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MECHANISM OF ALUMINUM CYTOTOXICITY IN MERISTEMATIC CELLS OF *HELIANTHUS ANNUUS* L.

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Aluminum toxicity of acid soils is an important growth limiting factor, which can reduce crop yields. Aluminum toxicity is a complex phenomenon having multiple effects on plant growth. The objective of present study is to analyze the effect of aluminum exposure on root tips of *Helianthus annuus* as the initial sites of aluminum toxicity, injury in root growth and reduction in rate of DNA synthesis. Results clearly showed a decrease in Mitotic index and an increase in abnormalities like bridges, stickiness, laggards etc alongwith the increasing Al concentrations.

Keywords: Aluminum; Chromosomal Abnormalities; *Helianthus annuus.*

Introduction

Aluminum is the most abundant metal and the third most common element in the earth's crust comprising approximately 7% of its mass[1]. The richest source is Aluminum trihydrate or bauxite, the ore from which Aluminum is derived.The naturally occurring forms of Aluminum are usually nontoxic and stable e.g. Al_2SiO_5, $Al(OH)_3$ and $AlPO_4$ and organically complex forms of aluminum are relatively non phytotoxic and amorphous precipitate that occur in many soils. Under certain acidic conditions (pH<5.0) however, aluminum can become soluble [Al^{+3}, $Al(OH)^{+2}$, $Al(OH)^{+}$], mobilized and easily available to plants[2,3] and become toxic for them.

The principal symptoms of aluminum toxicity include a rapid inhibition of root growth which has been proposed to be caused by a number of different mechanisms, including aluminum apoplastic lesion, interactions within the cell wall, the plasma membrane or the root symplasm[4].Aluminum can also react with other nutrients in soil such as P to form less available compounds. In addition, Al can interfere with the uptake and transport of mineral such as Ca, Mg, P, K and also causing water stress. Phytotoxic Al reduces DNA synthesis. It not only decreases the rate of DNA synthesis, but also decreases template activity[5] and causing possible interference with cell division.

Cytogenetic tests in plants are inexpensive and can easily be handled. Due to the size of their chromosomes, higher plants are suitable to cytological analysis and have shown good correlation with other bio-testing systems[6]. Plant root is extremely useful in biological testing. The root tips are often the first to be exposed to chemicals sprayed out naturally in soil or water[7]. Therefore, the observation of the root tip, constitutes a rapid and sensitive method for environmental monitoring. So experiments were performed on root meristems. *Helianthus annuus* L. (2n=34), belongs to family Asteraceae, is a heavy metal hyperaccumulator and it can tolerate metal toxicity in a better way than other plants. Root tips of *Helianthus annuus* have been utilized for the study of cytogenetical effects caused by Al. The present investigation aimed to analyse the cytogenetical responses and mechanisms of Al toxicity.

Materials and Methods

Healthy and uniform seeds of *Helianthus annuus* were selected and presoacked in distilled water for 12h and then germinated on wet filter paper in petridishes. Fresh roots, 2 cm long, were subjected to the six concentrations viz. 1, 5, 10, 15, 25 and 50 ppm of Aluminum salts (Al_2SO_4 diluted in Sodium citrate; pH=4) alongwith controls. The treatments have been arranged in a completely random design with 4 repetitions. Each repetition has been obtained from the average of 6 meristems. Excised roots of all treatments were fixed in freshly prepared fixative solution (1/3 v/v acetic alcohol) for 24 h. Squash technique was used for mitotic analysis using 2% acetocarmine as reagent. Mitotic index and percentage of mitotic abnormalities were also studied for all the treatments.

Results and Discussion

During the present investigation an increase in different chromosomal abnormalities was observed as the concentration of aluminum increases. In controls, (Fig. 1,2) 1.27% of chromosomal abnormality was observed, whereas at first treatment dose (1ppm) 1.93% abnormalities were registered which drastically increases upto 23.64% at 50ppm dose of Al salt (Table 1). However, the Mitotic index revealed contradictory results. There was a decrease in Mitotic index as the Al concentration increases. In

Table 1. Cytogenetical observation of different Aluminum concentration on meristematic cells of *Helianthus annuus*.

Dose of Treatment	Metaphasic (I/II) Abnormalities %				Anaphasic (I/II) Abnormalities %					MI %	T AB%
	St	Sc	Pr	Un	St	Br	Lg	Fr	Ot		
Con	0.74	-	-	-	-	0.47	-	-	-	14.67	1.21
1ppm	0.67	-	-	-	-	0.89	-	0.39	-	13.82	1.95
5ppm	1.26	0.58	1.0	0.77	0.39	1.72	-	-	-	10.54	5.72
10ppm	1.87	0.80	2.01	-	0.84	3.83	1.39	-	0.67	7.16	11.41
15ppm	2.69	1.72	2.35	-	2.07	7.01	-	0.67	-	6.11	16.47
25ppm	3.97	-	0.89	-	2.44	7.86	2.3	1.6	0.83	3.47	19.89
50ppm	4.04	1.50	2.46	1.50	4.57	5.13	3.48	-	0.96	1.03	23.64

Con=control; MI= Mitotic index; T Ab = total abnormality; St= stickiness; Pr = precocious movement; Sc = scattering; Un= unorientation; Fr = fragmentation; Br= bridge; Lg = laggard; Ot = other anaphasic abnormality.

controls Mitotic index was 14.67%, which decreases continuously as the dose of Al concentration increases (Table 1). Starting from 13.82% at 1 ppm it turned as 1.03% at 50 ppm. At 10ppm (7.16%) and 15 ppm (6.11%) there was a nominal difference in Mitotic index. The depression in Mitosis seems to have been caused by a physiological change in viscosity of cytoplasm, which might have inhibited the synthesis of hormones, enzyme and nucleic acid. According to Wallace and Anderson[8] aluminum inhibits DNA synthesis and interrupts entry of [H^3] thymidine. One another hypothesis to explain this reduction can be related to Al binding DNA. The double strands of DNA are captured by Al^{+3} and are unable to separate. In addition, chromatin fibers can be cross linked by the binding of Al^{+3} to DNA-phosphate between fibers which results in less active transcription [9].

Levan[10] and Liu *et al.*[11] have reported that Al causes severe cytological abnormalities in dividing cells of *Allium cepa* roots resulting from chromosomal stickiness. In our investigations however the chromosomal stickiness was found to be of second most prominent abnormality, whereas the bridge gained topmost position. Chromosomal stickiness (Fig. 5) is defined as chromosomal agglutination of unknown nature, which results in a pycnotic, or sticky appearance of chromosomes[12]. Gaulden[13] postulated that the stickiness might have resulted from the defective functioning of non-histone proteins involved in the chromosomal organization which are needed for chromosomal separation and segregation. The precocious movement (Fig. 3) of chromosomes might have been caused by the early terminalization, stickiness of chromosomes and or because of the movement of chromosomes ahead of the rest during anaphase[14]. C-metaphase or scattering (Fig. 4) observed in considerable number of cells was the consequence of inactivity of spindle apparatus connected with the delay in the division of centromere. Laggard was also observed much frequently (Fig. 6).

However, among all the abnormalities the clastogenic effects involve chromosomal and chromatid breaks resulting in formation of bridge and fragmentations at anaphase and ana-telophase of different treatments(Fig. 7, 9). Not only single but double and multiple bridges were also observed (Fig. 8) at higher concentrations of Al (15 ppm and 25ppm). This increase in number of bridges can be related to the disturbance caused by Al on spindle and DNA organization[15]. Frantzios *et al.*[16] reported that Al affects all the control mechanisms of the microtubules cytoskeleton organization. They affect the normal chromosomal movement carried out by the mitotic spindle. Decrease in the number of bridges at 50ppm (5.13%) can be justified by the low mitotic index observed, which hindered the observation of cells. Radicular growth inhibition also takes place in present study. It can be resulted from several possible reasons such as cell death, inhibition of cell division, inhibition of cell elongation or

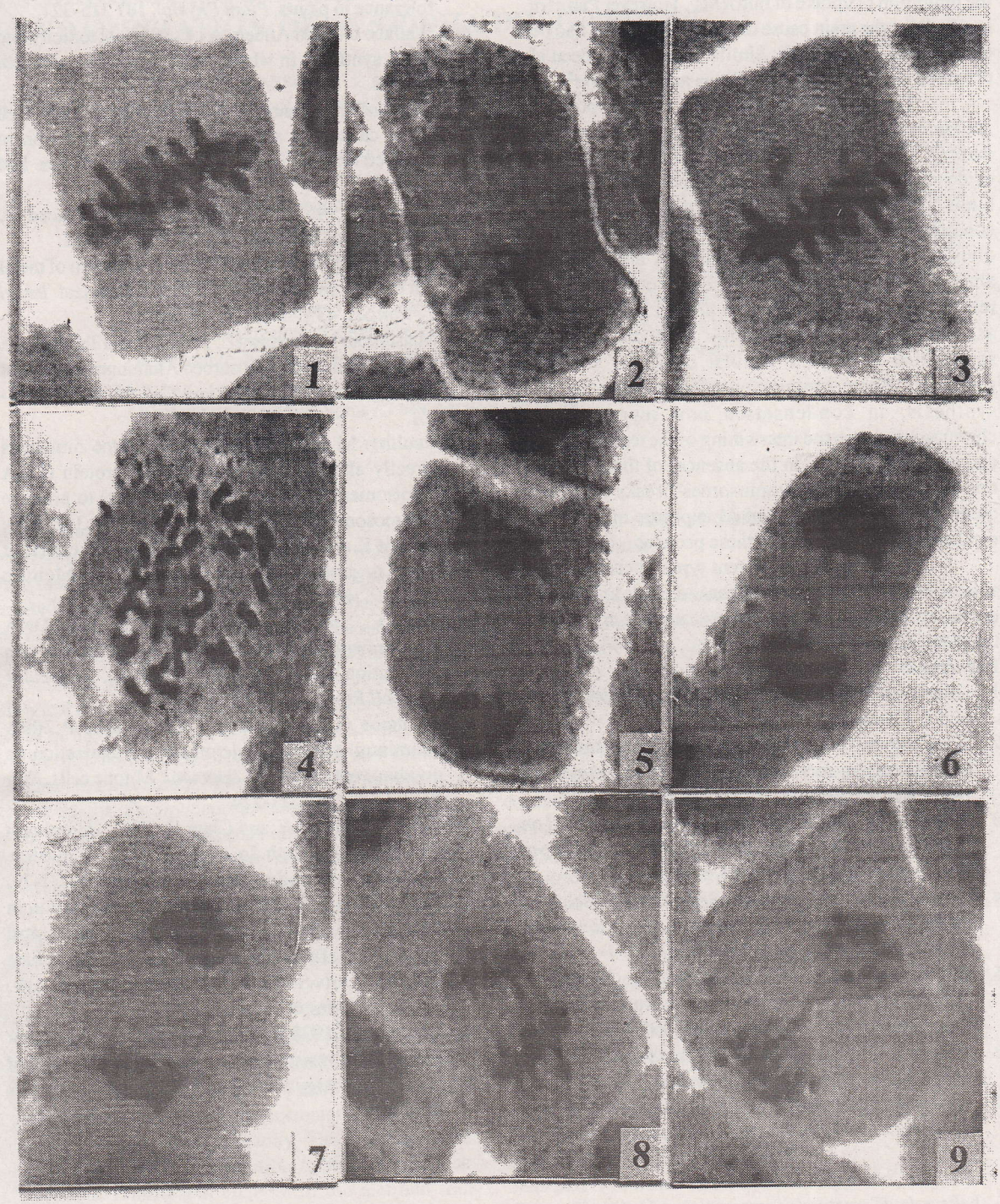

Fig. 1 – Normal Metaphase, **Fig. 2** – Normal Anaphase, **Fig. 3** – Precocious movement at Metaphase., **Fig. 4** –Scattering at metaphase, **Fig. 5** –Stickiness of chromosomes at Anaphase., **Fig. 6** –Sticky laggard at Anaphase, **Fig. 7** –Lateral sticky bridge at Anaphase.. **Fig. 8** –Multiple bridges at Anaphase., **Fig. 9** –Telophasic bridge.

the inhibition of uptake of nutrients[9]. However Pan *et al.*[17] verified that the main cause of growth inhibition had been the increase in cell death. Similar results have been also observed in Al treatment of *Allium* and *Zea mays*[2]

The differential response to Al exposure in *Helianthus annuus* root tip considering the intensity of damage and types of alterations observed, give us an evidence that different mechanisms can be involved in Al binds to DNA, unstabilizing the genetic material and consequently altering the process of replication and cell division[15-18]. Moreover the Aluminum also caused an abnormal distribution of ribosomes on endoplasmic reticulum, thus interfering with protein synthesis[19]. The association of some proteins with chromosomes segregation during the cellular cycle has also been suggested. These proteins could participate in DNA synthesis, in condensation and segregation of chromosomes or on the interwining of the replicated sister chromatids at mitosis. In the absences of these proteins, non-disjunction and chromosomes breaks have been observed[15]. It is possible that Al exposure may alter the mechanism of expression of these proteins.

On the basis of different types of chromosomal aberration our results supports that Al binds to DNA and creates chromotoxicity, which may cause the deviation of the plants from its parental lines.

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