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Effect of species and KIN on the formation and multiplication of peppermint plant nodes *Mentha* sp.

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Abstract

The study included the cultivation of the tips of mint plant branches for the wild species *Mentha pulegium* L. and the cultivated species *Mentha piperita* L. on MS medium supplemented with different concentrations of KIN. The results indicated that the highest rate of branching was 6.00 branches/plant part from the cultivation of nodes on MS medium supplemented with 0.5 mg/L KIN after 8 weeks of planting for the first species "wild". The highest rate of branching was 7.00 branches/plant part from the cultivation of nodes on MS medium supplemented with 1.0 mg/L KIN after 8 weeks of planting for the second species "cultivated". The highest rate of rooting was 4.00 and 2.50 roots/branch when planted on MS medium supplemented with 2.0 and 1.0 mg/L IAA for the wild and cultivated species, respectively.

Keywords: Mint (*Mentha* species), *Mentha pulegium*, *Mentha piperita*, MS medium, KIN (Kinetin)

Introduction

Tissue culture technology is one of the biotechnologies that has played, and continues to play, an important role in serving humanity, particularly in the field of propagating many plant species. This method is characterized by its advantages, the most important of which is the ability to obtain huge numbers of pathogen-free plants that are similar to the mother plant in a relatively short time and at any time of the year. Additionally, this technology is used in various research and applied fields, including plant breeding and improvement, the production of medicinal compounds and drugs, and rapid clonal propagation, which is one of the significant application carried out by following different methods of differentiation and morphological formation, such as the formation of adventitious buds, stimulating the growth of axillary buds, and inducing asexual embryos (somatic embryo), as well as studying the fundamental aspects of plant growth, development, and secondary metabolism (Gupta *et al.*, 2006; Kasumi *et al.*, 2004; Ford, 2000; Al-Kanani, 1987) ^[9, 11, 8, 3]. Peppermint is a green plant with a fragrant aroma that does not grow more than 30 cm above the ground. This plant contains volatile oils, proteins, fibers, vitamins, minerals, organic acids, and a volatile oil whose main components are menthol and menthone. It also contains flavonoids, with important compounds like luteolin and menthoside, and it contains phenolic acids (Al-Akri, 2002; Haikal and Abdul Razak, 1993) ^[4, 2]. Peppermint is considered beneficial in destroying cancer cells, preventing nausea, stomach pains, colic, and acidity, absorbing gases, and is useful in gout, itching, hemorrhoids, clearing phlegm from the chest, and relieving toothaches, with an antispasmodic effect (Carole, 1997) ^[7]. From the above, the importance of the possibility of producing seedlings locally becomes clear. Therefore, the current study was conducted on two types of mint plants: "wild" and "garden" with the aim of:

1. Stimulating the formation of branches from nodes by planting them in nutrient media containing different concentrations of kinetin (KIN).
2. Rooting the resulting branches by planting them in nutrient media containing different concentrations of indole acetic acid (IAA) and studying its effect on the characteristics of the resulting roots.

Materials and Methods

Fresh plant parts were taken, represented by nodes 0.5 cm in length from *Mentha* sp. L.

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plants, both wild and cultivated, grown in flowerbeds in the city gardens, with growths free from insect and disease infestations. They were then transferred to the laboratory, washed with regular detergent, and left under running water in a strainer for 10-15 minutes. Subsequently, they were moved to the planting table and treated with a 25% sodium hypochlorite (NaOCl) solution for 15 minutes (prepared from a commercial bleach solution containing 0.6% sodium hypochlorite). Afterward, they were washed three times with sterile distilled water for 5 minutes each time to remove the harmful effects of the disinfectant. Once the sterilization process was completed, the parts were transferred to sterile Petri dishes, making them ready for planting. They were then planted in MS medium prepared with different concentration of KIN (0.0, 0.5, 1.0 and 0.2 mg/L) for the initiation and multiplication stages. The branches resulting from multiplication were planted on full MS medium supplemented with 0.0, 1.0, and 2.0 mg/L IAA for rooting. Glass bottles with a capacity of 250 ml were used for planting the plant parts, containing 40 ml of the nutrient medium at pH 5.7, and the mouth of the bottle was covered with aluminum foil. The nutrient medium was sterilized using an autoclave at a temperature of 121°C and a pressure of 1.04 kg/cm² for 20 minutes. After planting the plant parts, the cultures were transferred to a growth room under a light intensity of 3000 lux, with a daily cycle of 16 hours of light followed by 8 hours of darkness, provided by white fluorescent tubes, and at a temperature of 25 ± 2°C. The Complete Randomized Design was used in the analysis of experimental data, and the means were compared using Duncan's multiple range

test at a significance level of 5% (Al-Rawi and Khalaf Allah, 1980) [1]. Each treatment included ten replications, and each replication contained one plant part.

Results and Discussion

Table (1) shows that the nodules of the wild mint species cultivated on MS medium with a treatment of 0.5 mg/L KIN resulted in the highest rate of the number of branches at 4.10 branches/plant part and the highest rate of the number of leaves at 6.13 leaves/plant part. When re-cultivated for another 4 weeks (multiplication stage) the nodules grown under the same treatment formed the highest rate of the number of branches at 6.00 branches/plant part, significantly surpassing the other treatments, and the highest rate of the number of leaves at 8.22 leaves/plant part, also significantly surpassing the other treatments.

The results in Table (1) regarding the multiplication of the cultivated parts can be explained based on the fact that KIN is one of the cytokinins that play a role in controlling apical dominance, thereby increasing the number of branches. As a result of achieving a balance between internal hormones and added growth regulators, the highest values were obtained, and increasing the concentration leads to a reduction in the values for the number of branches as its effect become inverse (Smith, 2000; HopKins and Hiiner, 2004) [14, 10]. The increase in length and number of leaves for the different treatments can be attributed to the effect of cytokinins on the division and elongation of cells, which in turn reflects on growth characteristics, in addition to its impact on the synthesis of nucleic acids (Wasef, 1995) [6].

Table 1: Effect of adding KIN on the growth and multiplication of the wild type nodes of the mint plant *Mentha pulegium* L. from its cultivation on solid MS medium after 4 and 8 weeks of cultivation.

Kin (Mg. L)	Stage of emergence (after 4 weeks)			Stage of multiplication (after 8 weeks)		
	Average number of branches	Average branch length (cm)	Average number of leaves	Average number of branches	Average branch length (cm)	Average number of leaves
0.0	1.00 d	2.50 c	2.00d	1.90d	2.93d	2.84d
0.5	4.10a	6.00 a	6.13a	6.00a	8.20 a	8.22a
1.0	3.00 b	5.00b	4.00b	4.33b	6.55 b	5.12b
2.0	2.00c	5.30b	3.00 c	2.91 c	5.43c	4.33c

Numbers with similar letters within the same column do not differ significantly from each other according to Duncan's multiple range test at a 5% probability level

Table (2) shows that the mint plant contract for the horticultural type grown in MS medium with a treatment of 1.00 mg/L KIN resulted in the highest average number of branches, which was 5.00 branches/plant part, and it gave the highest average number of leaf, which was 6.00 leaves/plant part, significantly superior to the other treatments. After replanting for another 4weeks

(multiplication stage), the plants grown under the same treatment formed the highest average number of branches, which was 7.00 branches/plant part, and the highest average number of leaves, which was 8.00 leaves/plant part, significantly superior to the other treatments. The results of Table (2) can be interpreted in light of the results of Table (1).

Table 2: Effect of adding KIN on the growth and multiplication of the garden type nodes of the mint plant *Mentha piperita* L. from its cultivation on solid MS medium after 4 and 8 weeks of planting.

Kin (mg.L)	Stage of emergence (after 4 weeks)			Stage of multiplication (after 8 weeks)		
	Average number of branches	Average branch length (cm)	Average number of leaves	Average number of branches	Average branch length (cm)	Average number of leaf
0.0	d2.00	b 5.77	b 5.10	c 3.11	b 7.00	b 5.50
0.5	c 3.00	c 3.50	b 5.20	b 5.00	6.00 c	b 5.60
1.0	a 5.00	a 7.00	a 6.00	a 7.00	a 9.50	a 8.00
2.0	b 4.00	c 3.53	c 4.00	b 5.55	d 5.11	b 5.00

The numbers with similar letters within the same column do not differ significantly from each other according to Duncan's multiple range test at a 5% probability level

Table (3) shows the effect of IAA on the rooting of branches of the wild mint plant obtained from tissue culture, where the highest rooting percentage of 100% was achieved at a treatment of 2.0 mg/L IAA. The same treatment also resulted in the best rate of root number at 4.00 roots/branch and the highest root length rate at 2.20, which significantly outperformed the other treatments. These results can be explained on the basis that IAA is one of the auxins that play an important role in activating cell division and increasing their size due to the polarization of nutrients and compounds that aid rooting, leading to the formation of root principles at the bases of the vegetative branch (Mohamed and Al-Rais, 1982) ^[5].

Table 3: Effect of IAA on the rooting of nodes of the wild mint plant *Mentha pulegium* L. resulting from tissue culture grown on solid MS medium after 4 weeks of planting.

IAA (mg.l)	Rooting (%)	Average number of root	Average root length (cm)
0.0	b 50	c 1.40	c 0.93
1.0	b 60	b 2.01	b 1.31
2.0	a 100	a 4.00	a 2.20

The numbers with similar letters within the same column do not differ significantly from each other according to Duncan's multiple range test at a 5% probability level

Table (4) shows the effect of IAA on the rooting of mint plant branches of the horticultural type produced from tissue culture. The highest rooting percentage of 100% was obtained at a treatment of 1.0 mg/l IAA. Additionally, the treatment at 1.0 mg/L IAA resulted in the best average number of roots at 2.55 roots/branch and the highest average root length of 3.11, which significantly outperformed the other treatment rates. These results are explained in light of what was mentioned in the interpretation of the results in Table (3).

Table 4: Effect of IAA on the rooting of branches of the garden plant *Mentha piperita* L. resulting from tissue culture grown on solid MS medium after 4 weeks of cultivation.

IAA (mg.l)	Rooting (%)	Average number of root	Average root length (cm)
0.0	c 40	b 1.00	c 0.60
1.0	a 100	a 2.55	a 3.11
2.0	b 80	b 1.11	b 1.00

The numbers with similar letters within the same column do not differ significantly from each other according to Duncan's multiple range test at a 5% probability level

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