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Phytotoxic Effect of Selenium on the Accessions of Wheat and Safflower

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ABSTRACT

Selenium (Se) is a metalloid and now becomes dietary important for human. The genotoxic potentiality and genetics of tolerance for this heavy metal is more or less lacking. The present communication deals with the cytological response of wheat (*Triticum aestivum*) and safflower (*Carthamus tinctorius*) to Se treatment. The 10^{-3} M concentration of SeO_2 and 10^{-4} M concentration of SeO_2 was used as a source of Se for treating wheat and safflower respectively. Control experiments were also conducted using Hoagland's solution and normal mitotic pattern was observed. *Triticum aestivum* had $2n = 42$ and *C. tinctorius* had $2n = 24$. The accessions exhibited differential cytological response to Se. Although, this heavy metal was mito-toxic to both wheat and safflower but genotoxicity was more pronounced in the accessions of wheat. In response to the Se treatment, both wheat and safflower were displaying more or less similar types of mitotic anomalies. Presented data will help to understand tolerance and effects of Se in plants.

Key words: *Carthamus tinctorius*, *Triticum aestivum*, genotoxicity, physiological, cytological

INTRODUCTION

Heavy metals like Pb, Hg, Se, As, Cd, Co, Cu, Ni, Zn and Cr are phytotoxic above their threshold levels. They infect the environment by affecting soil properties its fertility, biomass and crop yields and ultimately human health. Se, a chemical analog of S, is thought to be assimilated and volatilized by plants using the same enzymes of the S assimilation pathway (Brown and Shrift, 1982; Terry and Zayed, 1994). Controversy exists whether selenium is an essential plant micronutrient; however, there is evidence that trace amounts of selenium can enhance the growth of some plant species. Lower concentrations of selenium inhibit lipid peroxidation in *Lolium perenne* and this decrease coincides with increased growth (Hartikainen *et al.*, 2000). Se was also able to promote the growth of UV-stressed lettuce (*Lactuca sativa*) seedlings and delayed the death of plants subjected to severe UV-stress (Pennanen *et al.*, 2002). Low concentrations of selenium decreased the growth but increased the multiplication rate in *Lemna minor* (Severi, 2001). X-ray Absorption Spectroscopy (XAS) showed that wild-type Indian mustard treated with selenate accumulated Se, mainly in the form of selenate, in root and shoot tissue, whereas selenite- or SeMet-treated plants accumulated an organoselenium compound similar to SeMet (De Souza *et al.*, 1998; Zayed *et al.*, 1998). SeMet is volatilized much more readily than selenate or selenite; the rate of Se volatilization measured separately from roots and shoots of Indian mustard supplied with SeMet was 48 to 100 times higher than plants supplied with selenate or selenite (Zayed *et al.*, 1998).

Astragalus bisulcatus has the capacity to accumulate Se to high concentrations but it has a slow growth rate. It has been proposed that in Selenocysteine Methyltransferase (SMT) Specifically Methylates Selenocysteine (SeCys) to produce the nonproteinamino acid methylselenocysteine MetSeCys, which causes a reduction in the intracellular concentrations of SeCys and selenomethionine (SeMet), thus preventing their incorrect insertion into protein (Madaan, 2008). *Brassica juncea* over expressing the *A. bisulcatus* SMT gene, exhibited a greatly increased accumulation of MetSeCys and tolerance to Se compounds, in particular selenite (LeDuc *et al.*, 2004).

MATERIALS AND METHODS

During the present study (2006), 163 accessions of wheat (*Triticum aestivum*) and 234 accessions (*Carthamus tinctorius*) were tested for their cytological response to selenium (Se) treatment. At least fifty seeds each of both wheat and safflower were grown in separate sterilized petriplates lined with cotton pads, sandwiched between filter papers for raising different control and treated sets. Selenium dioxide (SeO₂) was used as source of Se. Control sets were raised in Hoagland's solution lacking selenium. The seeds of wheat were grown in 10⁻⁸ M whereas of safflower in 10⁻⁴ M solution of the SeO₂, prepared in Hoagland's solution. Both control and treated sets were raised in Calton's Seed Germinator in total darkness at 20°C. Root tip samples, collected from treated and control sets after the 48 h of the germination of seeds, were fixed in acetic alcohol (3 parts absolute ethanol+1 part glacial acetic acid) for at least 48 h and stored in 70% ethanol in refrigerator. Wheat root tips were smeared and squashed in 1.5% aceto-carmin while of the safflower in 1% aceto-orcein. Genotoxic effects of Se were quantified by working out mitotic index (MI), active mitotic index (AMI), type of mitotic anomalies and Total Mitotic Anomaly (TMA).

MI, AMI and TMA were calculated using the following formulae:

$$MI = \frac{\text{No. of cells destined to divide}}{\text{Total No. of cells}} \times 100$$

$$AMI = \frac{\text{No. of actively dividing cells (cells at metaphase and anaphase)}}{\text{Total No. of cells}} \times 100$$

$$\text{Mitotic anomalies} = \frac{\text{No. of cells showing anomalies}}{\text{No. of cells in active division}} \times 100$$

A parameter called Response Coefficient (RC) was calculated, using the following formula for estimating the toxicity imposed by Se treatment.

$$RC = \frac{VT-VC}{VC}$$

where, VT is value of treated set, VC is value of control set.

The negative value of RC's signified inhibition while positive values indicated stimulation. On the basis of RCs of the above mentioned parameters, accessions were categorized into five categories A-E. A=> -0.20; B = -0.20 to -0.39; C = -0.40 to -0.59; D = -0.60 to -0.79; E =< -0.80. Category A comprised of Tolerant (T), category E of Non-Tolerant (NT) and categories B-D of partially tolerant accessions.

RESULTS AND DISCUSSION

Wheat and Safflower exhibited differential cytological response against Se. The MIs could not be affected in both the crops. While in about 36% accessions of safflower Se could not adversely affect AMI, in wheat this could adversely effect only about 3% accessions. Further, about 8% accessions of safflower were having greater then 80% reduction in AMI but only about 4% accessions of wheat showed this behavior (Table 1).

Se could induce mitotic anomalies in 65% accessions of wheat while in safflower it could induce only on about 7% accessions; this clearly concluded that selenium was more toxic to wheat (monocot) than to safflower (dicot). Frequency distributions of the accessions (%) of wheat and safflower having different frequency (%) of TMA are shown in Fig. 1a and b. Different types of mitotic anomalies induced by Se in the two crops included: presence of c-metaphase (Fig. 2, 9), chromosomal erosion (Fig. 3), lagging of chromosomes (Fig. 4, 5), clumping of chromosomes at metaphase (Fig. 6, 8), chromatin bridge at anaphase (Fig. 7, 12), formation of groups of chromosomes at metaphase (Fig. 10), restitution nucleus formation (Fig. 11), presence of hyperploid cells (Fig. 13), formation of micro-nuclei at telophase (Fig. 14) and transcellular migration of chromatin material between neighbouring cells (Fig. 15).

Hershbach began growing plants like mustard that removed the toxic selenium compounds by concentrating them into its harvestable parts and thereby prevented the contamination of water sources. The strategy was later on named as phytoremediation. Phytoremediation is an

Table 1: Overall assortment (%) of the accessions (wheat and safflower) on the basis of AMIs to different classes of response coefficient for Se treatment

Crop	Parameters	A	B	C	D	E
Wheat	AMI	2.45	13.50	40.49	39.88	3.68
Safflower	AMI	36.32	11.11	20.51	24.36	8.12

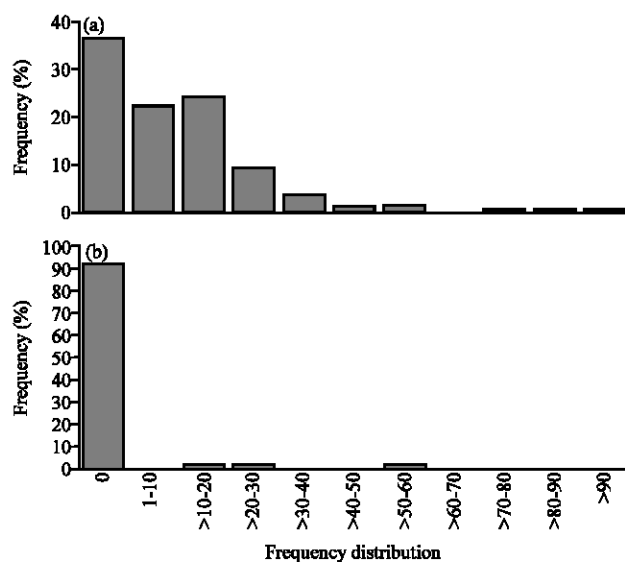


Fig. 1: (a, b) Frequency distributions of the accessions (%) of wheat and safflower having different frequencies of Total Mitotic Anomaly (TMA)

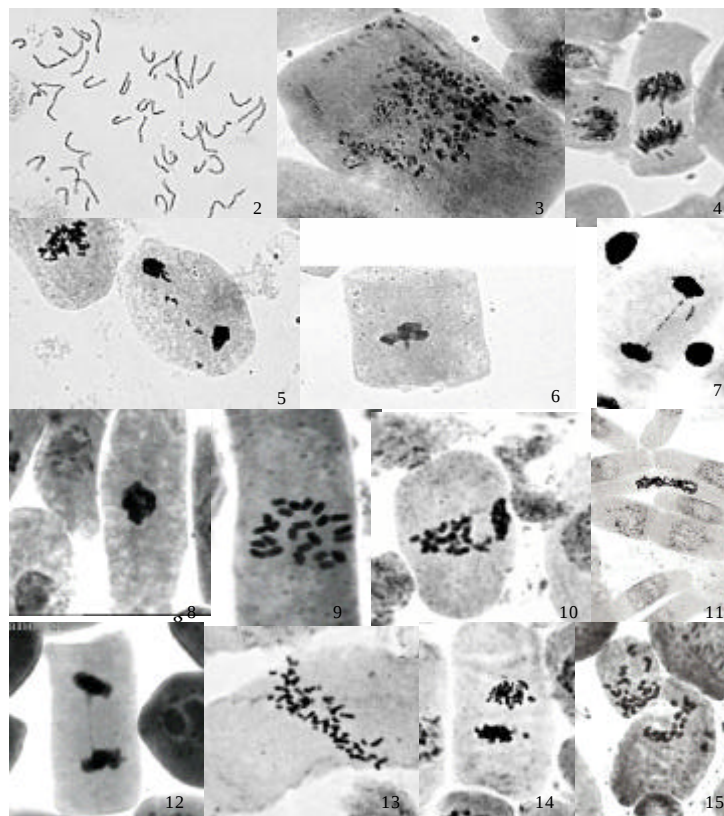


Fig. 2-15: Photomicrograph of mitotic anomalies induced by Se. (2-7 wheat) 2: C-metaphase, 3: Chromosomal erosions, 4-5: Lagging of chromosomes, 6: Clumping, 7: Chromatin bridge. (8-15 safflower) 8: Clumping, 9: C-metaphase, 10: Grouping at metaphase, 11: Restitution nucleus, 12: Chromatin bridge, 13: Hyperploid cell, 14: Micronuclei, 15: Transcellular migration

inexpensive, efficient and environment-friendly technology for the remediation of inorganic Se (Terry and Zayed, 1998). Selenium has been called as essential toxin, as it is required for certain cell processes and enzymes, but it becomes deleterious at greater doses (Russo *et al.*, 1997; Patterson and Lavender, 1997; Knekt *et al.*, 1998; Fleet, 1997; Shamberger, 1985; Young and Lee, 1999; Burguera *et al.*, 1990; Clark *et al.*, 1996; Combs and Liu, 2001; Burk, 2001; Combs *et al.*, 1997). It prevents the oxidation of Low-Density Lipoproteins (LDL) as it is an anti-oxidant, which further prevents the formation of plaque in the coronary arteries and thus saves from heart diseases (Ozer *et al.*, 1995; Lapenna *et al.*, 1998; Neve, 1996; Coppinger and Diamond, 2001). Body immune system naturally makes free radicals that can also harm healthy tissue. Se as an anti-oxidant, may help in controlling levels of free radicals and help to relieve symptoms of arthritis (Aaseth *et al.*, 1998). Indeed recent experiments with transgenic plants demonstrated that the proposed Se assimilation and volatilization pathway (Terry and Zayed, 1994) uses enzymes of the S assimilation and volatilization pathway. Phytoremediation through *Brassica juncea*, *Arabidopsis thaliana*, *Astragalus bisulcatus* (Pilon-Smits and Pilon, 2002; Mudgal *et al.*, 2010; Madaan and Mudgal, 2009) has proved useful.

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