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BIOMETRICAL STUDY ON SEVERAL *IN VITRO* REGENERANTS OF *MELISSA OFFICINALIS* L.

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INTRODUCTION

Melissa officinalis (lemon balm) is a medicinal plant used by herbal medicine for more than 2000 years for its positive therapeutically actions, mostly on the nervous and digestive systems. It was mentioned within the work of Plinius and Dioscoride due to its stimulating action on the nervous system, and in wound healing, migraine reliefs, spasms and digestive malfunctions. The species originates in the East-mediteranean and Western Asia, grown in southern, central and eastern Europe, Asia and North America. Lemon balm is widely cultivated in certain regions of our country, mainly in the counties of: Constanța, Giurgiu, Teleorman, Buzău, Prahova, and Timiș, (PÂRVU, 2000). The Romanian folk medicine commonly uses lemon balm herbal tea to treat insomnia, gastric pain caused by indigestion, disoentheria, to calm hysteria crises, ease toth and ear pain.

Melissa officinalis is frequently cultivated in the gardens to attract bees. The beekeepers use the plant in bloom to catch bee colonies (PÂRVU, 2000). The blooming time is between June and August. Its scent resembles the lemon one, a fact that triggers its popular common name. The lemon balm leaves are also used both by the modern, and by folk medicine as herbal tea or galenic preparates and are appreciated due to their healing action: sedative, carminative, antispasmotic, choleretic, antiviral, antibacterial and so on. Experimental research on human and mouse cells acknowledged the antioxidant effects of lemon balm oil, proving the antitumoral action on several cancer types. The extracts from this species improve memory, the plant may be therapeutically used to relieve the symptoms of the Alzheimer Syndrome (KENNEDY, 2003). A concentrated infusion of lemon balm may be used as an insecticide. The volatile oil extracted from this species is one of the most expensive and appreciated essences in the perfume and cosmetics industry, and aromatherapy as well.

Fig. 1 - *Melissa officinalis* L.

MATERIAL AND METHODS

Our complex research on the *in vitro* regenerants of *Melissa officinalis* L. comprised biometrical tests, as well. There were analyzed some growth parameters, immediately after the regenerants were transferred from the *in vitro* to the *ex vitro* environment. There were analyzed 30 plants/medium variant within three tests: I – plants aged 30 days; II – plants aged 50 days; III - plants aged 60 days. The investigated parameters were: root length, stem length, number of shoots growing from basal node, number of nodes/shoot, fresh biomass/plant.

The regenerants were transferred in field in spring, harvested in early September. At the harvesting moment, some parameters were analyzed: root length, stem length, number o branches/stem, number of nodes/stem, fresh biomass/plant. All the data were statistically processed and interpreted.

The root and stem length were determined by simple linear measurements, and the fresh biomass – by weighing with the OWALABOR scale. After acclimatization, to the *ex vitro* environment, the vitroplants were field grown on podzolic soil (Racova village, Bacău county). The biological material was harvested on a sunny weather conditions, in the morning between 10 to 11 AM. The next parameters were analyzed at the harvesting moment: stem length (cm), number of branches/stem, number of nodes/stem, fresh biomass/plant (g). The analyzed statistic indices were:

$\bar{x}$ = arithmetic mean

s = individual standard deviation from the mean

$s_{\bar{x}}$ = standard deviation

s% = variation index

$s_{\bar{x}}$% = standard deviation of the mean expressed in percents

RESULTS AND DISCUSSIONS

Several biometrical tests were effected on the regenerants of *Melissa officinalis* L., both at the moment of their transfer from the *in vitro* to the *ex vitro* environment, and on the *in vitro* provided regenerants grown on the experimental plot. The lemon balm shoots grown *in vitro,* during several stages, on the control medium variant (hormone free MS), and on some of its variants (enriched with growth regulators), were submitted to morpho – physiological indices' analyses at the moment of their transfer to the septic environment.

Three such tests were effected. The regenerants' age ranged between 30 and 60 days. The parameters that were tested are the following: the root and the stem length, the shoot number from the stem base, the number of nodes on the main stem and the individual fresh biomass.

There are some differences regarding the intensity of the growth processes between an experimental test to another, probably due to the explant origin and to the growth regulators within the nutritive medium.

For the 60 – day regenerants, the best root growth was registered on the BN_1 variant (158.75 mm), on which the regenerants provided the greatest number of basal shoots (4.0) and the highest individual biomass (1.59 g), compared to the control regenerants that registered the following values: 141.80 mm, 1.40 shoots and 0.75 g/neoplantlet. The plants generated on the hormone - free MS were characterized by a more intense stem growth (136.60 mm) and by a greater number of nodes/shoot (6.80). During the second experience, lacking the control variant, the values of the previously analyzed parameters were compared. As a result, for the regenerants aged 50 days it was noticed that: the longest roots (127.07 mm) were provided on KN_1, a medium variant that produced the greatest number of nodes/stem (5.08). The maximum values of the other parameters were registered on the BA_1 variant (stem length = 138.0 mm; 1.8 shoots at the stem base; 1.15 g of fresh substance/regenerant). Concerning the third test, effected on 30-day regenerants, it was noticed that the highest values of the other physiological parameters (but for the root and stem growth – that was higher in control plants) were provided by the medium variant enriched with BAP and GA_3 (BG_1): average number of 6.0 basal shoots, 5.0 nodes/stem, and 1.22 g of individual biomass (of each neoplantlet).

As one of our aims was the micropropagation in *M. officinalis*, these test results prove that the most appropriate medium variants to regenerate new plants in vitro are: BN_1 (provided 4 shoots/regenerant and 5 nodes/stem), B_{02} (provided 4 shoots/plant and 5.50 nodes/plant) and BG_1 (6.0 shoots/plant and 5 nodes/stem); these medium variants produce the highest number of explants/culture vial, and implicitly the highest number of *in vitro* regenerants. These nutritive medium variants can provide, using only one culture vial inoculated with nodes and shoot tips, between 100 (BN_1 medium) and 150 (BG_1 medium) *in vitro* regenerants, therefore the micropropagation turnover is very good in this species.

Biometrical measurements and tests were effected on the lemon balm plants provided *in vitro* and subsequently transferred in field (during the first year of vegetation) L. there were analyzed the following parameters: stem length, shoot number at the stem base, number of nodes/stem, and stem fresh biomass. The provided data were statistically processed.

The results obtained in lemon balm show that the stem length of the 5 experimental variants in field ranged between 60.06 cm in control plants and 82.04 cm in the in vitro regenerants provided on the culture medium enriched with 2 mg/l IAA (A_2 variant).

The plants regenerated on the medium variant with BAP and IAA (BA_1) were higher (80.46 cm) than the plants grown on other medium variants. Regarding the average number of branches from the stem base, the differences are insignificant, ranging between 1.0 (KN_1) and 1.10 branches/plant (BA_1).

Table 1. The value of some physiological parameters for the *in vitro* regenerants of *Melissa officinalis* L.

Var.	Medium	Growth	Root length	Stem length	No. of basal	No. of nodes/	Fresh biomass/
First test							
1	MS	60 days	141.80	136.60	1.40	6.80	0.75
2	B_{02}	60 days	65.87	102.25	2.43	4.68	1.08
3	BN_1	60 days	158.75	128.50	4.00	5.00	1.59
4	KN_1	60 days	73.00	115.00	1.30	5.40	1.07
Second test							
5	A_2	50 days	83.42	116.57	1.42	4.71	0.78
6	N_2	50 days	48.25	88.00	1.50	4.75	0.64
7	BA_1	50 days	75.33	138.00	1.83	3.66	1.15
8	KN_1	50 days	127.07	120.46	1.76	5.07	1.07
Third test							
9	MS	30 days	99.00	109.66	3.00	4.66	0.95
10	A_2	30 days	37.66	84.33	2.66	4.16	0.74
11	B_{02}	30 days	65.62	74.12	4.00	5.50	0.55
12	BG_1	30 days	64.00	105.60	6.00	5.00	1.22

Regarding the individual fresh biomass, the average values of this parameter ranged between 16.23 g on B_{02} and 21.74 g on A_2 medium variant. The regenerants provided on BA_1 showed a high average value of plant individual biomass (21.55 g). The value of the variation index (s%) prove that the stem length (regardless of the medium variant for the *in vitro* micropropagation) was an average variability parameter (s%=11.77-13.40); the number of branches from basal stem and the number of nodes/shoot were indices with an average to high variability (s% above 20); the fresh biomass/plant displayed a very high variability (s% sometimes exceeding 40). The value of $s_{\bar{x}}\%$ higher than 3 proves that the analyzed parameters are highly variable and that the number of analyzed plants might have been insufficient.

Table 2. The value of some physiological parameters in the *in vitro* regenerants of *Melissa officinalis* L. that were field grown (the first year of vegetation)

Medium variant	Parameter	n	$\bar{x}$	s	$s_{\bar{x}}$	$s_{\bar{x}}\%$	s%
MS	Stem length (cm)	50	60.06	8.05	1.13	1.88	13.40
	No. of branches/stem	50	1.06	0.23	0.03	2.83	21.69
	No. of nodes/stem	50	11.88	3.11	0.44	3.70	26.17
	Fresh biomass/individual (g)	50	18.91	9.04	1.27	6.71	47.80
BA_1	Stem length (cm)	50	80.46	10.67	1.50	1.86	13.26
	No. of branches/stem	50	1.10	0.41	0.05	4.54	37.27
	No. of nodes/stem	50	17.00	3.12	0.44	2.58	18.35
	Fresh biomass/individual (g)	50	21.55	6.18	0.87	4.03	28.67
KN_1	Stem length (cm)	50	64.13	7.55	1.06	1.65	11.77
	No. of branches/stem	50	1.00	0	0	0	0
	No. of nodes/stem	50	12.40	2.98	0.42	3.38	24.03
	Fresh biomass/individual (g)	50	17.74	9.50	1.34	7.55	53.55
B_{02}	Stem length (cm)	50	71.85	9.21	1.30	1.80	12.81
	No. of branches/stem	50	1.06	0.23	0.03	2.83	21.69
	No. of nodes/stem	50	13.96	4.27	0.60	4.29	30.58
	Fresh biomass/individual (g)	50	16.23	7.00	0.99	6.09	43.13
A	Stem length (cm)	50	82.04	10.52	1.48	1.80	12.82
	No. of branches/stem	50	1.06	0.23	0.03	2.83	21.69
	No. of nodes/stem	50	16.40	3.67	0.51	3.10	22.37
	Fresh biomass/individual (g)	50	21.74	9.82	1.38	6.34	45.17

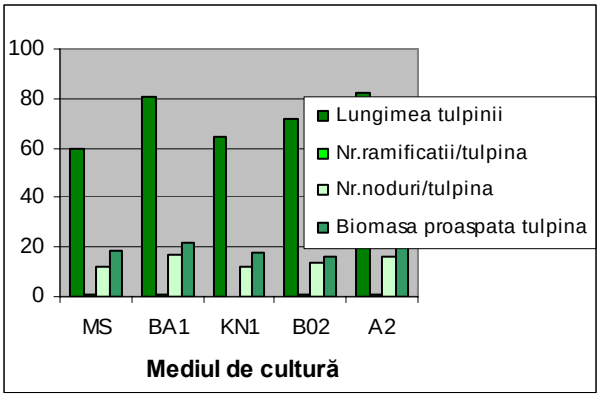

Fig. 2. The value of some physiological parameters for the *in vitro* regenerants of *Melissa officinalis* L., transferred in field (the first year of vegetation)

Fig. 3. Shoots of *Melissa officinalis* L. provided *in vitro* and transfered in field (first year of vegetation)

Fig. 4. Lemon balm regenerants on the experimental plot

Fig. 6. Regenerants of *Melissa officinalis* L. provided *in vitro* and field grown, during flower bloom, second year of vegetation

Fig. 7. Regenerants of *Melissa officinalis* L. provided *in vitro* and field grown, at the end of flower blooming (detail)

CONCLUSIONS

Considering that one of our goals was the *in vitro* micropropagation in *M. officinalis* L., these test results proved that the most appropriate medium variants plant regeneration are BN_1 (it provided 4 shoots/regenerant and 5 nodes/stem), B_{02} (4 shoots/regenerant şi 5.50 nodes/stem) and BG_1 (with 6.0 shoots/regenerant and 5 nodes/stem), variants that produced the highest number of explants/culture vial, and implicitly the highest number of plants that may be regenerated.

The lemon balm shoots obtained on the medium variant enriched with 2 mg/l IAA displayed the optimal field growth, reaching the highest stem length at the moment of flower blooming (30% superior to control plants) and the highest biomass (46.14% higher than the one of the regenerants provided on hormone free MS).

ABSTRACT

Melissa officinalis L. is a herbaceous, perennial plant of the *Lamiaceae* family, a native of the northern Mediterranean region. It is a xeromesophitic, moderate thermophile plant that is spread in sunny and also in shady places, and it is resistant to drought. It is sensitive to low temperatures, requiring mild winters. Its development is favourable on argillaceous earth and also on sandy, loamy ground. On vegetable soil its content of essential oils is lower. The aerial part of plant comprises 0.05 to 0.15% of volatile oil (that contains citronellal, citral, geraniol, linalool), polyphenols, tannins (3 to 6%), mucilages (12%), bitter substances etc. The seeds contain fat oil made up of linolenic, linoleic, oleic, palmitic and stearic acids (1-3, 5-8,10). The main action of its active principles, especially of volatile oil of *Melissa officinalis* is spasmolitic and sedative, recommended for gastro - intestinal spasms and cardiac neurosis. They are also known for an antiseptic, sedative, carminative, choleretic, mild laxative, stomachic, cicatrisant, galactagogue, and insecticide action.

Our complex research on the *in vitro* regenerants of *Melissa officinalis* L. comprised biometrical tests, as well.

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