Plant Cell, Tissue and Organ Culture **57:** 149–152, 1999.

Research note

Micropropagation of lemon balm

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Received 2 March 1998; accepted in revised form 25 August 1999

Key words: growth regulators, *Melissa officinalis*, organogenesis

Abstract

Traditional propagation of lemon balm *(Melissa officinalis* L.) is inefficient for establishing a good quality clonal population. Results of the presented experiments outline an effective method for micropropagation of this species. Following culture initiation from shoots of field-grown plants on growth regulator free Murashige–Skoog medium, rapid shoot multiplication with only rudimentary root formation could be achieved on media containing various concentrations of indole-3-acetic acid and 6-benzyladenine. The combination of 5.71 μM indole-3-acetic acid and 6.66 μM 6-benzyladenine resulted in the best multiplication. Transfer of propagules to media containing indole-3-acetic acid and kinetin did not result in shoot proliferation; however, single plantlets grown on media containing 5.71 μM indole-3-acetic acid and 13.9 μM kinetin developed more compact shoots and stronger roots than the control plants and were suitable for acclimatisation with an efficiency over 95%.

Abbreviations: BA – 6-benzyladenine; IAA – indole-3-acetic acid; MS – Murashige-Skoog (1962) medium

Lemon balm, *Melissa officinalis* L. (Lamiaceae), a native of the northern Mediterranean region is cultivated as a medical herb (Schultze et al. 1993). It is listed in a number of European Pharmacopoeia for its carminative, digestive, diaphoretic and stimulant activities (Gbolade and Lockwood, 1989) and also has reported antiviral actions (Herrman & Kucera, 1967; Dimitrova et al., 1993). It is also widely used for seasoning and flavouring purposes (Gbolade and Lockwood, 1989). Since the amounts of volatiles and other compounds differ significantly in each plant, the main goal of breeding is to select highly productive individuals and to propagate them vegetatively in order to maintain their valuable characteristics. Micropropagation can be an effective tool for mass cloning of such selected genotypes. Although earlier work has been reported the establishment and maintenance of cell cultures of *Melissa* sp. (Gbolade and Lockwood, 1989, 1992; Schultze et al., 1993), there is only one recent report describing multiple shoot formation from cotyledonary node explants of 10-day-old *Melissa* seedlings (Tavares et al., 1996); no information is available concerning micropropagation initiated from adult, field-grown plants. The aim of the present work was, therefore, to develop a high-frequency micropropagation technique which is applicable for field cultivated plants of *Melissa officinalis.*

Selected plants of three different clones designated as clones 36, 38 and 42 of field-grown (Experimental Station of the Department of Medical Plants, University of Horticulture and Food Industry, Budapest, Hungary) lemon balm (*Melissa officinalis* L.) were potted and transferred to the greenhouse. Two days before initiating sterile cultures, plants were treated with a 0.5% solution of the fungicide, Fundasol (Chinoin Co., Ltd., Hungary). Actively growing shoots were collected and washed for 20 min with tap water containing some drops of commercial household detergent. Surface sterilisation consisted of two steps: a 20-min washing in an 8% solution of sodium hypochlorite (commercial bleach diluted with distilled water to 1:1, v/v) plus 1 ml concentrated Tween 20

Table 1. Influence of media supplemented with different growth regulators on the growth parameters of balm cultures.

Treatment	Clone	Shoots/bud		Roots/bud	
		Length (mm)	Number	Length (mm)	Number
Growth regulator free	36	41.3 ± 5.3*	1	18.0 ± 5.7	8.6 ± 0.1
	38	37.9 ± 1.5	1	9.2 ± 2.0	7.9 ± 1.6
	42	55.7 ±11.8	1	41.8 ±10.8	9.9 ± 0.4
+ 0.5 mg l^{-1} IAA	36	20.7 ± 1.2	2.8 ± 0.2	7.8 ± 2.7	1.6 ± 0.5
+ 0.5 mg l^{-1} BA	38	25.0 ± 1.4	3.1 ± 0.3	2.1 ± 3.0	0.1 ± 0.1
	42	35.8 ± 3.6	3.3 ± 0.2	19.8 ± 9.8	2.9 ± 2.0
+ 0.5 mg l^{-1} IAA	36	20.0 ± 1.7	4.4 ± 0.5	9.0 ± 1.2	0.8 ± 0.3
+ 1.5 mg l^{-1} BA	38	24.7 ± 2.6	3.9 ± 0.3	3.7 ± 2.7	0.2 ± 0.1
	42	30.3 ± 0.4	4.1 ± 0.3	6.0 ± 8.4	0.2 ± 0.3
+ 1.0 mg l^{-1} IAA	36	23.5 ± 1.0	4.7 ± 0.1	5.0 ± 3.6	0.5 ± 0.5
+ 0.5 mg l^{-1} BA	38	21.5 ± 1.2	4.4 ± 0.3	6.3 ± 2.5	1.2 ± 0.5
	42	29.4 ± 1.4	4.1 ± 0.3	13.0 ± 1.2	4.0 ± 2.0
+ 1.0 mg l^{-1} IAA	36	18.8 ± 1.4	5.5 ± 0.3	2.0 ± 2.8	0.2 ± 0.3
+ 1.5 mg l^{-1} BA	38	23.6 ± 0.9	4.1 ± 0.5	7.5 ± 5.4	1.3 ± 1.1
	42	27.4 ± 1.6	4.5 ± 0.1	7.9 ± 5.8	0.9 ± 0.7
+ 1.0 mg l^{-1} IAA**	36	45.3 ± 8.4	1	27.1 ±19.0	4.7 ± 1.4
+ 0.5 mg l^{-1} kinetin	38	40.4 ± 4.9	1	22.4 ± 5.6	5.0 ± 1.4
	42	45.0 ± 2.6	1	46.1 ± 2.8	10.0 ± 4.5
+ 1.0 mg l^{-1} IAA***	36	28.4 ± 6.3	1	19.0 ±10.7	2.1 ± 2.1
+ 3.0 mg l^{-1} kinetin	38	23.9 ± 2.2	1	21.5 ± 9.8	4.4 ± 2.4
	42	26.0 ± 5.1	1	16.9 ± 0.6	5.7 ± 0.5

* s.d. values were calculated from 30 measurements in all cases.
**Plantlets were transferred from a medium containing 1.0 mg l^{-1} IAA + 0.5 mg l^{-1} BA.
***Plantlets were transferred from a medium containing 1.0 mg l^{-1} IAA + 1.5 mg l^{-1} BA.

followed by soaking in a 0.2% $HgCl_2$ solution for 10 min and finally, an intensive rinsing with sterile distilled water. Explants with a length of 10–15 mm were placed onto growth regulator free Murashige–Skoog media (Murashige and Skoog, 1962), and maintained as shoot tip and single node cultures until plant material was sufficient for further experiments.

Cultured shoot tips approximately 10 mm in length (two pairs of leaves) were placed onto Murashige–Skoog media without growth regulators as control and to the same media containing different growth regulators. Growth regulator treatments consisted of combinations of either IAA and BA or IAA and kinetin. Concentrations of the growth regulators were ranged as follows: 2.85 μM (0.5 mg l^{-1}) and 5.71 μM (1 mg l^{-1}) IAA combined with 2.22 μM (0.5 mg l^{-1}) and 6.66 μM (1.5 mg l^{-1}) BA or 5.71 μM (1 mg l^{-1}) IAA applied together with 2.32 μM (0.5 mg l^{-1}) or 13.9 μM (3 mg l^{-1}) kinetin.

Media were sterilised in an autoclave at 121°C, 1.2 bar for 20 min. Cultures were grown in 200-ml jars containing 30 ml of media. Environment conditions of the cultures were as follows: temperature was 23°C, constant illumination was supplied by cool white fluorescent tubes at 65 μmol m^{-2} $s^{-1}$for 16 h per day.

Cultures were transferred to fresh medium at 3-week intervals. The number and length of both shoots and roots were monitored as growth parameters. Data of three independent experiments represented by 10 individuals from each clone and treatment were evaluated and summarised.

Acclimatisation was carried out on benches in a greenhouse, in 6-cm plastic pots in a mixture of peat:

Figure 1. In vitro morphogenic responses of shoot tips of *Melissa officinalis* clone 36 after 30 days of inoculation. (A) Shoot clump developed on MS medium supplemented with 1.5 mg l^{-1} BA together with 1 mg l^{-1} IAA; (B) Shoot transferred to MS medium containing 0.5 mg l^{-1} kinetin plus 1 mg l^{-1} IAA.

garden soil: perlite (ratio 1:1:1). Since balm leaves are very sensitive to water loss and the loss of the water content of plantlets is irreversible, it was necessary to provide a high humidity environment. By placing the plants under a plastic foil tunnel and spraying them three times per day with water during the first week, the survival rate was increased by 20%.

On MS medium containing no growth regulators, only one shoot and 8 to 10 roots were produced from the explant (Table 1). Similarly to the results shown previously (Rech and Pires, 1986; Tavares et al., 1996), BA in the presence of IAA induced shoot development from numerous axillary buds in all conditions (Figure 1.). The different clones used in our experiments showed an increased number of shoots with changes of BA concentrations, when applied in combination with IAA. This is in accordance with the previous observations that the increase of BA concentration up to 1 mg l^{-1} gave a significant increase in shoot number (Yepes and Aldwinckle, 1994; Tavares et al., 1996). In the presence of growth regulators the length of shoots was significantly reduced in all cases with respect to the control. Root formation at these growth regulator concentrations was drastically reduced and the increased BA concentrations resulted in the formation of a few rudimentary roots only (cf. Figure 1 and Table 1).

After transferring shoots from media containing 0.5 or 1.5 mg l^{-1} BA and 1 mg l^{-1} IAA to media containing 0.5 or 3.0 mg l^{-1} kinetin in a combination with 1 mg l^{-1} IAA, shoot proliferation did not occur, shoots developed only from buds which had originally existed on the explant. On kinetin-containing media, the length of shoots was, however, increased especially at the lower kinetin concentration (Table 1).

Both kinetin concentrations applied resulted in a significant increase in both root number and length to compare to plantlets grown on BA at any concentrations investigated. On media containing 1 mg l^{-1} IAA plus 0.5 mg l^{-1} kinetin, cultures of all the three clones were vigorous and easy to handle. In comparison with plants grown on growth regulator-free media, these shoots were stronger and more compact; roots were also stronger and in most cases longer than observed on a medium without growth regulators. Plants having this appearance were suitable for transplantation to the greenhouse and the efficiency of their acclimatisation was between 95 and 98% in all cases.

Acknowledgements

This work was partially supported by the Hungarian National Foundation for Scientific and Technical Development (T 017697, T 026075 and T 026078), the Ministry of Culture and Education (MKM 351) and the Hungarian Academy of Sciences.

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