INFLUENCE OF ALUMINIUM TOXICITY ON PLANT GROWTH IN WHEAT

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Abstract

The effect of aluminium on seedlings of fifteen wheat cultivars varying in their sensitivity toaluminium was investigated with different aluminium concentrations ranging from 0µM to 5000µM and with different time periods from 0hrs to 72hrs at pH 4.2. Aluminum, in its Al^{3+}cationic form, is very inimical to agriculture, as it becomes toxic in nature at lower pH (below 5) which injures plant root cells and interfere nutrient and water uptake in crop plants; thus damage root system. With decrease in pH of the culture solution below 5, aluminium caused reduction in plant growth with cultivars RAJ 4120 and WH 711 exhibiting more pronounced alterations than RAJ 3077 and DBW 17.Similarly, when compared to 0hrs to 72hrs old plants and from lowest (10µM) to highest (5000µM) aluminium concentration, the seedlings of older age showed reduced net root/ shoot growth, reduced relative root elongation rate, accumulated higher levels of aluminium, decreased relative water contentunder aluminium stress, with cultivars of RAJ 4120 and WH 711 being more affected than RAJ 3077 and DBW 17. This paper reports the results of the influence of aluminium toxicity among the sensitive and tolerant wheat cultivars on the basis of their performance in some biochemical characteristics.

Key words: AluminiumToxicity, WheatCultivars,Biochemical Characteristics, Root System

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1. Introduction

50% reduction in the average yields of major crops worldwide is the result of salinity and drought which affect more than 10% of arable land (Boyer 1982; Bray 1997). Aluminum (Al) is not an essential element for either plants or animals. It is the third most abundant metal and represents approximately 8% of the totalmineral components in the earth's crust (Verstraeten*et al.*, 2008). Al toxicity is one of the major agronomic problems and is considered as the most important growth-limiting factor for plants in acid soils worldwide (Foy *et al.*, 1978; Foy, 1984; Carver and Ownby, 1995; Jayasundara*et al.*, 1998), thus, inhibiting plant growth and development.Although Al is present in all soils and the human environment but its toxic influence increases with decrease in pH.Its toxicity is the leading factor affecting crop production. In poor Ca and Mg soils, it becomes more severe (Vitorello*et al.*, 2005).

World constitutes about 2.6 billion ha of strongly acid soils with Al^{3+} toxicity (Car *etal.*, 1991).Acidification occurs because of atmospheric inputs of natural carbonic acid, anthropogenic acidic pollutants, and some fertilization practices (Marschner, 1995). The toxicity factors associated with acidic soils are principally high H^+ and Al^{3+} and, sometimes, low Ca^{2+}, Mg^{2+}, and phosphate in the soil solution (Wright, 1989). H^+ and Al^{3+} are intrinsic toxicants as they are directly intoxicating. Ca^{2+} and $Mg^{2+}$are the extrinsic ameliorants because they drive down H^+ and Al^{3+}activities at cell-surfaces and each meets an intrinsic nutrient requirement.

In acidic soils, which account for approximately 40% of the earth's arable land, Al^{3+} toxicity is a major factor limiting plant productivity (Gupta *et al.*, 2013). The root growth of many agriculturally important crops, including wheat (*Triticumaestivum*) and maize (*Zea mays*), are suppressed by even low (micro-molar) levels of Al^{3+} within minutes or hours. Therefore, it is important to understand the Al^{3+}tolerance mechanisms of plants to establish strategies, to increase crop productivity in acidic soils.

Biochemical responses of plants associated with toxicity and tolerance to various environmental factors have been studied (Tripathi and Gaur, 2004).Plants have developed various strategies to cope up with Al stresswhich includes Al resistance by preventing Al internalization, or Al tolerance, which represents the plant potentiality to accommodate Al after uptake (Nezames et*al.*, 2012).

Cereal crops are considered as a wonderful model for studying Al tolerance and resistancebecause of their importance in agriculture and day to day life and as well as they act as ample genetic resources, (Famoso*et al*., 2010). Wheat is grown on more land area than any other commercial crop and is the most importantly used to derive staple food for humans. It is observed that aluminium stress is controlled by a complex system in plants; however, some simple methods for screening tolerance in wheat may be useful (Taylor 1991, Tang *etal*., 2002). The objective of the present work was to examine the effects of excessive aluminium ions on some physiological characteristics of wheat cultivars in the form of net plant growth, relative root elongation rate, relative water content and relating parameters.

2. Material and Methods

2.1 Plant Material Collection and Growth

A primary screening experiment was conducted with 15 different wheat genotypes (*Triticumaestivum*),collected from IARI, Pusa Campus, New Delhi, India.Seeds of the test plant were surface sterilized, rinsed thoroughly with distilled water and thensoaked for overnight at 4^0C.Imbibed seeds were then germinatedin the dark room at 25^0Cfor 3-4 days.Seedlings with uniform root and shoot lengths were selected and 10 germinated seeds per pot were then transferred on a plastic net floating on 800mL plastic pot containing nutrient or Hoagland's media solution.

2.2Exposure to Stress (Al Treatment)

Aluminium treatment was given on the day of transferring of plants and would be added in the form of $AlCl_3$ (Aluminium Chloride anhydrous). As in previous studies recorded, different concentrations of Al would be added in nutrient media (0µM, 10µM, 25µM, 50µM, 100µM, 200µM, 300µM,500µM, 1000µM, 2000µM, 3000µM, 5000µM), having pH 4.2. Three replicates were taken for each concentration. All experiments were conducted in a growth chamber with controlled temperature (18-20ºC), and a photoperiod of 8-16 hours. Plants were kept in same media for next 72 hrs. Regular stirring of the media was done. Three days after Al treatment, the seedlings were harvested, and the fresh root material would be used for screening Al tolerant and sensitive wheat genotype.

2.3 Screening of Wheat Genotypes

To determine the Al tolerant and sensitive wheat cultivars from different selected lines, screening was done. As per the previous records, root and shoot growth measurement was used as a primary and essential parameter.

2.4 Physiological Parameters

2.4.1 Root- Shoot Length Determination

Being integral parts of a plant, shoot and root are highly dependent on each other for growth and survival.Root and shoot lengths of both control (0μM) and treated (Al exposed with above stated concentrations) seedlings were recorded manually (using ruler) at 0hrs, 24hrs, 48hrs & 72hrs.Control and Al stressed seedlings were sampled at the same time to avoid any variation in readings.

- NRG= Root Length after Al-treatment–Root Length before Al-treatment
- NSG = Shoot Length after Al-treatment– Shoot Length before Al-treatment

2.4.2 Relative Root Elongation Rate

The relative root elongation (RRE) can be defined as the percentage of root elongation after the Al treatment as compared to the Al-free control (Xu*etal*., 2012). The relative root elongation rate was calculated by using the formula as given by Li et al. (2009).

- $\text{RRER (\%)} = \frac{\text{Root Length Control – Root Length Treated x 100}}{\text{Root Length Control}}$

2.4.3 Root Growth Percent

Root growth rate was measured to quantify the inhibitory effect of Al on primary root growth. The root length of 10 seedlings from each replication was measured in cm before and after 72hrs treatment period in hydroponics. Root growth percentage was calculated as per the formula given by (Duressa*etal*., 2010)

- $\text{Root Growth (\%)} = \frac{\text{Root Length after treatment – Root Length before treatment}}{\text{Final Root Length Control – Initial Root Length Control}}$

2.4.5 Root Tolerance Index

Tolerance index, developed by Wilkins (1957) can be defined as the ratio of growth undertreatment divided by growth under control, has been extensively employed. The technique has been further reviewed by Wilkins (1978). Root length of plants was measured after 3 days of Al exposure. Al tolerance index of wheat plants was calculated using following formula.

- RootTolerance Index = Net Root Growth (NRG)
  Control Root Growth (CRG)

2.4.6 Relative Water Content (RWC) of Root

The relative water content technique, formerly known as relative turgidity, was originallydescribed by Weatherley (1950, 1951). Relative water content may be accurately estimated using the ratio of tissue fresh weight to tissue turgid weight thus,termed as relative tissue weight.

A composite sample of plant root was taken and the fresh weight was determined. It was then followed by dipping for up to 4hrs as the turgid water content being obtained by soaking the roots in water. The turgid weight was then recorded. Finally, the root was subsequently oven dried to a constant weight at about 70^0C. RWC was measured by using the formula as given by Weatherley, 1950.

- RWC = Fresh Weight – Dry Weightx 100
  Turgid Weight – Dry Weight

3. Results and Discussion

Different levels of Al toxicity were tested on 15 cultivars. Net Root Growth, Net Shoot Growth, Relative Root Elongation Rate, Root Growth Percent, Root Tolerance Index, Relative Water Content of rootwere recorded and finally two tolerant and two sensitive cultivars was screened out (Table 1).It was observed that Al toxicity caused stunted roots in susceptible cultivars but shoot growth was less affected The inhibitory effect was more pronounced in susceptible cultivar as compared to resistant cultivar. This reiterates that resistant cultivar obtained from

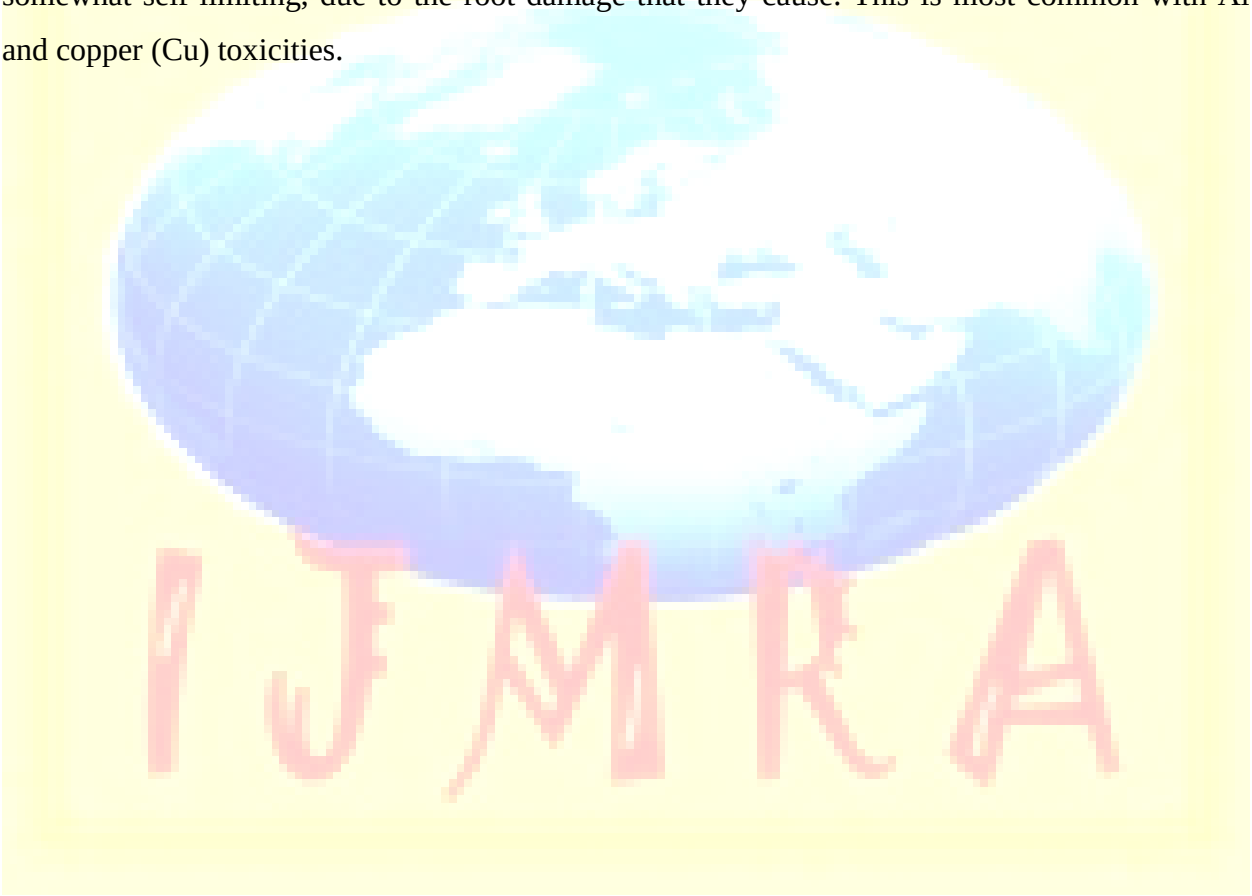
acidic soils can tolerate high soil Al levels and thus, they may be usedas source of breeding material.

Al toxicity had less effect on growth reduction in resistant cultivars. The reduction in shoot growth could also be due to nutritional imbalance caused by due to reduced availability of phosphorus (P), through the formation of Al-P compounds or reduced availability of sulphur (S), through the formation of Al-S compounds. Besides this, low pH could also reduce the availability of other nutrient cations through competitive interactions.

Root elongation is a complex process involving histo-anatomical modifications, as well ascell division and expansion changes. Root elongation of all genotypes dramatically decreased after 72hrs of exposure to different concentrations of $A1^{3+}$. However, RAJ 3077 and DBW 17 showed significant less reduction in root growth rate than Al-sensitive genotypes WH711 and RAJ 4120 under $A1^{3+}$ stress. Thus, $A1^{3+}$ tolerant genotypes had lower relative root elongation rate than sensitive cultivars. Similarly, whenRG% was observed for tolerant and sensitive cultivars, it was found that RAJ 3077 was reduced up to 12.68% withincreasing concentrationand whereas DBW 17, it was 11.64%. Whereas, WH 711 5.56% and RAJ 4120 was 8.66% (Table 2). Therefore, resistant genotypes have more capacity to tolerate $A1^{3+}$ toxicity, thus have amended root growth than the sensitive cultivars.

To evaluate the water status during the Al stress period, water deficit was observed in all the 15 genotypes with increasing $A1^{3+}$toxicity, which was calculated in the form of RWC in plant roots (Table 2, Graph 3). It is a useful indicator of the state of water balance of a plant (González and González-vilar 2001, Lata et al 2011). Thus in the presence of $A1^{3+}$stress, all the varieties lose much more water than under control condition. The ability of the plant to survive under water loss depends on its ability to restrict water transpiration. After 72hrs of stress exposure, RWC of RAJ 3077 was 83.67% and for DBW 17, it was 75.18% at 5000µM. While for WH 711 and RAJ 4120, it was about 33.35% and 23.29% respectively. Therefore, sensitive cultivars had shown more loss of water as compared to the tolerant varieties.

The soil pH is probably the single most important management factor controlling the amount of Al^{3+} in the soil solution. Although the primary damage caused by Al toxicity is to the root system but other above ground symptoms could likely occur, P-deficiency as one of the most common. Plants may also exhibit deficiency symptoms of calcium (Ca), magnesium (Mg), or other nutrients due to the Al-toxicity, that occurs in strongly acid soils. However, when the soil pH is too low, they might also show symptoms of manganese (Mn) toxicity. Finally, poor root development reduces the plants ability to absorb water and essential nutrients. It is quite difficult to diagnose root damaging plant problems with leaf analysis because the uptake of these toxins is somewhat self-limiting, due to the root damage that they cause. This is most common with Al and copper (Cu) toxicities.



Conc(µM) / Genotype	0	10	25	50	100	200	300	500	1000	2000	3000	5000	Mean
ROOT LENGTH													
RAJ 3077	5.018	4.974	4.947	4.888	4.868	4.843	4.814	4.793	4.756	4.724	4.713	4.678	4.835
DBW 17	5.565	5.404	5.358	5.308	5.283	5.219	5.133	5.103	5.032	4.883	4.820	4.759	5.155
WH 711	3.613	3.533	3.468	3.406	3.326	3.212	3.129	3.075	2.991	2.863	2.788	2.735	3.178
RAJ 4120	3.869	3.578	3.530	3.479	3.412	3.363	3.291	3.259	3.213	3.157	3.068	2.957	3.348
CD= 0.018, SE (m) =0.006													
SHOOT LENGTH													
RAJ 3077	5.201	5.170	5.14	5.095	5.048	4.990	4.919	4.872	4.789	4.658	4.578	4.503	4.913
DBW 17	5.532	5.416	5.363	5.226	5.146	5.048	4.977	4.911	4.846	4.754	4.687	4.598	5.042
WH 711	2.867	2.800	2.738	2.673	2.604	2.514	2.419	2.355	2.278	2.060	1.915	1.815	2.420
RAJ 4120	2.919	2.858	2.814	2.767	2.692	2.613	2.540	2.454	2.354	2.291	2.147	1.989	2.536
CD = 0.023, SE (m) = 0.008													
RELATIVE WATER CONTENT													
RAJ 3077	98.767	96.627	95.450	95.077	94.637	94.390	93.887	93.537	90.667	88.150	85.933	83.673	92.566
DBW 17	92.943	92.733	92.437	91.660	91.277	90.767	90.110	88.963	88.163	85.453	79.937	75.180	88.302
WH 711	82.650	79.577	72.357	67.687	64.250	60.947	57.660	53.243	49.453	44.350	40.170	33.353	58.808
RAJ 4120	95.180	92.307	88.850	81.730	74.463	68.267	61.473	53.047	48.657	41.430	32.847	23.29	63.462
CD = 0.046, SE (m) = 0.017													

Table1: Interaction between Genotypes and Al^{3+}Concentrations (µM)

Conc (µM)	NRG (cm)	RRE%	RG%	RRI	% Inhibition
0	0.71	0.00	100.00	1.00	0.00
10	0.62	13.15	86.85	0.87	13.15
25	0.50	29.58	70.42	0.70	29.58
50	0.47	33.33	66.67	0.67	33.33
100	0.44	37.56	62.44	0.62	37.56
200	0.39	44.60	55.40	0.55	44.60
300	0.33	53.52	46.48	0.46	53.52
500	0.26	63.38	36.62	0.37	63.38
1000	0.21	70.42	29.58	0.30	70.42
2000	0.15	78.40	21.60	0.22	78.40
3000	0.12	83.57	16.43	0.16	83.57
5000	0.09	87.32	12.68	0.13	87.32

Conc (µM)	NRG (cm)	RRE%	RG%	RRI	% Inhibition
0	1.70	0.00	100.00	1.00	0.00
10	1.46	14.31	86.19	0.86	14.31
25	1.32	22.35	78.11	0.78	22.35
50	1.27	25.10	75.35	0.75	25.10
100	1.20	29.41	71.01	0.71	29.41
200	1.13	33.33	67.06	0.67	33.33
300	0.94	44.51	55.82	0.55	44.51
500	0.88	48.43	51.87	0.52	48.43
1000	0.76	55.49	44.77	0.45	55.49
2000	0.51	69.80	30.37	0.30	69.80
3000	0.33	80.39	19.72	0.20	80.39
5000	0.20	88.43	11.64	0.12	88.43

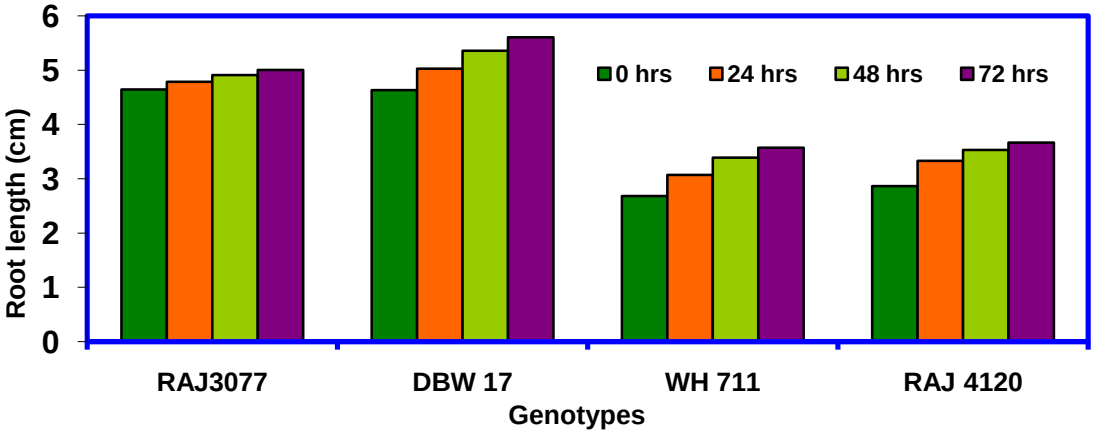
RAJ 3077 **DBW 17**

Conc (µM)	NRG	RRE%	RG%	RRI	% Inhibition
0	1.68	0.00	100.00	1.00	0.00
10	1.53	9.13	90.87	0.91	9.13
25	1.42	15.67	84.33	0.84	15.67
50	1.32	21.63	78.37	0.78	21.63
100	1.15	31.35	68.65	0.69	31.35
200	0.94	44.25	55.75	0.56	44.25
300	0.78	53.57	46.43	0.46	53.57
500	0.70	58.53	41.47	0.41	58.53
1000	0.55	67.46	32.54	0.33	67.46
2000	0.33	80.56	19.44	0.19	80.56
3000	0.20	88.10	11.90	0.12	88.10
5000	0.09	94.44	5.56	0.06	94.44

Conc (µM)	NRG	RRE%	RG%	RRI	% Inhibition
0	1.77	0.00	100.00	1.00	0.00
10	1.15	35.03	64.97	0.65	35.03
25	1.07	39.55	60.45	0.60	39.55
50	0.99	44.07	55.93	0.56	44.07
100	0.89	49.91	50.09	0.50	49.91
200	0.78	55.93	44.07	0.44	55.93
300	0.74	58.19	41.81	0.42	58.19
500	0.65	63.09	36.91	0.37	63.09
1000	0.59	66.48	33.52	0.34	66.48
2000	0.48	72.88	27.12	0.27	72.88
3000	0.37	79.28	20.72	0.21	79.28
5000	0.15	91.34	8.66	0.09	91.34

WH711 **RAJ4120**

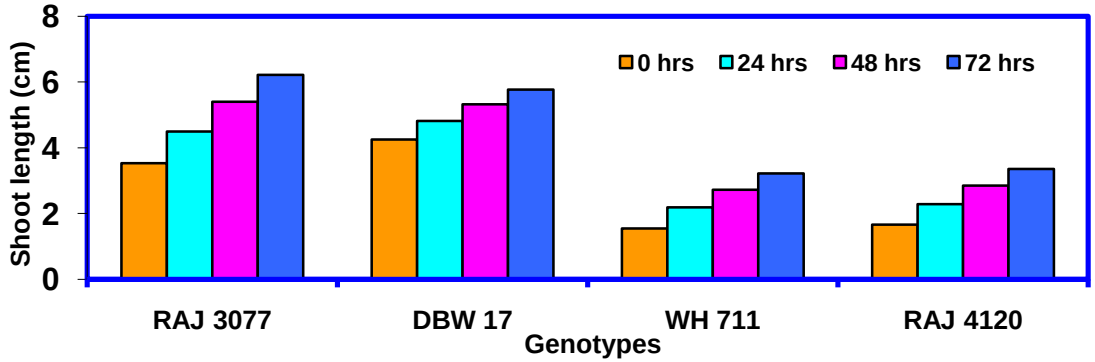
Table 2: NRG, RRE%, RG%, RRI and % Inhibition for genotypes RAJ 3077, DBW 17, WH 711 and RAJ 4120



Time(hrs) / Genotype	0hrs	24hrs	48hrs	72hrs
RAJ3077	4.646	4.785	4.907	5.001
DBW 17	4.631	5.027	5.357	5.606
WH 711	2.683	3.070	3.387	3.573
RAJ 4120	2.865	3.332	3.528	3.667
CD= 0.01, SE (m) = 0.004				

Graph 1: Effect of Al^{3+} on Root Length of 4 genotypes with increasing time period

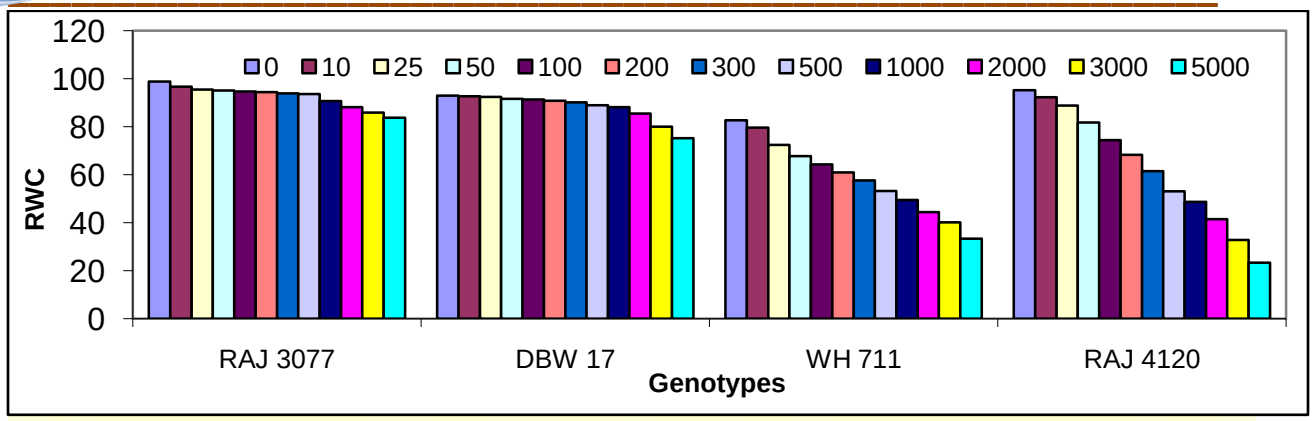
Table 3: Interaction between Genotypes and Time Period for Root Length



Time(hrs) / Genotype	0hrs	24hrs	48hrs	72hrs
RAJ 3077	3.529	4.496	5.406	6.223
DBW 17	4.254	4.822	5.323	5.769
WH 711	1.551	2.187	2.723	3.219
RAJ 4120	1.660	2.286	2.847	3.353
CD = 0.013, SE (m) = 0.005				

Graph 2: Effect of Al^{3+} on Shoot Length of 4 genotypes with increasing time period

Table 4: Interaction between Genotypes and Time Period for Shoot Length



Graph 3: Graph representing the effect of increasing Al^{3+} concentration on RWC

Many plant species secrete low molecular weight organic acids as chelators from roots in response to Al^{3+}(Gupta and Gaurav, 2014). For example, malate was released from the roots of Al^{3+} tolerant cultivars of wheat(Delhaize *et al*., 1993 a, b), and citrate from Al^{3+} tolerant cultivars of maize (Pellet et al. 1995). Some plant species accumulate Al^{3+} at high concentrations in aerial parts without showing Al^{3+} toxicity. There is evidence that Al-tolerant plant species have not only external but also internal Al^{3+}detoxification mechanisms, including chelation by organic acids. This will be presented in next paper.

4. Conclusion

A combination of proper use of fertilizers, amending of acid soils, sustainable management practices and Al tolerant varieties may improve crop yields and fertility in acid soils. Moreover, it is suggested that solid organic matter can hold Al by adsorption. Selection of proper genotypes is important for breeding for Al tolerance. Since Al is the most abundant element in the soil, but the soluble Al^{3+} is the toxic form, we need to know how much Al^{3+} is present in the soil and what controls its availability to plants. The availability of Al^{3+} is not completely understood, but certain soil factors are known to have a significant effect; 1) The total amount of Al present in a particular soil type, 2) the soil pH, 3)the types and amounts of clay in the soil and, 4) Soil organic matter. It was alsoconcluded that both tolerant and sensitive genotypes havea different strategy to control cell osmotic potential as sensitive cultivars shows more decrease of water content than in tolerant cultivars after the same concentration of $A1^{3+}$ stress and same time period.

5. References

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