STIMULATING EFFECT OF GAMMA IRRADIATION ON GROWTH AND PHYSIOLOGICAL ACTIVITY OF LAVENDER (*LAVANDULA MULTIFIDA*) PLANTLETS

Journal

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ABSTRACT

The plantlets of lavender were exposed to seven different dose levels of γ-irradiation, 0, 5, 15, 30, 45, 60, and 75 Gy. After that the irradiation plantlets were sectioned to start the multiplication stages, through subculturing every section four weeks intervals for three times. Data revealed that, γ-irradiation at low dose levels 5 and 15 Gy, increased all morphological, growth parameters and physiological characteristics of plantlets. The survival percentage of plantlets clearly showed that there were no differences between the dose levels 5 and 15 and the control plantlets, while the dose level more than 15 Gy decreased the survival percentage of plantlets. Dose level of 15 Gy increased significantly the length of plantlets in the three multiplication stages (M1, M2 and M3) and reached 8.00, 9.10 and 10.03 cm, respectively. Same trend was observed for leaves number. There were no significant differences in moisture content of plantlets in all multiplication stages except the dose level of 60 and 75 Gy which gave higher moisture content than control. Whereas, the dry matter content decreased with increasing irradiation dose level. It is worthy to mention that, the highest value of chlorophyll a,b and carotenoids were observed at the dose level of 15 Gy in the three multiplication stages. Overall, the results indicated that treatment for plantlets before multiplication stage with low doses of gamma rays could be used to enhance growth parameters and physiological characteristics of plantlets and that could be help the plants to overcome the environmental stress.

Key words: Gamma irradiation, chlorophyll, growth parameter, *lavandula multifida*

INTRODUCTION

The genus Lavandula contains many different species which belongs to the 'Labiatae' family that geographically grown in Mediterranean countries (**Baytop, 1984**). Labiatae are generally known for their multiple pharmacological effects such as anticonvulsant, sedative, antispasmodic, analgesic, antioxidant, local anaesthetic activity and it has been used for medicinal purposes **(Ghelardini et al., 1999; Hosseinzadeh et al., 2000 and Kovatcheva et al., 2001)**. Lavandula includes various species producing essential oils useful for perfume, cosmetic and medicine. It is consider one of the most important aromatic plants in the world. Usually, they are vegetatively propagated, e.g., stem cuttings. However, it is not so efficient to propagate them by means of cuttings because of poor rooting ability in some of them (**Gras and Calvo, 1996**). Accordingly, an alternative procedure is a requisite for propagating Lavandula plants efficiently. *In vitro* culture based on micropropagation technique has been successfully used for rapid and mass propagation of many medicinal plants (**Hassan and Roy 2005 and Biswas et al. 2009**). Gamma irradiation has been widely applied in medicine and biology in terms of biological effects induced by a counter intuitive switch-over from low doses stimulation to high-doses inhibition **(Charbaji and. Nabulsi 1999**). Radiation applications are often used on plants in developing varieties that are agriculturally and economically important and have high productivity potential (**Jain, et al., 1998**). Gamma irradiation can be useful for the alteration of physiological characters (**Kiong et al., 2008**). Previous studies have shown that relatively low-doses ionizing radiation on plants and photosynthetic microorganisms are manifested as accelerated cell proliferation, germination rate, cell growth, enzyme activity, stress resistance and crop yields (**Chakravarty and Sen 2001**). In addition, γ-irradiation at low doses revealed its stimulatory function on the growth of plants in their early ontogenetic stages (**Thapa, 2004**). The growth of Arabidopsis seedlings exposed to low-dose gamma rays (1 or 2 Gy) was slightly increased compared with control, while the seedling growth was noticeably decreased by the high-dose irradiation of 50 Gy **(Preussa and Britta, 2003).** Consequently, it is thought that seed irradiation may effect on some of the biochemical regulatory mechanisms involved in seed germination and plant growth. Irradiation treatments caused decrease in shoot dry weights with the increase of γ-irradiation dose levels (**Kiong et al., 2008**). Meanwhile, **Nassar et al. (2004)** found that gamma irradiation enhanced plant height and shoot. On the other hand**, Abdul Majeed et al., (2010)** reported that the growth parameters showed declining tendency with increasing doses of gamma irradiation. The stimulated growth of plants issued from seeds that have been exposed to low irradiation doses was reported (**Moussa, 2006**). Worth to mention that, **Irfaq and Nawab (2001**) opened a new era for crop improvement and now mutation induction has become an established tool in plant breeding that can supplement the existing germplasm and can improve plants in certain specific traits as well. To date there is no major report stating the use of gamma irradiation as a physical mutagen to alter the physiological characteristics of lavender. Considering the effects of γ-irradiation on plants, the present study was conducted to determine the effects of low doses of γ-irradiation on growth and some key of physiological and biochemical characteristics of lavender (*lavandula multifida*) through three multiplication stages.

MATERIALS AND METHODS

Plant materials:

Seeds of lavender plants *Lavandula Mulifida* were obtained from Experimental Farm, Faculty of Agriculture, Cairo University. The experiments of this investigation were carried out in the tissue culture laboratory of Natural Products Dept. National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo-Egypt and the tissue culture laboratory of Strawberry and Non-Traditional Crops Improvement Center, Faculty of Agriculture, Ain Shams University, Kalubia-Egypt.

Culture establishment:

Seeds of lavender were washed thoroughly with tap water. Under aseptic conditions, seeds were sterilized using 70% ethanol (30 sec) followed by 25% Clorox (15 min) and then rinsed several times using sterile distilled water. The seeds were cultured on MS medium (**Murashige and Skoog, 1962**). Culture jars were maintained in the growth chamber at 25±2ºC under 16-h/day photoperiod (1000 lux) and

8 hrs dark. The produced plantlets were used for the irradiation treatments.

The effect of growth regulators on micropropagation was evaluated on MS basal medium supplemented with 6-benzylamino purine (BA), different concentrations of 6-benzylamino purine (0.4 to 0.8 ppm) alone or in combination with indolacitic acid (IAA) and gibrilic acid were also tested. The produced plantlets were used for the irradiation treatments.

Irradiation treatments:

The produced plantlets divided into seven groups represents 50 gars for each, six of them were irradiated by Co^{60} gamma rays at dose levels (5, 15, 30, 45, 60, and 75 Gy) and the seventh group used as control for comparison. Radiation treatments were carried out in National Center of Radiation Research and Technology (NCRRT). Dose rate: 0.7 Gy/min, Atomic Energy Authorization, Nasr City, Cairo, Egypt.

Immediately after irradiation, plantlets were aseptically transferred into sterile fresh MS medium, placed in a growth chamber at 25±2 ºC under 16-h/day photoperiod (1000 lux) and 8 hours dark. Subculture has been done every 4 weeks for three times (M1, M2 andM3) after irradiation whereas, M1, M2 and M3 were the first, the second and the third subculture respectively. Meanwhile, M0 is the irradiated plantlets before subculture (zero time stage).

Plantlets samples were taken from M0, M1, M2 and M3 for the following determinations:

1. The morphological characteristics of plantlets:

Survival percentages of plantlets were recorded after 4 weeks from irradiation. Plantlet lengths (cm), leaves number and dry matter of plantlets (%) were recorded after each multiplication stage.

2. The chemical characteristics of plantlets:

2.1. Determination of moisture content:

Moisture content was determined by drying 2.00 g of sample using hot air up to 130°C for 1 h and repeated many times at the same temperature until constant dried weight. The dried samples cooled over $CaCl_2$ in a glass dissector. The moisture content was calculated according to **A.O.A.C. (1990).**

2.2. Determination of plant pigments:

Chlorophyll a, b and carotenoids were extracted and estimated according to **A.O.A.C. (1990)** and **Wettstein, (1957)** using the following procedure:

Fresh samples (0.5 g) were homogenized in a mortar with 85% acetone in the presence of washed dried sand and a little amount of $CaCo_3$ Ca (0.1 g) in order to neutralize organic acids in the homogenate of the fresh tissue. The homogenate was then filtered through sintered glass funnel. The residue was washed several times with acetone until the filtrate became colorless. The combined extract was completed to a known volume (50 ml). The optical density of obtained extract was determined using a spectrophotometer at 662, 644 nm for Chlorophyll a and Ch1orophyll b, respectively and 440.5 nm for carotenoids. The concentration of Chl a, Chl b, total chlorophylls and total carotenoid were expressed as mg g^{-1} FW.

Statistical analysis:

Data were subjected to statistical analysis using the analysis of variance method and the means of treatments were compared by using the least significant difference (L.S.D) at 0.05 level of probability according to **Duncan (1955)** multiple range test.

RESULTS AND DISCUSSION

Effect of growth regulators on micropropagation of lavender seeds:

Data presented in Plate (1) revealed that BA alone was the most effective cytokinin, where the highest multiplication rate of *Lavandula multifida* was obtained using MS medium full strength supplemented with 0.8 ppm BA. While, the lowest multiplication rate was noticed with all other medium under study. Multiplication rate was decreased by decreased BA concentration. The medium contain 0.2 ppm GA_3 gave very thin plantlets but the medium contain 0.2 ppm IAA + 0.8 ppm BA lead to callus formation.

These results are in agreement with **Jordan *et al.*, 1998** on *L. dentata*, **Andrade, *et al.*, (1999)** who found that the highest multiplication rate of *Lavandula vera* DC was obtained using MS medium supplemented with 1.0 mg 1^{-1} 6-benzylamino purine (BA) (2 μM). Other *Lavandula* species **(Nobre 1996, Andrade *et al.*, 1999)** and **Agostini and Echeverrigaray, (2006)** on *Cunila incise*.

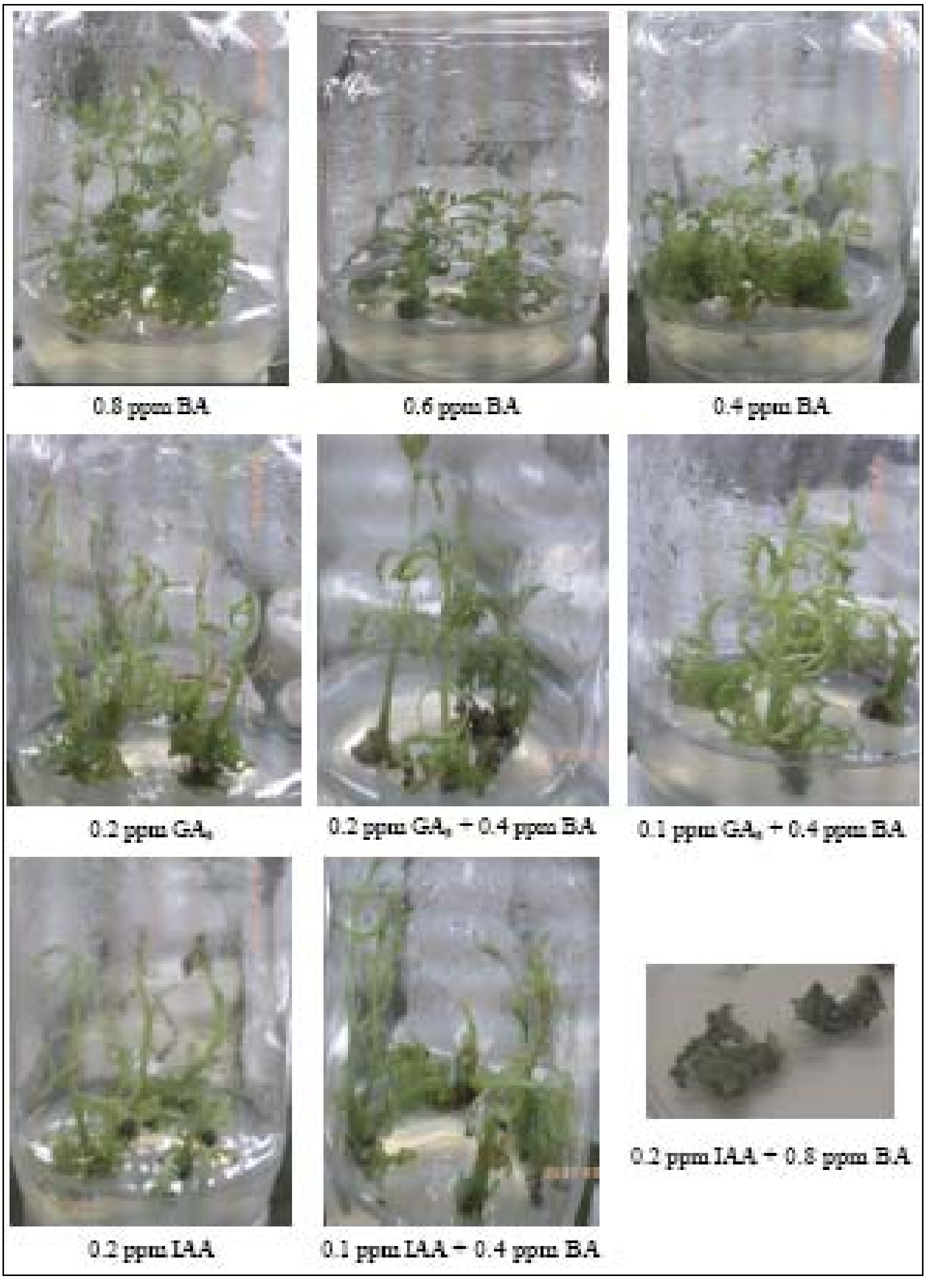

Plate (1): Effect of different growth regulators (BA, alone or in combination with IAA and GA_3) concentrations on multiplication rate of lavender during establishment stage.

1. The morphological characteristics of plantlets:

1.1. Survival percentage of plantlets:

The survival percentage of plantlets is exhibited in Table (1). The data clearly showed that the effects of γ-irradiation at different dose levels were varied from one dose to another in partial harmony. Increasing the tested dosage up to 15 Gy produced the highest survival percentage 100%. On the contrary, the dose level of 60 Gy gave the lowest percentage of survival 13.33% in comparison with the low doses and with the control which gave 100%. While, the high dose of 75 Gy didn't show any percentage of survival (00.00 %). These results were in accordance with the germination test done by **Melki and Dahmani (2009)** whereby there was no significant difference in germination and survival percentage of irradiated and non-irradiated seedlings of hard wheat. The results of **Kiong et al., (2008)** have shown that survival of plants to maturity depends on the nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with increasing radiation dose may be responsible for less germinability and reduction in plant growth and survival. In another study by **Kiong et al., (2008),** it was found that radiation increases plant sensitivity to gamma rays and this may be caused by the reduced amount of endogenous growth regulators, especially the cytokines, as a result of breakdown, or lack of synthesis, due to radiation. The stimulating causes of gamma ray on germination may be certified to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seeds irradiated (**Abdel-Hady et al., 2008**).

Table 4: Survival percentage of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

Irradiation treatments (Gy)	Survival%
Control	100.00 [a]
5.0	100.00 [a]
15.0	100.00 [a]
30.0	76.67 [b]
45.0	33.33 [c]
60.0	13.33 [d]
75.0*	0.00 [e]
LSD	7.09

* Lethal dose

1.2. Length of plantlets:

The length of the plantlets produced from irradiated and unirradiated lavender plantlets are presented in Figure (1) and Plate (2). Data indicated that applying irradiation dose level of 15 Gy produced the maximum plantlets length in the three multiplication stages since they reached 8.00, 9.10 and 10.03 cm, respectively, followed by 5 Gy treatments and reached 7.60, 8.20 and 9.13 cm, respectively. A gradually decline could be observed in the plantlet lengths by increasing the doses of radiations starting from 15 Gy tell reached to 60 Gy of which gave the shortest plantlet 2.93, 4.63 and 5.70 cm, respectively. It is clear that the dose level of 60 Gy exhibited the most inhibition effect on plantlets elongation.

1.3. Leaves number of plantlets:

The data presented in Figure (2) showed that the γ-irradiation at dose level 15 Gy gave the highest number of leaves per plantlet during the three multiplication stages (M1, M2, and M3) and the values were, 14.67, 15.33 and 16.67, respectively, followed by 5 Gy treatment (12.67, 14.76 and 15.33, respectively). On the other hand, the high dose level of 60 Gy significantly decreased the leaves number.

Physiological symptoms in a large range of plants exposed to gamma rays have been described by many researchers (**Kim et al., 2004; Kovács and Keresztes, 2002**). Our results are in agreement with **Youssef et al. (1998)** on *Melaleuca armillaris* who reported that, gamma irradiation at 20 Gy produced the tallest shootlets, and **Maraei, et al. (2006)** on strawberry mentioned that, the irradiation dose level 50 Gy, was the optimum dose, resulted in the tallest plantlets in the three multiplication stages. Also, same results were observed by **Hussein et al. (1995)** on *Datura meta* and **Maraei, et al. (2006)** on strawberry indicated that the highest number of leaves/plant were obtained by the dose of 50 Gy which might be attributed to a disturbance in the metabolic processes in shootlets cell divisions and enlargement. The stimulating effect of low doses of γ-irradiation on plant growth may be due to activation of cell division or cell elongation, alteration of metabolic processes that affect synthesis of phytohormones or nucleic acids (**Pitirmovae, 1979**). Radio-stimulation at low doses may be explained due to indirect role of radiation formation of free radicals. The hydroxyl (HO•) and superoxide anion (O2•-) radicals that are generated by radiation could modify the molecular properties of the proteins and lipids causing oxidative modifications of the proteins and lipid peroxidation (LP) (**Halliwell and Gutteridge, 1989**). Another role for stimulation was declared by **Bhatt et al. (2008)** who suggested that there might be two reasons of radiation effect on increasing endogenous hormones: Irradiation causes de novo synthesis of free hormone level to overcome the physical stress, or due to radiation effect, conjugated forms are converted to free form. Concerning low doses of irradiation on proteins can involve fragmentation, cross-linking, aggregation and oxidation caused by oxygen radicals which are generated by water radiolysis (**Cho and Song, 2000)**. In contrast, the growth inhibition induced by the high-dose irradiation levels has been attributed to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome**.** All these reactions depend on the amount of dose which consequently can activate or inhibit the plant growth (**Preussa and Britta, 2003**).

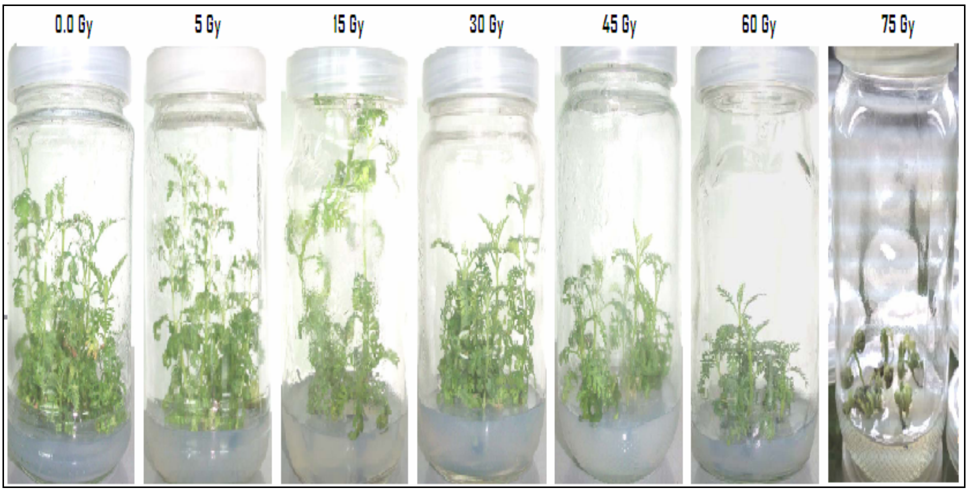

Plate (2): Length of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

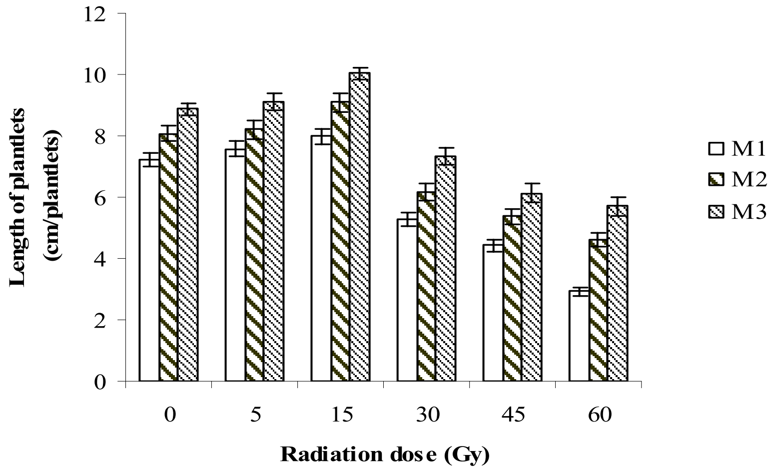

Figure (2): Length of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

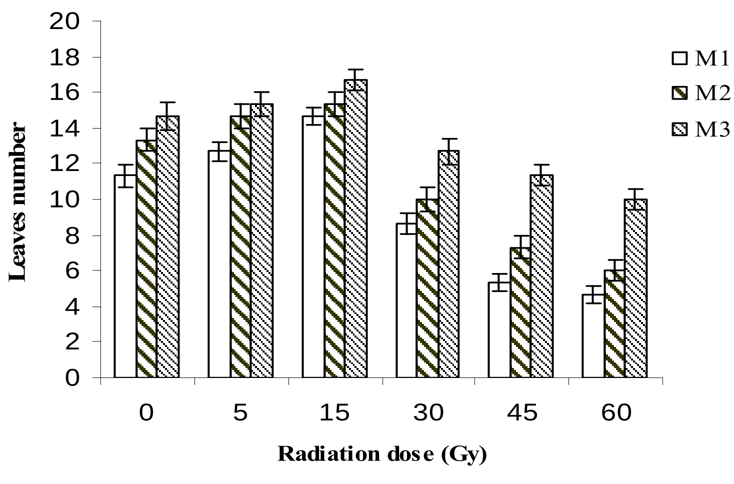

Figure (3): Leaves number of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

1.4. Plantlets moisture content and dry matter:

The effect of tested doses of gamma rays on moisture content and dry matter percentage of lavender plantlets is exhibited in Figures (4 and 5). Data showed that, no remarkable differences between the low doses and the control but the moisture% increased significantly at the high dose level of 75 Gy in zero time stage (M0) and 60 Gy in the other multiplication stages (M1, M2 and M3) compared with control samples in all multiplication stages. For the dry matter, data showed that, low dose of 5 Gy gave the maximum dry matter percentage in the three multiplication stages (M1, M2 and M3) 5.99, 5.60 and 5.53%, respectively followed by 15 Gy treatments which their values were, 5.49, 5.52 and 5.45%, respectively. On the other hand, dry matter decreased by increasing irradiation dose levels. However, the dose level of 60 Gy significantly decreased the dry matter percentage in the three multiplication stages 2.76, 3.17 and 3.34%, respectively. From the above results it could be generally stated that the dry matter decreased by increasing irradiation dose levels starting from 15 Gy. These results are in agreement with those observed by **Melki and Dahmani (2009)** on durum wheat (*Triticum durum* Desf.) who reported that the 20 Gy dose improved significantly the plants weight compared with the plants issued from non treated seeds.

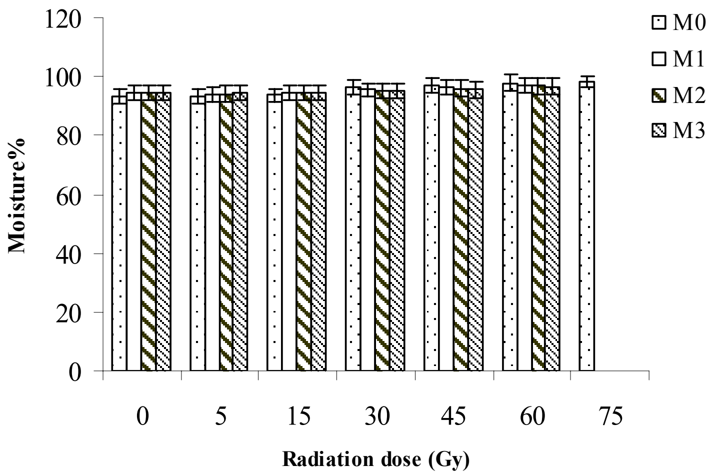

Figure (4): Moisture% of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

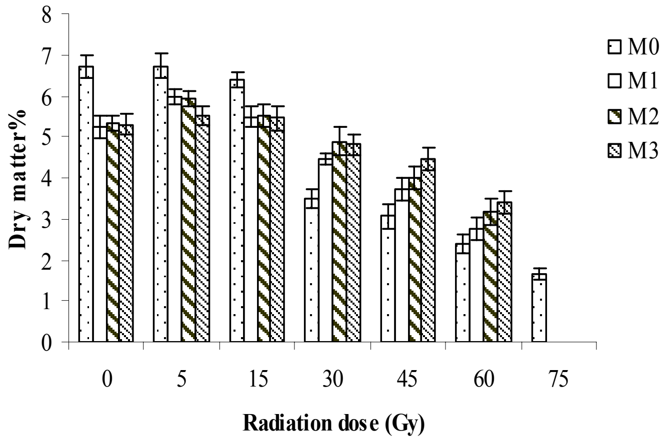

Figure (5): Dry matter% of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

Pigments content:

Photosynthesis is one of the most studied processes under the effects of gamma irradiation accompanied mainly by growth experiments. Despite the diversity of gamma ray targets in plants, it seems that the photosynthetic apparatus is among the main action sites of gamma rays (**Kiong et al., 2008).** Regarding the influence of γ-irradiation on the pigments content of the lavender plantlets which are presented in Figures (6-9), data showed that, in zero time stage there was no significant differences between treated and untreated plantlets. But in the others stages of multiplication (M1, M2, and M3) the data revealed that γ-irradiation treatments increased chlorophyll a by increasing γ-gamma irradiation dose levels. The highest value of chlorophyll a was observed by the application of dose level 15 Gy in the three multiplication stages 0.4797, 0.7361 and 0.3974 mg/g FW, respectively compared with their controls which gave, 0.3749, 0.3773 and 0.3903 mg/g FW. On the other hand, increasing the irradiation dose level more than 15 Gy decreased chlorophyll a content. The same trend was noticed also in the case of chlorophyll b, total chlorophyll and carotenoids. It seems that γ-irradiation at 15 Gy had the most enhancements dose the formation of chlorophyll b and carotenoids in comparison with the other applied doses. These results are in agreement with those pointed out by **Youssef et al. (1998)** on *Melaleuca armillaris,* and **Jia and Li (2008)** who reported that Chl *a*, Chl *b*, and Chl a+b contents decreased with elevated dose of γ-rays, in buckwheat (*Fagopyrum dibotrys* Hara). In another study by **Alikamanoglu et al. (2007),** however, an increase in chlorophyll a, b and total chlorophyll levels was observed in *Paulownia tomentosa* plants that were exposed to gamma irradiation. When compared to the non-irradiated plants, the total chlorophyll increased 20.6 % in *P. tomentosa* plants that were exposed to 80 Gy gamma dosage. Chlorophylls and carotenoids may be adversely affected by relatively high dosages of γ-irradiation, with carotenoids generally being less affected than the chlorophylls (**Pfundel et al., 1992**). Moreover, in the study on the effects of gamma irradiation on red pepper plants by **Kim et al., (2004),** plants irradiated at 16 Gy may have some significant increased in their chlorophyll content that can be correlated with stimulated growth. However, **Kim et al., (2004)** propoed that, chlorophylls are virtually insensitive to low dosage gamma irradiation whereas the levels of carotenoids decline in a dosage dependent manner at the same degree of irradiation. The decrease of chlorophyll concentrations may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation as well as for the damage caused to the photosynthetic apparatus **(Kim et al., 2004**). Furthermore, the reduction in chlorophyll b is due to more selective destruction of chlorophyll b biosynthesis or degradation of chlorophyll b precursors (**Marwood and Greenberg, 1996**).

Conclusion

In vitro propagation of *lavandula multifida* enables the mass production of the plantlets in short time. Gamma radiation applications are very important in mutation breeding in order to develop required features of plants and increasing the genetic variability. The results of this research showed that different doses of gamma irradiation has different effects on morphological, physiological and some biochemical plant characteristics such as increasing of chlorophyll content, stimulation plantlets growth. It could be concluded that under such conditions of the experiment, it is advisable to using 15 Gy of low gamma irradiation dose which was the best for making stimulation effect on many desirable characteristics. Also, this technique can be used for production of a mutant with ability for environmental stress tolerance.

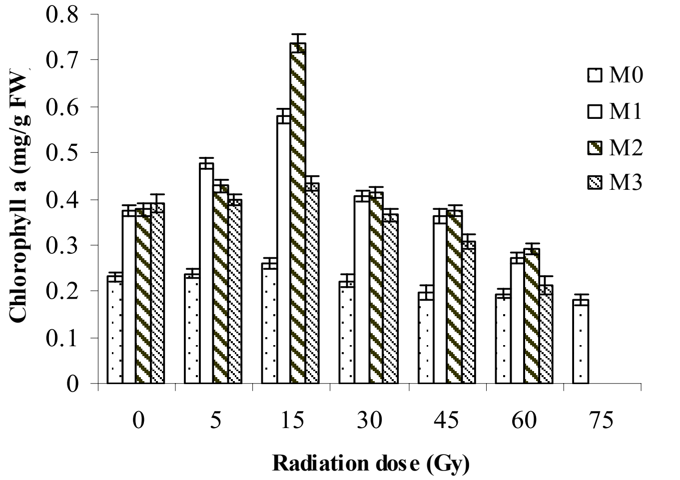

Figure (6): Chlorophyll a content (mg/g FW) of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

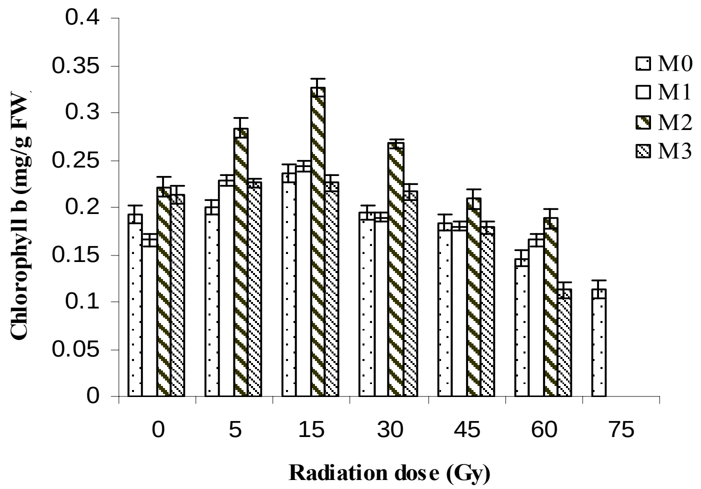

Figure (7): Chlorophyll b content (mg/g FW) of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

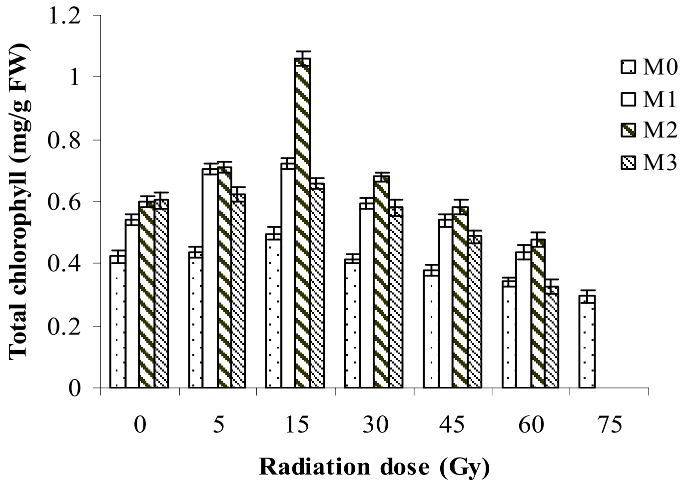

Figure (8): Total chlorophyll content (mg/g FW) of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

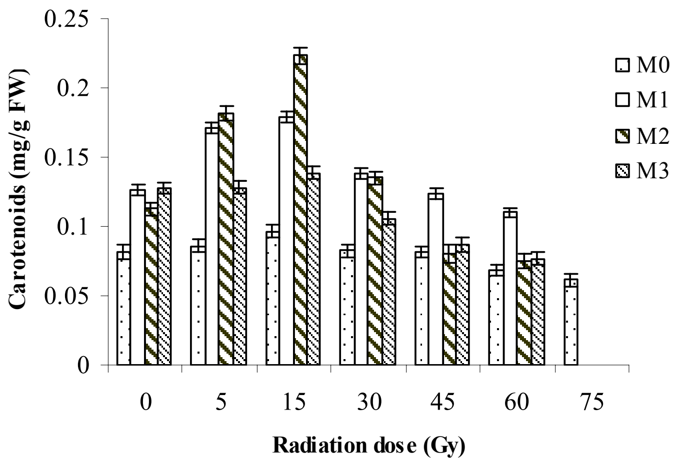

Figure (9): Carotenoids content (mg/g FW) of lavender plantlets (*Lavandula multifida*) produced from tissue culture of irradiated and unirradiated plantlets.

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التأثير المحفز لأشعة جاما علي النمو والنشاط الفسيولوجى لنبيتات اللافندر (*Lavandula multifida*)

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أجريت هذا ابحث لدراسة تأثير أشعة جاما على التضاعف والخواص الفسيولوجية لنبيتات اللافندر (*Lavandula multifida*). حيث تم تعرض نبيتات اللافندر الى سبعة جرعات مختلفة من أشعة جاما و هي ، ٠ ، ٥، ١٥، ٣٠، ٤٥، ٦٠، ٧٥ جراي . بعد ذلك تم تقسيم النبيتات لبدء عملية التضاعف كل اربعة اسابيع لثلاث مراحل متتالية (M1, .M2, M3) .

أظهرت النتائج :

ان الجرعات المنخفضة من أشعة جاما (٥- ١٥ جراى) أدت الي زياده النمو و الصفات المورفولوجية والفسيولوجية للنبيتات. لوحظ ان النسبة المئوية للبقاء فى الجرعات ٥ و ١٥ جراى لم يكن هناك اختلافات بينهما و بين الكنترول. بينما بزيادة الجرعة عن ١٥ جراى أدي الي نقص النسبة المئوية لبقاء النبيتات حية.

- الجرعة ١٥ جراي أدت لزيادة معنوية فى طول النبيتات خلال مراحل التضاعف الثلاثة (M1, M2, M3) و كانت النتائج علي التوالي هي ٨,٠٠ و ٩,١٠ و ١٠,٠٣ سم و كذلك لوحظ نفس الاتجاه حدث فى عدد الاوراق.

لم تكن هناك اختلافات معنوية في محتوى الرطوبة بالنسبة لنبيتات اللافندر في جميع مراحل التضاعف المختلفة فيما عدا الجرعة ٦٠ و ٧٥ جراي أعطوا أعلى محتوى للرطوبة عن الكنترول. بينما انخفض محتوى المادة الجافة بزيادة الجرعة الاشعاعية المستخدمة.

ومن الجدير بالذكر أنه لوحظت أعلى قيمة من الكلوروفيل أ، ب والكاروتينويد تم الحصول عليها بالجرعة ١٥ جراي خلال مراحل التضاعف الثلاثة.

وعموما أشارت النتائج الي ان معاملة النبيتات قبل مرحلة التضاعف بالجرعات المنخفضة من أشعة جاما يمكن أستخدامها و ذلك لتحسين النمو والخصائص الفسيولوجية للنبيتات والتي يمكن أن تساعد النباتات على التغلب على الأجهاد البيئي .