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# The Effect of Physical and Chemical Mutagen on Tomato Plant

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**Abstract:** Researches proved the strong ability of Ethyl Methanesulfonate (EMS) in producing chromosomal aberration and thus large variation in plant crops and depending on the concentration of EMS, it can have its positive or negative effect of the plant species. On the other hand, research proved the deleterious effect of UVB radiation and its ability to produce destructive mutations in plant corps which indicates its negative effect on growth of different terrestrial plant species. In this study, the effects of a physical (UVB radiation) and a chemical mutagen (EMS) was investigated on the tomato plant and how they could affect the plant growth in a positive or a negative manner. A bunch of tomato seeds were divided into three groups, the first group of the tomato seeds was treated with 0.07% EMS (Ethyl Methanesulfonate) solution as the chemical mutagen and the second group was left untreated and allow to grow and exposed to UVB soon after the first shoot appears and the third group was left to grow normally as control. The EMS turned to be a positive mutagen and it helped in speeding the growth rate and the overall plant size with a pale green leaves. On the other hand, the UVB rays were a negative mutagen because it caused a form of dwarfism into the tomato plants and it became smaller and shorter than the control and the EMS treated plant. The UVB treated plant had a pale yellow leaves which indicates a decrease in the chlorophyll content a well-known mutation caused in plant due to exposure to UVB light.

**Key words:** EMS (Ethyl Methanesulfonate) · UVB light (Ultra Violet Light) · Physical and the chemical mutagens · Tomato Plant

## INTRODUCTION

EMS is considered as one of the most important chemical mutagenic agents and it is considered as an ethylating agent that has a mutagenic effect on the DNA of many systems ranging from viruses to all mammals and it has a high carcinogenic effect on them [1]. This chemical compound is monofunctional in nature and its mutagenic affect is by alkylation action at the cellular nucleophilic sites by the SN1/SN2 reaction mechanism and on the DNA at the nitrogen position of the bases [2, 3]. In addition to that, EMS can induce alkylation on the O6 of the Guanine nucleotide and on the DNA phosphate group [4]. Apart of its alkylation action, EMS is capable of producing G/C to A/T and A/T to G/C transition mutations; it can cause a base-pair insertion, deletions as well as intragenic deletions. In the higher organisms, EMS is capable of breaking the chromosomes at different sites [5]. EMS was proved to causes a mutation in drosophila [6] and in the T2 phage [7] and it could cause mutation in Saccharomyces [8] and today, EMS is considered as the most used chemical mutagen on our planet [5].

In an experiment carried out on tomato plant to investigate the effect of EMS on it, the EMS treated seeds showed a positive correlation between M1- sterility and percentage of seedling mutants in M2 [9], however EMS is among mutagens that is used nowadays to improve yield and quality of the plant crops [10]. According to the finding of the experiment conducted by Akhtar *et al.* [11] MS has a developmental effects on the crops by affecting the meiotic features of these plants. In addition to that, he demonstrate how EMS treated seeds would develop a plant that has a better yield and quality with a better resistance to the harmful mutagens and the EMS is considered as a positive mutagens [11].

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On the other hand, very important physical mutagen is the ultra violet light (UVB light) which is will know for its mutagenic effect on many organisms [12]. The Ultraviolet radiation is the portion of the electromagnetic spectrum that is emitted from the sun, which is located between X rays and visible light that is between 40 and 400 nm and it has 5 types [13, 14]. UV light has enough energy to cause photochemical damage to cellular DNA which results in the occurrence of mutation within cells [15]. UV light has a serious effect on plants when they are exposed to it for a long period of time [16]. Many plant species are sensitive to UV light and especially to UV-B type which is responsible for the damage of the cellular DNA at the pyrimidine bases and that will results in reducing the growth of the plants, the photosynthesis activity and flowering rate [17]. UV light have the ability to effect the plant growth by effecting the plant photosynthetic activity by direct effects on photosynthetic enzymes, metabolic pathways or indirectly through effects on photosynthetic pigments or stomatal function [14].

The aim of this study is to examine the positive and negative effect of EMS and UV light on the tomato plant in order to develop new methods of cultivating tomato crops yield. The presented data in this research will help in exploring new possible ways to induce a positive mutation in the tomato plants that would help in increasing the crop yield and reduce the time needed for harvesting.

**Materials:** The requirements for the study includes fresh tomato seeds, MS media slants in 9 test tubes (Murashige and Skoog medium), 0.07% EMS solution (0.07 g in 100 ml), 0.01% Mercuric Chloride solution, UV light source, laminar air flow and plant tissue culture facility.

**Methodology**

- EMS Effect on the Tomato Plant

The fresh tomato seeds were dipped in the 0.01% mercuric chloride solution for 10 seconds to sterilize their surface and the cells were removed from the mercuric chloride and dipped in the sterile distilled water for 10 seconds to remove the access of mercuric chloride. The seeds were removed from the distilled water and were add to 70% alcohol for a second surface sterilization and keep for 10 seconds. Next to that, the seeds were added to distilled water to wash away the access of alcohol and soon after that dry the seeds on filter paper. A 0.07% EMS solution was prepared by adding 0.07 gm into 100 ml distilled water and incubate some of the seeds for 24 hours in the solution to induce the desired mutation. Seeds were then transferred to MS media slants prepared in different test tubes and incubate in highly sterile conditions to avoid any contamination in the media.

- UV light effect on the tomato plant

The remaining seeds were incubated in distilled water to use as a control and to use for inducing mutation using UV light. Slants of MS media were prepared in 9 test tubes in highly sterile conditions to avoid any contamination in the media. 0.01% solution of mercuric chloride was prepared by adding 0.01 g to 100 ml of distilled water to sterilize the surface of the seeds before they were planted in the media. Once the seeds start to germinates, the UV light labeled test tubes were taken and exposed to UV light in the Laminar Air Flow in alternative days for 15 minutes each time. Extra care must be taken not to increase the UV light exposure for more than 15 minutes each time.

In the first three test tubes (1$^{st}$ set) add one tomato seed (after surface sterilization by Mercuric Chloride) to each tube and label them as control. In the second three test tubes (2$^{nd}$ set) add one tomato seed in each tube (after surface sterilization by Mercuric Chloride) and label as UV light mutation. In the final set of the test tubes (3$^{rd}$ set), add one tomato seed from the seeds that were socked in EMS overnight and label as EMS mutation (no need to surface sterilize the EMS seeds because they got sterilized by the EMS).

All tubes were kept in the culturing room in a normal condition to allow them to grow and observe every day to check for any contamination in the plant tissue culture media. The reaming six test tubes (EMS and control labeled test tubes) were left in the plant tissue culture room and allowed to grow. The growth of the plants was observed on daily basis and the size of the leaves and the height of the plants stems were documented to confirm the effect of the mutagens.

- Molecular investigation of tomato plant to confirm mutation development

To detect the mutation on molecular basis, DNA isolation from the three plants (UV light plant, EMS plant and control plant) was perform and the DNA sample was analyzed using pulsed field gel electrophoresis [18]. In this method we separate the DNA bands and compared the difference between the normal plant and the mutant plant.

## RESULTS

**EMS Effect on the Tomato Plant:** Table 1 show the increase in the tomato plant stem height and leave size of the EMS treated seeds compare with normal and non-treated seeds of the tomato plants in duration of 8 weeks where the size and the height were measured in cm.

From the data presented in Table 1 and Fig. 1 indicated that by adding fresh tomato seeds to the 0.07% EMS solution and incubate them overnight, the growth rate and speed of the tomato plant was increased more than the normal plants (control) rate which indicated that incubating the seeds in 0.07% EMS solution caused a positive mutation which cause the tomato plant to grow faster with a bigger leaves and slightly longer stems.

**UV Light Effect on the Tomato Plant:** Data presented in Table 2 shows the idol duration of UV light exposure over time that is needed to induce and cause the desired mutation in the tomato plant. As we can see the suitable exposure time to UV light in order to induce mutation in the tomato plant is demonstrated in Table 2 which is 15 minutes in alterative days. Exposure period more than 15 minutes would cause damage to the tomato plant and prevents it from growing which is something that must be avoided since we want to observe the growth of the plant when it's exposed to UV light. On the other hand, less exposure time will not induce a mutation in the tomato plant.

Table 1 show the increase in the tomato plant stem height and leave size of the UV light exposed tomato plant compare with normal and non-exposed UV light tomato plants in a duration of 8 weeks where the size and the height was measured in cm.

From data presented in Tables 2, 3 and Fig. 3, indicated that by exposing tomato plant to UV light, the growth rate of the tomato plant was decreased visibly and the growth rate was almost two time slower than the normal rate of the control tomato plant. The results in Tables 2, 3 and Fig. 3 demonstrated that the exposure to UV light would cause a mutation in the tomato plant in the form of slow growth rate and growth limitations and that indicates the negative effect of the UV light as a physical mutagen.

Table 1: EMS effect on the tomato plant

| Date | Control Plant height (cm) | Control Size of the leaves (cm) | Test plat EMS tomato plant height (cm) | Test plant Size of the leaves (cm) |
|---|---|---|---|---|
| 1st week | 0 | 0 | 0 | 0 |
| 2nd week | 0 | 0 | 2 | 0 |
| 3rd week | 2 | 0 | 4 | .5 |
| 4th week | 3 | 0 | 5 | 1 |
| 5th week | 6 | 0.5 | 8 | 1 |
| 6th week | 8 | 0.5 | 12 | 1.5 |
| 7th week | 10 | 1 | 12 | 1.5 |
| 8th week | 12 | 1.5 | 13 | 2 |

Table 2: suitable UV light exposure to induce mutation

| Time of exposure to UV light | Results |
|---|---|
| 5 minutes in alternative days for 20 days | No mutation occurs. |
| 10 minutes in alternative days for 20 days | Mutation may occur but not with the desirable result. |
| 15 minutes in alternative days for 20 days | Mutation will occur with the desired results. |

Table 3: UV light effect on the tomato plant

| Date | Control tomato plant height (cm) | UV light exposed tomato plant height (cm) |
|---|---|---|
| 1st week | 0 | 0 |
| 2nd week | 0 | 0 |
| 3rd week | 2 | 1 |
| 4th week | 3 | 1 |
| 5th week | 6 | 2 |
| 6th week | 8 | 2 |
| 7th week | 10 | 3 |
| 8th week | 12 | 4 |

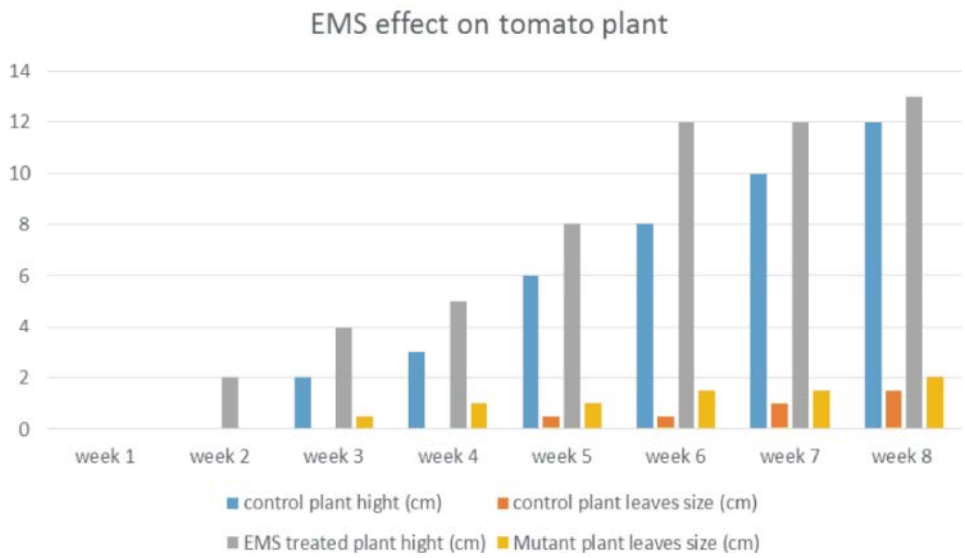


Fig. 1: EMS effect on the tomato plant

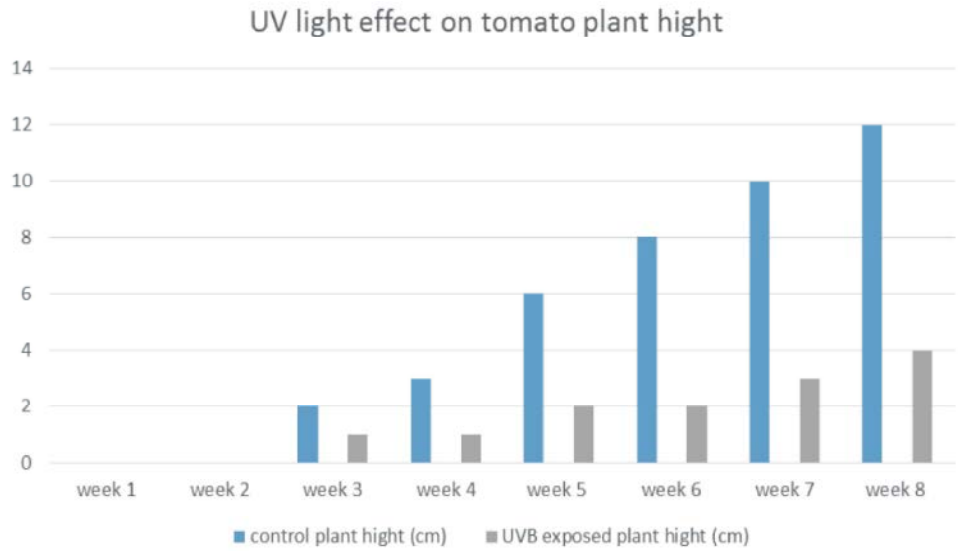


Fig. 2: UV light effect on the tomato plant

**The Detection of the Mutation in the Tomato Plant on a Molecular Basis:** After running pulsed field gel electrophoresis, the result of the DNA detection shows that mutation has occur in the EMS and the UV tomato plant due to the difference in the DNA bands between them and the control plant.

## DISCUSSION

The major issue in our project was how to determine the most suitable does to treat the tomato cells with and the accurate period of exposure to UV light and after several trails we found out that the most suitable concentration of EMS is .07 g (70 mg) and soak the fresh seeds in them for about 24 hours. It was found out that higher concentration caused a negative mutation and growth retardation took place in the plant. For the UV light source, we used the UV illuminator provided with the laminar air flow in our lab and we found out that the best time of exposure is 15 minutes in interval days. The daily exposure to UV light for 15 minutes did not allow the plant growth to observe the development of the mutation and instead it caused the plant to die soon. Finally, the last problem that we faced was the use of seeds, we first started with dried up seeds but we could not grow them on the MS media so we decided to go with the fresh media and luckily the growth was faster and steadier.

Plant exposure to UV light will cause some sort of growth retardation due to repressive action of UV light on the photosynthesis and that retardation will be in the form of decrease in the growth rate that is approximately 50% in the leaves when the plant is exposed for about 22 days. In addition to that, there will be a reduction in the plant total dry weight with the amount of chlorophyll remains the same [19]. On the other hand, the presentence of EMS during the seed germination and plant growth will have a clastogenic effect on the plant genome and that can cause different effects of the plant incubated with this chemical mutagen [20].

In our performed experiment, we show how both physical and chemical mutagens have the ability to cause positive and negative mutations which can be observed in the growth speed and rate [11, 21].

As the results of the UV light effect on the tomato plant, by exposing the tomato plant to UV light for 15 minutes in interval days and over a period of 8 weeks, the growth rate of the plant was twice less [22] than the normal plant (control plant) which indicates the negative mutagenic effect of the UV light [23] on the tomato plant and how it can cause growth retardation in the tomato crops [17].

On the other hand, by incubating the tomato seeds in a 0.07% EMS solution for overnight [5, 24], the growth rate of the plant was twice faster and the plant stem length and leaves size were twice bigger than the normal plant [11]. This indicates the positive effect of EMS on the tomato plant.

## CONCLUSION

EMS solution with a concentration of 0.07% have a mutagenic effect on the seeds of the tomato plant and cause a positive mutation on tomato plant in the form of fast growth rate and a taller plant and bigger leaves size. The exposure to UV light in alternative days will have a mutagenic effect on the tomato plant in the form of negative mutation which can be observed in the very slow growth rate and the growth retardation in the plant height. Both mutations are detected on a molecular level using DNA isolation techniques and pulsed field gel electrophoresis and the DNA bands from both mutant plants are compared to the control bands to insure that a change in the DNA composition changed due to the mutation. Further study should be conducted to use the date from this study to produce new methods that would help in improving tomato plant production and farming of tomato plants under controlled environment.

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