for the tissue culture propagation of *Eustoma*. Murashige & Skoog (MS, 1962) basal medium and the cultivar 'Dwarf Purple' was used in the experiment. Stem and shoot explants proliferated best on MS medium with 3 mg/l BA and 0.2

mg/l NAA; while leaf explants needed 3 mg/l BA without NAA. On the other hand, the resulting shoots were rooted on half-strength MS medium containing 2 mg/l IAA.

Damiano *et al.* (1989) used full-strength MS medium with 0.3 mg/l BAP during the multiplication and half-strength MS medium with 1 mg/l IAA to examine differentiation of roots, and 30 g/l sucrose, 8 g/l bacto agar was added in every cases. Multiplication rates ranged from 3.3 to 9.0 and the rooting percentage ranged from 84 to 100%.

Further experiments (direct shoot organogenesis and somatic embryogenesis) were carried out in 1990. Leaf fragments were cultured on liquid MS media containing zeatin, 2iP and BA in order to obtain regeneration. After four weeks shoot regenerations were found on media with zeatin (1.5 and 3.0 mg/l) or low concentrations of 2iP (2.5 and 5.0 mg/l), some globular formation (embryos) were found on medium with 10 mg/l 2iP. Unfortunately, plant regeneration from these embryos was not successful (Ruffoni *et al.*, 1990)

Farina & Ruffoni (1993) studied the effect of temperature during the micropropagation of *Eustoma*. Although too high indecision of temperature (24 °C diurnal and 18 °C nightly degrees) delayed or failed the flowering time, the highest multiplication range was observed in this case.

The effect of growth regulators (BA, IVS, IES and NES) was examined, too. During the multiplication, the higher

concentration of BA increased the number of shoots and the amount of vitrified plants with fragile, succulent leaves and spongy tissue. Differentiation of rooting was gone off on the medium containing IVS and IES, while NES was ineffective. Besides this, the number of stomas was examined. Although more stomas were observed from the *in vitro* propagated plants than the outdoor pieces, the size of stomas was the same. During the acclimatisation, the percentage of survival was 80%, while the adaptation of vitrified plants was a complete failure (Paek & Hahn, 2000).

Material and method

The experiments were carried out from 2003 to 2004, in the laboratory of the Department of Floriculture and Dendrology, CORVINUS University Budapest.

In the starting, shoot tips, axillary buds and flower buds were taken from 'Echo' F1 cultivars of *Eustoma grandiflorum*. At first, these explants were sterilized in 50% ethanol for 5 min, afterwards disinfected in 0.2% HgCl₂ for 5 min and rinsed in sterile water three times.

Multiplication of shoots was investigated on Murashige & Skoog (MS, 1962) basal medium with 11 g/l agar-agar and 20 g/l sucrose. To study the effect of BA three different concentrations were added: 0.10, 0.25 mg/l and a culture medium without BA.

Length of leaves (mm) and number of shoots were measured in this phase.

Differentiation of roots was examined on Jámbor-Benczúr & Márta (BM, 1990) basal medium with the same concentration of agar-agar and sucrose. For studying the rooting, NAA was added in different concentrations: 0.5, 1.0, 2.0 and 3.0 mg/l. Length of roots (mm), and the number of roots were measured and percentage of rooting (%) was determined in this case. The pH was adjusted to 5.6 in every case using KOH. The explants were placed in Erlenmeyer test-tubes and 220 ml bulk of bottles, and the cultures were grown at 22–25 °C in 8/16 hours dark/light cycles. The media used are summarized in Table 1.

Shoot regenerants were acclimated in the greenhouse and the after-effect of three different concentrations of BA (0.10, 0.25 mg/l and a medium without BA) was searched in this trial. Length of leaves (mm), height of plants (mm) were measured and the percentage of survival (%) was determined.

Results and discussion

Multiplication of shoots

During the multiplication, lower concentration (0.10 mg/l) of BA was better for every cultivar, except for the cultivar 'Echo White' because of the too many shoots with

Table 1 Growth regulator composition of the culture media

	Multiplication media (Murashige & Skoog, 1962)			Rooting media (Jámbor-Benczúr & Márta, 1990)			
BA (mg/l)	0.25	0.10	–	–	–	–	–
NAA (mg/l)	–	–	–	0.5	1.0	2.0	3.0
sucrose (g/l)	20	20	20	20	20	20	20
agar-agar (g/l)	11	11	11	11	11	11	11

too small leaves (Figures 1 and 2). This cultivar gave the worst results on the medium containing 0.10 mg/l BA; sometimes plants similar to moss were found (Figure 3). This cultivar formed the highest number of shoots (24.8) and shortest leaves (10.37 mm) in this case. The other three cultivars ('Echo Blue', 'Echo Rose', 'Echo Blue Picotee') developed the longest leaves on the medium with 0.10 mg/l BA. Fortunately, in the case of 'Echo White' cultivar, the number of shoots was reduced (9.75) and length of leaves was increased (11.12 mm) successfully on the medium without BA (Figure 4). Besides this, the cultivar 'Echo Blue Picotee' formed the greatest number of shoots on the medium containing 0.25 mg/l BA; this cultivar gave the best results with the longest leaves on every medium during the multiplication (Figure 5). Sometimes not only shoot regeneration but spontaneous rooting was observed during the multiplication (Figure 6).

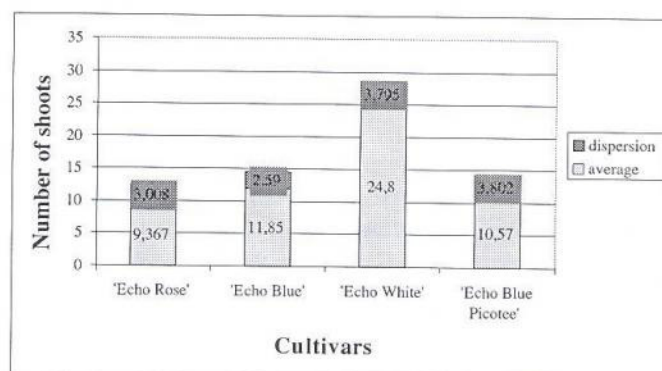


Figure 1 Multiplication: the effect of MS medium with 0.10 mg/l BA on the number of shoots

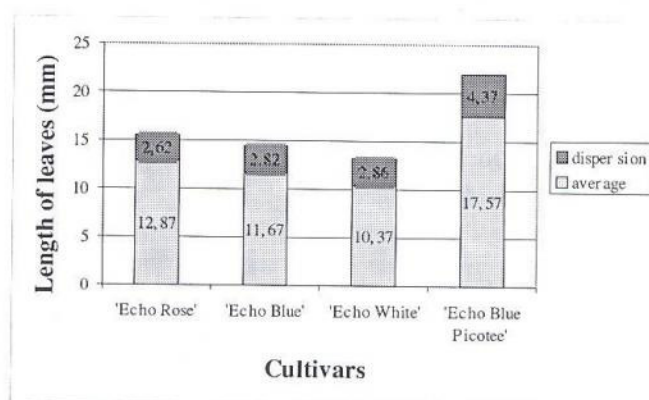


Figure 2 Multiplication: the effect of MS medium with 0.10 mg/l BA on the length of leaves (mm)

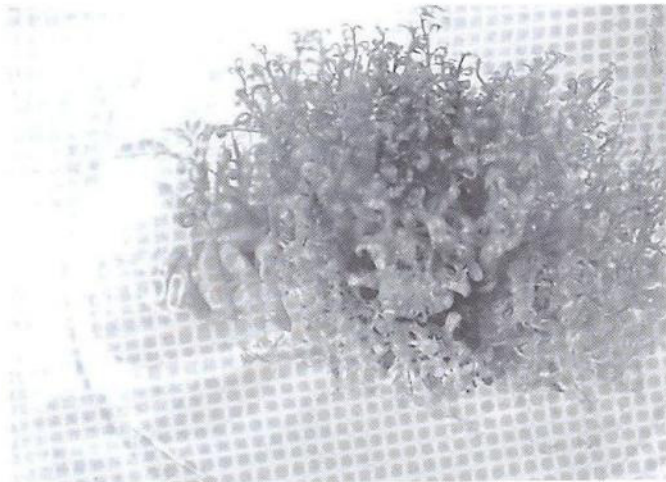


Figure 3 'Echo White' on medium with 0.10 mg/l BA



Figure 4 'Echo White' on medium without BA



Figure 5 'Echo Blue Picotee' on medium with 0.10 mg/l BA

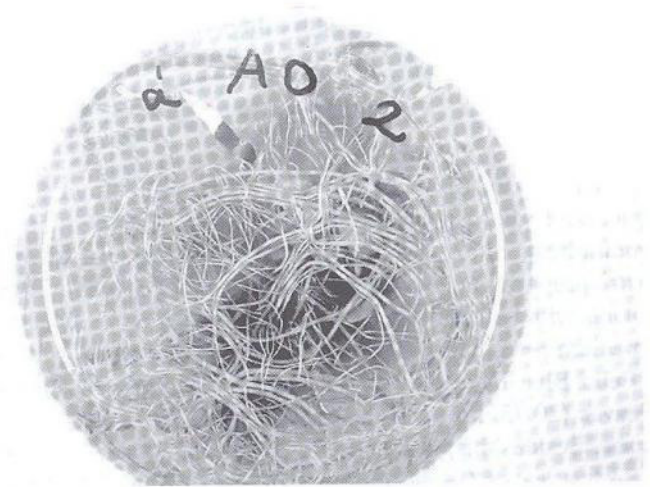


Figure 6 'Echo Blue' on medium without BA (spontaneous rooting)

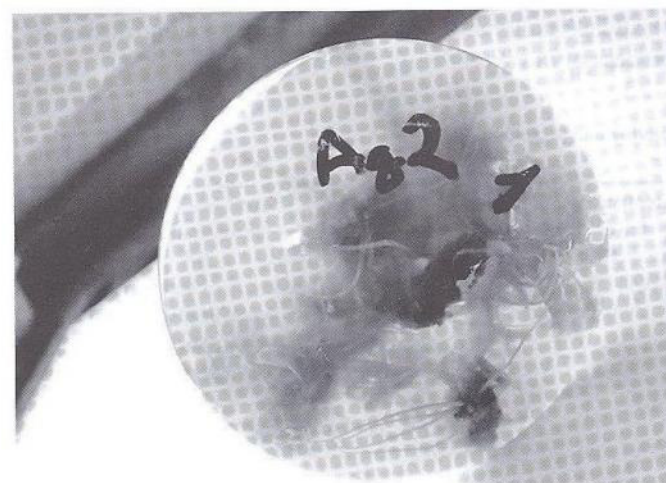


Figure 7 'Echo Rose' on medium with 0.10 mg/l NAA

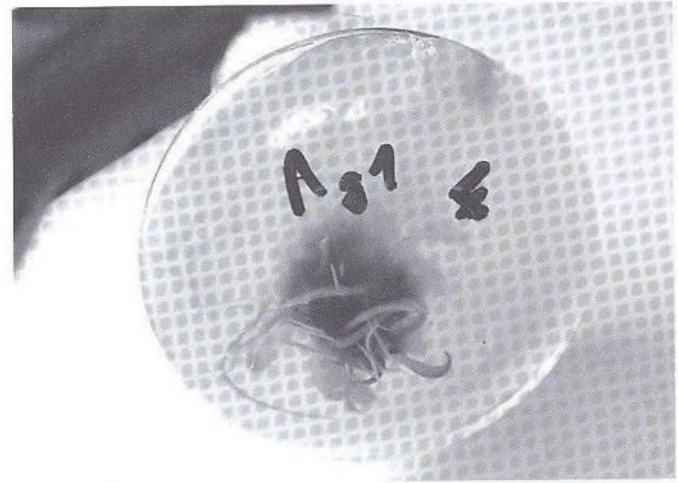


Figure 8 'Echo Blue Picotee' on medium with 0.5 mg/l NAA

Differentiation of roots

The highest percentage of rooting (97.3%) was found on the medium with 1.0 mg/l NAA and the cultivar 'Echo Rose' developed the highest number of roots (11) and shortest roots (11.8 mm) in this case (Figure 7). On the other hand, the

cultivar 'Echo Blue Picotee' developed the fewest (5.84) and longest (19.64 mm) roots. For this cultivar 0.5 mg/l NAA concentration proved to be the best, with the highest number of roots (9.8) on this medium (Fig. 8). By the way, higher concentration (2.0 and 3.0) of NAA already reduced the number of roots in every cases and the fewest number of



Figure 9 Acclimatised plants in platter

roots and lowest percentage of rooting (70.8%) was observed on the medium with 3.0 mg/l NAA.

Acclimatisation

The acclimatisation of every 'Echo' F1 cultivars was successful (the percentage of survival was 76.3%) after the multiplication (and not after the rooting), so the period of *in vitro* propagation was reduced (Figures 9 and 10). The tallest plants with longest leaves were found from the multiplication medium containing 0.25 mg/l BA. The most vigorous cultivar 'Echo Blue Picotee' gave the best results with the longest leaves (20.75 mm) and the tallest plants (16.04 mm) in this case. Besides this, the 'Echo Rose' was the weakest with the shortest leaves (14.54 mm) and smallest plants (10.42 mm) from the multiplication medium with 0.25 mg/l BA. The smallest plants with shortest leaves were found from the multiplication medium with 0.10 mg/l BA. The cultivar 'Echo Blue Picotee' formed the longest leaves (17.54 mm),

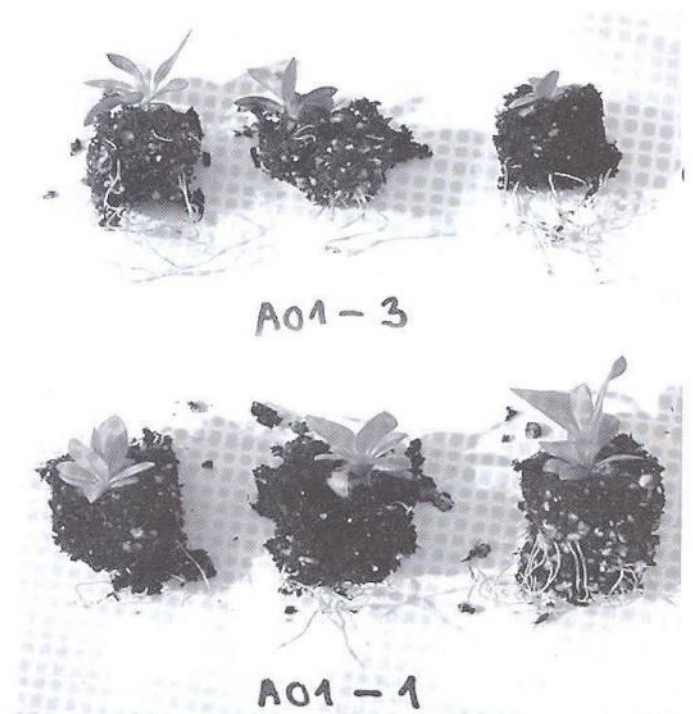


Figure 10 Acclimatised 'Echo White' and 'Echo Rose' (from medium with 0.10 mg/l BA)

the tallest cultivar 'Echo Rose' reached the highest degree (11.08 mm) and the weakest cultivar 'Echo White' formed the smallest plants (9.79 mm) with shortest leaves (13.92 mm) in this case. The cultivar 'Echo Blue Picotee' gave the best results (12.54 mm height of plants with 20.33 mm length of leaves) from the multiplication medium without BA. All of the results are summarized in Table 2.

To sum it up, the significant difference that was observed between the cultivars and suggested that the different kinds of 'Echo' cultivars needed a different medium during the process of *in vitro* propagation.

Table 2 Results of multiplication, rooting and acclimatisation

	Multiplication of shoots: the effect of BA			Differentiation of rooting: the effect of NAA				Acclimatisation: the after-effect of BA		
	A: length of leaves (mm)	B: number of shoots		C: length of roots (mm)	D: number of roots			E: length of leaves (mm)	F: height of plants (mm)	
BA (mg/l)	0.25	0.10	–	–	–	–	–	0.25	0.10	–
NAA (mg/l)	–	–	–	0.5	1.0	2.0	3.0	–	–	–
Percentage of rooting (%)	–	–	–	94.6	97.3	93.1	70.8	–	–	–
'Echo Rose'	A: 9.52 B: 8.58	12.87 9.36	10.7 8.2	C:10.28 D: 9.44	11.8 11.0	11.28 9.48	8.48 8.08	E:14.54 F: 10.42	16.13 11.08	16.25 11.42
'Echo Blue'	A: 10.32 B: 7.25	11.67 11.85	11.6 7.23	C:11.72 D: 8.72	12.64 7.76	13.48 8.16	9.32 7.16	E: 16.67 F: 13.00	14.71 10.96	13.46 9.13
'Echo White'	A: 10.45 B: 15.73	10.37 24.80	11.12 9.75	C: 9.96 D: 6.60	13.92 8.20	12.48 7.00	7.48 5.48	E: 16.17 F: 11.25	13.92 9.79	12.96 9.88
'Echo Blue Picotee'	A: 11.7 B: 18.1	17.57 10.57	14.07 6.98	C:17.96 D: 9.84	19.64 5.84	11.20 5.28	8.80 4.72	E: 20.75 F: 16.04	17.54 11.04	20.33 12.54

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