A Micropropagation Protocol for *Ecballium elaterium* (L.) A. Rich.

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Introduction: Squirting cucumber, *Ecballium elaterium* (L.) A. Rich. (Cucurbitaceae), is a wild medicinal plant found abundantly in the Mediterranean region. It has been utilized as a rootstock for many cucurbitaceous crops, mainly attributed to its resistance to abiotic as well as biotic stress (2). Important pharmacological uses (1, 9) are attributed to the bitter principles, cucurbitacins (5), which make the crop inedible. Micropropagation was aimed at determining the regeneration potential of this resistant rootstock.

Materials and Methods: *E. elaterium* seeds were obtained from immature fruit collected in the Southern region of Malta. The fruit were washed with tap water for 15 min., surface sterilized with 70 % ethanol for 30 sec, soaked in 10 % hypochlorite solution for 20 min and rinsed in three changes of sterile distilled water. Seeds were carefully removed under aseptic conditions, and placed on Murashige and Skoog (MS) basal medium (7). Two weeks from germination, node explants were taken for tissue culture.

The sectioned node explants were inoculated on MS medium containing different plant growth regulators (PGRs) or additives (Table 1), and every 4 weeks the surviving explants were either subcultured on the same medium or transferred to a different medium, in cases of impaired growth. The conditions for growth were 25 ± 1 °C and 3250 ± 250 lx. Bud multiplication, shoot elongation, root production and callus induction and proliferation were observed. The plantlets were transferred to Jiffy® pots (Sigma, U.S.A.) and closed in a Phytatray® (Sigma, U.S.A.) to maintain a high percentage of humidity. With the emergence of roots from the pot, the plantlets were transferred to larger pots until flowering.

The results were analyzed statistically by the one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test for equality of means. Only $p \leq 0.05$ were considered statistically significant.

Results and Discussion: *Effects of PGRs on explants*. The effects of the different PGRs or additives on the nodal explants are shown in table 1. The best responses for shoot multiplication were with NAA/BAP combination (Figure 1), followed by Ki ($p<0.05$, $v=10$). BAP responded synergistically with auxins unlike Ki. Nodal explants produced more than 5 shoots within 1 month especially with the NAA/BAP combination. In *Gomphrena* species, the index was three or more shoots per nodal segment after 1 month (8). A low auxin (0.1 mg/l NAA) and a high cytokinin (5 – 10 mg/l BAP) combination were optimum. For *E. elaterium*, decreasing the auxin concentration decreased the bud multiplication effect. As regards shoot elongation, the best and significant response was observed with Ki, BAP and GA_3. In their absence no elongation took place indicating that the plant in culture does not store or produce any endogenous cytokinins. Also cucurbitacins have anti-gibberellic activity (6) hence intrinsic gibberellins that may be possibly present are inhibited by these secondary metabolites. When NAA was completely omitted from the media, shoot elongation was noted in all treated shoot explants. Callus production was seen with all PGRs or additives except for IAA and charcoal. The 2,4-D/Ki combination showed significant effects on callus production with no effects on the other parameters. This goes in accordance with the observations made by Esaka (3) on *Cucurbita pepo* explants. Rooting was a parameter that posed several problems in the regeneration of *Ecballium elaterium* plantlets. In fact, the whole plantlet was not regenerated in tissue culture. Although IAA induced rooting, the low response might be due to the fact that IAA produces a response in the concentration range between 1 and 30 mg/l (4). Nevertheless, if the auxin had a higher activity, callus induction and proliferation might have

Fig. 1. Emergence of multiple shoots from nodal explants treated with the NAA/BAP combination

Fig. 2. Flowering and fruiting of the micropropagated *E. elaterium* plantlets.

Table 1. The overall effects of different media[z] on the different parameters studied.

	Percentage for each Stimulus (%)			
	Multiplication	**Elongation**	**Callus**	**Rooting**
IA/Ki	8.80	16.70[x]	5.76	8.73
NA A/BAP	26.39[x]	7.31	13.89	0.90
KI	23.09[x]	19.27[x]	3.60	6.55
2,4-D/Ki	0.00	0.00	14.39[x]	0.00
IBA	0.00	0.00	14.39[x]	26.19[x]
NA A/BAP (1/2)[y]	14.66	8.56	14.39[x]	0.00
MS	0.00	0.00	14.39[x]	26.19[x]
IAA	0.00	0.00	0.00	3.49
BAP	0.00	19.27[x]	14.39[x]	26.19[x]
Charcoal	16.50[x]	9.63	0.00	0.00
GA_3	10.56	19.27[x]	2.88	5.24

[z] The media contained MS medium and 1 mg/L of each PGR or additive listed: indole acetic acid (IAA), kinetin (Ki), naphthalene acetic acid (NAA), benzylamino purine (BAP), 2,4-dichlorophenoxyacetic acid (2,4-D) indole butyric acid (IBA) and gibberellic acids (GA_3).

[y]NA a?BAP (1/2) contains 0.5 mg/L of BNAA and 1 m g/L of BAP.

[x] p,0.05 (v=10).

The experiment was repeated three time with 15 replicates.

Table 2. Time (days) for rooting and repotting for the four treatments.

	IAA		**GA_3**	
	+R.H.[z]	**-R.H.**	**+R.H.**	**-R.H.**
Rooting in Jiffy® pots	10	23	46	63
Repotting	25	37	58	72

[z] Rooting hormone powder (1% NAA and thiram, Secto,UK).
The experiment was repeated three times with 10 replicates.

set in and hence posing a problem to the rooting process.

Transfer of explants. Based on the above findings, the shoot explant grown on GA_3 and IAA media were selected for pot trials, with the use of a rooting hormone (1 % NAA and thiram, Secto, UK). The best treatment was IAA cultures treated with rooting hormone (Table 2). For the IAA with rooting hormone treatment, flowering took place at approximately day 62 from transfer to Jiffy® pot. This was eventually followed by fruiting (Figure 2).

In conclusion, an ideal protocol would involve the germination of seeds on MS medium followed by the inoculation of node explants on NAA/BAP for three consecutive subcultures at 4-week intervals, elongation on GA_3 medium for another 4 week and an auxin (IAA) shock for 1 week, in tissue culture. Subsequently shoots should be treated with rooting hormone (NAA), transferred to Jiffy pots and after 3 to 4 weeks the plantlets should be repotted and acclimatized for another 4 to 5 weeks. Our results give a clear protocol for the regeneration of the squirting cucumber for possible use as a resistant rootstock for the edible cucurbitaceous crops.

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