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CYTOLOGICAL CHANGES IN *TRIGONELLA FOENUM-GRAECUM* (L.) UNDER THE ZINC STRESS

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ABSTRACT

The experiment was carried out to the study of cytological change in *Trigonella foenum-graecum* under the zinc stress. Different kinds of chromosomal abnormalities like chromosome bridge, laggard chromosome, micronuclei, vagrant chromosome, C-mitosis etc. were observed during cytological studies. The mitotic index decreased and percentage of aberrant cells increase as concentrations of zinc increases.

INTRODUCTION

Zinc at low doses is essential micronutrients for plants but at higher doses they may cause metabolic disorders and growth inhibition for most of plants species (Claire et al., 1991). Zinc toxicity on soybean (*Glycine max* (L.) Merr.) is reported by Tracy Shute and Sheila Macfie(2006). Rout and Das (2003) studies concerning the physiology and biochemistry with regard to zinc toxicity, uptake and transport of zinc showed that the major change was seen in the nucleus. The chromatin material was highly condensed and some of the cortical cells showed disruption and dilation of nuclear membrane. The cytoplasm became structure less, disintegration of cell organelles and the development of vacuoles were also observed; the number of nucleoli also increased in response to zinc. Cytogenetic effects of zinc sulphate were studies on somatic chromosome by Singh *et al*. (2007) and they observed different kind of chromosomal aberrations.

MATERIALS AND METHODS

The germination was carried out in petri dishes. Seeds were surface sterilized with H_2O_2 for the prevention of surface fungal/bacterial contamination. Different ppm solutions were prepared in pure distilled water in laboratory by using $ZnSO_4$ pure distilled water was used as control for the study. Ten seeds were placed on cotton in each petri dish and 40 mL solution of each concentration was supplied once for seed germination. Distilled water was applied every alternate day after this treatment. The petri dishes were monitored daily for fungal and other inspections. The cytological study was carried out 100ppm, 200ppm and 300ppm of zinc concentrations.

The staining procedure followed was that of Conger and Fairchid (1954) and Darlington and La Canr (1976). The staining with aceto orcine as well as 1:1 aceto orcine and aceto carmine was found suitable for present study.

Mitotic index was calculated by observing the slides of root tip of control and treated plants at 3rd, 5th and 7th days of growth. Total 100 cells were observed on slide. The cell in the stage of prophase, metaphase, anaphase and telophase were counted.

Photomicrographs were taken on Carl-Zesis pho-

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tomicroscope with planophotochromatic objectives using Kodak 100 ASA-35mm colour film. Daylight, yellow or green filter were used.

RESULTS

Trigonella foenum-graecum has 2n=16 number of chromosomes. When the roots of *Trigonella foenum-graecum* were treated with different concentration of zinc, the cells exhibited various abnormalities showing its toxic effect on mitotic divisions. A number of cytological abnormalities viz., chromosome bridge (Plate 1. A,C), tripolar divisions (Plate 1. F), micronuclei (Plate 1. B), vagrant chromosome (Plate 1. E), etc. observed in the treated plants. There was 'stickiness of chromosomes' in the metaphase (Plate 1. D), some cells were also seen with temporary chromosome bridge. Differences and decline of mitotic index, compared to controls were noticed in the cells of roots of treated plants.

The mitotic index and percentage of aberrant cells in control and zinc treated plants on 3rd, 5th and 7th day of growth in main roots as well as in side roots (Table 1, 2 and 3). At 3rd day mitotic index was reduced in all treatment, compared to control. The maximum reduction was observed in 300 ppm. At 7th day it was almost nil in 200 ppm and 300 ppm treatment. The percentage of aberrant cells increased with increase in the zinc concentration.

DISCUSSION

From these investigations it was revealed that zinc was mitodepressive and induced various types of

Table. 1 Effects of zinc on root tip cells of *Trigonella foenum-garecum* on 3rd day of growth.

	Concentration of zinc							
	Control		100 ppm		200 ppm		300 ppm	
	Main root	Side root	Main root	Side root	Main root	Side root	Main root	Side root
Mitotic index (%)	28.66±2.16	ND	18.66±1.22	ND	13.66±1.06	ND	11.00±0.77	ND
Aberrant cell (%)	4.64±0.46	ND	21.43±1.88	ND	31.69±2.96	ND	42.36±3.84	ND

All the values are means ± S.D., ND- Not developed during study period., NS- Not studied due to brusting and decay.

Table. 2 Effects of zinc on root tip cells of *Trigonella foenum-garecum* on 5th day of growth.

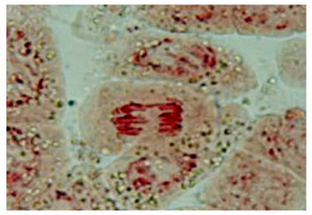
	Concentration of zinc							
	Control		100 ppm		200 ppm		300 ppm	
	Main root	Side root	Main root	Side root	Main root	Side root	Main root	Side root
Mitotic index (%)	27.33±1.48	ND	10.00±0.68	ND	6.33±0.48	ND	NS	ND
Aberrant cell (%)	4.86±0.60	ND	36.66±2.98	ND	47.39±4.55	ND	NS	ND

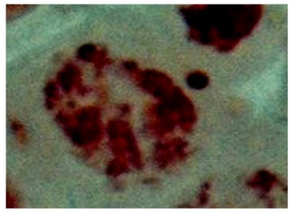
All the values are means ± S.D., ND- Not developed during study period., NS- Not studied due to brusting and decay.

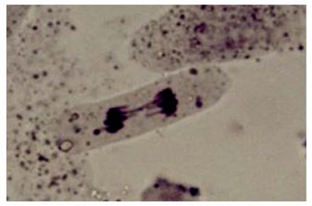
Table. 3 Effects of zinc on root tip cells of *Trigonella foenum-garecum* on 7th day of growth.

	Concentration of zinc							
	Control		100 ppm		200 ppm		300 ppm	
	Main root	Side root	Main root	Side root	Main root	Side root	Main root	Side root
Mitotic index (%)	25.66±1.33	21.66±1.12	8.33±0.54	17.33±0.80	5.66±0.38	12.66±0.66	NS	ND
Aberrant cell (%)	2.57 ± 0.42	1.52±0.28	42.26±4.08	3.66±1.67	53.00±4.62	23.69±2.14	NS	ND

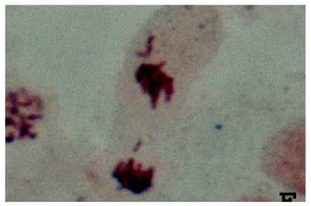
All the values are means ± S.D., ND- Not developed during study period., NS- Not studied due to brusting and decay.

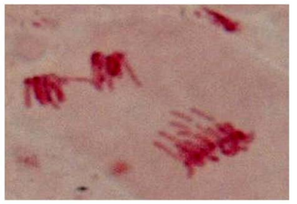

Chromosome bridge


Micronuclei


Chromosome bridge


Sticky metaphase


Vagrant chromosome


Tripolar anaphase

chromosomal aberrations in root tip cells of *Trigonella foenum-graecum*. Different concentration of zinc solution induced different types of chromosomal aberrations at various stages of cell division. Mitotic index was decreased with the increase in concentration of zinc.

Presence of more than one nucleus in proliferating following heavy metal treatment is a structural abnormality (Shylaja, 1994). In the present work the micronuclei was observed in *Trigonella foenum-graecum* due to the treatment of zinc. These micronuclei arose from acentric chromatid or chromosome fragments induced by heavy metals zinc and cadmium. Evans et al. (1959) studied the production of micronuclei after irradiating the *Vicia faba* roots. According to them all chromatid breaks, chromosome breaks, isochromatid breaks, asymmetrical exchanges and incomplete symmetrical exchanges would give rise to acentric fragments at mitosis. At the later stage of mitosis these fragments are frequently excluded from the two daughter nuclei and in the following inter phase they appear as micronuclei in one or both the daughter cells.

Stickiness of chromosome is a type of physical adhesion mainly involving the proteinaceous matrix of chromatin material and this aberration was observed by Stephen (1979) during the study of cytological causes of spontaneous fruit abortion in *Haemanthus katherinal* and suggested that sticky chromosomes could not move towards equator line or poles of anaphase and finally caused lethality of the cell. Bakale and Hadle (1981) revealed the thickening and swelling of chromosomes probably induced chromosomes to stick together, which formed a compact clump in *Euphorbia geniculata* by the treatment of herbicides 2,4-D, sodium arsenate and lasso.

The abnormalities such as vagrant were results of impaired spindle function (Shylaja, 1994). Laggards may produce micronuclei if they fail to reach the poles in time to be included in the main nucleus (Kumar and Rai, 2006).

Mitotic index decreased with increase in concentration of zinc and percentage of aberrant cells increased with increase in concentration of zinc. Reduction in mitotic index by the treatment of copper chloride on the root tips cells of *Helianthus annus* was

reported by Inceer *et al*. (2003). The effect of heavy metals on the mitotic index in Angiospermic plants was studied by several workers (Starova *et al*. 1994; Liu *et al*. 1995; Muller and Grill, 1996; Kristen, 1997; Dixon and Buschen, 1998; Muller *et al*. 1998; Kovalchuk *et al*. 1998; Krikland, 1998; Wonisch *et al*. 1999 and Ishido and Kunimoto, 2000). They all reported reduction in mitotic index. Zakia *et al*. (1990) reported mitodepressive nature in *Vicia faba* by the treatment of malathion and tamaron insecticides and suggested that the reduction of DNA synthesis which was in turn induced a substantial mitotic delay.

DISCUSSION

In conclusion, as has been stated above, zinc has harmful effects on the root tip cells of *Trigonella foenum-graecum*. In addition to these findings, the increase in soil and water pollution can lead to certain irreversible cytogenetic effects in plants and even in higher organisms.

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