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CYTOTOXICITY INDUCED BY ALUMINUM SULFATE IN CELLS OF ROOT MERISTEM OF *PISUM SATIVUM* CV. ARIKIL

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Abstract

Seeds of *Pisum sativum* var. Arikil were treated for 6 hrs with different concentrations of aluminum sulfate varying from 25, 50, 75, 100, 125 and 150 ppm. By observing the percentage of seed germination (PSG), radicle length (RL), mitotic index (MI) and chromosomal aberrations (CAs) in root tips of *P. sativum* the effect of Al was evaluated. Reduction in PSG and RL compared to that of part of control reveals that Al had substantial inhibiting effect on the root meristem activity of *P. sativum.* However, Al treatment also reduced MI in a dose-dependent manner when compared with control. With increasing concentrations of Al treatment, the overall percentage of aberrations usually increased. Sticky chromosome (STC), C-mitosis (C-M), Fragment (FR), Precocious Separation (PS) and Bridges (BR) were the most common aberrations observed in the present study.

Introduction

It is generally assumed that Al has no harmful effect on living organisms. Since Al rapidly forms insoluble compounds due to its reactivity, it is unable to penetrate into cells and tissues and so it is harmless to living organisms. However, in case of acid rains, Al may become soluble and react with DNA structure and thus can change their functions similar to calcium and magnesium (Wang and Kao 2004). Al is the most abundant metallic element in the earth crust after silicon and oxygen (Matsumoto and Motoda 2012, Silva 2012).

In the environment, main consideration should be given to assess potential Al toxicity. It is reported that in cultured human lymphocytes, Al treatment increased chromosomal aberrations, sister chromatid exchanges and micronuclei (Lankoff *et al*. 2006, Lima *et al.* 2007). Al treatment caused cell death in barley and *S. cerevisiae* (Pan *et al.* 2001, Zheng *et al*. 2007).

In order to identify the genotoxic effects of environmental pollutants, Plant bioassays are simpler and sensitive as compared to most other systems (Maluszynska and Juchimiuk 2005, Siddiqui 2012, 2015).

Pisum sativum (2n = 14) is a self-pollinated and dicotyledonous plant belonging to the family Fabaceae. It is a short duration crop and is used mainly as vegetable and manure. It is also used in the preparation of various ayurvedic medicines (Duke 1981, Davies *et al.* 1985). In India, it is used as an important source of protein in the diet. Though it is a multipurpose crop but only a few studies are available of the toxic effects of Al on *P. sativum* var. Arikil. In the present study, *P. sativum* var. Arikil was used to investigate the cytogenetic toxicity of aluminum sulfate in root tip of germinated seed.

Materials and Methods

Certified seeds of *Pisum sativum* var. Arikil were obtained from Agriculture seed bank, Govt. of Uttar Pradesh, Jhansi, India. Aluminum sulfate $Al_2 (SO_4)_3$, molecular weight (342.131 g/mol)

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was procured from Central Drug House (P) Ltd., New Delhi. Dry and healthy seeds of *P. sativum* of equal size and selected, were surface sterilized with 0.5% of sodium hypochlorite solution for 15 min and washed thoroughly with distilled water. They were finally soaked in double distilled water for 6 hrs. The seeds were then soaked in a glass beaker of 500 ml having 250 ml of Al solution of different concentrations {CN (Control), 25, 50, 75, 100, 125 and 150 ppm} for 6 hrs. Thirty seeds were taken from each group. The seeds were thoroughly washed 2 - 3 times in running tap water, in order to remove traces of Al sticking to the seed coat. The seeds were then spread over moist cotton which was kept in Petri dishes of 15 cm diameter. Next they were placed for further observation in a Biological Oxygen Demand Incubator (BOD) at 24 ± 2^0C. At every 24 hrs interval, the germination potential of seeds and radicle length were analyzed. This experiment was repeated thrice under similar conditions.

For cytogenetic analysis, root tips of germinated seeds were treated with different concentrations of Al. By using the method of Qian (1998) with minor modifications, chromosome preparations were made. The root tips were cut and fixed for 24 hrs in Carnoy's fixative (3 : 1, anhydrous alcohol : glacial acetic acid). They were transferred to 70% alcohol and stored in the refrigerator for further use. For 20 min, root tips were hydrolyzed at room temperature in 5N HCl. They were stained for one hr in 2% acetocarmine solution. By using squash technique as illustrated by Savaskan and Toker (1991), chromosome spreads were prepared. All slides were coded and examined blind, in order to overcome the observer biasness. For studying mitotic index (MI), from each preparation, a total of 500 cells was scored and it is expressed in terms of percentage. In minimum of 100 metaphase-anaphase plates, different types of chromosomal aberrations such as sticky chromosome, C-mitosis, fragment, precocious separation and bridges were studied.

By employing one way ANOVA test using GPIS software 1.13 (GRAPHPAD, California, USA), statistical analysis was performed in order to detect the significance of differences of variables. All values are expressed in terms of mean ± SE.

Results and Discussion

The effect of different concentrations of Al on germination of *P. sativum* seeds is shown in Fig. 1. A dose-dependent inhibitory effect on seed germination was found. At 48 hrs after the treatment, Al induced a significant reduction in seed germination at all the doses (75-150 ppm) compared to control group ($p < 0.05$ and $p < 0.01$). In group treated with (100 - 150 ppm) of Al, only 30% seeds germinated at 24 hrs. When the seed germination was observed at 24 and 72 hrs after treatment, a similar trend was observed, although at the lowest dose (25 ppm) it had a non-significant effect on seed germination. At 72 hrs after treatment 100% of the seeds germinated in control group which was only 93 and 90% in seeds treated with 100 ppm and 125-150 ppm of Al, respectively.

As shown in (Fig. 2) in untreated seeds (control group) of *P. sativum* radicle length increased with the increase in time interval and that were 0.3 ± 0.08 at 24 hrs, 1.26 ± 0.36 at 48 hrs and 3.22 ± 0.65 at 72 hrs. Al did not have any significant effect on radicle length when compared to control group at lower concentrations (25 and 50 ppm) at all time intervals (24, 48 and 72 hrs). A significant reduction was observed in radicle length when compared to control, at higher concentrations (100, 125 and 150 ppm) at all time intervals (24, 48 and 72 hrs). In treated seeds, at 25 ppm maximum radicle length was recorded (0.03 ± 0.06) at 24 hrs, (1.26 ± 0.36) at 48 hrs and (2.1 ± 0.52) at 72 hrs time interval. At 150 ppm, no radicle length was recorded at 24 hrs and minimum radicle length recorded (0.69 ± 0.20) was at 48 hrs and (1.12 ± 0.66) at 72 hrs time interval.

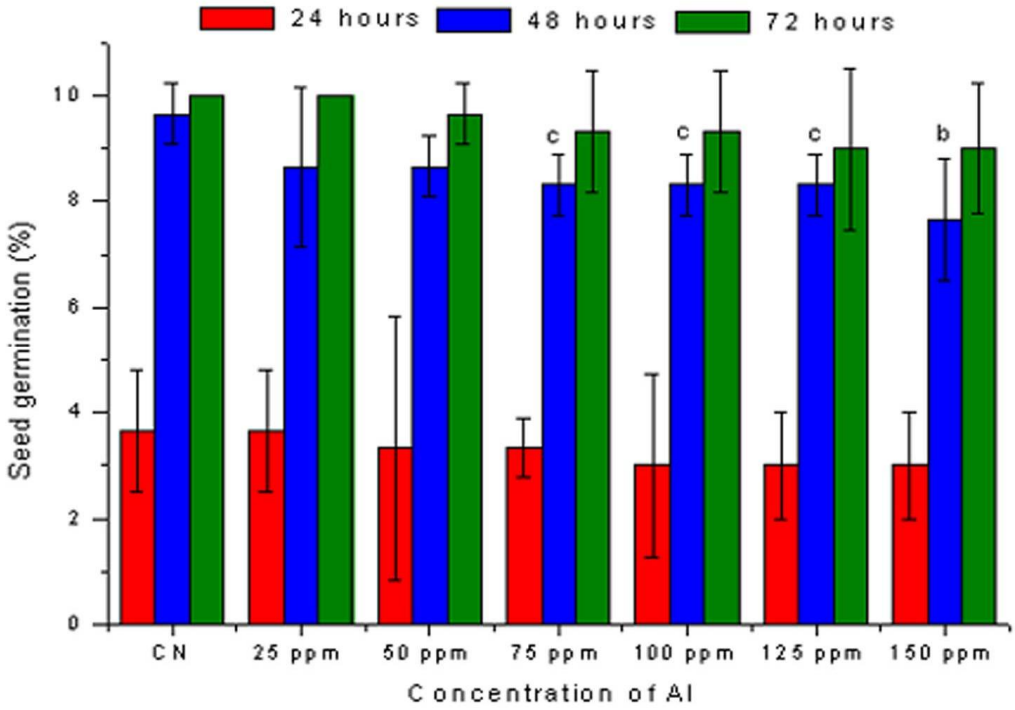

Fig. 1. Effect of different concentrations of Al on the germination of *P. sativum* var. Arikil seeds at various time intervals. b = $p < 0.01$ highly significant; c = $p < 0.05$ significant compared to control; CN = Control.

The cell division frequency is determined in the form of mitotic index (Fig. 3). The control group showed a mitotic index of 13.33 ± 2.3. In seeds treated with Al, mitotic index was observed to decrease with the increase of concentrations in a dose-dependent manner. As compared to control ($p < 0.01$), Al treatment resulted in a significant reduction in mitotic index in all concentrations (25 to 150 ppm). Mitotic index was five times lower than the control group (2.66 ± 1.15), when the seeds were treated with 150 ppm of Al.

Treatment of Al induced numerous mitotic aberrations in root tips of *P. sativum* at 72 hrs after treatment (Table 1). Several chromosomal abnormalities such as STC, C-M, FR, PS and BR were meta-anaphase plate in mitotic preparations from root tip cells of seeds treated with Al. In control, no such chromosomal abnormalities were observed. Increased prevalence of STC, C-M, FR and PS were observed after Al treatment (Fig. 4).

Increased frequency of STC (0.8 ± 0.04) was recorded in all concentrations except 125 ppm where it was absent. Maximum occurrence of C-M (0.8 ± 0.21) was observed at 150 ppm concentration of Al.

Maximum frequency of FR (0.8 ± 0.04) was found in 100 and 125 ppm of Al. Increased frequency of PS (0.4 ± 0.02) was obtained in all concentrations except 150 ppm where it was not found. BR was not found in all concentrations (25 to 100 ppm and 150 ppm) except 125 ppm (0.8 ± 0.24). Significant increase in occurrence of STC, C-M, FR, PS and BR was observed as compared to control.

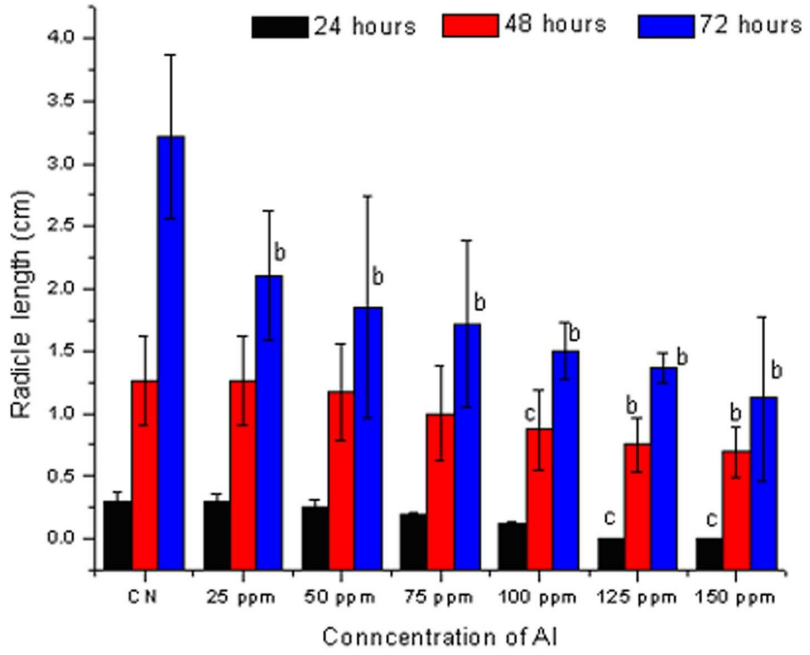

Fig. 2. Effect of different concentrations of Al on radicle length of *P. sativum* var. Arikil seeds at various time intervals. b = $p < 0.01$ highly significant; c = $p < 0.05$ significant as compared to control group; CN = Control. Data are mean of three replicates ± SE.

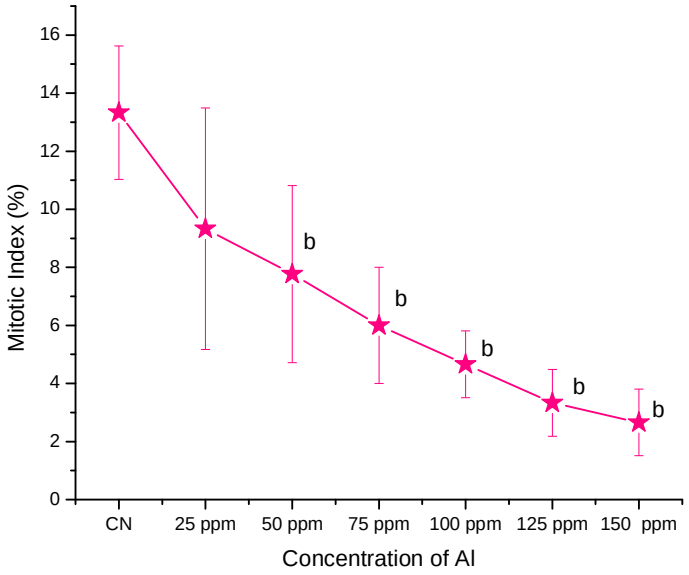

Fig. 3. Effect of Al treatment on mitotic index of root tip cells of *P. sativum* var. Arikil at 72 hrs after treatment. b = $p < 0.01$ highly significant compared to control; CN = Control. Data are mean of three replicates ± SE.

Table 1. Frequency of chromosomal aberrations at metaphase and anaphase stages of *P. sativum* var. Arikil seeds treated with different concentrations of Al at 72 hrs after treatment.

Aberration in 100 plates/cells	Concentration of Al (ppm)						
	CN	25	50	75	100	125	150
Stickiness	0.0±0.0	0.8±0.22	0.8±0.22[b]	0.8±0.24[b]	0.8 ±0.24[b]	0.0±0.00	0.8±0.32[b]
C-mitosis	0.0±0.0	0.0±0.00	0.0±0.00	0.4±0.02[b]	0.4±0.02[b]	0.0±0.00	00.8±0.21[b]
Fragment	0.0±0.	0.4±0.16[b]	0.4±0.03[b]	0.0±0.00	0.8±0.04[b]	0.8±0.22[b]	0.4±0.20[b]
Precocious Se.	0.0±0.0	0.4 ±0.01[b]	0.4±0.15[b]	0.4±0.02[b]	0.4±0.01[b]	0.4±0.02[b]	0.0±0.00
Bridge	0.0±0.0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.8±0.24[b]	0.0±0.00
Total aberrant plates	0.0±0.0	1.6±0.39[b]	1.6±0.40[b]	1.6±0.28[b]	2.4±0.31[b]	2.00±0.48[b]	2.0±0.73[b]

[b] = p <0.01 highly significant compared to control; CN = Control. Data are mean of three replicates ± SE.

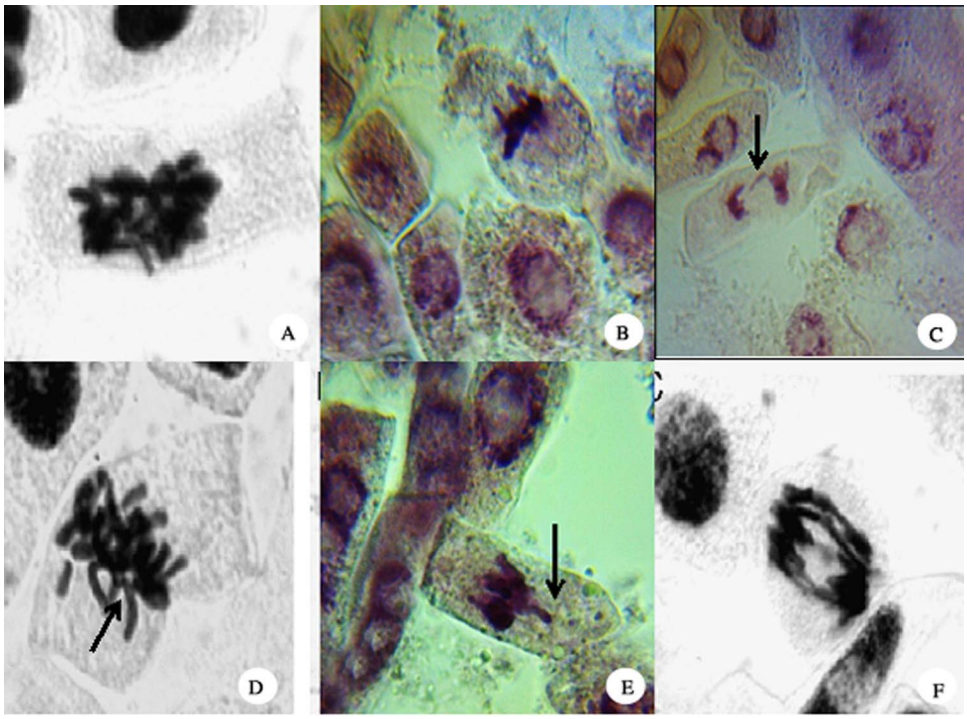

Fig. 4. A. Sticky metaphase chromosome, B. C-mitosis, C. Fragment at anaphase, D. FR Fragment at metaphase, E. Precocious separation and F. Bridge at late anaphase.

In different plants, inhibition in seed germination is shown by Al for example white spruce, pigeon pea and wheat (Nosko *et al.* 1988, Narayanan and Sayamala 1989, Lima and Copeland 1990). While working with different germplasms of wheat, Lima and Copeland (1990) accounted that seedling growth was more sensitive to Al than seed germination. Decrease in metabolic activity, inhibition of cell divisions, retarded water uptake and embryo enlargements may be the

reason leading to the failure of seed germination at high concentrations of Al treatment. Inhibition of the process of germination may be due to the blockage of any one of the phases.

After Al treatment, the growth inhibition of radicle length may be resulted from various possible mechanisms such as inhibition of cell elongation, cell division or uptake of nutrients (Delhaize and Ryan 1995, Siddiqui *et al.* 2009, Siddiqui 2015). Another hypothesis to elucidate this reduction may be related to Al binding with DNA, that may result in inhibition of cell division (Matsumoto *et al.* 1976).

A few authors have the opinion that the main reason for root growth inhibition was inhibition of cell elongation (Iamporova 2002, Zheng and Yang 2005). The main cause is that root growth inhibition may occur shortly in maize treated with Al (Llugany *et al.* 1995) whereas cell division is a bit slow process and it takes several hours to be completed.

Reduced mitotic index has been reported in current study. Reduction of mitotic index could be due to Al induced DNA synthesis blockage (Minocha *et al.* 1992, Mohanty *et al.* 2004). Total inhibition of mitosis in root meristematic cells of *P. sativum* reveals Al induced pycnosis formation (Rout *et al.* 2001). Al interrupts entry of ^{3}H-thymidine and inhibits DNA synthesis as reported by Wallace and Anderson (1984). In root tips of various species for example bean, wheat, barley and maize decrease of mitotic activity was reported as a result of Al treatment (Marienfeld *et al.* 2000, Frantzios *et al.* 2001, Doncheva *et al.* 2005, Li *et al.* 2008).

In the present study, STC, C-M, FR, PS and BR are reported in *P. sativum* root tips treated with Al. Latest study has confirmed that Al toxicity is related with production of reactive oxygen species (ROS) and mitochondrial dysfunction in plant cells (Yamamoto *et al*. 2002, Pan *et al*. 2001). Breaking of DNA single and double strands might be due to the interaction of reactive oxygen species with purine, pyriminide-bases and deoxyribose in DNA that may increase the possibility of the formation of chromosomal aberrations and micronucleus. Fragments and bridges were related to distrurbances caused by Al on spindle and DNA organization (Matsumoto *et al.* 1976, Frantzios *et al.* 2000).

Al treatment caused a considerable increase in fragments in *P. sativum* var. Arikil root tips which showed that Al is a clastogen which caused chromosome/chromatid breaks. Additional increase in Al treatment resulted in the reduction of structural chromosomal aberrations that might be due to the cytotoxic effect of Al metal which led to the suppression of cell division**.**

As found in the present study, the occurrence of different kinds of chromosomal abnormalities might be due to the fact that Al may reveal clastogenic effect on pea plant at higher concentrations. In crop plants for example pea, an advanced study of the mechanism of Al toxicity is essential.

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