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Research Article

ETHYL METHANE SULPHONATE INDUCED ALTERATIONS IN SEED GERMINATION AND SOME PHYSIOLOGICAL PARAMETERS IN SPINACIA OLERACEA L

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A B S T R A C T

Consequence of the chemical mutagen EMS (Ethyl Methane Sulphonate) has been studied on the vegetable crop Spinacia oleracea L. Mutagenic treatments were given in the form of 6 Hrs pre-soaking in EMS, after pre-soaking them in distilled water for 12 hrs. Various physiological parameters like proteins, carbohydrates were evaluated, along with chlorophyll content analysis. The level of amino acid proline was also assessed. It was found that treatment found to have significant inhibitory effect on seed germination. Strong reduction in parameters like proteins and chlorophyll was recorded. In the plants treated with EMS there was significant increase in the level of amino acid proline which is a stress induced amino acid. There was a considerable increase in amount of total carbohydtrates in plants with EMS treatments specially up to 50 mM dose of EMS. EMS found to effectively to induce changes in important physiological parameters.

INTRODUCTION

Plant breeding is a science of changing the traits of plants in order to produce desired characteristics (Sleper and Poehlaman., 1955). It has been used to improve the quality of nutrition in products for humans and animals (Hartung *et al*., 2014). Plant breeding can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation, to methods that make use of knowledge of genetics and chromosomes, to more complex molecular techniques.

The concept of induced mutagenesis for crop improvement developed dated back to the beginning of 20th century. During the past 80 years, mutation breeding has been successfully utilized for the improvement of crops as well as to supplement the efforts made using traditional methods of plant breeding (Amin *et al*., 2015). Induced mutation is the ultimate source to alter the genetics of crop plants that maybe difficult to bring through cross breeding and other breeding procedures (Khan and Wani, 2004).

As early as 1942, the first disease resistant mutant was reported in barley (Friesleben and Lein, 1942). This led to the further work on mutagenesis leading to the release of mutants in several crops. Among these varieties, 1468 were of cereals and 370 of legumes. In cereals majority of cultivars came from rice (434), barley (269) and wheat (197) (Maluszynski, 2000).

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Physical and chemical mutagens have been successfully used in plant breeding programs to artificially generate variation for the development of new varieties with improved traits such as increased yield, earliness, and reduced plant height, resistant to disease.

A majority of alterations in EMS mutated populations are GC and AT base pair transitions (Till *et al*., 2007). Physical mutagen is in the form of physical substance. The main physical mutagen is short wave rays which penetrate living cells and alter genetic material. Radioactive is a relative measure that gives an indication of the quantity of recognizable effects of the radiation exposure on the irradiated object. EMS has been shown to be very effective and efficient mutagen and has probably become the most popular chemical mutagen (Ibrahim, 2008).

It produces random point mutations in genetic materials. Mutation frequency, detected using various techniques, displays a wide range of variation according the EMS treatment conditions. Mutation frequency per gene differs from one crop to another starting from one mutation per Mb in barley, (Caldwell *et al*., 2004), to one mutation per 170 Kb in Arabidopsis (Greene *et al*., 2003), 1 mutation per 50 Kb in pepper (Pathirana, 2012), (Just *et al*., 2012) and 1 mutation per 25 Kb in the polyploid wheat (Slade *et al*., 2004).

Spinach (*Spinacia oleracea)* is a leafy green, cool season vegetable that is known for its nutritive value, and is concerned one of the most popular vegetables in the world. Spinach is a member of family Chenopodioideae and is related to Swiss chard, quinoa and sugar beet. It is believed to have originated from Persia where the earliest references to spinach

occurred between 200 to 600 A.D and was transported to India and Asia and then later to the Mediterranean countries and Europe (Wright , 2001). Many attempts have been made to improve the nutritive value of the crop plants previously. This experiment may be one of the efforts of improving the spinach nutrionally as well as to improve its chlorophyll content.

MATERIALS AND METHODS

The seeds of Spinacia oleracea L. variety Desi-palak collected from Krishi Kendra Amravati, treated with various concentrations of EMS and LD-50 was recorded which was 75 mM. Depending on LD-50 the three dosages viz- 10 mM, 25 mM and 50 mM were selected for the final analysis. Seeds of spinach were presoaked in water for 12 hours, and then transferred to the EMS solutions of various concentrations. In EMS solutions the seeds were soaked for 6 hours, washed thoroughly thereafter and sown in the soil. The 15 day old plants of the spinach were used for the assay.

Measurement of Seedling height

The seedlings were removed from the soil and any loose soil was washed off from the root. The seedlings were blotted gently with soft paper towel to remove moisture. The height of the seedling was measured from its base to its highest point. The ruler was set at the base of the seedling i.e. at the root and the height was measured using the ruler.

Calculation of Germination percentage

A germination test determines the percentage of viable seeds that germinated from the total seeds sowed. In the present study germination percentage was recorded to correlate it with physiological characters.

Estimation of Carbohydrate: (Anthrone method)

The total carbohydrate content was estimated by the method of Hedge and Hofreiter, 1962. The anthrone reaction is the basis of rapid and convenient method for the determination of hexoses, aldopentoses and hexuronic acids either free or present in polysaccharides. Carbohydrates are dehydrated by concentrated H_2SO_4 to form furfural. Furfural condenses with anthrone (10-keto- 9, 10-dihydroanthracene) to form a blue green colored complex which is measured colorimetric ally at 630 nm.

Estimation of Protein - (Bradford, 1976)

This assay is based on the ability of protein to bind Coomassie blue G 250 and form a complex whose extinction coefficient is much greater than that of the free dye and which absorbance is measured at 595 nm.

Estimation of Proline

The estimation is done by the method prescribed by Troll and Lindsey (1955). During selective extraction with aqueous sulphosalicyclic acid, proteins are precipitated as a complex. Other interfering materials are also presumably removed by absorption to the protein-sulphosalicyclic acid complex. The extracted proline is made to react with ninhydrin in acidic conditions (pH 1.0) to form the chromophore (red colour) and measured at 520nm.

Estimation of Chlorophyll

Chlorophyll is extracted in 80% acetone and the absorption at 663nm and 645nm are read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated.

RESULT AND DISCUSSION

Germination Percentage

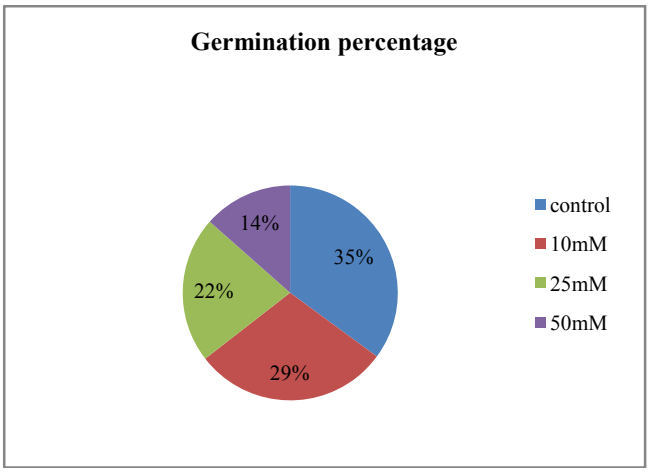

Diagram 1 Indicating the germination percentage estimated in various concentration of EMS.

Chlorophyll

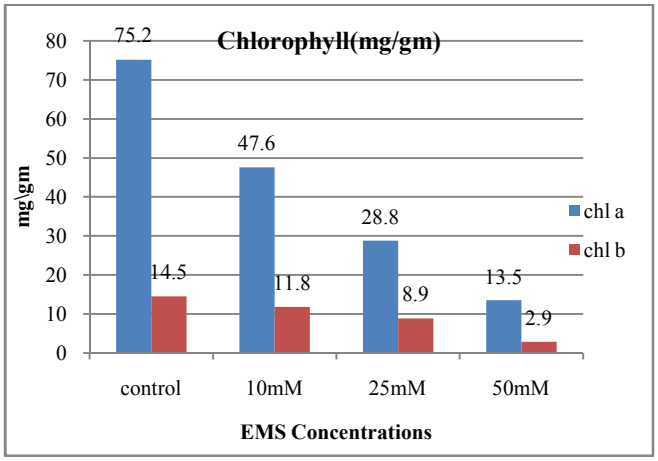

Diagram 2 Indicating the amount of chlorophyll estimated in various concentration of EMS after 15 days of treatment.

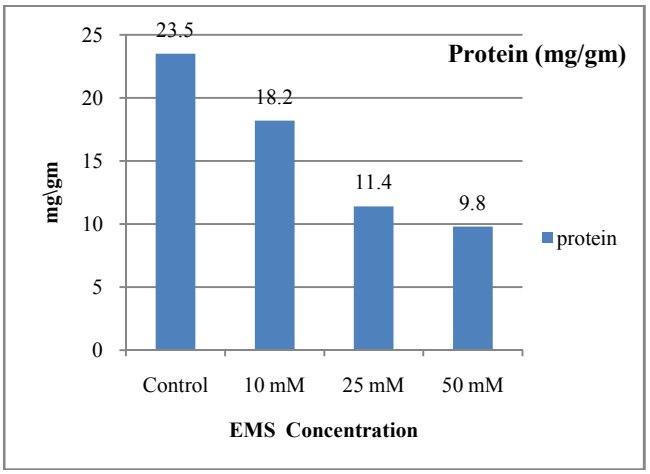

Diagram 3 Indicating the amount of Protein estimated in various concentration of EMS after 15 days of treatment.

Carbohydrates

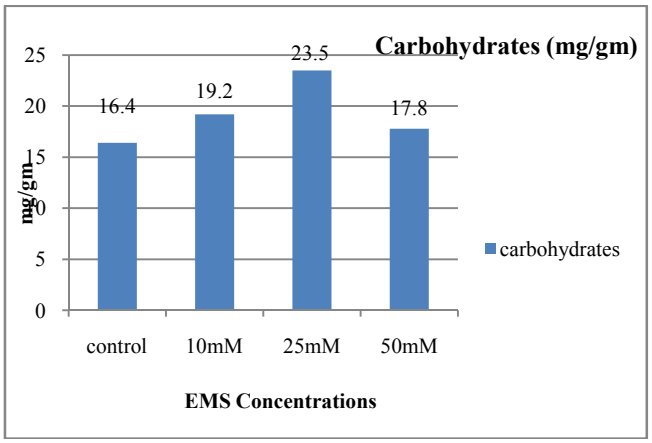

Diagram 4 Indicating the amount of carbohydrate estimated in various concentration of EMS after 15 days of treatment.

Proline

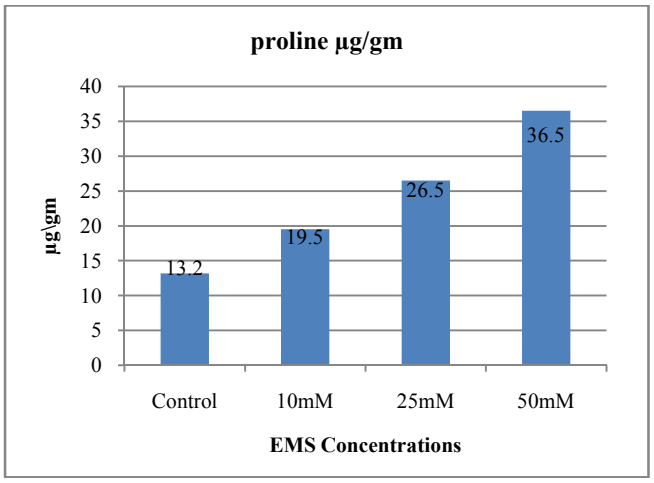

Diagram 5 Indicating the amount of proline estimated in various concentration of EMS after 15 days of treatment.

Based on the results that have been obtained in the course of the present investigation revealed that, the seeds of the spinach which were treated with different concentrations or doses of EMS (10Mm, 25Mm, 50Mm and 75Mm) had the inhibitory effect on the germination percentage and the seedling height of the plant as compared to the untreated control. After the 7 days of treatment, the highest germination percentage was observed in control i.e. 95.5% and the lowest were observed in 50mM concentration of EMS i.e. 16.6%.

Arisha *et al*. (2015) conducted study to enhance genetic variability in peppers (*Capsicum annuum*, cv B12) using ethyl methanesulphonate (EMS). The reduction in germination may be due to the seeds absorbing the mutagen, which subsequently reaches the meristemic region and affects the germ cell (Serrat *et al*., 2014). Also, a reduction in germination may be because of the damage of cell constituents (Kumar *et al*., 2013), alteration of enzyme activity or delay or inhibition of physiological and biological processes (Talebi *et al*., 2012). It was found that EMS reduced seed germination and survival ratio during the M1 generation and that effect continued in the second generation in peppers (Alcantara *et al*., 1996; Jabeen and Mirza, 2004) and Dianthus (Roychowdhury *et al*., 2012).Sonavane (2016) observed that the EMS treatment showed a gradual decrease in germination percentage of *Psophocarpus tetragonolobus* from lower concentration to higher concentration. In the present investigation, the seed germination percentage exhibited a declining trend with an increase in concentration of EMS and SA in both, II-EC-178313 and 2I-EC-38825 varieties of winged bean. All the treatments showed a marked tendency of reduction in germination at higher doses. Similar type of results were reported earlier by several researchers such as Gregory (1968) in peanut, Goud (1967) in wheat, Bajaj (1970) in *Phaseolus vulgaris*, Choudhary (2017) in cluster bean after the mutagenic treatment. An enhancement in seed germination gamma rays and EMS treatment was recorded by Rao (1983) in okra. Germination of seeds are controlled by the number of the various factors like temperature, humidity etc. The reduction in germination percentage may be due to the spinach seeds are not able to cope up with initial exposure to EMS.

The results of the present investigation revealed that, after 7 days of treatment of EMS concentrations on the spinach showed the significant decrease in the chlorophyll content of the plant as the EMS concentration increases. The untreatment control had the higher amount of chlorophyll content as compared to the different concentrations of EMS. The highest chlorophyll a and b content occurred in control of the spinach. The lowest chlorophyll a and b content occurred in 50mM of the EMS concentration. Whereas there were some fluctuations in the chlorophyll content after the 15 days of the treatment, the highest chlorophyll a and b content occurred in control (15 days). The lowest chlorophyll a occurred in 75 mM and the lowest chlorophyll b occurred in 50mM. M. Aruna (2012) observed that the values of chlorophyll contents decreased in higher concentrations. Decreased chlorophyll content with increasing concentrations of chemical mutagen confirmed the results obtained by earlier workers (Rosen, *et al*. 1961). Chaudhari *et al*, (2015) observed that when the seeds of P. corylifolia were exposed to 15Mm, 30Mm, 45Mm and 60Mm of EMS the chlorophyll content decreases with higher doses of EMS.

The plants were grown in germination chamber in the laboratories. Therefore, due to less light exposure chlorophyll amount may get decreased. It may be also an step towards the adjustment with mutagens.

The results of the present investigation revealed that, the proline content increased significantly as the EMS concentrations increases. After the 15 days of treatment the highest proline content occurred in 50mM of EMS concentration i.e, 36.5µg and lowest proline content occurred in control i.e, 13.2µg. Kavikishore *et al*. (1995) and Nanjo *et al*. (1999) found that the proline content increased significantly with increasing EMS concentrations. Proline has been shown to act as a compatible osmolyte and its increased production confirms osmo-tolerance in plants.

The result of the present investigation revealed that 50mM concentration showed the maximum amount of protein content after 15 days of treatment. The highest protein content was observed in untreated i.e. control and lowest protein content is present in 50mM.

More and Borkar (2016), observed a positive shift of mean value in leaf and seed protein content in majority of viable mutants. Total estimated protein content in leaf and seed was slight higher than control after mutagenic treated with EMS, Gamma rays and combination. The similar result was reported by many researchers. According to them leaf protein content

were estimated in two varieties of chickpea after Gamma radiation then leaf protein content were slightly decreased in some doses and leaf protein content was increased at higher doses. (Arulbalchandran and Mullainathan, 2009) in Black gram. Seed protein content was gradually increased with increase in dose or concentration of EMS and Gamma ray treatments against control. Total protein content in leaf and seed was slight higher than control recorded Mutagenic treatments produced recessive mutations and these mutations have affected a number of associated quantitative characters which improve yield and proteins. The result of the present investigation revealed that the higher amount of carbohydrate content occurred in non treated control. The highest carbohydrate content occurred in control and the lowest carbohydrate content occurred in 10mM concentration of the EMS.

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