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EFFECT OF SOME GROWTH REGULATORS ON THE MULTIPLICATION AND STIMULATING THE PRODUCTION OF THE VOLATILE OIL OF *ROSEMARY OFFICINALIS IN VITRO*

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Abstract

The experiment was conducted at the plant tissue culture laboratory of the Department of Horticulture and Garden Engineering - College of Agricultural Engineering Sciences, University of Baghdad, in order to study the effect of some growth regulators on propagation an stimulation production of volatile oil compounds of rosemary plant *Rosmarinus officinlis* using two vegetative parts (apical and lateral buds). Factorial experiment was implemented in completely randomized design with twenty replications. The results indicated that culturing the apical meristem on the medium Murashige and Skoog (MS) media with 0.5 mg.l^{-1} (BA) with 0.1 mg.l^{-1} of NAA gave the highest response rate of 100%. As for the doubling stage, the levels of BAA and IAA (Indole acetic acid), and their interaction showed a significant effect on the number and length of branches, fresh and dry weight. The treatment of 0.5 mg.liter $^{-1}$ of BA with 0.0 mg.liter $^{-1}$ of IAA gave the highest number of branches (5.9 branches.plant^{-1}), and fresh and dry weight (4272and446.2 mg), respectively. Whereas the treatment of 1.5 mg. liter $^{-1}$ of BA with 0.3 mg. liter $^{-1}$ of IAA gave the highest length of doubled branches (5.2 cm). The use of BA at a concentration of 0.5 mg.liter^{-1} was found to increase the active compounds in the volatile oil compared to the MS media free of growth regulator. The best rooting rate of branching was achieved in MS media with complete and half the strength of salts supplied with IBA at a concentration of 0.5 mg.liter^{-1} or at a concentration of 1 mg. liter $^{-1}$, where it reached 90%. In addition, the highest number of roots and their lengths in MS media achieved in half of the strength of salts supplied with IBA at a concentration of 0.5 mg.liter^{-1} reached 5 root. rooted branch^{-1} and 5.30 cm, respectively. The relative survival rate of the adapted plantlet was 90%.

Key words : Cytokinins, auxins, active compounds, rosemary, in glass.

Introduction

Rosemary (*Rosmarinus officinalis* L.) is an important economic medicinal plant, that follows the Lamiaceae family, the English name Rosemary and the Arabic name "Iklil al-Jabal". The miracle herb, Hasaliban, Hashishat Al- Arabs and others. Rosemary is a native to South Europe and the Mediterranean Sea , and spread from there to the world, such as Tunisia, Algeria, Morroco, Middle East, France, Italy, Russia, Portugal, Spain, Turkey and the United States (Peter, 2004). It is an evergreen shrub plant. It is known as the herb of memory, which was used by the Greeks since ancient times to activate memory. It is anti-oxidant and anti-bacterial, which is used to preserve foods and prevent deterioration of their quality (Fernendes *et al*., 2005). It is used to treat rheumatism, activate blood circulation, improve digestion, treat headaches and colds, and is used as a diuretic, anti-cirrhosis, anti-inflammatory, asthma, cough, tonic, anti-diarrhea, and a good gas remedy and anti-carcinogenic (Chevallier, 2001).

Plant tissue culture technology helps in the rapid production of secondary metabolites without being restricted to the planting season and throughout the year.As well as reducing the area required for agriculture in addition to the high purity of the materials produced as compared to those manufactured (Lila, 2005). Thus, growing interest in tissue culture technology as an alternative to traditional agriculture. The process of propagating vegetative parts (outside the living body) is like branches as one of the technologies of tissue culture,

and one of the main objectives in the production of secondary compounds, because callus cultivation and cellular suspension are in a meristematic state which are undifferentiated and adversely affect the production of secondary materials because they accumulate normally in high-differentiation tissues (Khairallah, 2015).

Plant growth regulators play an important role in the growth and detection of cells and tissues. They are divided into auxins, cytokinins, gibberellin, ethylene, Abscisic acid and others (Rifai and Shobaki, 2002). In addition, plant growth regulators affect plant metabolism, levels and accumulation of metabolites in plants (Ramwat, 2004). Auxins have a significant role in apical dominance, organ development and formation, root and buds formation, seed germination, flower formation (Skhinner, Liang, 2004). Cytokinins have many effects, such as breaking apical dominance, increasing the speed of cell division, and encouraging branch formation from adventitious buds, callus, leaves, roots and stem (Hopkins, 1999). Where it works to build chlorophyll and proteins, increase cell division and activate cell enzymes. The vital pathways of cytokanin build-up are combined with some vital pathways for the production of secondary compounds (Verpoort, 2000). In a study by Zhao *et al*. (2001), cultivating parts of the *Catharanthus roseus* plant where he succeeded in obtaining an increase in secondary compounds through the use of combination between NAA and KN.Xu et al (2008) produced rosemarnic acid from giant hyssop (*Agastache rugosa*) cultivation outside the living body (*in vitro*). Which significantly exceeded the treatment of combination of 2,4D and BA in obtaining the highest amount of Rosemarnic acid. In a study by Affonso *et al*. (2009). In a study by Affonso *et al*. (2009) to demonstrate the effect of plant growth regulators on the production of volatile compounds of thyme (*Thymus vulgaris*) in vitro, they found an increase in the production of some major compounds such as Terpinene, P-cymene, Thymol as compared to the control treatment. In a study by Santoro *et al*. (2013) to demonstrate the effect of plant growth regulators on the production of secondary compounds at the propagation of thyme *in vitro*, it was observed that the production of secondary compounds was affected only when adding cytokinins, where the results indicated that there was an increase of 40% of the total yield of essential oil and also observed an increase in the main compounds of essential oil such as Menthone, Menthol, Pulegone and Menthofuran.Where it was noted that the addition of growth regulators caused an increase in biomass, which coincided with an increase in Terpenoids.In a study by Hamoud (2017) to demonstrate the effect of some plant growth regulators on the production of total alkaloids of the winter cherry (*Solanum capsicastrum*) plant, there was an increase in the quantitative content of total alkaloids when adding growth regulators compared to the control treatment. The objective of this study is to clarify the effect of plant growth regulators in increasing the active substances of the rosemary plant *in vitro*.

Materials and Methods

The study was carried out in the plant tissue culture laboratory, Post Graduate, College of Agricultural Engineering Sciences, University of Baghdad. The plant was obtained from the Research Unit of Medicinal and Aromatic Plants of the College of Agricultural Engineering Sciences, University of Baghdad. Apical and lateral buds (vegetative parts) were taken from two-year-old plants for the purpose of propagation. The plant parts were sterilized using bleaching agent (Fas) containing sodium hypochlorite with active ingredient concentration was 6% in the concentrations 0.3, 0.6, 0.9 and 1.2% for 15 minutes. After that, three times was washed with sterilized distilled water every five minutes. Planting done on MS media free of growth regulators and the pollution was calculated after 10 days of planting. The following experiments were carried out: - 1 - Initiation stage: - Cultured of sterile parts from the previous step in the center of the initiation of the media of MS added to the BA concentrations 0, 0.5, 1 and 1.5 mg. $Liter^{-1}$ in addition to NAA concentrations 0 , 0.1 , 0.3 and 0.5 mg. $Liter^{-1}$, after a month the results were taken to calculate the percentage of response and by 20 replication for each treatment. 2. The multiplication phase: The vegetative growths obtained from the best treatment from the previous experiment were cultured on MS media with different concentrations of BA (0, 0.5, 1 and 1.5 mg. $Liter^{-1}$) with IAA in concentrations (0.1, 0.3 and 0.5 mg. $Liter^{-1}$). After a month of planting, the results of the number and length of branches and fresh and dry weight were taken, beside the measurement of the proportion of some major compounds of volatile oil. 3- The rooting stage: The resulting branches from the best treatment were transferred from the multiplication phase to MS media, which was supplied with different concentrations of IBA 0.0, 0.3 and 0.5 mg. $Liter^{-1}$ and in combination with a different strength of MS salts ¼ X, ½ X and X, with ten replicate for each treatment. Plantlets were incubated s in the same environment mentioned above for the purpose of encouraging the rooting and then calculated and recorded data represented in percentage of rooting by the following formula:

Percentage of rooting = (number of rooted branches/ total number of branches) × 100

Number of roots and their length were measured after 4 weeks. 4. The Acclimatization stage: - Extraction of homogeneous rooted plantlets as possible from culturing vessels and washed with ordinary running water for the purpose of disposal of the remnants of Agar attached to its roots, which may be a good media for the growth of organisms because it contains the sucrose , and then roots were flooded in a solution containing a quarter of the strength of MS salts for the purpose of Acclimatization of the plants, then the plantlets were transferred to the soil and left for a week in the growth chamber (Salman, 1988). After that, the culture media prepared using a 1: 1 mixture of silt and peat moss sterilized in the autoclave for 30 minutes at 121°C and pressure of 1.04 $kg.cm^{-2}$. The roots of the plantlets were immersed in the fungicide (Pentanol) with a concentration of 2 ml. The plantlets were then planted in a 7 cm diameter fliny pots, then watered and covered with a plastic cover to preserve the moisture. The lid was gradually lifted after a week of planting. The rate of plantlet success was recorded 8 weeks after planting.

Statistical analysis

Statistical analysis of the data was performed by using ANOVA. We applied Factorial in completely randomized design with twenty replications. Least Significant Differences (LSD) test was used to compare the experimental results of the treatments means, significance was defined by a probability level of $p<0.05$ (El-Sahooki, 1990).

Results and Discussion

Effect of BA and NAA and their interaction in vegetative response to initiation four weeks after planting on MS media

The results revealed that the plant part has a significant effect on increasing the percentage of initiation of plantlets (table 1). The apical meristem significantly exceeded the lateral buds, giving the highest percentage of 62.2% while the lateral buds gave 42.5%. The effect of the BA concentrations had a clear effect on the increase in the response rate. The concentration of 0.5 $mg.liter^{-1}$ significantly higher than other concentrations, the highest response rate was 82.5%, while the control treatment gave the lowest response rate of 21.9%. The high levels of the BA resulted in a decrease in growth rates, which is evident in the concentration of 1.5 mg. Liters -1, giving a response rate of 42.5%.

The results indicated a significant decrease in the response rate with a higher concentration of NAA. The concentration of 0.1 gave the highest response rate of 60%, while the concentration of 0.5% gave the lowest response rate of 44.4%. The results showed that all two-way interactions had a significant effect on the response rate. The highest rate of response achieved in the treatment of apical meristem with the BA at a concentration of 0.5 $mg.liter^{-1}$, which gave 92.5%, and

Table 1 : Effect of BA and NAA and their interaction in vegetative response (Apical and lateral buds) for initiation four weeks after planting on the MS media of the rosemary plant.

EX BA	NAA				BA	Vegetative Part (E)
	0.5	0.3	0.1	0		
31.3	20	30	40	35	0	Apical bud
92.5	85	90	100	95	0.5	
72.5	65	70	80	75	1	
52.5	45	50	60	55	1.5	
12.5	50	10	20	15	0	Lateral bud
72.5	65	70	80	75	0.5	
52.5	45	50	60	55	1	
32.5	25	30	40	35	1.5	
13.7	27.4				LSD	
	44.4	50	60	55	meansNAA	
E means	9.7				LSD_{NAA}	
	ExNAA					
62.2	54	60	70	65	Apical bud	
42.5	35	40	50	45	Lateral bud	
6.8	13.7				LSD	
	NAA X BA					
BA means	NAA				BA	
	0.5	0.3	0.1	0		
21.9	12.5	20	25	25	0	
82.5	75	80	85	85	0.5	
62.5	55	60	65	65	1	
42.5	35	40	45	45	1.5	
9.7	19.4				LSD	

the lowest response rate was in lateral buds with BA at concentration of 0 mg.liter^{-1}. The interaction between the plant part and the NAA concentration, showed that the highest mean response rates of 70% achieved in at the treatment of the apical meristem with NAA at a concentration of 0.1 mg.liter^{-1} and the lowest was 35% when treated on a lateral budswith NAA at 0.5mg.liter^{-1}. Whereas, the interaction between BA and NAA, indicated that the highest mean response rate was in the treatment of BA-0.5 mg.liter^{-1} and NAA concentrations at 0 and 0.1 mg.liter^{-1}, which was 85% and the lowest (12.5%) in the BA-treatment of 0 mg.liter^{-1} with NAA at a concentration of 0.5 mg.liter^{-1}.

As for the effect of three-way interaction, the results indicates that the highest response rate (100%) achieved in the treatment apical meristem with BA at concentration of 0.5 and NAA of 0.1 mg.liter^{-1}, followed by the treatment of apical meristem with BA at a concentration of 0.5 and NAA at a concentration of 0 mg.liter^{1} and the treatment of apical meristem with BA at a concentration of 0.5 mg.liter^{-1} and NAA at a concentration of 0.3 mg.liter^{-1}. The response rate in these treatments were 95 and 90% respectively, while the lowest response rate (10%) In the treatment of axillary bud with BA at a concentration of 0.0 mg.liter^{-1} and the NAA at a concentration of 0.3 mg.liter^{1}.

The extent of response of cultured vegetative part is determined by the addition of appropriate concentrations between cytokanine and auxins, increasing the rate of detection (Mohamed and Younis, 1991).

As a result of the non-response of the lateral budsat the initiation stage experiments compared with the apical meristem, the apical meristem were adopted in the experiments of initiation and other propagation stages because the apical meristem possess a number of axillary buds, which have a greater chance of survival and rapid growth (George *et al*, 1984 and Rasool *et al*, 2013). The reason for the superior response of the branches may be due to the presence of auxins in the apical meristem of the branches more than in the single node because the apical meristems of branches is the main center for the manufacturing auxin in the plant and thus the effect on the division of cells and elongation more than in the ends of branches (Abdol, 1987; Hartmann *et al*, 2002). The superiority of the apical meristems is attributed to the rapid division of its cells as non-specialized and undifferentiated cells and in the initial developmental stages (Hammoud, 2017). This results are consistent with Salman *et al.* (1994), Al-Obeidi *et al.* (2001), Aswath *et al*. (2003), Aswath, Warzeen (2004), Tan *et al.* (2007), Kumar, Kanwar (2008), Gantait *et al*. (2010), AI-Ibrahimi (2011), Kumar and Minerra (2013), who found that apical meristems were more responsive than lateral buds.

The obvious effect of BA concentrations is due to the catalytic action of cytokinins in stimulating the cells of branches that are cultured on differentiation and differentiation. The result is the differentiation of the buds of vegetative branches. Moreover, many researchers have pointed out the role of cytokinines in appropriate concentrations in tissue culture (Zeiger and Taize, 1991). This is consistent with the report of Bhatt and others (2012) and Soni and others (2011), who found that Cytokinins have an important role in the formation of plants and are not consistent with Kumar *et al*. (2016).

The low rates of growth at high levels of BA are caused by the disruption of biological processes within the plant tissues, which has led to hormonal imbalance and thus reduced rates of growth of plant parts. This decline is not necessarily the death of a cell but usually may be the result of developmental disability (Devlin and Withman, 1998). The decrease in the response rate, with the increase of auxin concentrations to the role of auxins which inhibits the growth of lateral buds and promotes apical dominance, as high concentrations inhibit cell division through growth inhibitor (Zeiger and Taize, 2010 and Khafaji, 2014).

Multiplication phase

Effect of BA and IAA and their interaction on the number of branches

The results revealed that the BA concentrations have a significant effect on the increase in the number of branches (table 2), with a concentration of 0.5 mg.liter^{-1}produce the highest number of branches (5.20 branches. Vegetative part^{-1}) as compared to the control treatment, which gave the lowest number of branches, reached 0.25 branches.vegetative part^{-1}. As for the effect of the IAA, the results of the same table indicate that the highest rate of the number of branches reached 3.23 branch. vegetative part^{-1} for the control treatment, which significantly exceeded the rest of the treatment while the treatment of the concentration 0.5 mg. liter^{-1} produced lowest number of branches (2.03 branch. vegetative part $^{-1}$), which did not differ significantly from the treatment of concentration 0.3 mg.liter^{-1}, which gave 2.25 branches .vegetative part^{-1}.

As for the interaction between BA and IAA concentrations, the results indicates that the treatment of the concentration of 0.5 mg. liter^{-1} for the BA with 0.0 mg.liter^{-1} for the IAA gave the highest number of branches (5.90 branches. vegetative part^{-1}), which did

Table 2 : Effect of BA and IAA and their interaction in average branch number of Rosemary (branch.plant^{-1}).

IAA	BA				IAA Means
	0	0.5	1	1.5	
0	1.00	5.90	4.00	2.00	3.23
0.1	0.00	5.40	3.50	1.60	2.63
0.3	0.00	5.00	3.00	1.00	2.25
0.5	0.00	4.50	2.60	1.00	2.03
LSD	0.62				
BA means	0.25	5.20	3.28	1.40	
LSD	0.31				

Table 3 : Effect of BA and IAA and their interaction in average multiplied branch length of Rosemary (cm).

IAA	BA				IAA mean
	0	0.5	1	1.5	
0	0.00	2.25	3.20	4.20	2.41
0.1	0.00	2.35	3.30	4.50	2.54
0.3	0.00	2.65	3.35	5.20	2.80
0.5	3.50	2.00	3.10	3.75	3.09
LSD	0.89				
BA Mean	0.88	2.31	3.24	4.41	
LSD	0.45				

not differ significantly from The treatment of 0.5 mg.liter^{-1} of BA with a concentration of 0.1 mg.liter^{-1} of the IAA, which was 5.40 branches. vegetative part^{-1} but differed significantly with the rest of the treatments.

The treatments containing 0.0 mg.liter^{-1} of the BA and 0.1, 0.3 and 0.5 mg.liter^{-1} of the IAA did not give any branches (0 branches.plant^{-1}), but were turn to form callus. The reason is that treatment with auxin will affect the physiological state of the cells and change the differentiation in the cells that respond to it, as treatment with auxin made the differentiated cells for the plant part lose differentiation and accelerate the division to form callus tissue (George *et al*., 2008 and Neumanm *et al*., 2009).

The results of the current study showed that the use of BA in the low concentrations was the best in stimulating the multiplication of the branches and increasing their number, which are consistent with Bertaccini *et al*. (1986) and Taha (2002), they indicated that the low concentrations of BA have led to get large numbers of branches. The use of the BA in doubling vegetative stage for many plants in tissue culture as a source of cytokinins due to its effectiveness in the liberation of the axillary buds without having to cut the apical dominance because they believes

Table 4 : Effect of BA and IAA and their interaction in average fresh weight of Rosemary (cm).

IAA	BA				IAA mean
	0	0.5	1	1.5	
0	1307	4272	2933	1346	2465
0.1	0	3742	2271	1163	1794
0.3	0	3676	2226	794	1674
0.5	0	3285	1796	749	1457
LSD	N.S				256.6
BA Mean	327	3744	2306	1013	
LSD	256.6				

Table 5 : Effect of BA and IAA and their interaction in average dry weight of Rosemary (cm).

IAA	BA				IAA mean
	0	0.5	1	1.5	
0	135.5	446.2	304.7	139.9	256.6
0.3	0	389.1	235.8	120.9	186.4
0.5	0	382.2	231.2	82.3	173.9
1	0	341.5	186.5	77.5	151.4
LSD	53.27				26.64
BA mean	33.9	389.8	239.6	105.2	
LSD	26.64				

that cytokinins stimulates the formation of xylem tissue in buds and stem and thus facilitates the transmission of nutrients and water and thus stimulates axillary buds (Mohamed and Younis, 1991).

As Sutter (1996) points out, the importance of adding cytokinins is to stimulate cell division and thus stimulate the formation and growth of axillary and adventitious buds. As a result, the increase in the number of branches is due to the stimulation of cytokinins in encouraging cells to divide and differentiate, resulting in the differentiation of buds planted in vitro to vegetative growth. Many researchers have pointed out that the use of cytokanins at appropriate concentrations in vitro to their role in breaking the apical dominance and form nutrient attractions sites and thereby stimulate the growth of buds. The results of the present study showed that the use of BA alone gave the highest number of branches. This is consistent with the findings of Sharma and Sen (1991), Irshad *et al.* (2013), Hamoud (2017).

Table 6 : Effect of BA in the concentration of active compounds of volatile oils for rosemary plant.

Sabinene	Terpinene	Linderol	merycenen	linalool	Cymene	camphor	Penene	Limonene	camphenen	
0.44	0.09	0.44	0.82	0.30	0.19	0.66	0.39	0.06	0.26	Growth regulator
0.23	0.02	0.23	0.33	0.04	0.10	0.34	0.12	0.02	0.14	No growth regulator
0.000***	0.000***	0.000***	0.000***	0.011*	0.000***	0.000***	0.000***	0.008***	0.000***	P-value

*,**,*** significant at 0.05, 0.01, and 0.001 primality level using independent t test.

Effect of BA and IAA and their interaction in the length of multiplied branches (cm)

The results indicate the significant effect of the different concentrations of BA in the length of branches (table 3). The results showed that the concentration of 1.5 mg.liter^{-1} significant exceed other concentration and gave the highest length of branches (4.41 cm), whereas the control treatment produced the lowest branch length (0.88 cm).

The results of effect of IAA in average branch length indicates that the treatment with concentration of 0.5 mg.liter^{-1} significantly exceed other concentration with branch length 3.09 cm, while the lowest branch length recorded for control treatment (2.41 cm), which is not significantly different from concentration of 0.1mg.liter^{-1} (2.54 cm).

The results of the interaction between BA and IAA revealed the superiority of the concentration 1.5 BA and 0.3 mg.liter^{-1} IAA treatment with 5.20 cm branch length , which is not significantly different from treatment with 1.5 BA and 0.3 mg.liter^{-1} IAA (4.50 cm). Whereas, the lowest branch length (2.0 cm) recorded by 0.5 BA and 0.50 mg.liter^{-1} treatment.

The increase in the length of branches is due to the stimulation of cytokinins in encouraging cells to divide and differentiate, resulting in the increasing their number (table 4), as a result of increasing number of branches led to the competition on the available nutrient which led to shorten their length, and that in consistence with finding of Dabbagh and Salman (2000).

Effect of BA and IAA and their interaction in the fresh weight of vegetative growth (mg)

The results in table 4 revealed significant effect of BA concentrations on increasing the average fresh weight of the vegetative growth. The concentration of 0.5 mg.liter^{1} significantly exceed other concentration with fresh weight of 3744 mg, while the control treatment showed the lowest fresh weight of 327 mg.

The additions of IAA result in decrease in average fresh weight. The control treatment gave the highest average fresh weight (2645 mg), while the concentration of 0.1 and 0.3 mg.liter^{-1} gave 1794 and 1647 mg respectively and the significant low fresh weight resulted from concentration of 0.5 mg.liter^{-1} (1457 mg).

The results of the interaction between BA and IAA revealed the superiority of the concentration 0.5 BA and 0.0 mg.liter^{-1} IAA treatment with 4272 mg fresh weight,

Whereas, the lowest fresh weight (749 mg) recorded by 1.5 BA and 0.50 mg.liter^{-1} treatment. The cytokinins have an important role in increasing multiplication led to increase in branch number (table 2) then increase the biomass, which was reflected in the increase of the fresh and dry weight of this mass (Mohamed and Younis, 1991).

Effect of BA and IAA and their interaction in the dry weight of vegetative growth (mg)

The results in table 5 revealed that the BA has a significant effect on the increase in the dry weight; with a concentration of 0.5 mg.liter^{-1} exceed the rest of the concentrations with highest dry weight of 389.8 mg, while the control treatment showed the lowest dry weight of 33.9 mg. The results of the same table indicate that the different concentrations of IAA caused a decrease in the dry weight. The control treatment gave the highest dry weight rate of 256.6 mg while the concentrations of 0.1 and 0.3 mg.liter^{-1} gave 186.4 and 173.9 mg respectively and the significant low dry weight resulted from concentration of 0.5 mg.liter^{-1} (151.4 mg).

The results of the interaction between BA and IAA revealed the superiority of the concentration 0.5 BA and 0.0 mg.liter^{-1} of IAA treatment with 446.2 mg dry weight, Whereas, the lowest dry weight (77.5 mg) recorded by 1.5 BA and 0.50 mg.liter^{-1} of IAA treatment.

Effect of BA in the concentration of the main compounds of the volatile oil of Rosemary

The treatments with growth regulators significantly exceed the treatment without growth regulators in the percentage of major compounds of volatile oil (table 6) at the probability level of 0.001 for camphene, limonene,

penne, camphor, cymene, merycenen, linderol, terpinene and sabinene, while linalool significantly exceed control treatment (free of growth regulators) at probability level 0.05 with concentration of 0.30 and 0.04%, respectively.

The increase in the level of active substances when adding the BA to the nutrient media is due to the increase in the number of branches and the fresh and dry weight of the vegetative parts (tables 2, 4 and 5). It is also due to the role of growth regulators in the enzymatic activity of cells (Verpoort *et al*., 2000). These results are consistent with the results of Hamoud (2017) and showed an increase in quantitative content of alkaloids when adding BA to MS media.

Rooting stage

Effect of the strength of MS salts and IBA concentrations and their interaction on percentage of rooting

Addition of IBA to the media of rooting increased the rooting ratio to 86.7 at the concentration of 0.5 and 1 mg.liter^{-1} (table 7), which significantly exceeded the concentration of 0.3 mg.liter^{-1} (50% rooting) and the control treatment, which is failed to form roots, as for the effect of the strength of salts MS did not any significant effect.

As for the effect of the interaction between the strength of MS salts and the concentrations of IBA, the result indicates that the treatment of branches with 0.5 mg.liter^{-1} of IBA + ½ salts strength, 0.5 mg.1iter^{-1} of IBA + whole salt strength, 1 mg of IBA + ½ salts strength and 1 mg of IBA + of whole-strength salts, gave the highest rooting percent (90%), which differed significantly from the treatments of the concentration 0.3 mg.liter^{-1} + ¼ and the whole salt strength were 40% and 50%, rooting, respectively. The treatment of 0.0 mg.liter^{-2} of IBA by with the all strengths of MS salts fail rooting.

Effect of salinity of MS salts and IBA concentrations and their interaction on the average number of roots (root.rooting branch^{-1})

The results of table 8 shows that the addition of IBA at different concentrations has a significant effect on the increase in the number of roots with a maximum of 4.40 root.branch^{-1} at the concentration of 0.5 mg.liter^{-1}, which was significantly higher than the rest of treatments. The lowest number at concentration of 0.3 mg.liter^{-1} with a mean of 2.83 after the control treatment which fail to give rooting, on the other hand, the results indicated a significant differences between the strength of MS salts, where the treatment ½ exceeded ¼ strength which amounted of 3.05 and 1.85 root.branch^{-1}, respectively, while it is not significantly different from the whole strength treatment of MS salts (3.30 root. branch^{-1}).

As for the effect of the interaction between the concentrations of IBA and the strength of the MS media salts, the results revealed significant differences between the treatments and the largest number of roots (5 roots.branch^{-1}) obtained from treatment with 0.5mg.liter^{-1} of IBA with half strength of MS salts and the lowest number of roots (1.80 root.branch^{-1}) from treatment of 1 mg.liter^{-1}of IBA with a quarter of the strength of MS salts.

Effect of MS salts and IBA concentrations and their interaction in mean root length rooted branch^{-1}(cm)

The IBA supplementation had a significant effect on the length of the root. The results of table 9 showed superiority of a concentration 0.5 mg.liter^{-1} of the IBA, (4.07 cm) on the concentration of 1 mg.liter^{-1} (3.17 cm). The lowest length of roots at the concentration 0.3 mg.liter^{-1} of the IBA (2.13 cm) after control treatment (0 cm).

The results indicated a significant effect of the strength of MS salts on root length (table 9) with highest length at 1/2 and whole strength with (2.89 and 2.46 cm)respectively, and the lowest root length (1.68 cm) for ¼ strength of MS salt.

As for the effect of the interaction between the concentrations of IBA and the strength of the MS media salts, the results revealed significant differences between the treatments and the higher root length (5.30 cm) obtained from treatment with 0.5 mg.liter^{-1} of IBA with half strength of MS salts, and the lowest root length (1.60 cm) from treatment of 0.3 mg.liter-1of IBA with a quarter of the strength of MS salts.

The results of the tables 7, 8, 9 shows that the reason for the superiority of ½ strength of MS salts in the percentage of rooting and increasing the mean number and length of root is due to the reduction in the salinity levels of the media which mean the decrease in the level of nitrogen in the media and the reduction in its internal level in the branches leading to an increase in the proportion of carbohydrates to nitrogen (C/N), which leading to an increase in root formation and number (Gawel *et al*., 1990; Hartmann *et al*., 2002).

As for the role of reducing the salt strength by half, lead to an increase in the length of roots due to the phenomenon of food tropism and competition of roots for food because of the decrease in the concentration of salt by half compared to the full concentration, which stimulated roots to spread far beyond the nutrient media

Table 7 : Effect of the strength of MS salts and IBA and their interaction in percentage (%) of the branch rooting of rosemary after four weeks of culturing.

IBA	MS			IBA Mean
	¼	½	1	
0	0	0	0	0.00
0.3	40	60	50	50.00
0.5	80	90	90	86.70
1	80	90	90	86.70
LSD	32.00			18.49
MS mean	50	60	57.5	
	N.S			

Table 8 : Effect of the strength of MS salts and IBA and their interaction in number of roots of rosemary after four weeks of culturing.

IBA	MS			IBA mean
	¼	½	1	
0	0	0	0	0
0.3	2.10	3.20	3.20	2.83
0.5	3.50	5.00	4.70	4.40
1	1.80	4.00	3.30	3.03
LSD	1.69			0.98
MS mean	1.85	3.05	2.80	
	0.85			

Table 9 : Effect of the strength of MS salts and IBA and their interaction in length of roots of rosemary after four weeks of culturing.

IBA	MS			IBA mean
	¼	½	1	
0	0	0	0	0
0.3	1.60	2.35	2.45	2.13
0.5	2.70	5.30	4.20	4.07
1	2.40	3.90	3.20	3.17
LSD	1.37			0.79
MS mean	1.68	2.89	2.46	
	0.68			

to compensate for the shortage in the amount of nutrients (Mohamed and Elriss, 1982). This results are consistent with results of Obaid (2009).

Several studies have shown that the IBA has a key role in rooting the branches in vitro as auxins plays a large role in stimulating the emergence of adventitious roots through its physiological effect in the loss of differentiation of the specialized parenchyma cells and then its return to its condition in the process of loss of differentiation (Dedifferentiation), which divide forming the initiatives of the roots, which continues to grow and develop into the roots and then grow outside the stem tissues, forming adventitious roots (Hartmann *et al*., 2002). While the failure of rooting of cultured branches on the IBA-free MS media could be explained on the basis that the level of auxins in the resulting branches of the compound was not sufficient to stimulate root formation (Paek *et al*., 1987). While the failure of rooting of cultured branches on the IBA-free MS media could be explained on the basis that the level of auxin in the resulting branches of the multiplication was not sufficient to stimulate root formation (Paek *et al*., 1987).

Plantlet acclimatization stage

The acclimatization of plantlets process was carried out in pots filled with a mixture of 1 silt :1 peatmoss, the silty soil was a good media for the growth and spread of roots and prevented suffocation, Because it has good pores for ventilation. While peat moss is a good medium to provide the nutrients necessary for growth and helps retain the moisture necessary for growth.The success rate was 90%. It should be noted that transparent plastic caps were used during the process of acclimatization and were lifted after completely after three weeks after planting. These results are consistent with the findings of Ameri (2000), Abeed (2009) and Al-Hameedawi (2016), who found that cultivation in a media of silt and peat moss increases the success rate of cultivated plantlets.

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