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Research Article

ASSESSMENT OF PHYSICAL AND CHEMICAL MUTAGENIC EFFECTS OF SODIUM AZIDE ON M1 GENERATION OF *TRIGONELLA FOENUM-GRAECUM* L

Bansod P.G., Shrivastav S. R and Athawale V. A

Department of Botany, Vidya Bharati Mahavidyalaya Amravati – 444602

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ABSTRACT

Consequence of the chemical mutagen Sodium azide has been studied on the vegetable crop Trigonella foenum-graecum L. Mutagenic treatments were given in the form of 6 Hrs pre-soaking in Sodium azide, 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 enhancement in parameters like proteins and chlorophyll was recorded. In the plants treated with Sodium azide 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 Sodium azide treatments.

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INTRODUCTION

Mutation breeding supplements conventional plant breeding by virtue of increasing genetic variability that could confer specific improvement without significantly altering its acceptable phenotype (Ojomo, 1979). The prime strategy in mutation breeding has been to upgrade the well-adapted plant varieties by altering one or two major traits which limit their productivity or enhance their quality (Novak and Brunner, 1992). Mutations have played a great role in increasing world food security. Since new food crop varieties embedded with various induced mutations have contributed to the significant increase of crop production (Kharkwal and Shu.2009).

Mutation induction offers the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evaluation. Treatment with mutagens alters genes or breaks Chromosomes. Gene mutations occur naturally as errors in DNA replication. Most of these errors are repaired but some may pass to the next cell division to become established in the plant offspring as spontaneous mutations. Gene mutations without phenotypic expressions are usually not recognized. Consequently, genetic variation appears rather limited and breeders have to resort to mutation induction (Novak and Brunner, 1992). Chemical mutagenesis is regarded as an effective and important tool in improving the yield and quality characters of crop plants.

In general alkylating agents are very effective mutagens in higher plants. However, Sodium azide has also improved its worth as chemical mutagens to induced genetic variability. Thus, this chemical mutagen has become important tool to enhance agronomic traits of crop plants. The role of mutation breeding in increasing the genetic variability for quantitative traits in various crops plants have been proved (Khan et. al. 1998). Physical and chemical mutagens induce physiological damage (injury), gene mutation (point mutation) and (chromosomal aberrations) in the biological material in M1 generation (Gaul, 1970). However, Azide treated seeds show complete apparently normal growth in M1 except for M1 sterility and a high frequency of M1 chlorophyll chimaeras. To enhance the mutagenic effectiveness and efficiency of Sodium azide and especially the metabolite, more knowledge about the effect of time, pH value, temperature, seed seeaking and various concentration are required (Khan *et al*. 2009).

Sodium azide (NaN_3) is the least dangerous and the most efficient mutagen and has been repored to be mutagenic in several crop species (Adamu and Aliyu, 2007; Mostafa, 2011). The frequency of chromosome breakage caused by Sodium azide is relatively very low. The issues concerning metabolism,

Corresponding author:* **Bansod P.G
Dept. of Botany, Vidya Bharati Mahavidyalaya Amravati – 444602

activity, cytotoxic and mutagenic effect of Sodium azide and comparison with other mutagens are presented. (Gruszka, *et al*., 2014). Sodium azide (SA) is a well-known inhibitor of heavy metal enzymes with influences on metabolism and respiration of living cells. It is metabolized in vivo to a powerful chemical mutagen in many plant species, including barley, rice, maize and soybean (Owais and Kleinhofs, 1988; Szarejko and Maluszynski, 1999).

The mutagenic effect of Sodium azide is mediated through the synthesis of an organic metabolite, which was identified in bacteria and barley as an amino acid analogue L-azidoalanine [N3-CH2-CH(-NH2)-COOH]. A free amino acid group is essential for mutagenic activity, when compared to the carboxyl group. Synthetic D-azidoalanine displays very low mutagenic activity, indicating that a stereo-selective process is involved in azidoalanine mutagenicity. The production of this metabolite is dependent on the enzyme O-acetyl serine sulfhydrylase inhibited in vitro by cysteine. (Sadiq and Owais, 2000).

Trigonella foenum (fenugreek) commonly known in India as methi plant, is a dicotyledonous plant of the fabaceae, subfamily Papilinoideae. It is a self pollinated crop short duration and multipurpose (Vegetative, condiment, manure and medicinal) cash crop of India. Induced mutation have played an important role in genetic improvement of cultivated plant by producing genotype with higher yield, altered flowering, disease resistant and maturation characteristic (Wenzel., 1985)

The effect of several physical and chemical mutagens an morphological character in Trigonella species are reported, however, there is not much information on the physiological changes induced by Sodium azide in the methi plant. Keeping this in mind, we studied the effect of Sodium azide at different concentration on the various physiological activities and seed germination in T. foneumgraceum.

MATERIALS AND METHODS

The seeds of Trigonella foenum-graecum L. variety *Rajendra Kranti* collected from Krishi Kendra Amravati, treated with various concentrations of Sodium azide and LD-50 was recorded which was found 4.0 mM. Depending on LD-50 the three dosages viz- 1.5 mM, 2.0 mM and 2.5 mM were selected for the final analysis. Seeds of spinach were presoaked in water for 12 hours, and then transferred to the Sodium azide solutions of various concentrations. In Sodium azide solutions the seeds were soaked for 6 hours, washed thoroughly thereafter and sown in the soil. The 7 days and 15 days old plants of the Trigonella 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.

Germination percentage

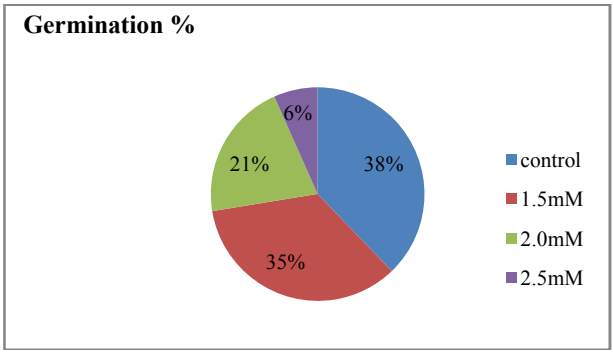
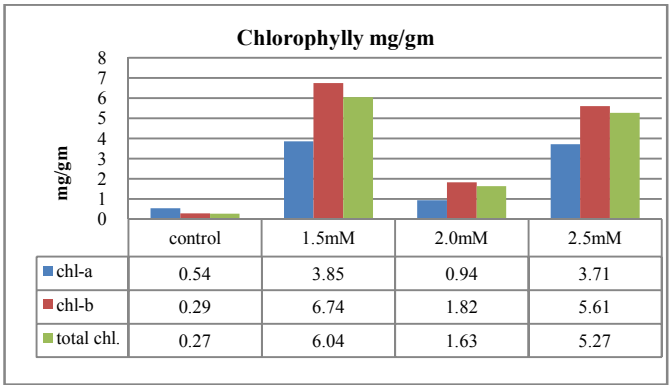


Diagram no.1 Indicating the Germination Percentage Estimated in various concentration of SA



	control	1.5mM	2.0mM	2.5mM
chl-a	0.54	3.85	0.94	3.71
chl-b	0.29	6.74	1.82	5.61
total chl.	0.27	6.04	1.63	5.27

Diagram no 2 Effect of Sodium azide on chlorophyll content of Trigonella foenum in 7 day old plant

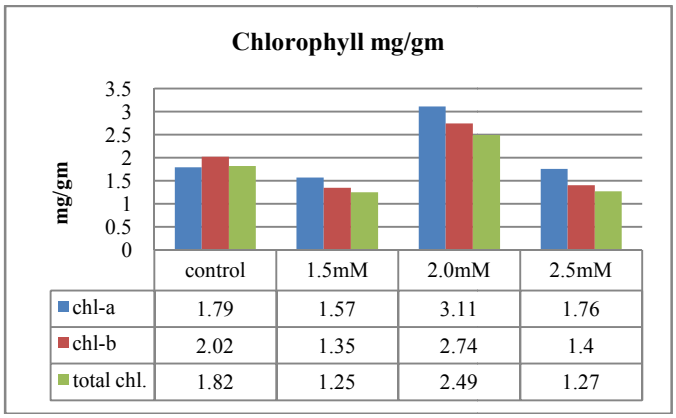


Diagram no 3 Effect of Sodium azide on chlorophyll content of Trigonella foenum in 15 day old plant

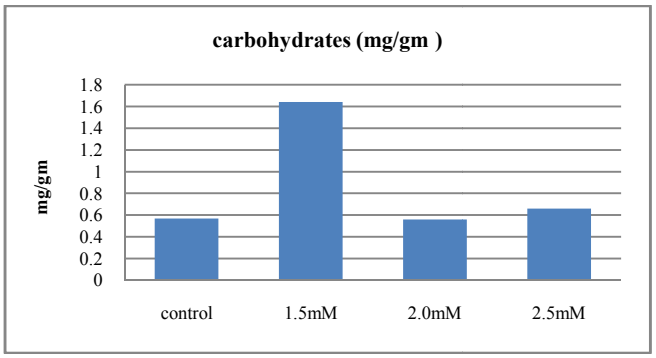


Diagram no 4 Effect of Sodium azide on total carbohydrate contents of Trigonella foenum in 7 day old plant.

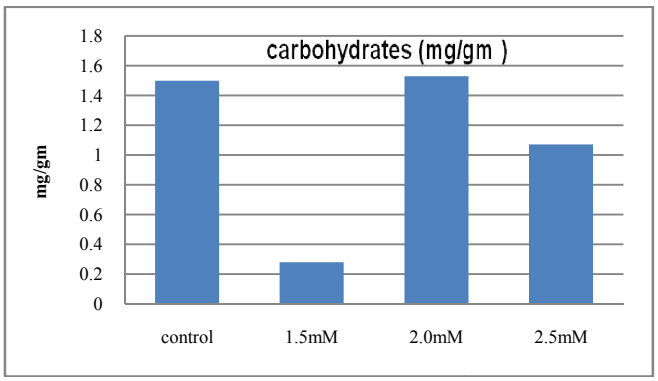


Diagram no 5 Effect of Sodium azide on total carbohydrate contents of Trigonella foenum in 15 day old plant.

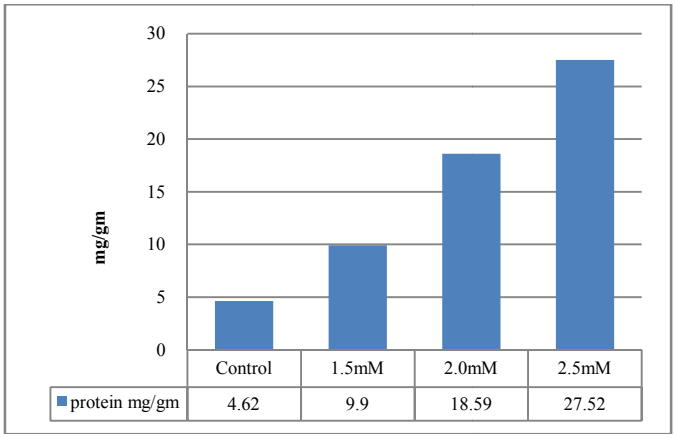


Diagram no 6 Effect of Sodium azide on protein contents of Trigonella foenum in 7 day old plant

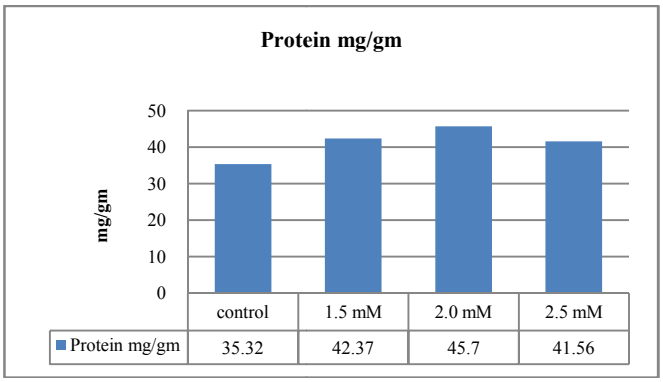


Diagram no 7 Effect of Sodium azide on protein contents of Trigonella foenum in 15 day old plant

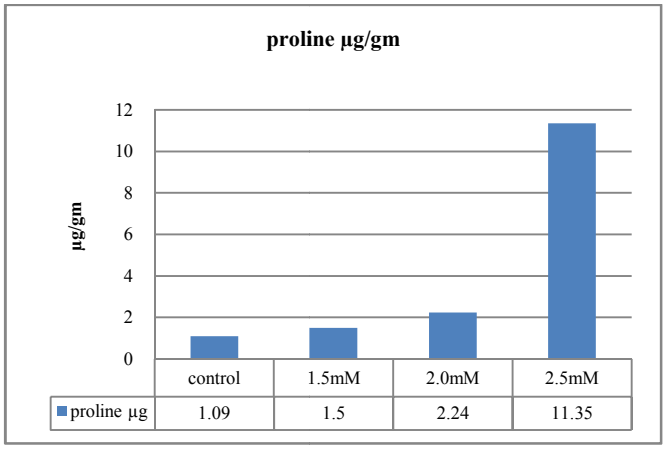


Diagram no 8 Effect of Sodium azide on proline contents of Trigonella foenum in 7 day old plant

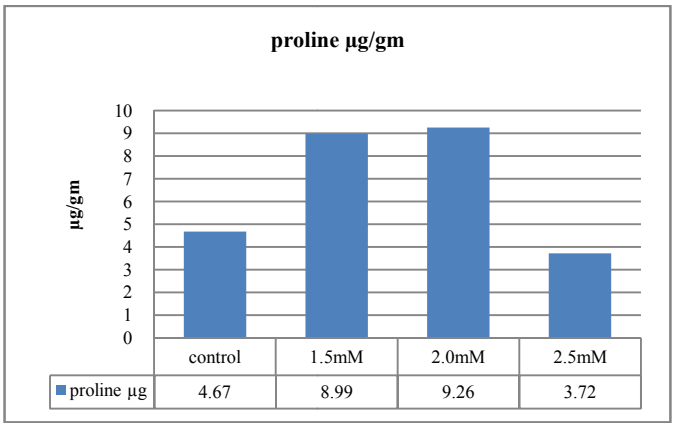


Diagram no 9 Effect of Sodium azide on proline contents of Trigonella foenum in 15 day old plant

In the present investigation, as the Sodium azide concentration increases the chlorophyll content also increases. The highest chlorophyll a and b content occurred in 1.5mM (7 days) i.e. 3.85 mg\gm and 6.74 mg\gm of the trigonella respectively. The lowest chlorophyll a and b content occurred in control of the Sodium azide concentration i.e. 0.54 mg\gm and 0.29 mg\gm of the trigonella respectively. The highest chlorophyll a and b content occurred in 2.0mM (15 days) i.e. 3.11mg\gm and 2.74 mg\gm respectively. The lowest chlorophyll a occurred in 1.5mM i.e. 1.57mg\gm and the lowest chlorophyll b occurred in 1.5mM i.e. 1.35mg\gm of the trigonella respectively.

Also it has been found that the highest proline content occurred in 2.5mM of Sodium azide concentration (7 days) i.e, 11.35µg

and lowest proline content occurred in control i.e, 1.09µg. The highest proline content is present in 2.0mM of Sodium azide concentration (15 days) i.e, 9.26 µg and lowest proline content occurred in 2.5mM i.e, 3.72µg. Proline has been shown to act as a compatible osmolyte and its increased production confirms osmo tolerance in plants (Mensah *et al*., 2016).

In the present investigation,, the highest protein content is recorded in 2.5mM of Sodium azide concentration(7 days) i.e, 27.52 mg/gm and lowest protein content occurred in control i.e 4.62mg/gm. The highest protein content is present in 1.5mM of Sodium azide concentration(15 days) i.e, 42.37mg/gm and lowest protein content is present in control i.e 35.32mg/gm.

Similarly, Saddam Hussain at al., (2017) in his investigation observed that the effect of various concentration of SA on Protein Percentage of Brassica Napus L. showed that with the high concentration of SA increase the protein contain i.e. 0.2 SA (27.8%), 0.6 % SA (27.0%), 0.8% SA (28.5%), except 0.4% SA which show deviation and decrease protein contain as compare to control. The similar result reported by Bashir *et al*. (2013).

In this study, the highest carbohydrate content occurred in 1.5 mM (7days) i.e. 1.64 mg and the lowest carbohydrate content occurred in 2.0mM concentration i.e. 0.56%mg of the trigonella. The highest carbohydrate content occurred in 2.0mM(15 days) i.e. 1.53%mg and the lowest carbohydrate content occurred in 1.5Mm concentration i.e. 0.28%mg of the Trigonella.

Saad-Allah *et al*.,(2014) studied The impact of treatments of NaN_3 on seed germination, some growth criteria, photosynthetic pigments, some metabolic activities and cytological behavior in the growing seedlings, the yield parameters as well as the variation in the protein profile of the yielded seeds of the selected mutants grown from the seedlings. The germination percentage, shoot height, root depth, leaflets area, chl. a, ch. b, the initial level of fluorescence (Fo) were decreased with NaN3 treatments and these decreases were directly proportional to the dosage and duration of treatment.

Sodium azide (NaN3) is a chemical mutagen and has been one of the most powerful mutagens in crop plants. The Mutagenisity is mediated through the production of an organic metabolite of azide compound. This metabolite enters into the nucleus, interacts to DNA and creates Point Mutation in the genome. Several factors such as properties of mutagens, duration of treatment, pH, pre and post treatment, temperature and oxygen concentrations etc influence the effect of mutagens. The dose of a mutagen applied is an important consideration in any mutagenesis programme. Generally, it was observed that higher the concentrations of the mutagen greater the biological damage. Sodium azide is perhaps the least dangerous and the most efficient mutagen in that high yields of mutations are achieved at moderate M1 Sterility rates. Although, in some cases it has been reported the treatments with Sodium azide, the physiological effects of azide are wead, few chromosomal aberrations are induced and it delays germination and growth.

From the present study, it is observed that the germination percentage of the Trigonella foenum decreased as the Sodium azide concentration increases but the germination of seed are controlled by no. of factors like temperature, humidity, etc. but the reduction in germination percentage can be due to the Trigonella foenum seed were not able to cope up with the initial exposure to Sodium azide.

The plants were grown in germination chamber in the laboratories. Therefore, due to less light exposure Chlorophyll amount may be decreased. It may be also as step towards the adjustment with mutagen.

The Proline, Chlorophyll, Carbohydrate content showed significant increase in the higher concentrations of Sodium azide other than control. Therefore, further investigation regarding this aspect may be performed to support the induced mutagenesis of crop plants.

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