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EFFECT OF GAMMA IRRADIATION AND ADDITIVES ON THE PRODUCTION AND CHEMICAL COMPOSITION OF SWEET BASIL THROUGH TISSUE CULTURE

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ABSTRACT

In vitro propagated plantlets of *Ocimum basilicum* L. were placed on different thidiazuron (0.0,0.1,0.2 and 0.3mg/l), different additives (adenine sulfate, malt extract, casein hydrolyzate and yeast extract) for two months after culturing on MS medium supplemented with 30 g/l sucrose. Growing explants of *Ocimum basilicum* L. cultured on MS medium with 0.2 mg/l thidiazuron gave the highest length and the greatest number of shoots than other thidiazuron treatments. The medium containing malt extract produced the greatest values of callus maturation and the highest number of shoots. Plantlets were cultured on MS medium containing BA at different concentrations to select the best BA concentration to induce the best proliferation and then plantlets were gamma irradiated at doses 0.0, 0.5, 1, and 15 Gray, and placed on the same conditions. Irradiated plantlets induced changes in shoot propagation and leaf size of plantlets. Using gamma irradiation at level of 1 and 1.5 Gray stimulated either leaf shape or the size of stem. Essential oil contents increased with increasing some doses of gamma irradiation.

***Key words**: chemical composition, gamma irradiation, sweet basil, tissue culture.*

1. INTRODUCTION

Ocimum basilicum (common basil), *O. americanum* and *O. micranthum* are members of the family Lamiaceae. These plants, and oils from them, have received attention for their potential medicinal properties. Of these plants, *O. basilicum* is the most widely used. It is used in cosmetics, liqueurs, medicines, and perfumes. There are many compounds in Ocimum spp.,the dried leaves of *O. basilicum* contain 0.20-1% essential oil. The major compounds in the oil are linalool and methylchaviol. Both the essential oil, leaves, seeds, flowers, and roots of Ocimum spp. are used as medicines. The essential oil has been shown *in vitro* to have antibacterial activity against *Staphylococcus aureus, Salmonella enteritidis* and *Escherichia coli,* antiseptic activity against *Proteus vulgaris, Bacillus* and *Salmonella paratyph,* and antifungal activity against *Candida albicans, Penicillium notatum,* and *Microsporeum Grayseum.* Choudhary (1991) on rose , reported that MS medium supplemented with 2.5 mg/L BA caused the greatest proliferation. Nathan (1992) on Agloanema plants, found greatest axillary branches with BA at 26.6 Um. Kozak and Dabski (1995) on *Hypocyrta glabra,* found that shoot proliferation was greatest with MS medium containing 1mg/L BA., that the famous tissue culture medium is Murashige and Skoog (1962); it is very popular and most plantlets react to it favourably. Sakane and Katano (1997) on *Enkianthus perulatus* found that adding sulfate in Gamborg medium was adequate for shoot tip growth than MS medium.

Malathy and Pai (1998) working on *Ixora singaporensis* showed that adding adenine sulfate to MS medium was the best for shooting. Eapen (1976) reported that exposing cells to gamma irradiation at doses 0.5-2 Gray was able to produce callus and regenerated buds of tobacco.

Revishvili (1969) on geranium plants, showed that the chemical components decreased at higher doses of gamma irradiation. Ochatt (1991) on *Lonicera nitta,* found that addition of 250 mg/l casein hydrolysate promoted rhizogenesis in callus. Radojevic and Subotic (1992) on *Iris setosa,* reported the formation of embryogenic callus with MS medium supplemented with 250 mg/l casein hydrolysate. Singh and Sehgal (1999) found that cutting young inflorescences of *Ocimum sanctum* on MS medium containing TDZ produced only non-morphogenic callus. Binder *et*

al., (1999) on *Mellisa officinalis* tissue culture, reported the presence of alcohols and acetate esters using headspace analysis. Zheljazkov *et al.,* (1996) on *Mentha piperatae*, found that the oil was increased after the treatment with 0.2 and 1 Gray of gamma irradiation. Faure *et al.,* (1998) on spearmint and peppermint, found that adding 0.5 mM TDZ to MS culturing media enhanced regeneration to 78% and 49%, respectively. Hussen *et al.,* (2004) found that adding TDZ on the culture media after BA produced increasing frequency of shoot regeneration and shoot number of *Tamarindus indica in vitro.*

2. MATERIALS AND METHODS

This investigation was carried out in the Plant Tissue Culture Lab. of the National Products Departement, the National Center for Radiation Res. and Technology, Nasr City, Cairo. during 2006. Shoot tip explants of *Ocimum basilicum* L. cultured in jars containing basic MS medium (Murashige and Skoog,1962) supplemented with TDZ concentrations (0.0 ,0.1 ,0.2 and 0.3 mg/l) and 30 g/l sucrose to produce shoots and callus formation, then incubated in a growth room at 25±2 °C and light (16/8 L/ D) for 30 days to select best growth. After 4 weeks *in vitro,* growing plantlets were transferred to MS culture media contaning different additives (adenine sulfate, malt extract, casein hydrolysate and yeast extract) to detect the most effective additive that maximize growth of sweet basil plants, and then plantlets were cultured on MS medium containing different BA concentrations, and then exposed to gamma irradiation from Co 60 source at the National Center for Radiation Research and TechnoloGray, Atomic EnerGray Authority, Nasr City, Cairo. Gamma treatments were used at 5, 10, 15 Gray at dose rate of 1.9Gray/min. Plantlets were subcultured three times and after the third subculture, the regenerated plantlets were selected to determine the essential oil contents.

2.1. Statistical analysis

The obtained data were subjected to analysis of variance and statistically analyzed according to (Duncans, 1955) at 1% level.

3. RESULTS AND DISCUSSION

3.1. Effect of TDZ on *in vitro* parameters of *Ocimum basilicum* L.

As shown in Table (1), the results showed an increase in callus formation with adding TDZ at the maximum concentration (0.3mg/l). Survival percentage was decreased with increasing TDZ concentrations to the lowest percentage (70%) with 0.3 mg/l. Number of shoots and length of shoots increased to the highest values (3.55 and 4.21), respectively with 0.2 mg/l TDZ., while the number of leaves was increased to the highest number of leaves with 0.1 mg/l TDZ and then decreased to the lowest number of leaves (1.50) with 0.3 mg/l TDZ. These result are in agreement with the findings of Faure *et al.,* (1998) on spearmint and peppermint.

3.2. Effect of different additives on *in vitro* parameters of *Ocimum basilicum* L.

As shown in Table (2), it is quite, evident that adding malt extract to the culture medium produced the highest values of callus formation than other additives. Also these additives reached the maximum number of shoots; 4.53 per callus and the highest regenerated plantlets (6.8), while adding adenine sulfate and yeast extract on the culture medium gave the lowest values (1.30 and 2.31) regenerated plantlets. These results mean that malt extract is considered the best additive. These results are in harmony with the findings of Ochatt (1991) on *Lonicera nitta.*

3.3.Effect of BA concentrations on *in vitro* parameters of *Ocimum basilicum* L.

As shown in Table (3), the results indicate that the control treatment recorded the highest survival percentage (100%) in comparison with all BA treatments which showed a slight or moderate decrease from 96-90 %. The data also showed increase in the number of shoots, length of shoots and number of leaves with increase BA in the culture media to 0.5 and 1 mg/l than the other treatments, while callus formation was increased with adding 2 mg/l BA to MS culture medium. These results are in line with Choudhary (1991) on rose.

3.4. Effect of gamma irradiation treatments on *in vitro* parameters of *Ocimum basilicum* L.

The data in Table (4) showed that increasing gamma irradiation caused a decrease in survival percentage to the lowest value (95%) with 10 and 15 Gray than untreated control plantlets. Data also showed that the number of shoots recorded highest value with control also length of shoots (6 cm). While the number of leaves was increased with 5 and 10Gray to greatest number of leaves (6) than other treatments. These results are in line with those of Eapen (1976) on tobacco cells .

Table (1): Effect of thidizuron (TDZ) on *in vitro* parameters of *Ocimum basilicum* L.

TDZ Treatments mg/l	Survival %	Callus formation	No. of shoots	Length of shoots	No. of leaves
0	98A	+	1.32C	1.00D	3.11A
0.1	90B	+	1.85B	2.13C	3.22A
0.2	80C	++	3.35A	4.21A	2.01B
0.3	70D	+++	1.15C	2.50B	1.50C

Table (2): Effect of different additives on callus formation and growth of *Ocimum basilicum* L. *in vitro*.

Treatments	Survival	Callus formation	No. of shoots	No. of regent plants
Adenin sulfate	90A	+	1.20C	1.30D
Malt ext.	85B	+++	4.53A	6.80A
Casen hydrolysate	70C	+	2.01B	4.12B
Yeast ext.	50D	+	1.30C	2.31C

Table (3): Effect of BA concentration on growth and proliferation of *Ocimum basilicum* L. *in vitro*.

BA conc. Mg/l	Survival %	Callus formation	No. of shoots	Length of shoots	No. of leaves
0	100A	+	1.40C	1.20C	1.50C
0.5	96A`	+	3.00B	4.00A	2.50B
1	95A	++	3.70A	2.70B	3.00A
2	90B	+++	1.90C	1.50C	1.70C

Table (4): Effect of gamma irradiation on growth and proliferation of *Ocimum basilicum* L. *in vitro*.

Gamma rays	Survival %	No. of shoots	Length of shoots	No. of leaves
0	100A	4A	6.0A	5B
0.5K	98A	2C	4.0B	6A
1K	95B	2C	4.5B	6A
1.5K	95B	3B	2.5C	4C

Table (5): Effect of gamma irradiation on chemical composition of *Ocimum basilicum* L. *in vitro*.

Treatments	1-8 cineole	Linalool	Methyl chavicol	Eugenol	Beta caryophylle	Cadinol
0	1.31	13.39	52.48	11.71	2.37	1.18
0.5K	1.43	12.84	62.10	12.11	10.93	1.23
1K	1.21	11.93	53.20	10.07	2.10	1.11
2K	1.14	11.91	43.86	10.11	2.11	1.13

3.5. Effect of gamma irradiation treatments on chemical composition of *Ocimum basilicum* L.

Data in Table (5) revealed that the control treatment reached the highest value of linalool content (13.39), but the treatment 15Gray gave the lowest content (11.91 mg/100g), also the use of 5 Gray produced the highest values of 1-8 cineole, methyl chavicol,eugenol,beta caryophyllin, and cadinol conttents (1.43, 62.1, 12.11, 10.93 and 1.23) respectively. The chemical composition was decreased with high doses of gamma irradiation treatments. The treatment of low gamma irradiation doses (5Gray) is considered the best which improved the essential oil contents of *Ocimum basilicum* plants. These results are in harmony with those of Revishvili (1969) on geranium plants.

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تاثير اشعة جاما والاضافات علي الانتاج والتركيب الكيميائي لنبات الريحان بزراعة الأنسجة

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ملخص

تم دراسة اثراستخدام بعض الاضافات الطبيعية والكيميائية وكذلك اشباه السيتوكينين والتعرض لجرعات مختلفة من اشعة جاما علي تطور النمو وانتاج نباتات الريحان، كما تم دراسة التغيير الذي حدث في نسبة المركبات الثانوية الموجودة ومكونات الزيت العطري داخل النبات .

وقد اوضحت النتائج المتحصل عليها ما يلي :-

- ادي تحضين النباتات المنزرعة علي بيئة موراشيجي وسكوج المزودة بتركيزات مختلفة من السيتيزرون الي وجود اختلافات في النمو في مختلف التركيزات.
- زيادة نسبة النموات المتكونة من نبات الريحان وزيادة اطوال النموات المتكونة مع استخدام تركيز ٠,٢ مليجرام /اللتر بالمقارنة بالتركيزات الاخري .

- زيادة اعداد النموات واعداد الاوراق المتكونة من نباتات الريحان عند اضافة المولات الي بيئة الزراعة المستخدمة وكذلك زيادة نسبة الكالس المتكون بالمقارنة بباقي الاضافات.
- زيادة عدد النموات المتكونة واعداد الاوراق باضافة البنزايل ادنين الي بيئة الزراعة المستخدمة وذلك بتركيز ١ ملليجرام/ لتر بينما ادي استخدام تركيز ٢ ملليجرام/ لتر الي زيادة الكالس المتكون بالمقارنة بباقي المعاملات .
- ادي تشعيع النموات باشعة جاما بتركيزات ١٥ جراي الي زيادة سمك الاوراق والسيقان للنموات المتكونة مقارنة بالنموات غير المعاملة بينما ادت المعاملة بتركيز ٥ ، ١٠ جراي الي زيادة اعداد النموات والاوراق المتكونة.
- زيادة النسبة الكلية للمركبات الثانوية خاصة مع ٥ جراي التي اعطت اعلي نسبة للميثايل شافينيكول والايوجينول (٦٢,١جرام و ١٢,١١/١٠٠ جرام) للنموات المتكونة.
- اظهرت النتائج وجود نقص في بعض المركبات الثانوية في النموات المعاملة بالاشعاع بجرعة ١٠ جراي وكانت النسبة اقل من النموات غير المشععة في بعض المركبات الموجودة.