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EFFECT OF ALUMINIUM ON *IN VITRO* WHEAT PLANTS

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ABSTRACT

The present investigation was designed to understand the *in-vitro* effects of Al on various morphological, physiological parameters in wheat plants. The study also aims to explore the effect of Al on some biomarkers of oxidative stress in order to study relationship between Aluminium toxicity, oxidative stress and detoxification responses.

Keywords **:** In-vitro, Oxidative Stress, Aluminium Toxicity, Detoxification

Introduction

The toxic metals at very low doses can result in the impairment of biological functions. Toxicity occurs when capacity of an organism to regulate the internal concentration of metals lost and abstract normal growth in organisms. Generally, organisms can metabolize and regulate both essential and non-essential metals in their internal systems with exclude, take up, accumulate and excrete metals. They show the physiological responses such as essentiality, toxicity and tolerance. Several toxic metals for instance Aluminium, Barium, Mercury, Cadmium, led do not meet the requirement of essentiality and usually accumulate, disrupt physiological processes.

Aluminium, isn't an essential element for either plants or animals. So before going into further details about Aluminium effect on plants growth, it is better to know more about how soil acidity develops and how it influences plant growth. Soil acidity is a major environmental and economic concern. It affects the availability of nutrients for plants.

Aluminium is the third most common element on the Earth's surface (Carpenter, 2001) after Oxygen and Silicon. The Al abundance in the Earth's crust is 7.28%, which is more when compared to its concentration in the ocean, which is below 1µg/L. Aluminium toxicity is a serious factor limiting crop productivity in acid soil. Al dissolves in the various ionic forms, among these Al^{+3} cation is more toxic to plants.

Because of this profitable market, opportunities may be reduced. Crop plants and grass plants which grown on acidic soil yield less crop production.

The effects of Al on Wheat plants were studied in the present investigation under *in vitro* growth conditions. The doses of Al i.e., 1.5 milliMolar, 3.5 milliMolar, 5.5 milliMolar, 7.5 milliMolar and 9.5 milliMolar were used as a treatment in the present study.

Materials and Methods

Raising of Plants

The experimental plants, Wheat (*Triticum aestivum*) belongs to the family Poaceae (Graminae) is important crop for food security in semi-arid and arid regions of the World due to high nutritional quality. The seeds of Wheat were collected randomly from the research field of Agricultural University, Polasa, Jagityal, Karimnagar and Telangana, India. Seeds with uniform size were chosen for experimental purpose.

MS medium was used as a basal medium in present investigation. Composition of MS media is given in the following tables. Initially prepared all the stock solutions required for the MS medium for the accuracy and for time saving purpose.

Composition of MS medium:

I. Macro stock (MS-I) in 1000 ml.

S. No.	Nutrient Name	Quantity in g (10 × 100)	Volume of Stock (in ml/L)
1.	KNO_3	19.0	100.0
2.	NH_4NO_3	16.5	100.0
3.	KH_2PO_4	1.7	100.0
4.	$MgSO_4.7H_2O$	3.7	100.0

The above-mentioned salts were taken into a 2-liter beaker, dissolved initially in 400 ml distilled water (H_20). Finally, the volume was made up to 1000ml by adding distilled water. Then this solution was labeled as stock- I solutions and stored at 4°C.

II. Calcium stock (MS-II) in 1000 ml.

S. No.	Nutrient Name	Quantity in g (10 × 100)	Volume of Stock (in ml/L)
1.	$CaCl_2 .2H_2O$	4.4	100.0

4.4g of $CaCl_2.2H_20$ was taken into a 2.0 liter beaker, then dissolved with 400ml distilled water, and finally made up the volume 1000ml by adding distilled water (H_20). Stored at 4ºC. This is the Stock – II.

III Micro stock (MS-III) in 1000 ml.

S. No.	Nutrient Name	Quantity in g (10 × 100)	Volume of Stock (in ml/L)
1.	H_3BO_3	62	100.0
2.	$MnSO_4. H_2O$	168.9	100.0
3.	$ZnSO_4.7H_2O$	86	100.0
4.	KI	8.3	100.0
5.	$Na_2MoO_4.2H_2O$*	2.5	100.0
6.	$CuSO_4.5H_2O$**	0.25	100.0
7.	$CoCl_2.6H_2O$**	0.25	100.0

*Prepared separately and then added

**Prepared 100 mg in 100 ml DDH_2O and then added required quantity (for 1000 ml of 100X-25 ml)

In a 2.0 liter beaker, dissolved all the salts sequentially in a descending order with 400ml of double distilled H_20 and finally made up the volume up to 1000ml by adding distilled H_2O and labeled it has stock- III solution and stored at 4^0C.

IV. MS Iron EDTA stock (MS-IV) in 1000 ml

S. No.	Nutrient Name		Quantity in g (10 × 100)	Volume of Stock (in ml/L)
1.	Na_2	EDTA.	3.73	100.0
	$2H_2O$			
2.	$FeSO_4. 7H_2O$		2.78	100.0

1000ml double distilled water was taken in a 1000 ml of amber colored bottle and warmed the water up to near boiling. Then added Na_2 EDTA. $2H_2O$ while stirring under magnetic stirrer. After it is dissolved added $FeSO_4$ gradually and mild stirring was done using magnetic stirrer. Then closed the bottle immediately and kept on stirring for an hour. And then labeled it as MS-IV and stored at 4^0C.

V. Vitamin stock (MS-V) in 1000 ml.

S. No.	Nutrient Name	Quantity in g (10 × 100)	Volume of Stock (in ml/L)
1.	Myo- inositol	1000	100.0
2.	Glycine	20	100.0
3.	Thiamine HCL	1	100.0
4.	Nicotinic acid	5	100.0
5.	Pyridoxine HCI	5	100.0

400 ml double distilled water was taken in a 2 liter beaker and then added every salt sequentially in a descending order and kept on dissolving the salts using magnetic stirring, and finally made up the solution volume to 1000 ml by adding double distilled water. Later labeled it as MS-V and stored at 4^0C.

VI. Growth regulator stocks:

Growth regulator (Auxin and Cytokinins) stocks were prepared at a concentration of 1.0 mg/ml.

MS Medium preparation:

Mixing of all stock solutions:

MS Medium was prepared by adding the above said all stock solution in a sequential manner. The below given table gives details about how much quantity of each stock solution is needed to prepare the MS Medium.

S. No.	Stock	Quantity in ml or g per liter.
1.	MS-I (40X)	25.0
2.	MS-II (40X)	25.0
3.	MS-III (40X)	25.0
4.	MS-IV (40X)	25.0
5.	MS-V (40X)	25.0
6.	Sucrose (gm)	20.0
7.	Calcium pentatinate	2.00

Preparation of 1 liter of MS basal Medium:

For preparation of 1 liter of MS Basal Medium, all the above mentioned stock solutions were added sequentially in about 400 ml of double distilled water. And then added 20 g of sucrose and dissolved it with the help of magnetic stirrer.

Then IAA and 6-BAP the medium growth regulators were added and volume of the medium was made up to 1000 ml by adding distilled water. pH of the medium was adjusted to 4.5 using 0.1 NaOH or 0.1 N HCL before autoclaving the medium.

Preparation of semi-solid medium:

Different amounts of agar were used (i.e., 8.0g, 9.0g, 11g, 14g, and 22g), because increasing concentration of Al may interfere solidification of medium. Medium was transferred into six conical flasks containing different concentrations of Al (1.5 mM, 3.5 mM, 5.5 mM, 7.5 mM, and 9.5 mM). Then boil the medium until the agar is dissolved completely. Later sterilize this medium by autoclaving at dispense the medium aseptically in sterile culture vessels.

After the autoclave, the medium was dispensed into the sterile culture vessels. Wait until the medium gets solidify, mean the time, seeds were surface sterilized with $HgCl_2$ for 3 min, followed by washing with double distilled water for several times and under laminar air flow. Then these seeds were cultures on MS Medium containing different concentrations of Al. Control was maintained along with other treatment tubes except without adding Al.

All culture tubes were maintained in a growth chamber at 24^0C, 70% RH and irradiance of 40-60 µmol m^{-2} s^{-1}T with 16 hours photoperiod. All the aseptic conditions were maintained throughout the period of seed germination.

Growth conditions

Seeds were hence surface sterilized with 0.001 Molality mercuric chloride for a couple of minutes and thoroughly washed with water several number of times. Sterilized seeds were cultured on MS medium containing different concentrations of $Al_2(SO_4)_3$ entailing 1.5 mM, 3.5 mM, 5.5 mM, 7.5 mM, 9.5 mM and solidified with different amounts of agar (9, 10, 13, 18, 22 grams) respectively. Since increasing Aluminium concentrations affected solidification of media. The pH of media was adjusted to 4.0 before autoclaving. All cultures were maintained in a growth chamber at 24^0c, 70% relative humidity and irradiance of 40-

60 µmol $m^{-2}s^{-1}$T (cool white fluorescent tubes) with 16 hours photoperiod.

Root and shoot length

The seedlings were disjoined into the roots and shoots after sampling, using a scale, the length of every part was measured. Shoot length was thus measured starting from the junction of the root to the tip of the leaf having the highest length.

Percent phytotoxicity:

Was hence calculated using the formula which is formulated by Lin and Chou in the year 1976.

$$\text{Percent Phytotoxicity} = \frac{\text{Radical length of control - Radical length of test}}{\text{Radical length of control}} \times 100$$

Assay of Lipid Peroxidation

Estimation of the lipid peroxidation (Malondialdehyde or MDA content) was hence measured by colorimetric method, which was formulated by Stewart and Bewley, in the year 1980.

Procedure

0.2 grams of leaf specimens were integrated in 5ml of distilled water. An equal volume of 0.5% thiobarbituric acid which in short known as TBA in 20% trichloroacetic acid solution was added and the sample was thus incubated at 95°C for 20 minutes. The reaction was seized by placing the reaction tubes in an ice bath. The specimens were then centrifuged at 18,000 revolutions per minute for 20 minutes.

The supernatant was detached, absorption was read at 532 nm, and then the amount of non-specific absorption was read at 600 nm and then deducted from this value. Then the amount of MDA present is determined from the extinction coefficient of 105 $mM^{-1}cm^{-1}$. Enzyme activity and MDA content of specimens were recorded in triplication, and then expressed in terms of nM/gr.fr.wt.

MDA (nM gr^{-1}fr.wt.) = [(A532 – A600) × V× 1000/ ε] × W.

Where ε is the specific extinction coefficient (105mM Cm^{-1}), W is the fresh weight of leaf, V is the volume of crushing medium, A532 is the absorbance at 532 nm wave length and A600 is the absorbance at 600 nm wave length.

Results and Discussion

***Wheat* plants were grown in M.S. medium supplemented with Al ions.**

Details of Aluminium treatments, sampling days and the various concentrations of Al applied to the culture medium were provided in the materials and methods chapter. After treatment, the plants were observed for the development of anydetail's visual symptoms of toxicity. Fig. 1-3 shows details of the symptoms exhibited, and morphological changes if any at various stages of plant growth.

Phytotoxicity symptoms

Wheat plants treated with Al were observed at regular intervals for phytotoxicity symptoms, if any, at all stages of plant growth. In our experiment, Al toxicity affected root, shoot growth decreasing linearly with increasing Al in the growth medium. Inhibition of root and shoot growth is a visible symptom of Al toxicity. The earliest symptoms concern roots.

A general retardation in the roots and shoots were observed with increase in Al treatment. Delayed germination of *Wheat* seedlings was observed with Al treatment when compared to control (Fig No 1.). Aluminium does not affect the seed germination, initial development of new root and seedling establishment. Inhibition of root growth was detected 2–4 days after the initiation of seed germination.

Inhibition of root elongation is one of the primary and most distinct symptoms of Al toxicity. The root growth of *Wheat plants*is depicted in Table 1. In the current study, the root length was determined on the tenth day, twentieth day and fortieth day of growth. We noticed significant reduction of root length as the first symptom of the Al toxicity. Marschner, 1995, demonstrated that Al shows negative impact on plants, which grow in low pH soils. The root growth inhibition is the primary symptom Al toxicity (Samac; Tesfaye, 2003; Hartwig et al., 2007; Massot; Poschenrieder; Barcelo, 1992) & reported in Wheat and Barley (Teraoka *et al.,* 2002*;* Zakir *et al.,* 2003). Matsumoto (2001); Matsumoto H and Motoda H (2012) reported that the Al toxicity reduces the root growth.

Table 1 : Application of various Aluminium concs on root length (cm $plant^{-1}$) of *Wheat* (*Triticum aestivum)*at different stages of plant growth.

Aluminium in milli Molar Conc	Sampling days		
	10	20	40
Control	4.6±0.23	11.4±0.17	15.7±0.18
1.5 Mm	3.2±0.12	3.9±0.11	7.4±0.21
3.5 Mm	3.5±0.24	2.6±0.16	6.2±0.09
5.5 Mm	2.9±0.31	1.9±0.01	5.4±0.21
7.5 Mm	2.1±0.27	1.3±0.12	3.4±0.41
9.5 Mm	0.5±0.41	0.6±0.07	0.7±0.06

Data expressed as Mean ± SE, (n = 3). Al treatment*, Sampling days*

(* significant at $P < 0.05$).

Fig. 1: 10th day old *Wheat* plants treated with different concentration of Al Concentration of Al expressed in mM.

Table 2 : Application of various concs of Aluminium on shoot length (cm plant^{-1}) of *Wheat* (*Triticum aestivum)*

Aluminium in milli Molar Conce	Sampling days		
	10	20	40
Control	7.2±0.42	8.4±0.31	9.2±0.27
1.5 mM	3.9±0.23	4.2±0.21	8.6±0.01
3.5 mM	1.2±0.07	3.7±0.07	7.2±0.08
5.5 mM	2.3±0.32	3.1±0.06	5.8±0.51
7.5 mM	1.3±0.16	2.6±0.13	3.3±0.47
9.5 mM	0.9±0.01	1.4±0.03	1.8±0.06

Data expressed as Mean ± SE, (n = 3). Al treatment*, Sampling days* (* significant at P < 0.05).

Shoot Length

The effect of Al toxicity on shoot growth of *Wheat* plants is depicted in Table 2. The shoot growth of the plants was significantly affected with all concentrations of Al on Tenth day, Twentieth day and Fortieth day of plant growth. At elevated levels, Al is known to inhibit root length by inhibition of cell elongation (Gupta *et al*., 2013). Butler *et al.*, 2001 observed a significant decrease in plant growth with increased Al concentrations in the soil.

Lipid Peroxidation in Roots

We next performed Lipid Peroxidation analysis to assess to what extent Al toxicity influence its effect on Malondialdehyde (MDA) levels in the roots of Wheat plants.

Fig No. 2 shows that the Al treatment results in a significant increase in MDA level, an indicator of lipid peroxidation.

We estimated MDA content in 10-day-old plants, which were treated with different Al concentration. Our analysis revealed that the MDA levels were recorded high in Al treated plants when compared to control plants. The MDA levels were noted gradually increased with different concentrations of Al.

These levels were 0.65 % at 1.5 mM, 32.31% at 5.5 mM, 78.71% at 7.5 mM and 126% at 9.5 mM of Al treatment when compared to control.

Further, we estimated MDA levels in 20-day-old plants. As expected, the MDA content was increased at all concentrations of Al. The Increased levels of MDA were 1.20 % at 1.5 mM, 73.23% at 7.5 mM and 131.8% at 9.5 mM of Al treatments compared to the control.

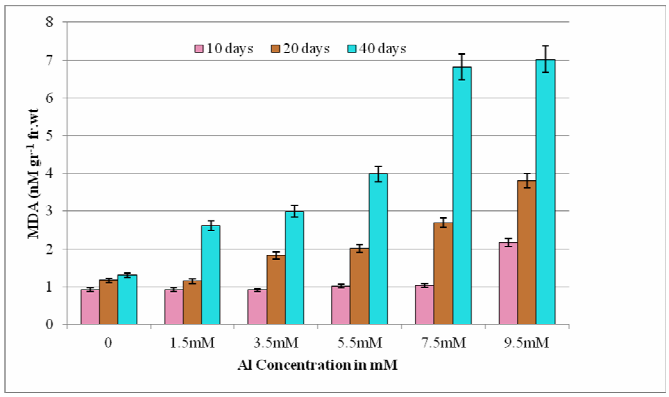
Furthermore, we estimated MDA levels in 40-day-old plants. In this analysis, we found a remarkable increase in lipid per oxidation with different concentrations Al treatment. There was a marked difference between different Levels of Al treatment and the control levels. At 1.5 mM, the significant increase in MDA content was 118% and this further increased to 469% at 7.5 mM and furthermore to 485% at 9.5 mM.

Oxidative stress is a well known mechanism of cellular injury that occurs with increased lipoperoxidation of cell phospholipids and that has been implicated in various cells dysfunctions (Sies, 1991a, b; Catala, 2006).

The metal induced Lipid peroxidation is mostly attributed to increased production of ROS especially. OH^- Radicals in plant systems (Halliwell and Gutteridge, 1984). Excess Al promoted Lipid peroxidation with excessive production of MDA content over untreated plants in concentration and age regulated manner.

Al increases super oxide anion and the concentrations of H_2O_2 and MDA by binding to sulphydryl groups of membrane proteins and increased the permeability. There by inducing Lipid peroxidation (Peixoto *et al.,* 1999; Xiao *et al.,* 2003) in Longam leaves.

Altogether, Lipid peroxidation showed significant (P < 0.05) increase in all sampling days of 10 days, 20 days and 40 days old age plants.



Vertical bars represent ± SE, (n = 3). Aluminium treatment *, Sampling days* (* significant at P < 0.05)

Fig. 2 : Effect of Al on Lipid Peroxidation activity (IU/ gr.fr.wt.) in the Roots of *Wheat* (*Triticum aestivum)* at different stages of plant growth.

Conclusion

It is evident that root growth was quickly and adversely affected within 2 days of treatment with Aluminium in Wheat plants. The effect of Al on lipid peroxidation was increased in higher doses. In general, acid soils limit crop production on large parts of agricultural land globally, primarily due to Aluminium toxicity. Therefore, understanding the effect of Aluminium toxicity on plants helps to improve new scientific and regulatory approaches.

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