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Effect of Different Doses of Sodium Azide on Cytogenetical and Biochemical changes in Fenugreek (*Trigonella foenum graecum*)

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ABSTRACT: Sodium azide is a popular plant mutagen. The present study was conducted to assess the mutagenic/cytogenetic changes in root tip cells in the local variety of fenugreek (*Trigonella foenum graecum*). Seeds of *Trigonella* were treated with various concentrations of sodium azide (0.1%, 0.2%, 0.3% and 0.4%) at room temperature for 3 hours and were germinated. The treated root tip cells exhibited abnormalities such as stickiness, laggards, unequal separations and bridges. The other parameters of the study included biochemical variations like protein, carbohydrate and proline variations.

Keywords: Chromosomal abnormalities, fenugreek, stickiness, biochemical variations, chemical mutagen.

INTRODUCTION

Fenugreek (*Trigonella foenum graecum*) also known as methi is an economic value herb. It is an important, short duration; multipurpose cash crop of India belongs to Family Fabaceace, subfamily Paplinoideae. The cultivation of the herb is carried out around the world for seed, vegetable and fodder production. The seeds of plant are grown as pot herbs and used as a spice or as herbal medicine in most of the parts of Asia (Bashir *et al.,* 2013 and Lust, 1986). *Trigonella* have potential properties as antiulcer; wound healing, CNS stimulant, antioxidant, antidiabetic, antineoplastic and antipyretic drugs (Zargar, 2014 and Srivastava and Srivastava 2018).

Genes are the building blocks and basis of growth and development of plant. To study the nature and function of genes, mutations are being used as important tool. By this genetic improvement of economic important crops takes place (Adamu *et al.,* 2007, Bashir *et al.,* 2013). In the present scenario mutational breeding helps in a bigger manner of genetic variability. This genetic variability induced by chemical and physical mutagens. These can be used for getting the desired characters. Mutational breeding helps in improving one or more character and the rest of the genotype does not change (Rajoriya *et al.,* 2016). Higher mutation rates are found in chemical mutagens treated plants as compared to physical mutagens. Chemically induced mutagenesis gives higher efficiency, more efficient for morphological and genetical variability and greater specificity of mutation (Dixit *et al.,* 2013, Bhosle and Kothekar 2010, Goyal and Khan 2010, Prabha *et al.,* 2010a and b, Avijeet *et al.,* 2011, Bhat *et al.,* 2005 and Srivastava *et al.,* 2019). The present study was undertaken to investigate the effects of sodium azide at different concentrations in *Trigonella foenum graecum* for mutagenic exploitation of the crop.

MATERIALS AND METHODS

Healthy *Trigonella* seeds of uniform size were selected. Seeds were surface sterilized with teepol. The seeds were then soaked separately in solutions of 0.1%, 0.2%, 0.3% and 0.4% of Sodium azide for 3 hours. Control groups were soaked in double distilled water. Seeds of various treatment groups were placed in petri dishes on moistened cotton.

The root tips after cutting were fixed in Carnoy's fixative and transferred in 70% alcohol after 24 hours and stored at room temperature. The squash technique as described by Savaskan and Toker (1991) was used to make the chromosome spreads.

Lowry's (1950) method was used to determine the protein content. The results are expressed in unit mg^{-1} protein. Carbohydrate content was estimated by Anthron's method. Proline activity measured as explained by Bates *et al.* (1973). The supernatant was read at 520nm by spectrophotometer.

RESULT AND DISCUSSION

A. Cytological analysis

The application of plant mutagen is of significant importance in improving the field, quality and economic value of the plant. Sodium azide is one of the most commonly used and most efficient plant mutagen. Mitotic investigations in the control root tips exhibited no irregularities in the structure and behaviour of chromosomes and revealed normal chromosomes at metaphase and anaphase stage (Fig. 1).

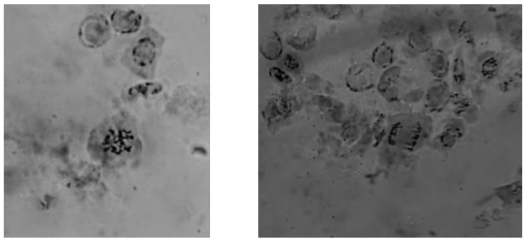
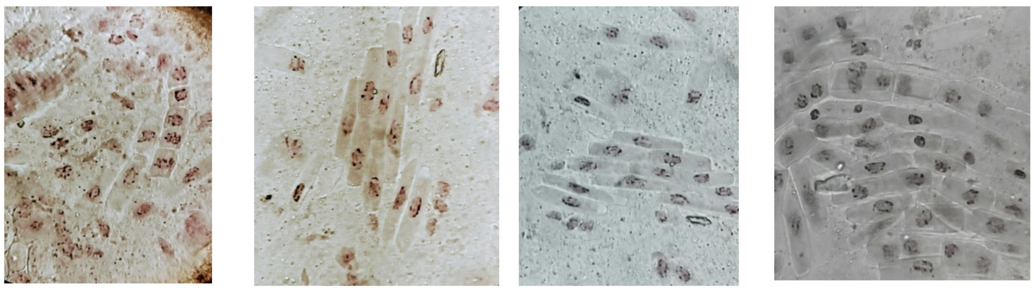


Fig. 1. Normal Metaphase and anaphase.

However, sodium azide treated plants projected various chromosomal abnormalities during mitosis. The chromosomal abnormalities included the formation of laggards, bridges, stickiness and unequal separation of chromosomes (Fig 2a-d). The sodium azide treated populations showed dose dependent increase in the percentage of root tip cells with abnormal metaphase. Maximum incidence of abnormal behavior was reported in 0.3 % and 0.4% SA. Increased incidence of stickiness and laggards was increase in dose. Stickiness may be due to nucleic acid de-polymerization caused as a result of mutagenic treatments or due to nucleo-proteins partial dissociation and alterations in their pattern of organization (Evans, 1962). Patil and Bhat (1992) suggested that stickiness is basically a type of physical adhesion which involves mainly the proteinaceous matrix of chromatin material. Jayabalan and Rao (1987) suggested that stickiness may result due to disturbances in cytochemically balanced reactions. Different types of chromosomal aberrations followed by treatment of chemical has also been reported by Bashir *et al.,* 2013, Dhulgande *et al.,* 2015, Siddiqui *et al.,* 2007, Kapoor and Srivastava 2010a and b, Dixit *et al.,* 2013 and Rajoriya *et al.,* 2016.



(a) (b) (c) (d)

Fig. 2. (a) Unequal distribution (b-d) stickiness at 0.3% and 0.4%.

B. Protein content

In the present investigation, the highest protein content was recorded in seeds treated with 0.3% of sodium azide concentration and lowest protein content was reported in seeds treated with 0.4% of sodium azide concentration (Fig. 3). Our results so obtained were in correlation with the similar findings of work done by Dahot *et al.* (2011) on sorghum; Gnanamurthy *et al.* (2013) on *Zea mays*; Saad-Allah *et al.,* 2014, on *Pisum sativum* and *Vicia faba*; Animasaun *et al.,* 2014 on *Arachis hypogea* L.; Hussain *et al.,* 2017 on *Brassica napus* L.; and Bansod *et al.,* 2019 on the effect of various sodium azide concentration on protein percentage of *Trigonella foenum-graecum* L. Their findings were similar to our results showing that the protein was lowest in the seeds treated with 0.4% sodium azide concentration.

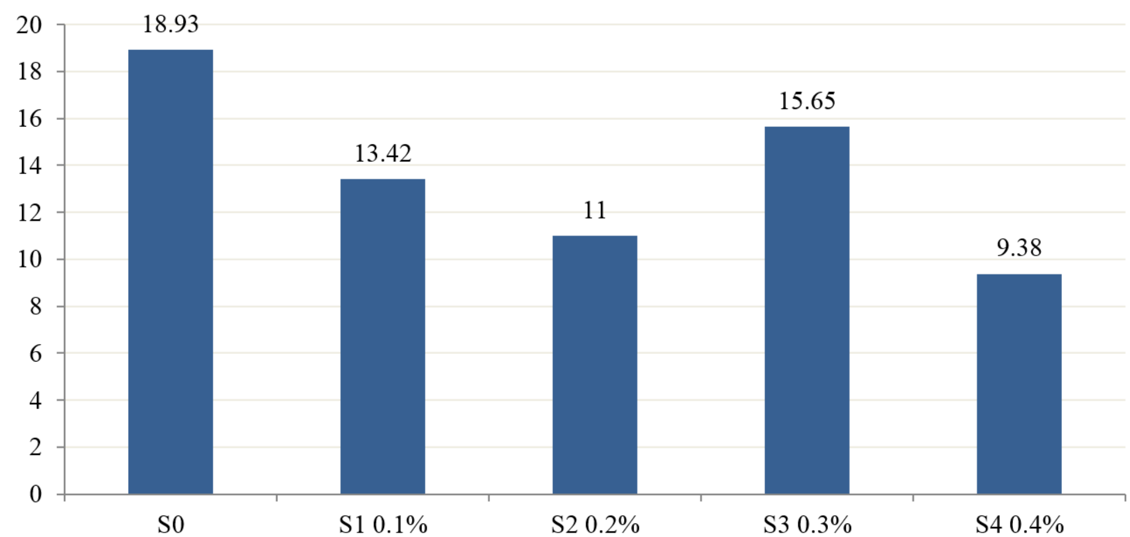


Fig. 3. Protein content (Percentage).

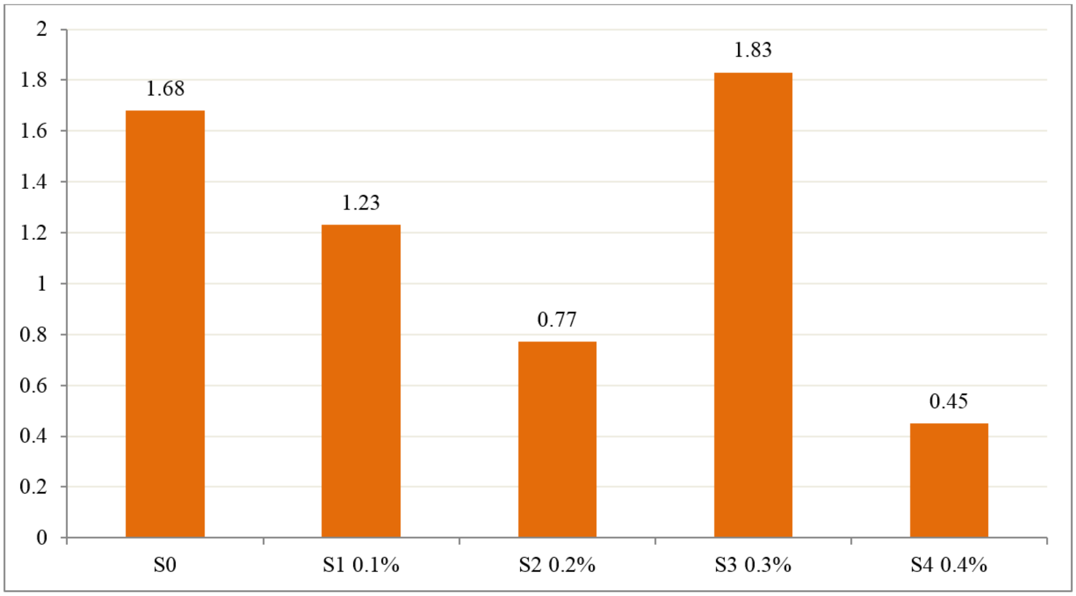


Fig. 4. Carbohydrate content (Percentage).

C. Carbohydrate content

In this study, the highest carbohydrate content was reported in seeds treated with 0.3% of sodium azide concentration and the lowest carbohydrate content was reported in seeds treated with 0.4% of Sodium azide concentration (Fig. 4). The results similar to the findings obtained by Saad-Allah *et al.,* 2014 in *Pisum sativum* and *Vicia faba*; and Bansod *et al.,* 2019 on *Trigonella foenum-graecum* L. According to El-Shafey *et al.,* 2009 this increase in the carbohydrate content can be a part of another defensive mechanism, as a result of pre-exposure to the mutagen which causes certain genes to get activated leading to the expression of enzymes responsible for accumulation of osmolytes.

D. Proline content

In the present investigation the highest proline content was reported in the seeds treated with 0.4% of sodium azide concentration and lowest proline content was reported in seeds treated with 0.1% sodium azide. The proline content is shown to increase with increasing concentration of sodium azide (Fig. 5). Similar results are shown in works of Kisk *et al.,* 2016 on Grand Nain banana plants and Bansod *et al.,* 2019 on *Trigonella foenum-graecum* L. According to Mensah *et al.,* 2006 proline is known to act as a compatible osmolyte and its enhanced production confirms osmo-tolerance in plants.

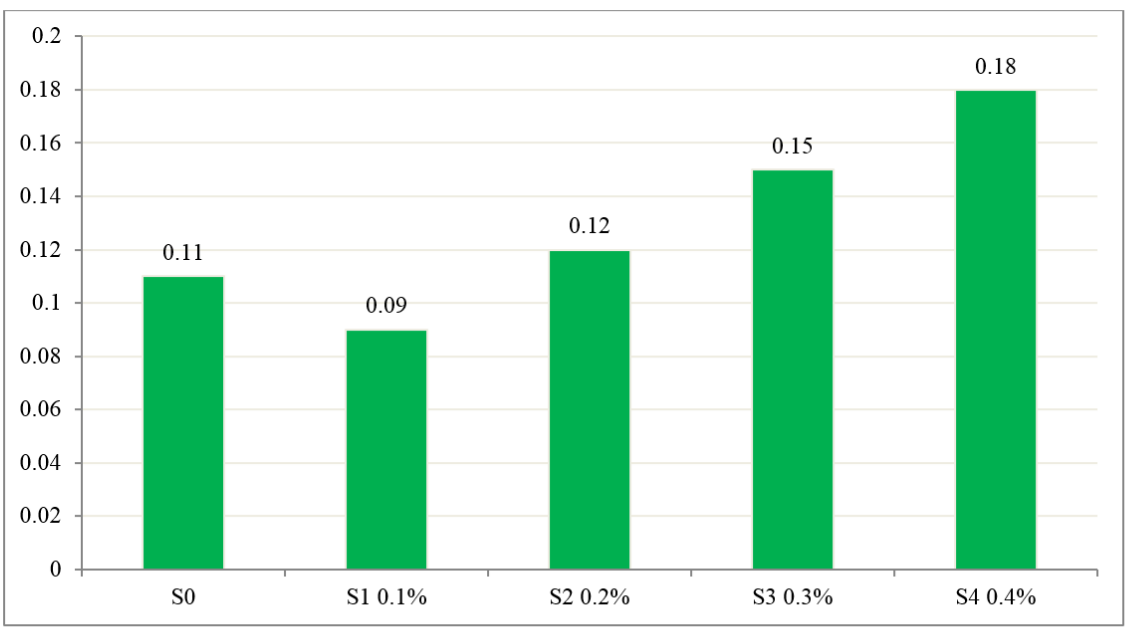


Fig. 5. Proline content (Percentage).

CONCLUSIONS

The present study considered the mutagenic effects of sodium azide on germination percentage, chromosomal structure and biochemical variations like protein, carbohydrate and proline content in the plant- *Trigonella foenum graecum*. The results showed that sodium azide mutagen is effective in inducing various abnormalities such as stickiness, unequal separations, laggards and bridges in *Trigonella foenum graecum*, with an increase in the dose of sodium azide, which increased the number of root tip cells showing abnormal metaphase. Similarly, the increase in the proline content also shows a dose dependent increase. The study positively concluded that sodium azide could be employed to improve protein and carbohydrate content in *Trigonella foenum graecum* at a particular concentration. Sodium azide, as a mutagen is effective in inducing various mutagenic effects. These mutagenic effects can be used to study the nature and function of genes so as to improvement the commercial applicability of cash crops. With a future perspective, these mutagenic improvements can be brought about by the increase in the nutritional composition of the plants and also other economical aspect like increase in oil, starch, sugar content in plant to name a few.

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