Mitoclastic responses of biofertilizer and chemical fertilizer in root meristems of *Trigonella foenum graecum* L.

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Received June 9, 2011; accepted August 4, 2011

**ABSTRACT.** To increase crop yield and soil fertility, crop producers use agricultural chemicals and artificial fertilizers. As a result plants become prone to fertilizer and soil infertility increases. A substitute to inorganic fertilizer could be biofertilizer. Biofertilizer is very beneficial for plants as it promotes growth and yield of plants without causing any ecological imbalance. The present investigation has been carried out to access the cytological effects of fertilizer urea and biofertilizer agrozyme on root tips of *Trigonella foenum graecum* L. It has been found that fertilizer turns out to be more chromotoxic and mito-inhibitory at higher doses in comparison to biofertilizer. High concentrations caused gradual decrease in mitotic index in case of fertilizer while in case of biofertilizer mitotic index is increased parallel to the increasing doses. Various chromosomal abnormalities were also observed at high concentration. In case of biofertilizer the abnormalities were lesser in comparison to fertilizer. The treated system showed various cytological irregularities viz. stickiness, precocious movement, unorientation, bridges, and forward movement. Although both share common range of aberrations but stickiness is predominant among them.

**KEYWORDS:** Agrozyme, Biofertilizer, Chromosomal abnormalities, Fertilizer, *Trigonella foenum graecum* L., urea

According to the United Nations report, population of world is projected to reach nine billion by 2050 (U. N. Pop. Div., 2010). Along with the increase in world population the arable lands are gradually shrinking. Food availability for mankind is rapidly declining because of overgrowth in world population and decrease in agricultural area. To deal with the problem, producers focus on genetic improvement and artificial fertilization for increasing per unit plant yield. However, excessive fertilizer application can cause lot of problems (Mason 1980). Fertilizers are soil amendments applied to promote plant growth. It has been reported that use of synthetic nitrogen fertilizers has increased steadily in last 50 years rising almost 20 fold to the current rate of 100 million tones of nitrogen per year (Glass 2003).

More than 90% of the world production is destined for the use of fertilizer. Chemical fertilizers have come to increase the output of agricultural product and to meet ever increasing demand of human population. The problem is further compounded in several areas due to excessive use of chemical fertilizers which resulted into considerable deterioration in the quality of indigenous soil (Himadri and Dharmvir 2007). Though fertilizers have increased productivity, yet these can become lethal to some of beneficial insects and animals (Preap et al. 2002). A substitute to chemical fertilizer could be biofertilizer; organic medium. Organic farming is proposed to protect environment and natural sources to replace harmful ecological balance, to protect flora and fauna and to maintain biological diversity (Tabur and Oney 2009).

Biofertilizer is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey 2003). Biofertilizer is natural and organic in nature that helps to provide and keep in the soil all the nutrients and microorganisms required for the benefits of the plants. Bio-fertilizers also secrete growth substances and antifungal chemicals, as well as improve seed germination and root growth. Thus, the use of bio-fertilizers will effectively enrich the soil and are better to use than chemical fertilizers.

In present study *Trigonella foenum graceum* L. (*2n=16*) was used as test system for the assessment of cytogenetical effect of fertilizer and biofertilizer on the somatic cells of the plant.

**MATERIALS AND METHODS**

For the study of mitosis, germinated seeds of *Trigonella* were treated with aqueous solution of urea and agrozyme. The duration for the treatment was 3 hrs for 4 different concentrations viz 0.25, 0.50, 0.75 and 1%. Controls were maintained simultaneously by treating the seeds with distilled water only. The treated seeds were washed thoroughly with distilled water. Then the treated seeds were fixed in 3:1 acetic acid and absolute alcohol solution. Slides were prepared using chromosome squash technique with 2% acetocarmine. Slides were analyzed under Olympus light microscope with 40x resolution and photographs were taken using PCTV photography software.

**RESULTS**

The present study on root meristems of *Trigonella foenum graecum* L. (*2n=16*) gave us a comparative cytogenetical effect of chemical fertilizer and biofertilizer. The entire analysis and their variation trends with the different treatment doses of chemical fertilizer and biofertilizer are shown in Table-1. Mitotic index value in root meristems
of seeds destined control was 14.10\% (Table 1). While in case of the system of urea, the AMI showed dose dependent variation. AMI first displayed an increasing trend at 0.25 and 0.50\% concentrations giving the values 14.94 and 15.83\% respectively. However further increase in concentrations i.e. at 0.75\% it roughly coincides with AMI of control i.e. 14.14\%. At the highest dose of 1\% it decreases to 13.69\% (Table 1). On the other hand with the increase in the concentration of biofertilizer the AMI increases this shows that it acts as a mitoenhancer. Along with rise in concentration, the AMI increased until the highest dose with values 15.68 and 17.84\% for 0.25 and 1\% concentrations respectively (Table 1).

The abnormalities percentage (Ab \%) increases with the increase in the concentration of both the fertilizer and biofertilizer meaning thereby that the abnormalities in root tips of *Trigonella foenum graecum* are dose-dependent. The various abnormalities accounted are presented in Figure A-I. Different chromosomal abnormalities recorded at various doses were stickiness at anaphase (Fig. 1C), stickiness at metaphase (Fig. 1F), precocious movement (Fig. 1E). Besides these; unorientation (Fig. 1D), multiple bridge (Fig-H), forward movement with stickiness (Fig. 1I) are also observed. Frequency of metaphase stage was increased in treated seeds, a condition known as Metaphase arrest. This indicates that artificial fertilizers should be used by suitable methods and in recommended doses. The trends of variation in AMI\% are shown in Fig. 2 and comparative account of total abnormality\% is shown via Fig. 3.

### DISCUSSION

The variation in AMI\% with the increasing treatment doses of chemical fertilizer suggests its mitoclastic effect in the root meristem. At lower concentration of chemical fertilizer doses there is an increase in AMI\% but as the concentration doses is increased the AMI\% shows a downfall (Fig. 2). This indicates that chemical fertilizer should be used by suitable methods and in recommended doses. The system treated by biofertilizer shows an unheeded increase in AMI\% (Fig. 2) indicating its remarkable positive effect, but the margin of increase is shortened as the concentration increases. This suggests that at very high concentration mitoclasticity will prevail. The Lowering of AMI can be attributed to inhibition of DNA synthesis at S-phase (Sudhaker *et al.* 2001). The chromosomal abnormalities show unheeded increase with the increase of concentration in both the systems (Fig. 3). Thus, fertilizer produced mitoclastic well as chromotoxic effects.

During the present investigation an increase in different chromosomal aberrations was observed as the concentration of urea and agrozyme increased. In case of urea the abnormality \% was 5.55, 12.12, 15.38, 21.51 at 0.25, 0.50, 0.75, 1.0\% concentrations respectively (Table 1). Stickiness was the most dominant abnormality 5.8\% at 1\% concentration. In case of biofertilizer the abnormalities do occur but lesser in comparison to fertilizer 4.2 at the highest dose 1\% (Table 1).

The chromosomal stickiness is defined as chromosomal agglutination of unknown nature, which results in a psychotic or sticky appearance of chromosome. The stickiness might have resulted from the defective functioning of nonhistone protein involved in the chromosomal organization, which are needed for chromosomal separation and segregation (Gaulden 1987). Bridges at anaphase may be suspected to be due to the stickiness of chromosome at metaphase (El-Khodary *et al.* 1990) and their failure to separate freely at anaphase or due to breakage and reunion of chromosome (Haliem 1990). The precocious movement of chromosome might have been caused by the early terminalization, stickiness of chromosome or because of the rest during anaphase (Permjit and Grover 1985). Unequal separation, unorientation etc, may be attributed
to the disturbance in spindle formation (Haliem 1990).

Artificial fertilizers become injurious by accumulating in plants structure. These pass from plant to animal and human directly or indirectly. Recently, artificial fertilizers unconsciously used, have threatened human health (Verhoog et al. 2003). Excessive nitrogen fertilizer applications can also lead to pest problems by increasing birth rate, longevity and fitness of certain pests (Jahn 2004).

Organic farming therefore is proposed to excel inorganic farming. They protect environment and natural sources, repair harmful ecological balance, to protect flora and fauna and maintain biological diversity. Thus, the use of biofertilizer is better for all the systems. The study concludes that artificial fertilizer proved harmful unless these are used by suitable methods, dose or amounts advised. On the other hand the use of biofertilizer effective and safer in comparison to fertilizer.

ACKNOWLEDGEMENTS. Special thanks to the Botany Department for providing the necessary facilities. Thanks are due to all the members of Plants Genetics Laboratory for their valuable help.

LITERATURE CITED


