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THE USE OF GAMMA RAYS FOR INDUCTION OF USEFUL MUTATIONS AND MICROPROPAGATION OF MARJORAM (*MAJORANA HORTENSIS* MOENCH)

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ABSTRACT

The present study aimed to induce useful mutations and increase variability in marjoram population, as well as trial to micropropagation of marjoram. Seeds of Marjoram (*Marjorana hortensis* Moench) were irradiated at three γ-ray doses (50, 100 and 150 Gy). The effect of gamma ray dose for induction of genetic changes was decreasing with the increasing in the dose at M_2 generation. The 100 Gy of gamma ray consider as a favorable dose for induction of changes in marjoram. Four morphological mutants were isolated at M_2 generation; that possessed hairless, dark-green leaves and large leaves. The mutant line No.3 consider as a promising mutant for its high oil content and shoot dry weight followed by mutant No.4. The 50 Gy dose of gamma rays was the most effective dose in inducing of useful mutations for oil content (h^2 = 0.80). Leaf explant was more suitable than stem explant for callus induction and the hormone balance (3.0 mg/L BAP + 0.5 mg/ L NAA) followed by (1.0 mg/L BAP + 0.5 mg/L NAA) consider as more suitable for callus induction. In the second trial, stem explant was more suitable than leaf explant for callus induction No relationship between explants and hormone concentration for callus induction was found (chi-square was non-significant). The medium 3.0 mg/L BAP + 0.5 mg/L NAA only, which gave plant regeneration (shoot formation) in the same medium.

Keywords: Mutations, γ-rays, micropropagation, *Marjorana hortensis* Moench

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INTRODUCTION

Majorana hortensis Moench, commonly known as 'sweet marjoram' is a member of the family Lamiaceae. It is a perennial herb native to Cyprus and eastern Mediterranean countries (Letswaart, 1980). The plant is propagated by seeds and tender stem cuttings. This plant is characterized by a strong, sweet, spicy pleasant odour. The leaves are used fresh or dried and are highly esteemed as a condiment for seasoning food products. The aerial parts of the plants are used for the isolation of oil, which has many uses in the flavour, perfumery and pharmaceutical industries. In the food industry, it is mainly used as a spice in sausages but its use in backed goods, processed vegetables, condiments, soups, snack foods and gravies has also been reported (Burdock, 1995). In addition to this, marjoram is well known for its medicinal value (Chevallier, 1996). The plant is also reported to possess anticancer (Hartwell, 1969), antioxidant and antifungal properties (Pruthi, 1980).

Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like morphology, cytogenetics, biotechnology and molecular biology etc. Mutation breeding has become increasingly popular in recent as an effective tool for crop improvement (Acharya, *et al.*, 2007) and an efficient means supplementing existing gemplasm for cultivar improvement in breeding programs (Dubinin, 1961).

A change in composition of the oil may be induced through mutations that would favourably alter the quantity of terpenes and minor constituents (Srivastava and Tyagi, 1986). The generation of new variability is necessary and new techniques are needed to circumvent the present barriers for improvement. Mutation breeding is most promising for the induction of hereditary metabolic blocks suppressing the conversion or degradation of economically important active principle (Srivastava and Tyagi, 1986). Numerous investigations have pointed to the usefulness of induced genetic variation in the biosynthesis of alkaloids and other secondary metabolites (Hegnauer, 1975; Levy, 1982; Borssman and Wilcox, 1984 and Srivastava, 1984).

Endemic plants greatly contribute to the richnes and diversity of the flora of Turkey. Bajaj *et al.* (1988) have been pointed out the growing interest worldwide in medicinal and aromatic plants. Conservation of endemic, endangered, medicinal and aromatic plants is beyond regional scope and becomes of global significance (Iucn, 2001). They should be protected by different methods including in vitro culture. *In vitro* propagation is a suitable method for plant regeneration, micro propagation and long-term storage of plant material.

There is a few information about *in vitro* culture of *Origanum* species in the current literature (Kumari and Saradhi, 1992; Crutis and Shetty, 1996; Iyer and Pai, 2000; Tisserat and Vaughn, 2004; Minnas; 2001). Plants belonging to genus *Origanum* L. (Lamiaceae) are represented by 25 taxon in Turkey and 16 of them are endemic (Davis *et al.*, 1982; Kitiki, 1996 and Duman *et al.*, 2000). Some species of these plants are well known in Anatolian folk medicine and widely used as spices and herbal tea (Baytop, 1983). They are great economic importance which is not only elated to their use as in fact, as recent studies have pointed out, oregano is used traditionally in many other ways as their essential oils have antimicrobial, antifungicidal cytotoxic, antiviral maticidal and antioxidant properties (Lagouri *et al.*, 1993; Sivropoulou *et al.*, 1996).

Therefore, the present study aimed to induction of useful mutations and increase of variability into marjoram population, as well as trial to micropropagation of marjoram.

MATERIALS AND METHODS

This investigation was carried out during seasons 2007, 2008 and 2009 at green house of Faculty of Agriculture, Zagazig University.

Materials

Plant variety

French variety of marjoram (*Marjorana hortensis* Moench) was used in the present study.

Mutagen

Gamma rays as physical mutagens were used by (50,100 and 150) Gray (Gy).

Methods

Mutation induction in marjoram

Procedures of mutagens treatment

A sample of dry well filled seeds from marjoram (*Marjorana hortensis* Moench) variety was arranged in monolayer in polyethylene bags and subjected to actual doses 50 Gy, 100 Gy and 150 Gy. The exposure time was exactly adjusted to allow the seeds to receive the predetermined dose.

Post- treatment handling of the material

The irradiated seeds of each treatment along 200 untreated control seeds were planted into small plot. After three months, the M_1 seedlings were transplanted into the field at the rate of one plant per hill with a close spacing of "15 × 25" cm in the season of 2007. Fertilization; irrigation and other ordinary field practices were done.

Harvesting M_1 plants and raising M_2 families

After discarding all most plants exhibiting drastic morphological changes or complete sterility, the remaining M_1 plants were harvested separately in each treatment from the respective control material only 50 randomly chosen plants were individually harvested.

In M_2 generation, the seeds of each M_1 plant constituting M_2 families were sown in small plot in a nursery in the field in the season of 2008. After three months the survived seedlings were transplanted into family rows. The control of each variety was represented by 50 plant families.

Screening of mutation for M_2 generation

The M_2 plants were individually screened in the season of 2008 for any apparent morphological change. Screening was done periodically the entire growing season up to maturity. The morphological changes observed were those affecting plant height, change in colour of plant and any morphological changes were assessed after harvesting the seed for each mutant were collected in the laboratory.

Generally, the following criteria were studied for each induced morphological mutants at M_2 generation: Plant height, fresh

weight, dry weight, No. of branches/plant and oil content.

Raising the progenies in M_3 generation

Seeds of mutant lines (four mutant lines) and 10 plants from each treatment were grown and transplanted in the field in the season of 2009, after three months the seedling for all lines were transplant at two replicates as one row in each line. Field practices were similar to those observed in growing of M_1 and M_2 generations.

Studies on mutant progenies

The mutant progenies were carefully and continuously observed in the field in order to confirm their breeding behavior.

These mutants were studied for following characters: Plant height, fresh weight, dry Weight, oil content and change in the leaf colour.

Marjoram micropropagation

Tissue culture was carried out at plant Biotechnology laboratory, Department of Genetics, Faculty of Agriculture, Zagazig University.

***In vitro* propagation (Tissue culture) procedure**

1- Stem and leaves were collected from adult plants grown in the field segments 3-4 mm long were sampled from each node (axillary bud).

2- Stem and leaf samples were sterilized by immersion in 70 % ethanol for 10 sec. followed by three washes using sterile distilled water then immersed in 10% of commercial Clorox solution for 15 min, these stem and leaves were sub sepuently rinsed several times with distilled sterile water. Three segments of air draining stem and leaves were cultured aseptically on modified MS-medium. The PH of these media was adjusted to 5.8 by addition of 0.1 HCL or NaOH. All cultures were solidified by 0.7 agar added prior autoclaving at 1.2 Kg/Cm^2 for 15 min.

Enhancement of different morphological responses

The following content ratios of 2.4-D (dichloro phenoxy acetic acid) and kinetin (N-6-furfuyl adenine) or NAA (nephthalene acetic acid) and BA (6-benzyamino purine) were added to culture medium at the following

concentrations for enhancement of different morphological responses

1- MS medium free hormones.
2- MS + 0.5 mg/L 2.4 D + 1 mg/L kin
3- MS+ 0.5mg/L 2.4 D + 2 mg/L kin
4- MS+ 0.5mg/L NAA + 1 mg/L BA
5- MS+ 0.5mg/L NAA + 3 mg /L BA
6- MS+ 0.5mg/L NAA + 5 mg/L BA

Cultures were incubated under light condition 16h/day photoperiod at intensity of 3000 lux from cool light fluorescent lamb for 30 days and maintained at 26 ± 1 ° C. Five replicates from each treatment were set up, the percentage of morphological responses for calli and calli differentiation were recorded.

Rooting medium:

MS Medium + 0.2 mg/L IBA

All branches resulting from directly or from callus differentiation were transplanted into rooting medium under more intensity of light.

Statistical analysis

Analysis of variance (hierarchical design) (Table1) was used for estimation of genotypic and phenotypic variation, estimation of heritability (h^2) and selection genetic gain (Gs) at M_3 generation.

Chi-square test was applied for micropropagation study for estimation of differences between media and explants as callus induction response.

RESULTS AND DISCUSSION

The Use of Gamma Rays for Induction of Mutation

The average mean of M_2 generation for plant height, No. of branches per plant, shoot fresh weight per plant, shoot dry weight per plant and oil content under control and three γ-ray doses (50, 100 and 150 Gy) were showed in Fig. 1. As general, 100 Gy does (10 kr) γ-ray consider as an optimum dose for increasing plant height, shoot dry weight and oil content. In contrast, 150 Gy from γ–ray were decreasing plant height, No. of branches per plant, shoot dry weight and oil content. Therefore, the 100 Gy from gamma ray consider as favorable dose, for induction of changes in marjoram.

Table 1. Analysis of variance and expected mean sum of squares

Replication of variance	d.f	S. S.	M. S.	Expected M. S.	F. value
Replication	r-1	$Zx_{g.}/\ nq\text{-}X^2../nqr=S^2$			
Between lines	n-1	$ZX_{i.}/\ rq\text{-}X^2../nqr=S^2{}_2$	V_1	$O^2{}_w + qO_1{}^2$	$\frac{V_1}{V_2}$
Within lines	rn(q-1)	$S^2{}_3\text{-}(S^2{}_1 + S_2{}^2)$	V_w	$O^2{}_w$	
Total	**rnq-1**	$\mathbf{ZX^2{}_{igk}\text{-}\ X^2../\ nqr=\ S_3{}^2}$			

Where:
r = number of replicates
n = number of lines.
q = number of plants in each line within replicate.
Xg = total of g_{th} replicate.
$\mathbf{X_i}$ = total of i_{th} line.
$\mathbf{X_{igk}}$ = observation of K_{th} plant in i_{th} line of g_{th} replicate.

The effect of gamma ray dose for induction of genetic changes was decreased with increasing the dose at M_2 generation (Fig. 1). These results confirmed with the findings of Girija and Dhanavel (2009) who studied the mutagenic effectiveness and efficiency of gamma rays in cowpea, they confirmed that mutagenic effectiveness and efficiency increased with the decreased in dose or concentration. In the other investigation, Srivastave and Tyagi (1986) studied the effects of seed irradiation on yield and quality of essential oil in palm arose (*Cymbopogon martimii* stapf), they reported that the yield and quality of oil at 10 kr and 15 kr doses were enhanced significantly and the results on high yield and quality of oil as induced by gamma irradiation were discussed form the point of view that a gene may have been altered by mutagen treatment to produce a metabolic block between geraniol and geranyl acetate on the biosynthetic pathway or that modifier genes controlling the expression of key precursor molecule may have been father modified to give rise to a geraniol rich chemotype in palmarose.

Very important morphological mutants were isolated at M_2 generation (Fig. 2). These mutants possessed hairless, dark-green leaves and large leaves. Four mutants were isolated at M_2 generation and grow as family for each mutant at M_3 generation. The behavior of these mutants at M_2 and M_3 generation were recorded in Table 2. From Table 2, the mutant line No.3 consider as a promising mutant whereas, it possess high oil content and shoot dry weight, followed by mutant No.4. These mutants require to more studies and conservation of them until new promising lines and cultivars.

Analysis of variance, heritability (h^2) and expected genetic advance (Gs) for four studied characters in M_3 families of marjoram (Table 3) confirmed that significant difference between lines for all studied characters, i.e., plant height, shoot fresh weight, shoot dry weight and oil content at three different doses of gamma-rays, while, the control was insignificance. These results confirmed that the gamma rays succeed in induction more variation in the studied important

criteria, which permit to selection at following generations for genetic improvement of yield characters, especially oil content. Moreover, heritability in brood-sense and expected genetic advance supported the above conclusion, whereas the high heritability estimates were recorded for all studied characters and confirmed that the importance of 50 Gy of gamma rays dose for induction of variation in oil content ($h^2 = 0.80$), as well as high expected genetic advance for all studied traits, especially oil content. These results confirmed the importance of physical mutagens as gamma rays for induction of new variation in marjoram as medicinal plants, for acceleration of genetic improvement of these important plants as local consumption and export.

The average mean for different families at M_3 generation to different treatments for oil content were showed in the Table 4. These results confirmed that same lines consider as promising lines for improvement of oil content as line No.8, and No. 6 in the doses of gamma rays, i, e, 50, 100 and 150 Gy, respectively. As well as the general mean for lines from three treatments was higher than the average mean of oil content of control.

Marjoram micropropagation

Micropropagation of marjoram as medicinal plant consider as main objective in these plants, for solving of mass production of medicinal plants, which suffering from nature propagation by seeds or cutting especially, to trial of chemotype marjoram production as a demanded pharmaceuticals at present and future . Callus formation from different explants (stem and leaf) was recorded by using MS medium and different concentrations of benzyl amino purine (BAP) and naphthalene acetic acid (NAA) (Table 5 and Fig. 3). Leaf explants consider as more suitable for callus formation than stem explant, whereas, highly significant difference between them. In the same way, hormone concentration appeared highly significant difference. Also, the hormone balance (3.0 mg/L BAP + 0.5 mg/L NAA) followed by (1.0 mg/L BAP + 0.5 mg/L NAA) consider as more suitable for callus induction (table 5 and fig. 3). Highest value from BAP consider as unsuitable dose for callus formation. The medium 3.0 mg/L BAP + 0.5 mg/L NAA only, which

gave plant regeneration (shoot formation). No relationship between explants and hormone concentration for callus induction (chi-square was insignificant) was found. These results confirmed with findings of Dudu őzkum, (2007) who studied *In vitro* shoot regeneration of oregano (*Origum. minutiflorum* O. Schwarz & Davis) and used different explants and different concentrations of BAP and NAA. MS medium supplemented with 2.0 mg/L BAP + 0.1 mg/L NAA was the most effective medium for shoot formation. As well as, the single nodel segments were the most successful explant in all used hormone concentrations. Multiple shoots were obtained from sweet marjoram (*Marjorana hortensis* Moench) nodel stem explants where cultured on Murashiga and Skoog (MS) medium supplemented with 6-benzylaminopurine at 2.0 mg/L only (Iyer and Pai, 1998).

Other trial for callus induction by using other hormone balance (table 6) reflect behavior on first trial stem explant under different concentration of hormones. Stem explant was more suitable than leaf explant for callus induction (Table 6 and Fig. 4), but two concentrations of hormone [(0.5 mg/L 2,4-D + 1.0 mg/L kin) and (0.5 mg/L 2, 4-D + 2.0 mg/L kin)] were insignificant between them for callus formation. No interaction between explants and hormone balances (chi- square for independence was not significant) also.

The formed callus in all media were transferred to MS medium supplemented by 3.0 mg/L BAP + 0.5 mg/L NAA and obtaining of shoot formation (Fig. 5). The formed shoots were transferred into rooting medium (0.2 mg/L IBA) and formation of roots (Fig. 6).

In the same way, it recorded in the study of Iyer and Pai (2000) where, callus was induced in stem explants in medium containing 0.4 mg/L 2,4-D. This callus showed organogenesis in MS medium containing 3.0 mg/L BAP and 0.2 mg/L IBA. Other study (Arafeh *et. al,* 2003*)* where, micropropagation of wild Syrian marjoram highest shoot proliferation was obtained when kinetin (0.4 mg/L) and BAP (0.8 or 1.2 mg/L) were used. TDZ failed to promote shoot proliferation and induced callus. In the same way, Borovec, 1988 found that the explants of lateral buds were successfully cultured on MS medium with 4.0mg/L BAP and 0.1 mg/L NAA and regenerated shoots formed a well-developed root system.

Table 2. The behaviors of selected mutants at M_2 and M_3 generations for studied characters

No. of mutants	Length (cm)		Fresh weight (gm)		Dry Weight (gm)		No. of Branches	Oil content (ml)
	M 2	M3	M 2	M3	M 2	M3	M 2	M3
control	44	34	20.11	19.9	5.44	7.18	15.0	0.8
1	46	35	21.18	23.48	4.59	9.18	12.0	1.04
2	48	30	69.40	27.47	26.84	11.54	36.0	0.66
3	42	37	65.36	35.75	23.63	16.59	27.0	1.26
4	32	41	67.33	50.03	24.34	19.74	30.0	1.20

Table 3. Analysis of variance, heritability and expected genetic advance for plant height, shoot fresh weight, shoot dry weight and oil content in M_3 families for marjoram (*Majorana hortensis* Moneach)

Mutant lines	source of variation	d.f	plant height			shoot fresh weight			shoot dry weight			oil content		
			M.S	h2	Gs	M.S	h2	Gs	M.S	h2	Gs	M.S	h2	Gs
control	Between Lines	18	55.144	—	—	198.147	—	—	56.033	—	—	0.502	—	—
	Within Lines	40	39.433			194.289			32.467			0.205		
5 GY	Between Lines	18	38.996**	0.67	4.69	569.218**	0.87	21.3	87.55667**	0.7	7.2	0.62253**	0.8	0.67
	Within Lines	40	5.5			26.464			11.1455			0.079		
10 GY	Between Lines	18	48.46**	0.67	5.29	261.582**	0.87	12.4	26.74889**	0.7	4.43	0.32128**	0.62	0.42
	Within Lines	40	6.566			32.988			1.98475			0.074		
15 GY	Between Lines	18	57.04**	0.71	5.89	465.652**	0.85	18.8	49.16833**	0.78	5.86	0.31301**	0.50	0.35
	Within Lines	40	6.9			26.812			4.13325			0.104		

** Highly significant at 0.01

Table 4. The oil content (mean ± SD) for different families (random selected) at M_3 generation to different treatments over replication

No. of family	Control	Treatment 50	Treatmen100	Treatment 150
1	1.345±0.083	1.313±0.0847	1.235±0.226	1.193±0.085
2	1.265±0.209	1.281±0.521	0.496±0.141	0.738±0.552
3	0.865±0.270	1.006±0.698	0.680±0.234	1.056±0.0357
4	0.796±0.289	1.548±0.687	0.584±0.037	0.684±0.315
5	0.352±0.126	1.398±0.360	0.904±0.341	0.545±0.355
6	1.101±0.265	1.979±0.503	1.055±0.425	1.445±0.215
7	1.484±0.068	1.143±0.340	0.830±0.304	0.812±0.130
8	1.266±0.331	1.565±0.337	1.351±0.120	1.084±0.005
9	0.171±0.254	1.539±0.290	1.471±0.220	1.290±0.360
10	0.0079±0.004	1.195±0.325	0.848±0.0920	0.511±0.314
General mean ± SD	0.865 ± 0.190	1.397 ± 0.415	0.945 ± 0.214	0.936 ± 0.237

Table 5. Callus induction frequencies of different explants and different hormone concentrations of marjoram (*Majorana hortensis* Moneach) at first trial

Growth regulator (mg/L)	Callus induction frequencies from		total
	Stem	Leaf	
0.5 NAA+ 1.0 BAP	3.0	25.0	28.0
0.5NAA +3.0 BAP	1.0	31.0	32.0
0.5 NAA+5.0 BAP	1.0	5.0	6.0
total	5.0	61.0	

	Computed	Tabled
χ^2 explants:	47.5	3.84
χ^2 media:	17.8	5.99
χ^2 independence	1.97	5.99

Table 6. Callus induction frequencies of different explants and different hormone concentrations of marjoram (*Majorana hortensis* Moneach) at second trial

Growth regulator (mg-L)	Callus induction frequencies from		total
	Stem	Leaf	
0.5 2,4-D +1.0 kin	19.0	6.0	25.0
0.5 2,4-D+ 2.0 kin	20.0	15.0	35.0
total	39.0	21.0	

	Computed	Tabled
χ^2 explants:	7.4	3.84
χ^2 media:	1.66	3.84
χ^2 independence	2.27	3.84

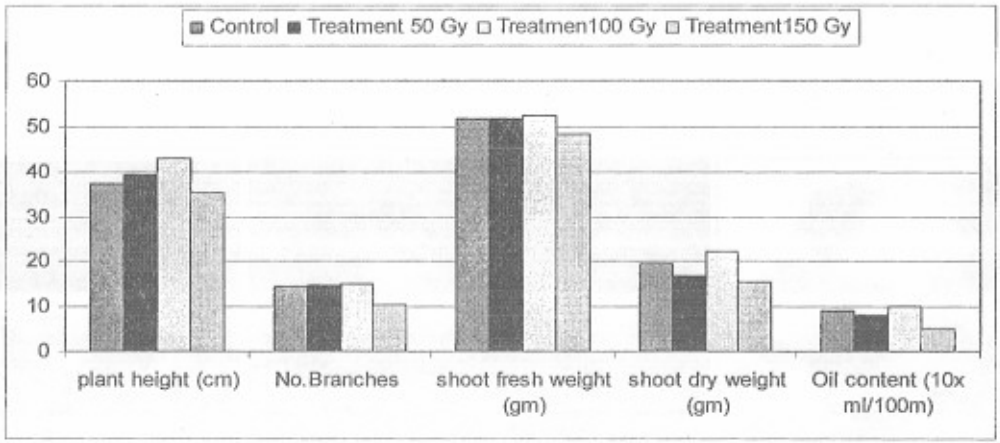

Fig. 1. The average mean of M_2 generation for plant height, No. of branches per plant, shoot fresh weight per plant, shoot dry weight per plant and oil content under control and three γ- ray doses (50, 100, 150) Gy

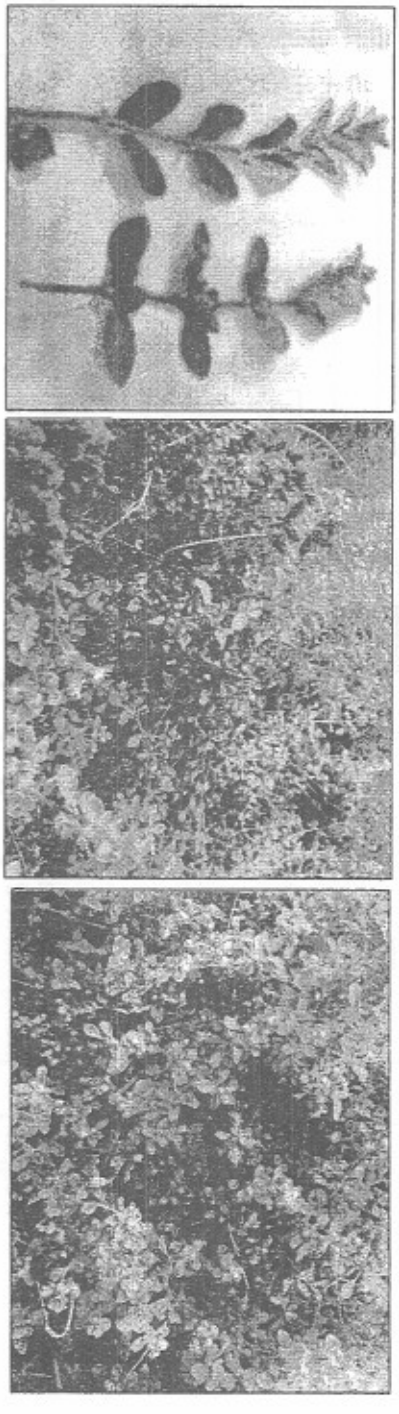

Fig. 2. Morphological mutants; hairless, dark-green leaves and large leaves comparison with normal type

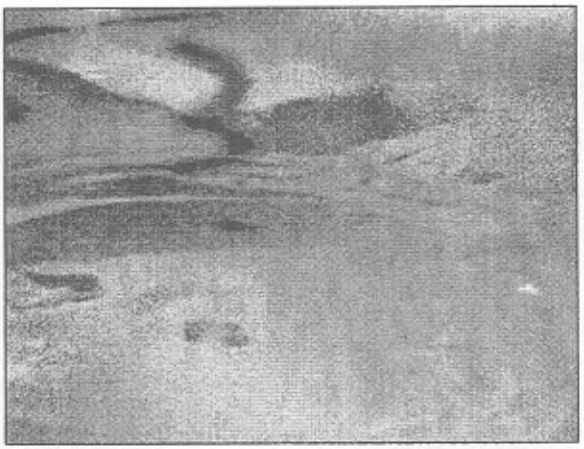

Fig. 3. Callus formation from leaf explant by using M.S medium and hormone balance (3.0 mg/L BAP + 0.5 mg/ L NAA)

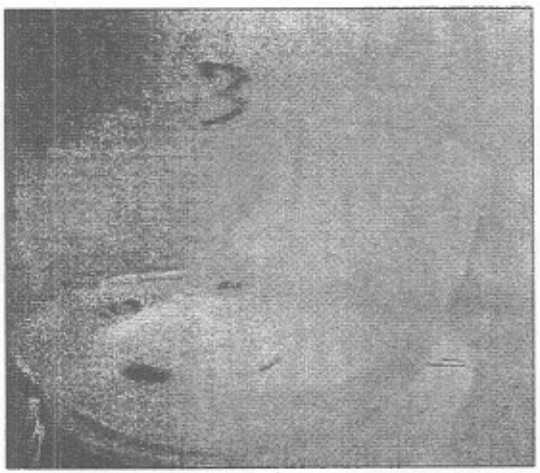

Fig. 4. Callus formation from stem explant by using M.S medium and hormone balance (0.5 mg/L 2, 4-D + 2.0 mg/ L Kin)

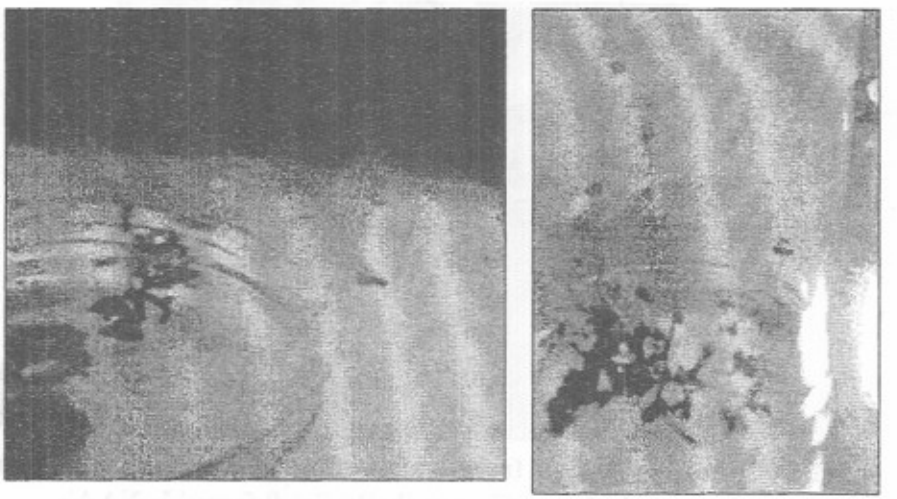

Fig. 5. Shoot formation by using M.S medium and hormone balance (3.0 mg/L BAP + 0.5 mg/ L NAA)

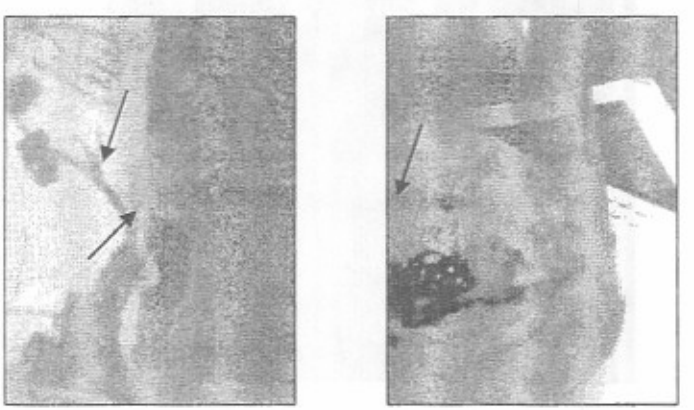

Fig. 6. Root formation into rooting medium (0.2 mg/L IBA)

REFERENCES

Acharya, S. N., J. E. Thomas and S. K. Basu 2007. Improvement in the medicinal and nutritional properties of fenugreek (*Trigonella foenum-graecum* L.). In: Acharya SN, Thomas JE (eds) Advances in medicinal plant research, Research Signpost, Trivandrum, Kerala, India.

Arafeh, R. M, M. S. Mahmoud and R. A. Shibli 2003. In vitro seed propagation of wild Syrian marjoram (*Origanum Syriacum* L). Advances in horticultural science.17(4):241-244.

Bajaj, Y. P. S, M. Furmanowa, and O. Olszowska 1988. Biotechnology of the micropropagation of medicinal and aromatic plants. p.. In: Y.P.S. Bajaj (Ed.) Biotechnology in Agriculture and Forestry 4. Medicinal and Aromatic Plants, I: 60-103

Baytop, A. 1983. Farmasotik Botanik, İstanbul Univ. Eczacılık Fak. Yayınları, 36, 282-285.

Borovec, v. 1988. Micropropagation of clones of marjoram (*Origanum Vulgare L*) under in vitro conditions. Vyzkumny a slechtitelsky ustav zelinarsky Olomouc.32 (4), 49-54.

Borssman, D. and J. R. Wilcox, 1984. Induction of genetic variation for oil properties and agronomic characteristics of soybean. Crop Sci. 24: 783-787.

Burdock, G. A. Ed. 1995. *Fenaroli's handbook of flavour ingredients Vol. 1: Natural flavours,* 3rd Edn., CRC Press, Boca Raton, FL, USA.

Chevallier, A. 1996. *The encyclopaedia of medicinal plants*, Dorling Kindersley, London, UK,.

Crutis, O. F. and K. Shetty, 1996. Growth medium effects of vitrification, total phenolics, chlorophyll and water content of *in vitro* propagated oregano clones. Acta Hort., 426, 489-497.

Davis, P. H., R. R. Mill and K. Tan, (Ed.) 1982. Flora of Turkey and the East Aegean Islands, Edinburgh Univ. Press, Edinburg, Vol. 7, pp. 186- 187 and pp. 307-308

Dubinin, N. P., 1961. Problems of radiation genetics. Oliver and Boyd, London.

Dudu őzkum 2007. *In Vitro* Shoot Regeneration of Oregano (Origanum minutiflorum O. Schwarz & Davis) Hacettepe J. Biol. & Chem., 35 (2),97-100

Duman, H., L. Origanum , A. Guner, , N. zhatay, T. Ekim and, K. H. K. Başer 2000. Flora of Turkey and East Aegean Islands, Edinburg University Press, Edinburg, Vol 11, pp. 207- 208.

Girija, M. and D. Dhanavel, 2009. Mutagenic effectiveness and efficiency of gamma rays ethyl methane sulphonate and their combined treatments in Cowpea (*Vigna unguiculata* L. Walp). Global Journal of Molecular Sciences 4 (2): 68-75

Hartwell, J. L. 1969. *Plants used against cancer. Asurvey*, Vol. 3(3), Lloydia, , p. 247.

Hegnauer, R. 1975. Secondary metabolites and crop plants. In: Frankel, O. H. and J. G. Hawkes (Eds), Crop genetic resources for today and tomorrow. Cambridge University Press, London, p. 249.

Iucn, 2001. Red List Categories: Version 3.1. Prepared By IUCN Species Survival Commission, IUCN, Gland Switzerland and Cambridge, UK,.

Iyer, P. V. and J. S. Pai. 1998. Micropropagation of sweet marjoram (*Majorana hortensis* Moench).Journal-of-spices and Aromatic Crops.7:1, (3)47-49.

Iyer, P. V. and J. S. Pai, 2000. In vitro regeneration of Majorana hortensis Moench from callus and nodel stem segments .Journal-of-spices and Aromatic Crops.9:1, (9), 47-50.

Kitiki, A. 1996. Status of Cultivation and Use of Oregano in Turkey , Proceedings of the IPGRI International Workshop on Oregano, CIHEAM, Valenzano (Bari), 122-132,.

Kumari, N. and P. P. Saradhi 1992. Regeneration of plants from callus cultures of *Origanum vulgare* L. Plant Cell Rep., 11 (9), 476-479.

Lagouri, V., G. Blekas, M. Tsimidou, S. Kokkini and D. Boskou 1993. Composition and antioxidant Activity of essential oils from oregano plants Grown wild in Greece. Z. Lebensm. Unters. Frosch,. 197: 20-23.

Letswaart, J. H. 1980. A *taxonomic revision of the genus*

Origanum (Labiatae), Leiden University Press, Leiden.

Levy, A. 1982. Natural and induced genetic variation in the biosynthesis of alkaloids and other secondary metabolites. In: Improvement of oil seed and industrial crops by induced mutations. IAEA, Vienna, pp. 213-222.

Minnas, G. J. 2001. Certain dittany apical meristem micropropagation in vitro, Miscellaneous-Reports. Agricultural Research Institute Ministry of Agriculture, Natural Resources and the Environment, No. 80, 7pp.

Pruthi, J. S. 1980. Spice and condiments: chemistry, microbiology, technology, Academic Press, New York, USA, p. 221.

Sivropoulou, A., E. Papanicolaou, C. Nikolaou, S. Kokkoni, T. Lanaras, and M. Arsenakis 1996. Antimicrobial and cytotoxic activities of Origanum Essential oils. J. Agric. Food Chem. 44: 1202-1205.

Srivastava, H. K. 1984. Prospects for genetic engineering in medicinal and aromatic plants. In: Y. S. Murti Commemoration Volume, Meerut University, Trends Pl. Res. 1: 327-341.

Srivastava, H. K and B. R. Tyagi 1986. Effects of seed irradiation on yield and quality of essential oil in palmarose (Cymbopogon Martinii Stapp),Euphytica 35 :369-380.

Tisserat, B. and S. F. Vaughn, 2004 .Techniques to improve growth, morphogenesis and secondary metabolism responses from Lamiaceae species in vitro. Proc. XXVI IHC-Future for Medicinal and Aromatic Plants Eds. L.E.Craker et al., Acta Hort. 629, ISHS, 333-339.

استخدام أشعة جاما لاستحداث طفرات مفيدة و الاكثار الدقيق للبردقوش

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تهدف هذه الدراسة الى الحصول على طفرات مفيدة و زيادة التنوع الوراثى فى البردقوش و كذلك الاكثار الدقيق له باستخدام زراعة الأنسجة. تم معاملة بذور البردقوش (الصنف الفرنسى) *Marjorana hortensis* Moench بثلاثة جرعات من أشعة جاما (50, 100 and 150 Gy) وحصلنا على الجيل الطفرى الأول M_1 و فى الجيل الطفرى الثانى M_2 أكدت النتائج أن تأثير أشعة جاما فى انتاج التغيرات الوراثية يقل بزيادة الجرعة المستخدمة من الأشعة و أن الجرعة 100 Gy أنسب الجرعات لانتاج طفرات فى البردقوش. تم الحصول على أربعة طفرات مورفولوجية تتميز بأوراق خضراء داكنة و عريضة و خالية من الشعيرات hairless و كذلك تم الحصول على طفرة مبشرة (No.3) ذات محتوى عالى من الزيت و الوزن الخضرى الجاف يليها الطفرة (No.4) وكانت الجرعة 50 Gy من أشعة جاما أنسب الجرعات للحصول على طفرات مفيدة محتواها عالى من الزيت. أكدت نتائج الاكثار الدقيق بزراعة الأنسجة فى المحاولة الأولى أن الأوراق هى أفضل منفصل نباتى explant عن أجزاء الساق فى انتاج الكالوس callus وكان أنسب اتزان هرمونى لذلك هو (3.0 mg/L BAP + 0.5 mg/ L NAA) يليه الاتزان الهرمونى (1.0 mg/L BAP + 0.5 mg/L NAA) و فى المحاولة الثانية كانت أجزاء الساق كمنفصل نباتى أفضل من الأوراق لانتاج الكالوس وبالتحليل الاحصائى باستخدام مربع كاى لوحظ عدم وجود علاقة بين المنفصل النباتى المستحدم فى الاكثار الدقيق و التركيز الهرمونى عند انتاج الكالوس. البيئة المحتوية على الاتزان الهرمونى (3.0 mg/L BAP + 0.5 mg/ L NAA) هى الوحيدة التى أعطت كالوس و نبات جديد على نفس البيئة.