

Creation of Point Mutation by Means of EMS Mutagen in Lemon Balm (*Melissa officinalis*)

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Abstract – The plant (*Melissa officinalis*) is a type of medicinal plants that has many uses in hygiene and drug industries because of its many precious essences. Mutagenic material like EMS make distinct form resulting from genetic mutation in the plants and eventually led to emergence of new phenotype. In this research any type of EMS concentration induced on MS medium without hormones. The effect of time and concentration of EMS were 24 & 48 hours and 0, 0/05%, 0/01%, 0.005% respectively. The results show that induction of mutagenic material like EMS could be effected on peppermint. Various morphological factors including the stem length, root length, germinations per stem, the count of progressive lateral roots, and also the leaf count were analyzed and showed a significant response from the explants in this study (5%,1% level). Concentration of 0.01% EMS has excellent result on it. Some of peculiarity like length of stem, number of leaf and number of lateral bud has been changed. Extracted of essence and comparison with prototype plant have been showed no differences in essence.

Keywords – EMS, In Vitro, *Melissa Officinalis*, MS Medium.

I. INTRODUCTION

Lemon balm (*Melissa officinalis*) is a perennial grass from the mint family (*Lamiaceae*) that is known by different names in Iran, such as *bad-rang-booyeh*, *varang-boo*, *balengoo* and mountain basil. This plant is native to southern Europe, Middle East, and growth wild in southern parts of North America [13]. *Melissa* is spread throughout all Mediterranean countries, including the coastal areas of Turkey, and in northern Iran [3], [11]. *Melissa* has been cultivated for more than 2000 years in Europe, the Caucasus, Iran, Turkmenistan, the temperate foothills of the Himalayas, India, Moldova, Indonesia and, in modern times, across the world [20].

Melissa grows in any soil, but soils with a rich structure of calcium compounds and nutrients are appropriate for it. The best soil pH for *Melissa* is 4.8-8 [14]. *Melissa* requires warmth and light during growth. The seeds can sprout at 10-12 °C but the best temperature for sprouting is 18-20 °C. Buds on the rhizomes produce stallions (right roots) and other root branches of a light brown color [14].

Plant tissue culture is one of the techniques to remove these limitations. Plant tissue culture techniques have currently become as powerful tool for removal of the basic and applied problems in plant biology. On many occasions, we employ explants to start growth in an

artificial culture medium for plant tissue culture where differentiated and undivided passive cells and growing explants are initially undergone by some changes when they enter into meristematic state [2].

Mutation simply consists of a change in nucleotide sequence of a DNA molecule [5]. Term “Mutation” is derived from a Latin word that stands for an essential, basic, and sudden change. Mutagen is general name for materials that induce artificial mutation. In other words, they are the materials which their biological effects for mutation induction are much more than amount that occurs in situ or randomly [8],[4].

Chemical mutagens: These mutagens are included Ethyl Methyl Sulfonate (EMS), Acridine, Proflavines, Basic Analogs, Diethyl Sulfur (DES), Ethyl Amine (EL), Nitrous, N Methyl Urethane (NMUT) etc [7],[8].

Chemicals mutagenesis is very important in vivo, because chemical reactions have been identified as responsible factor for mutagenesis in many cases and this knowledge can be used to produce specific types of mutations. In addition, many chemicals are less toxic for plant against irradiation and also frequency of their mutagenesis is higher. Hence, mutagenic chemicals are useful as research tools to produce wide type of mutations [16].

Meftahzadeh et al (2010) reported that NAA, IAA and IBM auxin cause simulation of root in lemon balm and 96% root has formed in 1 mg/lit concentration of NAA after 25 days while IBA caused induction of 64% root with the same concentration and time and root length was obtained 3.32±0.045 cm in average. Mean number of stems in explants had index 1.08 that was in medium with 3 mg/lit concentration of BAP and 0.5 mg/lit NAA.

MeftahiZadeh et al (2010) reported that auxins such as NAA, IAA, and IBA induce root growth in *Melissa*. At a 1 mg/l NAA concentration after 25 days, 96% of the root developed; IBA under the same condition induced 64% root growth and an average root length of 3.32 ± 0.045. The average stem count in the explant that was in the environment with 3 mg/l BAP with 0.5 mg/l NAA had an index of 1.08. They placed the cotyledons from a 20-day old sapling in B5 and MS cultivation environments without growth regulators but did not achieve favorable results and the samples did not germinate. The MS cultivation area with 3 mg/l and 1-2 mg/l NAA was the best compound for growth initiation of the shoot tip explants.

During studies that were conducted on barley Asouit (1967) stated that gamma ray and EMS treatments have increased genetic variation related to clustering and panicle length in M2 generation families.

Fuji (1968) studied EMS effect on germination of Einkorn wheat. The mutation concentration rates, which he used in this test, were 0.1%, 0.3% and 0.5%. He declared that germination is decreased under EMS treatment as mutation concentration increases and its reduction rate has been very high in the F1 given hybrids. Also he stated that gamma ray had approximately no effect on germination of this wheat variety. According to his report, combined treatment of EMS and gamma ray had more osmotic effect than their separate treatments. During his studies about mutations effect, Bhan (1972) has reported that as osmosis rate increases, the number of seedling and formed sterile seeds also increases in M1 generation.

Hikar and Takishi (1981) examined MNU and EMS mutants in germinated and dry seeds and found that MNU produced little mutation in dry seeds, but produced a maximum effect on germinated seeds.

EMS, however, produced its maximum effect on dry seeds. Williams *et al* (1992) reported that the mutations created in hexaploid wheat by means of seed treatment with EMS produced mutant lines that were resistant to the 13-15 races of stem wheat rust. Saliskowa and Rapopot (1993) reported that mutation improvement plan in 1965 in which chemical mutagens were used, led to 499 mutant varieties from farming crops out of which 134 varieties had been released during 1977&1992 and 25% of them were mutants of fall- grown wheat, spring and fall- grown barley, and corn.

II. MATERIALS AND METHODS

This research was performed in plants breeding laboratory of Sari Agricultural Sciences and Natural Resources University from December 2012 until July 2013. Lemon Balm (*Melissa officinalis*) was provided from Mazandaran Province (Jihad-E-Daneshgahi) Academic Center and transferred to laboratory after the genus and species identification and confirmation of scientific name by experts to derive explants. Success of plant tissue culture is strongly influenced by used materials in culture medium. Thus, at first step stock solutions and then artificial medium should be prepared (Gong *et al*, 2001).

After explants cultivation that was included lateral and terminal buds, grown head branch in MS medium without hormone was treated by 0.0% (control group of tissue culture), 0.05%, 0.01% and 0.005% concentrations of EMS at 24 and 48 hours.

III. APPLICATION METHODS OF EMS TREATMENTS IN PLANT WITH CULTURED TISSUE

Healthy and pollution- free tissue culture samples were separated and transferred into culture chamber with EMS stocks until treatments application do under hood. First,

they opened the tubes door and pressed several terminal leaves (bottom leaves) by sterilized pincer to create scratch and EMS enter to plant system from location of creating these wounds. Then 0.05% EMS stock was picked up by a syringe and poured into cuvette so far created scratch place at leaf (4 repeats for 24 hours treatment and 4 repeats for 48 hours treatment) and we quickly fastened their door by parafilm and note treatments characteristics on tubes. For 0.01% and 0.005% concentrations of EMS, this operation is also done the same as the previous procedure. Finally we transferred test tubes to incubator.

After passing the given time, we washed and cleaned the samples of EMS as follows. Treated tissue culture samples were transferred to culture chamber and first we opened their lids under hood and removed EMS solution of test tube and poured the sterilized distilled water into test tube several times to be completely washed, and then we poured some drops of MS medium liquid in previous culture medium to compensate nutrients shortage and closed their doors quickly with parafilm to prevent from contamination.

IV. RESULTS AND DISCUSSION

The results of explants growth in MS culture medium showed that using of this medium without hormone might play a critical role in production of this medicinal plant (Figure 1) and also it could be considered as appropriate method for inducing regeneration.

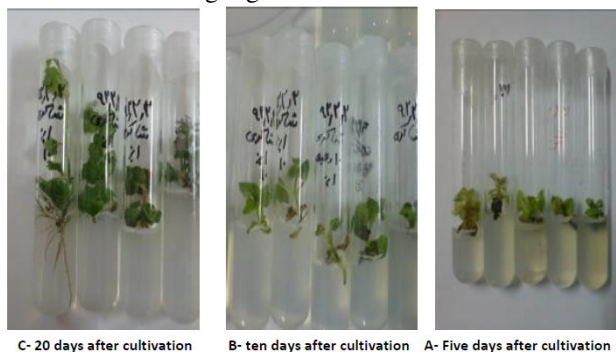


Fig.1. Plant growth stage development in MS medium

V. EMS EFFECT ON MORPHOLOGICAL CHARACTERISTICS OF PLANT

Accurate evaluation of EMS effect on plant and selection of most appropriate concentration and time of treatment requires wide domain of concentrations and times. To this end, in this research several concentrations of materials were used, as it described in Materials and Methods chapter. Tissue culture samples were destroyed at concentrations greater than 0.05% EMS, hence all data with more than 0.05% were omitted and data, which derived from ANOVA, were not included in this study. After EMS application, as it explained in materials and methods chapter, morphological characteristics were examined and measured every 8 days for 40 days and data analysis was done after this period.

VI. EMS CONCENTRATION AND TIME EFFECTS ON QUANTITY

According to obtained results of this research and after data analysis, the given results have shown that EMS mutation concentration and time could not create the significant effect on measured characteristics. The results indicated that dose, time and their interactive impact had no significant effect on root length and root number and EMS mutation could not cause the changes in root area of plant.

Another result of this research was EMS mutation effect on simulation of lateral buds growth so that both of EMS doses had significant effect at 5% level and among these two concentrations, 0.01% dose was more effective and we observed the fast growth in lateral buds.

To investigate these results with findings from other researchers, we studied EMS mutation effect on different plants. Fujii studied EMS effect on wheat germination and stated that the germination is decreased as mutation concentration increases and we also came to this result that increasing in concentration causes destruction of varieties. So far few studies have been performed about EMS effect on medicinal plants under field-cultivation and or tissue culture conditions or no report has been presented in this regard while most of studies have been carried out more on the effect of this mutation on important plant seeds such as wheat and rice.

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A-Day treatment B- Ten days after treatment C- One month after treatment



A-Day treatment B- Ten days after treatment C- One month after treatment

Picture 1: Overview of the EMS effect on plant tissue culture

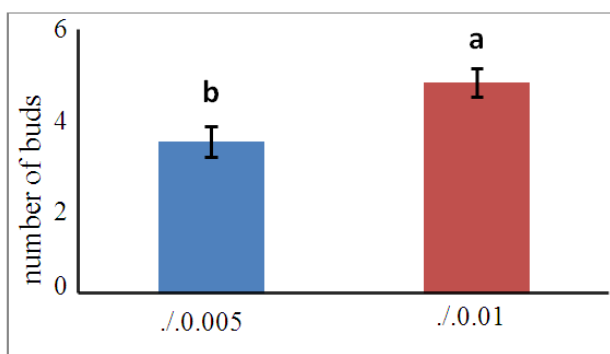


Fig.2. EMS dose and time effects on number of buds



Table 1: Morphology result of EMS exposure on the tissue culture treatment

S.O.V.	MS							
	df	Length of Stem	Length of Root	Number Leaf	Number Roots	Number of Buds	Lateral Root	Root
Concentration	3	0.0025 ^{ns}	3.7378 ^{ns}	0.04 ^{ns}	9 ^{ns}	5.0625*	0.0625 ^{ns}	2.9756 ^{ns}
Time	1	28.09 ^{ns}	2.0069 ^{ns}	0.0625 ^{ns}	4 ^{ns}	0.5625 ^{ns}	0.0625 ^{ns}	0.1406 ^{ns}
Concentration * Time	3	0.3025 ^{ns}	0.5378 ^{ns}	0.09 ^{ns}	0 ^{ns}	0.5625 ^{ns}	0.0625 ^{ns}	0.1406 ^{ns}
Error	12	9.4454	1.9451	0.0288	7.4167	0.6458	17.396	3.7003

ns,*,** : non-significant, significant at $p < 0.05$ and $p < 0.01$, respectively.