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STUDY THE ROLE OF ADENINE SULFATE, SUCROSE AND PLANT GROWTH REGULATORS FITTED TO THE MEDIA PHYSIOLOGICALLY IN MICROPROPAGATION OF MELISSA OFFICINALIS L. PLANT IN VITRO

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Abstract:

Susceptibility of a number of factors were tested in the stages of propagation of the lemon balm plant *Melissa officinalis* under controlled conditions in the laboratory of plant tissue, Horticulture and Gardening department , Agriculture college, Karbala university, shoots culture of Melissa were received from planting the ends of the branches with a length one cm on MS medium that have been prepared with different concentrations (0.0, 0.5, 1.0, 2.0) mg l^{-1} of adenine sulfate and (0.0, 1.0, 2.0, 3.0) mg l^{-1} of benzyl adenine with fixed con. 0.5 mg l^{-1} of IBA, The rooting experiment included the cultivation of vegetative branches with a length of two cm obtained from the multiplication stage on a nutrient medium prepared for rooting, which includes different con. (20, 30, 40) g/l of sucrose and (0.0, 0.5, 1.0, 1.5) mg l^{-1} of IBA with a constant con. 0.5 mg l^{-1} IBA, results indicated that superiority of adenine sulfate at con. one mg l^{-1} in investigation the top average length of vegetative branches was 5.40 cm, while the con. two mg l^{-1} was superior in achieving the highest average of shoot number, fresh and dry weight of total shoot was 31.75 branches $plant^{-1}$, 4.51 and 0.95 mg consecutively, and about the influence of BA on the average of the studied traits of the vegetative group, it was superior at the con. two mg l^{-1} in achieving the highest average length of branches and dry weight of total vegetative was 3.61 cm. and 0.80 mg consecutively. whereas the con. of 3 mg / liter achieved the highest average branches number and the fresh weight of the vegetative total was 30.24 branches $plant^{-1}$ and 4.19 mg, respectively, and on the effect of sucrose on the studied root traits characteristics, the con. 40 g l^{-1} exceeded in height average, roots number, wet and dry weight of rooting group reached 4.00 cm and 27.03 roots $branches^{-1}$, 2.35 and 0.57 mg respectively, as the results indicated the superiority of the IBA with a concentration of 1 mg / Liter in the mean root mean rate was 4.00 cm, 24.66 roots / branches, 2.34 and 0.51 mg, respectively.

Keywords: Melissa officinalis, micropropagation, adenine sulfate, sucrose, growth regulators, in vitro.

INTRODUCTION

Lemon balm or honey plant, or it is sometimes known as Melissa, or aching herb, is a perennial herbaceous plant, belongs to the family of Lamiaceae. and about the name of Melissa, it is a Greek word meaning bees. This name is due to the ability of this plant to attract bees to it (1), the plant reaches a height of approximately 1 cm, its soft leaves with a light fluff are cardiac in shape with serrated edges and white flowers that are composed in simple flowering clusters (`2), the regions of southern Europe, North Africa, and the Mediterranean are the original home of this plant and currently it is grown all over the world for its medicinal importance as the plant contains many secondary metabolism compounds, where the sources indicated the presence of both phenolics compounds, volatile oils, flavonoids, triterpines, tannins and alcoholic glycosides . And other substances of pharmaceutical interest (3 and 4), lemon balm was used in the treatment of bedsores resulting from severe cold (5) and in the treatment of depression and anxiety and rapid heartbeat and is useful in cases of Stomach and stomach cramping disorder (6), as used as a bacterial, viral, anti-inflammatory and antioxidant (7), and its leaves were used in the manufacture of spices, drinks, perfumes and cosmetics

because they contain volatile oils with a very acceptable lemon scent (8). the increasing use and great trend in recent years of the use of herbs, medicinal substances with its urgent need become a major threat to plants and natural resources, as a result of their removal and sabotage of the environment, so, attention in practical applications of biotechnology has increased. Tissue culture is one of the more important and developed of them, and it depends on simple methods that do not require complicated and expensive laboratory equipment. It also has many applications, the most important of which is the laboratory vegetative propagation, that is, the production of plants in the laboratory instead of the field through the development of tissue or individual plant cells on a suitable sterile food environment, and researchers have a great interest in the field of vegetative propagation of plants by this technology, due to the scarcity of some of them, or Of its nutritional value, or the ability of some to produce substances of high pharmacokinetic value in the production of prescription drugs (9, and 10), so, this biotechnology is an indispensable way to produce medicinal substances derived from plants, it also has great benefits in terms of type, quantity and controlled production without being restricted by environmental conditions (11). The growth and disclosure of plant tissues in the laboratory can be controlled by the type and structure of the medium, growth regulators are among the critical components of the media that have a direct impact on the growth and development of the planted plant part, as they play a role in increasing cell division and multiplication and then increasing the production of metabolic compounds (12), Several researchers also stressed the importance of adding Adenine sulphate to the nutritional medium in order to obtain the best growth for plant parts growing in vitro due to its positive role in stimulating and regulating growth, so it was widely used for the purpose of increasing and elongating the multiplying branches (13). From the above, and given the lack of studies on the accurate multiplication of the lemon balm plant, the study aimed to employment tissue culture technology in the plant's micropropagation, and to study the effect of adding both adenine sulfate and sucrose and growth regulators in different concentrations to the medium in the stages of plant propagation in vitro.

MATERIALS AND METHODS

The seeds of Melissa were sterilized after soaking them with a concentration of 3% of commercial minor NaOCl for 10 minutes, after that it was washed with sterile water 3 times to get rid of the effect of the sterilization substance, then transferred to culture tubes equipped with solid food medium (MS)(14) free of growth regulators, the plants were kept in the growth room at a temperature of 25°C with a luminous intensity 1000 lux for 16 hours day^{-1}. The multiplication experiment include: culturing the shoot tips with a length one cm received from initiation stage on MS medium prepared with different con. (0, 1, 2, 3) mg liter^{-1} of BA and adenine sulfate in concentrations (0, 0.5, 1, 2) In the presence of a fixed con. of 0.5 mg L^{-1} IBA, the plants were incubated in the same storage conditions as before, study indicators were taken after 30 days, that include average number and length of branches, average Fresh and dry weight ratio of shoot, the rooting experiment included: Cultivation of branches obtained from the multiplicity stage with a length of 2 cm on media prepared with various con. (20, 30, 40) g l^{-1} sucrose and different con. (0, 0.5, 1, 1.5) mg l^{-1} IBA with fixed con 0.5 mg l^{-1} BA, the cultures were incubated in the same conditions as before, and the study indicators were taken after 30 days of storage in the incubation room, that included average number and length of roots, fresh and dry weight of roots system, this study was carried out as factorial experiments using the complete random design (CRD), then the averages of the coefficients were compared according to the LSD test under the probability level of 0.05 (15) with ten replications.

RESULTS AND DISCUSSION

Laboratory culture using plant tissue culture techniques in vegetative propagation has previously been shown as the best way to obtain homogeneous vegetative strains away from genetic differences between members of the resulting offspring, in addition to obtaining high yields for propagation compared to traditional methods. In order to reach this level and for this method to be economically feasible, all stages of propagation must be successful, and this depends on the materials added to the food medium, as the effectiveness of these materials varies according to their type, concentration and interaction with each other**.** The outcomes recorded in the table 1 indicate that con of adenine sulfate has significant effect on rate of branches length, as the con 1 mg l^{-1} achieved the highest rate of 5.40 cm, then the response decreased by increasing the concentration to 2 mg l^{-1}, which amounted to 4.17 cm, Whereas, the neutral experience achieved the lowest rate was 2.19 cm, the results of the same table also showed the superiority of the concentration of 1 mg l^{-1} of BA in achieving the highest average length of the vegetative branches of 5.10 cm, then the response decreased by increasing the concentration to 2 and mg l^{-1}, which amounted to 3.61 and 3.49 cm, sequentially, the neutral experience achieved the lowest rate, which was 2.86 cm, . As for the effect of the interaction, the nutritional medium with a con. of 1 mg l^{-1} of adenine sulfate and benzyl adenine outperformed in achieving a highest rate of 6.85 cm, Whereas the neutral treatment gave a lowest con. was 1.20 cm. The increase in BA concentrations in the food media to more than 1 mg l^{-1} led to a decrease in the average length of branches, the reason may be attributed to the fact that the increase in cytokinein concentrations in the food media reduces the role of auxien accumulated inside the branch responsible for elongating stem cells towards the longitudinal axis and then reducing the length of the branch (16), the result agreed with the findings (17) when high con. of BA supplied to media prepared for propagation of Melissa plant. Regarding the effect of adenine sulfate and BA con. on the average number of branches, the data indicated in Table (2) showed that the con. of 2 mg l^{-1} adenine sulfate achieved the highest rate of 31.75 branches /plant, which differed significantly from the other concentrations, while the control treatment gave the lowest rate was 15.04 branches plant^{-1}, the con. 3 mg l^{-1} of BA achieved an average was 30.24 branches plant^{-1}, which did not differ significantly from the con. of 2 mg l^{-1}, which gave an average was 29.09 branches plant$^{-1,}$ whereas the control treatment gave a lowest was 17.25 branches plant^{-1}. As for the effect of the bilateral interaction, the media supplied with a con.

2 mg l^{-1} adenine sulfate and benzyl adenine outperformed in achieving a highest rate of 35.17 branches $plant^{-1}$, Whereas, the neutral treatment gave the least average was 1 branch $plant^{-1}$ .The reason for the increase in the number of branches when a increment the con. of benzyl adenine supplied to the media and to reach the optimum con, maybe come back to the stimulatory action of Cytokinines in urging cells to divide and differentiate, and this results in the differentiation of the buds grown outside the vivo into vegetative branches. Many researchers pointed to the role that Cytokinines play in appropriate concentrations in tissue culture in terms of breaking the apical dominance and creating areas of attraction in the lateral buds, and this stimulates the speed of the transfer of nutrients to them, which results in stimulating the growth of buds (18), these results are in agreement with (19) when micro propagation of banana (Musa spp.) and with (20) when propagation vegetative branches of *Catharanthus roses* in vitro and with (21) when propagation vegetative branches of *Digitalis lanata* in vitro.

Table 1 effect of adenine sulfate, benzyl adenine con. and the interference between them on average of branch length (cm).

Adenine sulfate con. (mg l^{-1})	Con. of BA (mg l^{-1})				
	0.00	1.00	2.00	3.00	mean
0.0	1.20	2.65	1.80	3.14	2.19
0.5	2.17	4.70	2.50	3.86	3.30
1.0	4.20	6.85	5.90	4.65	5.40
2.0	3.88	6.21	4.25	2.34	4.17
L.S.D 0.05	1.12				1.05
mean	2.86	5.10	3.61	3.49	
L.S.D 0.05	1.02				

Table 2 effect of adenine sulfate, benzyl adenine con. and the interference between them on average of branches number (branch $plant^{-1}$).

Adenine sulfate con. (mg l^{-1})	Con. of BA (mg l^{-1})				
	0.00	1.00	2.00	3.00	mean
0.0	1.00	15.70	18.30	25.18	15.04
0.5	18.40	20.98	32.15	30.45	25.50
1.0	22.50	22.13	30.75	30.23	26.40
2.0	27.12	29.60	35.17	35.12	31.75
L.S.D 0.05	1.66				1.20
mean	17.25	22.10	29.09	30.24	
L.S.D 0.05	1.36				

As for the role of adenine sulfate in the soft rate of the vegetative total, the data recorded in Table (3) indicated that there is a plus in average when increased the con. fitted to the media, reaching the con. 2 mg l^{-1}, that gave a significant superiority was 4.51 mg, whereas control experiment gave the least average was 1.15 mg, the data of the same tables showed that BA was significantly superior at concentration 3 mg l^{-1} in giving highest average was 5.19 mg compared to other con. except the con. 2 mg l^{-1} that gave average was 4.11 mg, whereas the control experiment gave the least average was 1.69 mg, regarding the effect of the interaction, the media supplemented with a con. 2 mgl^{-1} adenine sulfate and benzyl adenine in achieving a highest fresh weight rate of 5.65 mg compare with control experiment gave the least average was 0.10 mg.

The outcomes of table 4 indicated that the various con. of adenine sulfate had a superiority effect in dry weight rate of shoot, where the con. of 2 mg l^{-1} excelled in giving the highest amended was 0.95 mg compare with control experiment that gave the lowest average was 0.19 mg, and the effect of BA at con. of 2 mg l^{-1} was superior in achieving a highest rate was 0.80 mg and did not differ significantly on the con. of 3 mg l^{-1} that gave average was 0.78 mg, whereas the control treat gave a lowest rate was 0.32 mg, and about the interaction effect, the con. was 2 mg of adenine sulfate was superior with BA the highest rate of 1.35 mg was achieved, while the lowest rate was achieved when the control treatment was 0.03 mg. The reason to excel of benzyl adenine treatment in a average of branches number, it may be due to the increase in the live mass, which was reflected in the fresh and dry weight of this mass, the reason for the increase in multiplication and then the increase in the rates of fresh and dry weight may be due to the important role of cytokinein in cell division, especially in the case of its presence with auxien, which means that the effect increases when they are present together (22), these results are agree with the findings of (23) and with (24) when conducting the vegetative multiplication experiment of *Melissa officinalis* and measuring the soft and dry weight of vegetative shoots. It was also shown from the results presented in the previous tables on the effect of BA on the rate of the studied traits that the comparison treatment achieved the lowest average of the concentrations mentioned above. Vascular connection between the vascular tissues of the axillaries buds and the vascular tissues of the stem, which leads to the lack or lack of passage of nutrients from the stem tissues to the buds, and then the lack of their growth and elongation (25), or the reason may be due to the role of auxien in encouraging apex dominion and preventing a growth of side

branches (26), it was also noted from the results of Tables 1 to 4 that the presence of adenine sulfate in the food media had a clear and positive effect on the average length and number of branches, which was reflected on the average fresh and dry weight of the shoot, the reason may be due to the fact that adenine sulfate contains the nitrogenous base (adenine), which is naturally involved in the synthesis of Cytokinines, in addition to its entry into the construction of nucleic acids, DNA and RNA, forming proteins and enzymes, while also entering into the construction of energy compounds such as ATP and ADP, and all this will have effect on the process of cell division, increasing its size and differentiation, and then increasing the number of branches formed (27), or, the reason may be due to the role of indirect adenine sulfate in stimulating cell division and stimulating the formation of vascular tissue for shoots and branches, facilitating the transfer of water and nutrients, which leads to an increase in the length of branches (28), therefore, this substance was used in interaction with Cytokinines and within certain concentrations in several studies conducted in the field of tissue culture and on different plants for the purpose of increasing and raising the level of vegetative multiplication, such as the study conducted by((29) when propagating the branches of *gardenia Jasminoides,* and the study done by (30) when propagating *Kalanchoe blossfeldiana* and (31) when propagating *Stevia rebaudiana in plant tissue culture*.

Table 3 effect of adenine sulfate, benzyl adenine con. and the interference between them on the average of fresh weight (mg).

Adenine sulfate con. (mg l^{-1})	Con. of BA (mg l^{-1})				
	0.00	1.00	2.00	3.00	mean
0.0	0.10	1.08	1.18	2.26	1.15
0.5	1.23	1.13	4.60	4.75	2.92
1.0	2.04	2.10	5.01	4.60	3.43
2.0	3.40	3.83	5.65	5.17	4.51
L.S.D 0.05	0.56				0.22
mean	1.69	2.03	4.11	4.19	
L.S.D 0.05	0.28				

Table 4 effect of adenine sulfate, benzyl adenine con. and the interference between them on an average of dry weight (mg).

Adenine sulfate con. (mg l^{-1})	Con. of BA (mg l^{-1})				
	0.00	1.00	2.00	3.00	mean
0.0	0.03	0.11	0.20	0.45	0.19
0.5	0.29	0.24	0.73	0.80	0.51
1.0	0.38	0.40	0.93	0.70	0.60
2.0	0.60	0.68	1.35	1.17	0.95
L.S.D 0.05	0.22				0.18
mean	0.32	0.35	0.80	0.75	
L.S.D 0.05	0.16				

The results shown in Table (5) indicate the effect of sucrose and IBA concentrations and the interaction between them on the average root length. It was noted that the concentration was 40 g of sucrose gave the highest rate of root length was 4.00 cm, which was significantly superior to the other concentrations, whereas control treat gave a lower in rate was 2.19 cm. It is also noted from same data that the con. of IBA had active influence on rate of root length, where the con. 1 mg l^{-1}outperformed in achieving the highest rate was 4.00 cm, which did not differ significantly from the concentration 1.5 mg l^{-1}, which achieved a rate was 3.79 cm, while the control treatment achieved the lowest average was 2.44 cm, as for the influence of the interference between the con. of sucrose and IBA, it is noted from the data of the same table that the medium prepared for rooting with concentrations of 30 gm of sucrose and 1.5 mg $l^{-1}$of IBA was significant in a average length of root was 4.85 cm compare with control treat that gave the lower average was 1.43 cm. Regarding the impact of sucrose with IBA con on a average of roots number, the data referred to in table (6) showed that the con of 40 g of sucrose was superior in achieving the highest rate was 27.03 roots $plant^{-1}$, which significantly differ from other con, while a control treatment achieved the lowest rate was 13.60 roots $plants^{-1}$, at the same time, the con 1 mgl^{-1} of IBA had significant influence on the rate of roots number, which significantly differ from other con, amounting was 24.66 roots $plant^{-1}$, while comparison experience gave the lowest rate was 16.46 $root.plant^{-1}$. 40 gm of sucrose and 1 $mg.l^{-1}$ IBA achieved the highest rate was 31.11 root. $Plant^{-1}$, while the control treatment achieved the lowest rate was 10.13 root. $plant^{-1}$.

Table 5 effect of sucrose, IBA con and the interference between them on average of root length (cm)

Sucrose con. (gl^{-1})	Con. of IBA (mgl^{-1})				
	0.00	0.50	1.00	1.50	mean

20	1.43	2.08	2.60	2.66	2.19
30	2.25	2.17	4.80	4.85	3.51
40	3.64	3.91	4.60	3.86	4.00
L.S.D 0.05	0.44				0.20
mean	2.44	2.72	4.00	3.79	
L.S.D 0.05	0.28				

Table 6 effect of sucrose, IBA con and the interference between them on average of root number (root plant^{-1})

Sucrose con. (gl^{-1})	Con. of IBA (mgl^{-1})				
	0.00	0.50	1.00	1.50	mean
20	10.13	13.27	20.70	10.33	13.60
30	20.75	18.30	22.17	26.87	22.02
40	18.50	30.02	31.11	28.52	27.03
L.S.D 0.05	2.01				1.17
mean	16.46	20.53	24.66	21.90	
L.S.D 0.05	1.24				

And about the effect of sucrose concentrations added to the medium prepared for rooting on the characteristic of the fresh weight rate of the root group, the data recorded in Table (7) indicated that there is a increment in average when increasing the con additive to the media, reaching the con of 40g, which gave a significant superiority was 2.35 mg, whereas the control treat gave the less average was 1.29 mg, also the same table data showed the significant superiority of IBA at the con of 1 mgl^{-1} in achieving the highest rate was 2.34 mg compared to other concentrations, whereas the control treat gave less average was 1.49 mg, and about the impact of interaction, the prepared medium with 40 g sucrose and 1 $mg.l^{-1}$ IBA achieved the highest fresh weight rate was 3.09 mg compared with the control treatment that achieved the lowest rate was 1.10 mg.

The results of Table (8) also showed that the presence of sucrose in the food media with its different concentrations had an superior impact on a rate dry weight of root system, where the concentration of 40 g was superior to achieving the highest rate was 0.57 mg in comparison with the control treat that gave the lowest average was 0.16 mg, either regarding the effect of IBA, the con of 1 mgl^{-1} was superior in achieving a highest rate was 0.51 mg, whereas control treat that gave the lowest average was 0.21mg, and regarding the effect of the interaction, the food medium prepared with a concentration of 40 g of sucrose was outperformed by the interaction with IBA at 1 $mg.l^{-1}$ in the highest rate was 0.93 mg, while the lowest rate was achieved when the control treatment was 0.10 mg. From the foregoing presentation of the results shown in the rooting tables, the increase in sucrose concentrations led to the improvement of rooting of the branches, and this may be due to the fact that carbohydrates are a source of energy and carbon as well as they regulate the osmosis in the medium, and the concentration of 40 g. l^{-1} was optimal in increasing the formation and growth of roots (32), this result was agreement with (33) when rooting the shoot of *juvenile avocado* and (34) when rooting the shoot of *Lilium longiflorum*, as well as (35) when rooting the shoot of *Billbergia zebrine in vitro*. The results of the table probably interpreted depends on the IBA is one of the auxien which encourages the division and elongation of cells, and then stimulates the formation of roots in the cut areas, and that the addendum of plant growth perform to a plus in the rates of length and root number to get optimal con and any increase in con over that may be leads to opposite effects. (36), the reason for rooting in control plants may be due to the plant's internal content of hormones, including auxin (37), the result agreed with (38) when rooting the shoot of *Santalum album* and with (39) when rooting the branches of *Ocimum basilicum* and with (40) when rooting the shoot of *Melissa officinalis in vitro.* and with (41) when rooting *Digitalis lanata* in vitro.

Table 7 effect of sucrose, IBA cons and the interference between them on average of fresh weighing of root system (mg)

Sucrose con. (gl^{-1})	Con. of IBA (mgl^{-1})				
	0.00	0.50	1.00	1.50	mean
20	1.10	1.15	1.83	1.11	1.29
30	2.00	1.25	2.12	2.28	1.91
40	1.37	2.70	3.09	2.25	2.35
L.S.D 0.05	0.80				0.20
mean	1.49	1.70	2.34	1.88	
L.S.D 0.05	0.22				

Table 8 effect of sucrose, IBA con and the interference between them on average of dry weighing of root system (mg)

Sucrose con. (gl-1)	Con. of IBA (mgl^{-1})				
	0.0	0.5	1.0	1.5	mean
20	0.10	0.18	0.28	0.10	0.16

30	0.30	0.20	0.33	0.45	0.32
40	0.25	0.66	0.93	0.44	0.57
L.S.D 0.05	0.10				0.06
mean	0.21	0.34	0.51	0.33	
L.S.D 0.05	0.04				

CONCLUSION

It was found through the research that was conducted on the propagation of lemon balm plant *in vitro*, that the addition of adenine sulfate and cytokinein to the medium prepared for micropropagation had an effective role in stimulating the axillaries and transverse buds within appropriate concentrations, and it was also found that the addition of sucrose and auxien to the rooting medium had an effective effect. In the emergence of adventitious roots, so it is possible to rely on the technique of plant tissue culture with the addition of these materials for the purpose of improving the efficiency of vegetative propagation of ornamental and medicinal plants for pharmaceutical purposes.

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