

Original Research Article

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Impact of Aluminum Toxicity on Physiological Aspects of Barley (*Hordeum vulgare* L.) Cultivars and its Alleviation through Ascorbic Acid and Salicylic Acid Seed Priming

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ABSTRACT

The effect of Aluminum toxicity on seed germination and other biochemical parameters of two varieties of Barley (RD2052 and RD2552) differing in their sensitivity to aluminum toxicity were studied. In present study different concentrations of Al (Control, 2mM, 4mM and 6mM) were used to impose Aluminum toxicity under *in vitro* condition and ameliorating role of Salicylic acid and Ascorbic acid by seed priming method was studied. The complete experimental set was classified into three categories viz. (i) unprimed seedlings with Aluminum treatment; (ii) Ascorbic acid Primed seedlings with Aluminum treatment and (iii) Salicylic acid primed seedlings with Aluminum treatment. The seeds were germinated under *in vitro* condition for six days. After six days of germination, seedling parameters (Root length, Shoot length, Plant height, Fresh matter, Dry matter), Photosynthetic pigments (Chl a, Chl b, Total Chl, Carotenoids), biochemical parameters (Total sugar, Reducing sugar, Total soluble protein), enzymes of carbohydrate metabolism (Invertase, Sucrose synthase and α -amylase) and enzymes of Protein metabolism (Nitrate Reductase and Protease) were analyzed. RD2052 was more affected under Al stress due to its susceptible nature, while RD2552 showed better result and performed tolerant nature against Al toxicity. All data were analyzed by the one way analysis of variation (ANOVA).

Keywords

Hordeum Vulgare L., Germination, Al toxicity, Ascorbic acid, Salicylic acid.

Article Info

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Introduction

Al toxicity is the primary factor limiting crop production in acid soils all over the world (Kochian, 1995). Soluble forms of Al [Al^{3+} or $Al(H_2O)_6^{3+}$] inhibit roots and shoot as well as most of the plants leading to reduced growth and production. Toxic effects of Al lead to several physiological and biochemical changes in plants (Alvarez *et al.*, 2012). Aluminum also confers negative effects on photosynthetic pigments; Cai *et al.*, (2011) observed that Aluminum affects the quantity of chlorophyll pigments and suppression of

photosynthetic activities at the photosynthetic apparatus. Wan (2007) suggested that the reduction in total sugars in Al stressed is related with arrested growth rate and reduction in photosynthetic pigments. Al also reduces the enzymatic activity of carbohydrate metabolism. Sucrose synthase and Invertase are important enzymes that convert sucrose into hexose (Sun *et al.*, 1992). Al can cause harmful effects in the assimilation of nitrogen in the plants (Pal'ove-Balang and Mistrík, 2011). Toxic effect of Al

causes a reduction in nitrate concentration (Souza *et al.*, 2014). Al alters protein and amino acid content due to changes in enzymes of protein metabolism (Azmat *et al.*, 2015). Nitrate reductase and Protease are important enzymes for protein metabolism. This study was designed to investigate the protective role of Ascorbic acid (AA) and Salicylic acid (SA) in two barley varieties under Al stress by studying the seedling parameters, photosynthetic pigments, biochemical parameters, enzymes of carbohydrate metabolism and enzymes of protein metabolism.

Materials and Methods

Study area and Plant material

The present work was carried out in School of life Sciences, SIILAS Campus, Jaipur National University, Jaipur, Rajasthan, Barley (*Hordeum vulgare* L.) varieties (RD2052 and RD2552) were collected from Rajasthan Agriculture Research Institute Durgapura, Jaipur, Rajasthan.

Procedure of seed germination and priming with Ascorbic acid and Salicylic acid

Seeds were surface sterilized using 0.1% HgCl₂ for 5 minutes and washed with distilled water repeatedly for three times. This study was targeted to analyze the effects of two plant growth regulators (AA and SA) seed priming in presence of Al toxicity. Seeds were primed according to the method given by Ansari and Sharif-Zadeh (2012). Seeds were soaked in salicylic acid (250µM) and ascorbic acid (2mM) solutions at 25 °C for 12 h. The imbibed seeds were dried on filter paper at 25±2°C for 24 h and then germinated in glass petri dishes with different concentrations of aluminum (C, 2mM, 4mM and 6mM) in ¼ strength Hoagland solutions

at pH4. Seeds were allowed to germinate at 25±2°C for six days in growth chamber. After six days the average seedling parameters (Root length, Shoot length, Plant height, Fresh matter and dry matter) were recorded.

Estimation of Chlorophyll pigments

Chlorophyll pigment was estimated according to the method given by Coombs *et al.*, (1985). 0.2 g fresh leaves were homogenized in 14 ml of 80 % acetone followed by centrifugation at 10,000 rpm for 10 min. The absorbance of the supernatant was recorded at 647 nm, 664 nm and 470 nm against 80 % acetone as blank for determination of Chlorophyll a (Chl a), Chlorophyll b (Chl b) Total Chlorophyll and Carotenoid).

Anthocyanin was estimated according to the method given by Swain and Hillis (1959).

0.1 g fresh leaves were homogenate with 5ml 80% ethanol and centrifuged at 10000 rpm for 10 min. 1 ml of the alcohol extract was transferred into a test tube. 3 ml of aqueous methanolic HCl (0.5 N HCl in 85% methanol) and 1 ml of anthocyanin reagent (1 ml of 30% H₂O₂ mixed with 9 ml of methanolic HCl) were added. The blank tube was prepared in the same manner by adding 1 ml of aqueous methanolic HCl solution instead of anthocyanin reagent. All the tubes were kept in the dark for 15 min and measured the absorbance at 525 nm against the blank.

Estimation of Carbohydrate and Free amino acid

Extract preparation- The dried leaves (0.05g) were homogenized in 10 ml hot ethanol (80%) and centrifuged at 2000 rpm for 10 min. and supernatant was pooled and three ml of ethanol (80%) was add to residue and recentrifuged and supernatant was pooled again in the same vessel and evaporate to

dryness in china-dish on boiling water bath. The residue was eluted with 5 ml of 20% ethanol and subject to analysis for total sugars, reducing sugars and free amino acids.

Total sugar was estimated according to the method given by Yemn and Willis (1954).

4 ml of chilled anthrone reagent (Anthrone reagent 0.2% was dissolved in 95% chilled Sulphuric acid), 50 μ l of ethanol extract and 950 μ l of 20% ethanol was added. These was then covered with glass marbles and immediately placed in boiling water bath for 10 min. and cooled in ice bath. The absorbance of blue green color solution was read at 625 nm in spectrophotometer against blank containing 20% ethanol.

Reducing sugar was estimated by the method given by Sumner (1935).

1 ml of DNSA (dinitro-salicylic acid) reagent (1g of DNSA was dissolved in 50 ml distilled water, 1.6 g sodium hydroxide was added and dissolved 30 g of sodium potassium tartarate was added and thereafter the final volume was made up to 100 ml with distilled water), ethanol extracts (250 μ l) and 20% ethanol (750 μ l) was added. The tubes of reaction mixture were kept at 100°C for 12 minutes in boiling water bath. 2 ml of distilled water was subsequently added and absorbance was recorded at 560 nm against blank containing 20% ethanol in place of ethanol extract.

Amino acid estimation estimated by the method was given by Lee and Takahashi (1966).

3.8 ml Ninhydrin reagent (ninhydrin, 0.5 M citrate buffer and pure glycerol) was added to 1 ml of ethanol extract and the content was shacked vigorously. The mixture was heated in boiling water bath for 12 min and cooled to room temperature in running tap water. The

absorbance of the color solution was read at 570 nm against a blank containing 20% ethanol.

Total soluble protein estimation estimated by the method given by Bradford (1976).

Fresh leaves 0.1 g was homogenized in 1.5ml of 0.1 M phosphate buffer (pH7.5) and transferred to eppendorf tubes. The homogenate was centrifuged at 8000 rpm for 10 min. 0.1 ml of supernatant was taken in tube and diluted by 1 ml by 0.1M phosphate buffer (pH7.5). Then 5 ml of Bradford reagent (0.01 mg of Coomassie Brilliant Blue G-250 was dissolved in 50 ml of ethanol and to this 100 ml of 85% phosphoric acid) was added and mix thoroughly. Absorbance was recorded at 595nm against the blank.

Determination of enzymes of Carbohydrates Metabolism

Invertase activity was estimated according to the method given by Hawker and Hatch (1965).0.1g fresh plant material was homogenized in 1.5ml of chilled sodium acetate buffer (0.2 M pH4.8) containing polyvinyl pyrrolidone and centrifuged at 10,000 rpm at 4°C for 10 minutes and supernatant was used as enzyme extract.

Reaction mixture was prepared by adding 0.6 ml of (0.2M) acetate buffer pH4.8, 0.3 ml of (0.4M) sucrose solution (0.4 M sucrose solution in 0.2 M Sodium Acetate buffer pH4.8) in 0.1 ml of enzyme extract. In control tubes, sucrose was added only when enzyme preparation was inactivated by boiling for 5 min., after incubation at 30°C for 30 min. 1 ml of DNSA (2.5 g of DNSA with 150 ml distilled water containing 4.0 g of sodium hydroxide, 75 g of sodium potassium tartrate and made up the final volume up to 250 ml with distilled water) was added to reaction mixture. Thereafter, tubes were placed in

boiling water bath for 10 min. and then cooled at room temperature. The entire sample was diluted to 5 ml and absorbance was recorded at 560 nm.

Sucrose synthase activity was assayed by the method of Hawker *et al.*, (1976).

0.2 g fresh leaf tissue was homogenized in 1.5 ml of ice cold 50 mM sodium phosphate buffer, containing (10mM MgCl₂, 1mM EDTA, 10mM ascorbic acid, 2.5mM DTT and 1g Polyvinyl Polypyrrolidone), Centrifuged at 12,000 rpm for 15 minutes at 4°C and supernatant used for assay. 0.5 ml of 50mM Hepes buffer pH 8.5 containing (15mM MgCl₂, 0.2 ml of 10mM Fructose, 0.2 ml of 10mM UDP-Glucose solution) in 0.1 ml of enzyme extract, and incubated for 30 min at 30°C. The reaction was stopped by adding 0.5ml 1N NaOH. The concentrations of Sucrose Synthase were obtained by measuring optical density at 495 nm.

α- Amylase activity was assayed by the method of Shuster and Gifford (1962).

0.1 g fresh plant material was homogenized in 1.5 ml ice cold extraction buffer (.1M phosphate buffer pH7) and centrifuged at 40°C at 10,000 rpm and supernatant was used as enzyme extract. 1 ml of freshly prepare starch substrate (150 mg potato starch was dissolved with 600 mg KH₂PO₄ and 20 ml of anhydrous CaCl₂ in 100 ml of distilled water, boiled for one minute, cooled and filter) was added to 0.5 ml of enzyme extract. At zero time 0.2 ml of aliquot was removed from this and 3 ml of iodine solution (254 mg I₂ and 4 gm of KI dissolved in one liter of distilled water) was added. The absorbance was recorded at 620nm. Then the reaction mixture was incubated at 25°C. Then after every 30 min. removed the aliquot and repeated the color developing process by adding iodine solution. The enzyme activity was expressed in terms of decreased in O.D at 620 nm per unit-time (min).

Enzymes for Protein metabolism

Nitrate reductase activity was estimated according to the method given by Bordon (1984). Leaf tissue was homogenized in cold 50mM phosphate buffer containing 0.5% KNO₃ centrifuged at 12000 rpm for 10 minute at 4°C. 0.3 ml of extract was treated with 0.2 ml 1% Sulphanilamide and 0.5 ml 5% (1-Naphthyl)-ethylene diamine and left at room temperature for 20 minute. The absorbance was recorded at 542 nm.

Protease activity was assayed using the method of Ainous (1970). leaf tissue was homogenized in cold 50mM phosphate buffer containing 1% NaCl centrifuged at 12000 rpm for 10 minute at 4°C. 0.2ml supernatant was treated with 0.2 ml 1% Casein and 0.4 ml of 40% TCA solution and then 0.2ml of 0.5% Folin phenol reagent were added and absorbance was recorded at 570nm.

Data analysis

The data were determined by the one way analysis of variance (ANOVA), the design was completely randomized design (CRD). Data analysis was carried out using SPSS software. Vertical bar represent standard error.

Results and Discussion

The one way analysis of variance (ANOVA) for all data determined that there were highly significant variation between both varieties (P<0.01). According to (table 1-5) the marginal mean of the RD2052 and RD2552 treated with AA showed highest root length, shoot length and plant height compared to unprimed and SA primed seedlings. These results indicate that both AA and SA were ameliorating the AI affect successfully but AA priming was more effective in ameliorating the stress. These results

confirmed the earlier statistical analysis that primary target of Al toxicity are roots and similar observations were observed in maize (Bell and Edward, 1986) and barley (Foy, 1996). The present results of barley seedling parameters grown under Al are in agreement with the reports on maize (Malekzadeh *et al.*, 2015), Rice (Bidhan and Sanjib, 2014) and Flax (Saritha and Vasantha, 2016). All these studies reported drastic effects of Al on various growth parameters.

AA and SA both are self produced in plant, play crucial role in plant growth, also show ameliorative effect against various biotic and abiotic stresses. Similar observations were reported by Wang *et al.*, (2014) in Tomato seedling where Salicylic acid (SA) ameliorated its toxicity through activation of antioxidant system. Batool *et al.*, (2012) reported stimulatory effect of Ascorbic acid (AA) on sugarcane seedlings.

The photosynthetic pigments (Chl a, Chl b, Total Chlorophyll, Carotenoid) content significantly decreased, while Anthocyanin content increased with increased Aluminum concentration (graph 1-5). The more declined photosynthetic pigments were recorded in RD2052 barley variety that depicts its susceptible nature in comparison to RD2552 tolerant variety. Similar results of Al toxicity on photosynthetic pigments have been reported in Citrus (Jiang *et al.*, 2009) and *Brassica napus* (Zahra *et al.*, 2015). Pereira *et al.*, (2006) showed that, Al caused decrease in Chl synthesis by inhibiting the activity of aminolevulinic acid (ALA) dehydratase enzyme responsible for the formation of monopyrroleporphobilinogen, which is a part of the Chl molecule as well as the cytochromes and also impaired plant growth. Vetorello *et al.*, (2005) also reported that Al toxicity resulted in declined chlorophyll content due to cellular and ultrastructural modifications of leaves, reduction of stomatal opening, decreased photosynthetic activity,

chlorosis and leaf necrosis. Priming with AA and SA PGRs ameliorated the adverse effects of Al toxicity and resulted in the maintained Photosynthetic pigments. Comparable results have been reported for SA and AA applications in various other crop plants e.g., Sorghum (Mahendranath *et al.*, 2012), Tomato (Varalakshmi *et al.*, 2014) and Flax (Belkhadi *et al.*, 2010). Salicylic acid was reported to protect photosynthesis and stomatal regulation of plant under salinity and drought stress (Arfan *et al.*, 2007). Zhou *et al.*, (1999) reported that photosynthetic pigments increased in corn with SA application.

Increased Anthocyanin concentrations were observed in RD2052 than RD2552 at high Al concentration. Comparable results were reported in *Vigna radiata* (Sevugaperumal *et al.*, 2012) for high anthocyanin content under Al toxicity. Anthocyanins are water-soluble pigment which exhibits defense against ultraviolet radiation, herbivores, drought and cold temperatures (Hatier and Gould, 2008). Priming with SA and AA, mitigated the Al effect and further improved the Anthocyanin content. Decreased photosynthetic pigments lead to the impaired photosynthesis and this may lead to the declined assimilation product concentration.

According the graph no.6-7 the total sugar and reducing sugar concentration decreased significantly with increased Al concentration. The decrease in total sugar and reducing sugar content was more in susceptible (RD2052) barley variety in comparison to tolerant (RD2552) barley variety with aluminum treatment at 6mM concentration. Similar findings were reported in Barely (Abdalla, 2008) and Sunflower (Najmeh *et al.*, 2014) that decline in sugar content with increase in Al concentrations. Sugar content in AA and SA primed seedlings of both varieties showed better results than unprimed with less

decreasing percentage. In both RD2052 and RD2552 primed with AA showed highest sugar content at control in comparison to unprimed and SA primed barley varieties. Amira and Abdul (2014) reported that ascorbic acid treatments improved plant tolerance against water stress and sugars approached near its normal condition. Increasing amount of sugars and thus the osmosis gradient in plant tissues treated with ascorbic acid would lead to the resistance against losing water, protect chloroplasts and accelerate plant growth under stress conditions in Okra (Amin *et al.*, 2009). Similarly, mitigating effects of SA was discussed by Umebese and Fabiyi (2015), who reported that Aluminum decreased total sugar content but SA significantly alleviated Al toxicity and maintain total sugar content in *Abelmoschuses culentus* var.

Free amino acid concentration increased significantly with imposed Al toxicity. The free amino acid in susceptible variety (RD2052) increased up to 60.6% and in tolerant (RD2552) increase was up to 51.75% with aluminum treatment at 6mM concentration compared with control (graph no.8). In the same way it was noticed that AA primed RD2052 showed 42.15% increased, while RD2552 AA primed showed 26.63% increase, similarly SA primed RD2052 showed 38.86% increase, while SA primed RD2552 showed 20.33% increase at 6mM Aluminum concentration compared to control. According to Luma *et al.*, (2016) increased in total soluble amino acid content may have probably been caused by the increase in the activity of proteases enzyme, which break the reserve proteins according to the exposition of a plant to any injury, in this case the effect of aluminum toxicity. Treatment with AA and SA less increased concentration of free amino acid was noticed compared to unprimed variety. AA and SA alleviated the Al toxic effect by regulating the protease activity in

comparison to unprimed. AA and SA cause accumulation amino acid under stress through maintaining an enhanced level of ABA in seedlings (Hamada *et al.*, 2000; Hamed and Ahmed, 2013). Total soluble Protein content (graph no.9) decreased significantly with increased Aluminum concentration. The susceptible variety RD2052 showed highest decrease% (64.96%) in Protein content, while in tolerant RD2552 it was 41.46% under aluminum treatment at 6mM concentration. According to Cruz *et al.*, (2011) during the stress caused by aluminum, this element acts as a limiting factor for the assimilation of nitrogen, once there is a reduction in the nitrate reductase activity, and the low supply of nitrogen would cause a reduction in the synthesis of protein. With AA and SA priming the decrease in the total protein content was comparatively less at different Al levels compared to unprimed seedling. Dolatabadian *et al.*, (2010) reported that ascorbic acid scavenged reactive oxygen species and prevented protein oxidation and degradation. Azooz *et al.*, (2011) reported an increase in soluble proteins, due to foliar spray with SA leading to increase in broad bean growth.

Invertase, Sucrose synthase and α -Amylase was significantly decreased in both varieties RD2052 and RD2552 with increased Aluminum concentration (graph no. 10-12), but susceptible variety showed more reduction of all three enzymes than tolerant over control at 6mM aluminum treatment, But AA and SA treatment improved the activity and proved that they alleviate Al toxicity, So both primed varieties performed better and showed less decreased enzyme Invertase, Sucrose synthase and α -Amylase activity at 6mM Aluminum concentration. Similar results were observed in tomato (Simon *et al.*, 1994); barley (Mona, 2008); rice (Muthukumaran and Vijaya, 2014) against aluminum toxicity.

Table.1 Effect of different concentrations of Aluminum on Root length (cm) of RD2052 and RD2552 with and without AA and SA treatment at pH4

VarXConc	C0	2mM	4mM	6mM	Var.Mean	Decrease%
RD2052	9.82	5.50	4.04	3.03	5.61	69.14
RD2052AA	10.72	9.01	7.98	6.03	8.44	43.75
RD2052SA	10.97	8.59	6.21	5.42	7.80	50.63
RD2552	7.40	5.33	4.27	3.21	5.05	56.62
RD2552AA	9.88	9.06	7.48	6.54	8.25	33.81
RD2552SA	8.67	7.83	6.25	5.66	7.1	34.72
Conc. Mean	9.57	7.55	6.04	4.93	7.06	
S.Em±0.19		C.D.5%= 0.53		C.V%=9.16	P= 2.231E-18	

Table.2 Effect of different concentrations of Aluminum on Shoot length (cm) of RD2052 and RD2552 with and without AA and SA treatment at pH4

VarXConc	C0	2mM	4mM	6mM	Var. Mean	Decrease%
RD2052	11.57	9.22	6.29	5.66	8.18	51.08
RD2052AA	12.77	11.15	9.18	8.43	10.38	34.01
RD2052SA	12.36	10.43	8.93	7.36	9.52	35.21
RD2552	7.85	6.20	5.06	4.63	5.94	41.02
RD2552AA	10.32	10.05	7.81	7.22	8.85	30.04
RD2552SA	9.13	8.62	7.57	6.93	8.06	24.1
Conc. Mean	10.50	9.28	7.47	6.71	8.5	
S.Em±0.22		C.D.5%= 0.61		C.V%= 9.7	P =2.311E-20	

Table.3 Effect of different concentrations of Aluminum on Plant height (cm) of RD2052 and RD2552 with and without AA and SA treatment at pH4

VarXConc	C0	2mM	4mM	6mM	Var.Mean	Decrease%
RD2052	21.06	14.72	10.33	8.69	13.7	58.74
RD2052AA	23.49	20.17	17.16	14.46	18.82	38.44
RD2052SA	23.33	19.02	15.14	12.78	17.32	42.74
RD2552	15.01	11.53	9.33	7.84	10.93	47.77
RD2552AA	20.20	19.11	15.30	13.76	17.09	31.9
RD2552SA	17.79	16.45	14.82	12.6	15.42	29.17
Conc. Mean	19.98	16.83	13.68	11.7	15.55	
S.Em±0.26		C.D.5%= 0.75		C.V% =5.95	P= 2.203E-25	

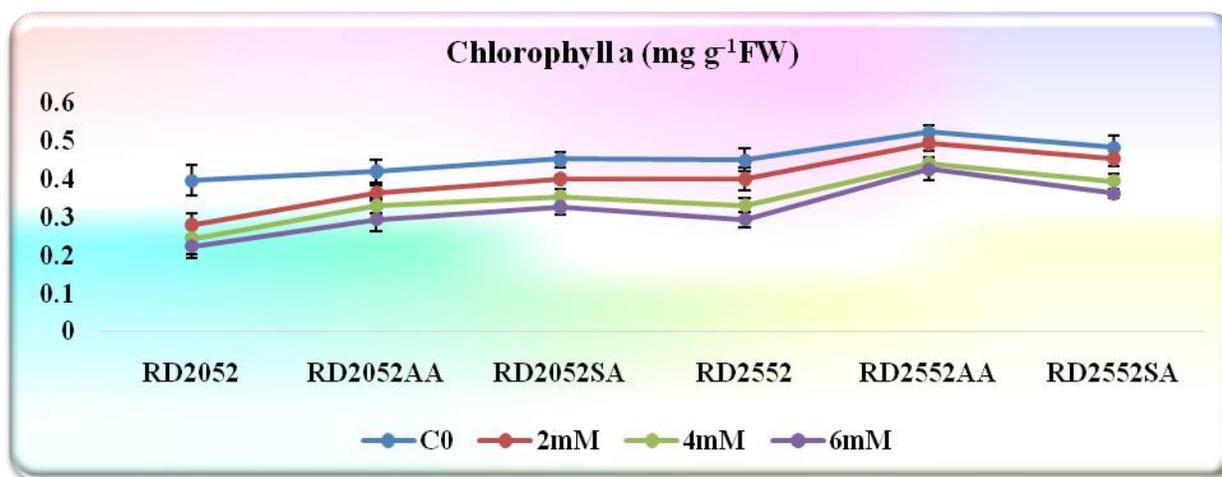
Table.4 Effect of different concentrations of Aluminum on Fresh matter (g) of RD2052 and RD2552 with and without AA and SA treatment at pH4

VarXConc	C0	2mM	4mM	6mM	Var. Mean	Decrease%
RD2052	0.653	0.433	0.327	0.250	0.416	61.72
RD2052AA	0.823	0.737	0.603	0.523	0.672	36.45
RD2052SA	0.803	0.703	0.623	0.482	0.653	40.1
RD2552	0.520	0.457	0.357	0.3	0.408	42.31
RD2552AA	0.637	0.597	0.517	0.450	0.550	29.36
RD2552SA	0.597	0.550	0.463	0.410	0.505	31.32
Conc. Mean	0.672	0.579	0.482	0.402	0.534	
S.Em±0.008		C.D.5%= 0.021		C.V%= 4.864	P= 2.121E-32	

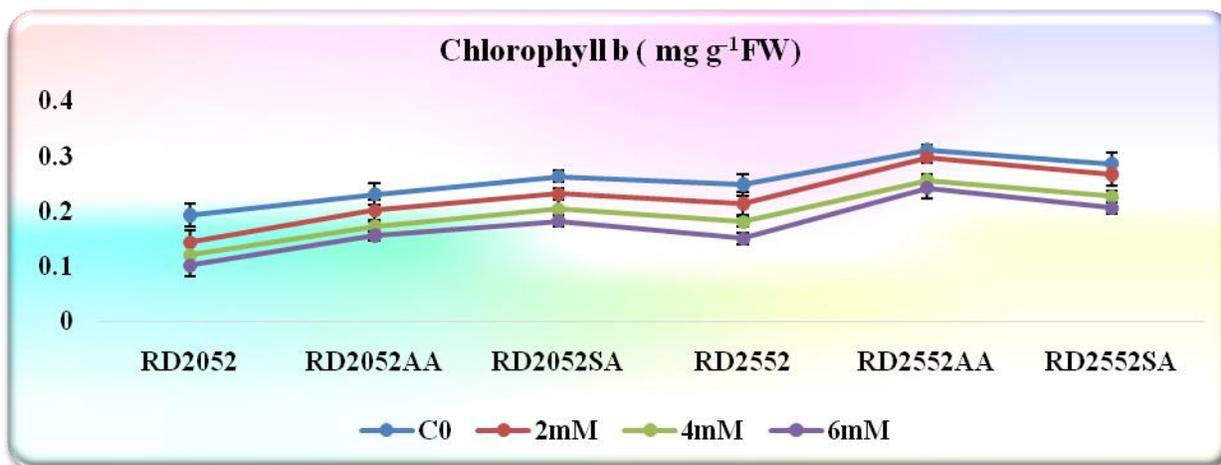
Table.5 Effect of different concentrations of Aluminum on Dry matter (g) of RD2052 and RD2552 with and without AA and SA treatment at pH4

Means	C0	2mM	4mM	6mM	Var. Mean	Decrease%
RD2052	0.073	0.053	0.037	0.03	0.048	58.9
RD2052AA	0.107	0.087	0.073	0.064	0.083	40.2
RD2052SA	0.103	0.083	0.063	0.056	0.076	45.63
RD2552	0.077	0.057	0.043	0.037	0.054	51.94
RD2552AA	0.093	0.093	0.07	0.066	0.078	29.03
RD2552SA	0.087	0.077	0.064	0.058	0.072	33.33
Conc. Mean	0.090	0.075	0.058	0.052	0.068	
S.Em±0.002		C.D.5%= 0.005		C.V%= 8.496	P= 2.433E-21	

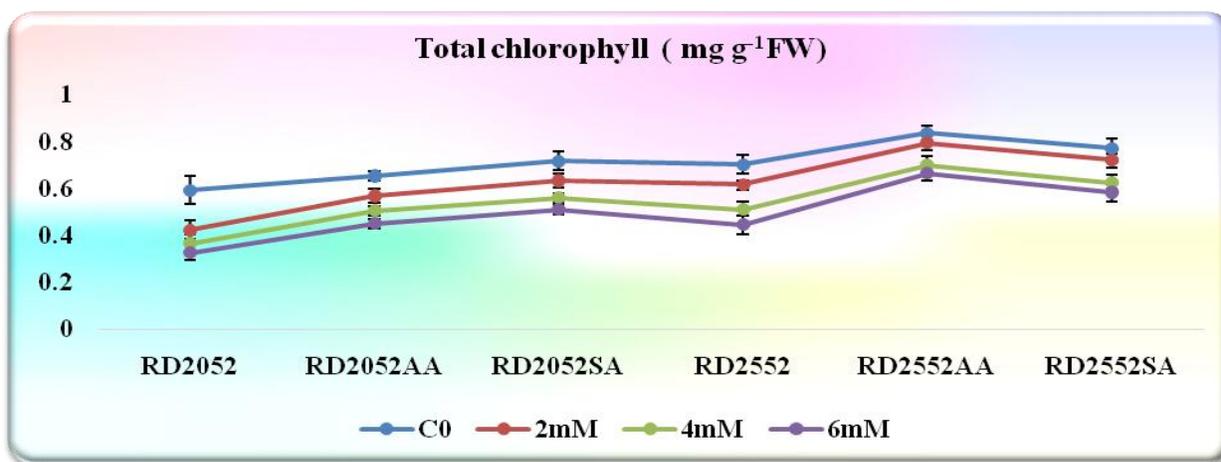
Graph.1 Chl a (mg g-1FW) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



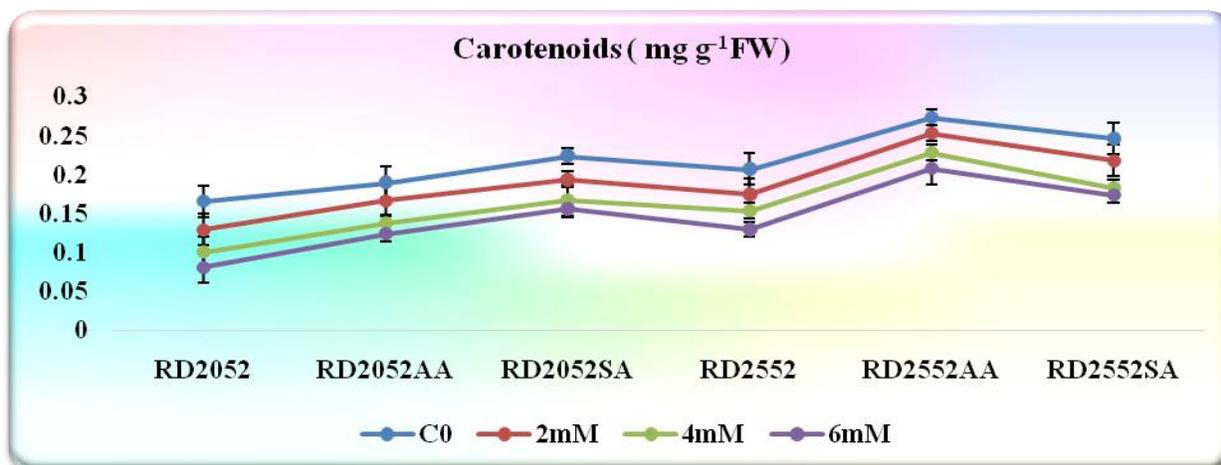
Graph.2 Chl b (mg g⁻¹FW) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



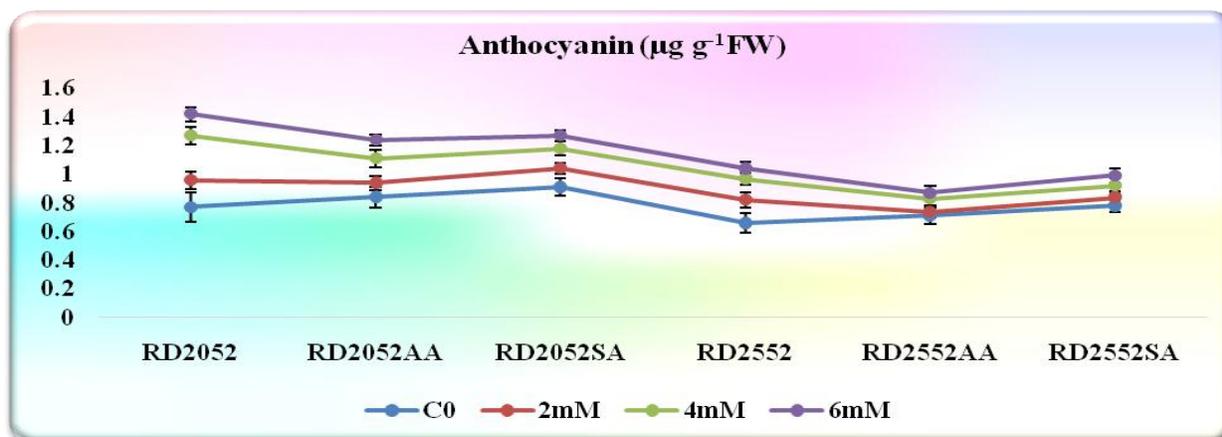
Graph.3 Total Chlorophyll (mg g⁻¹FW) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



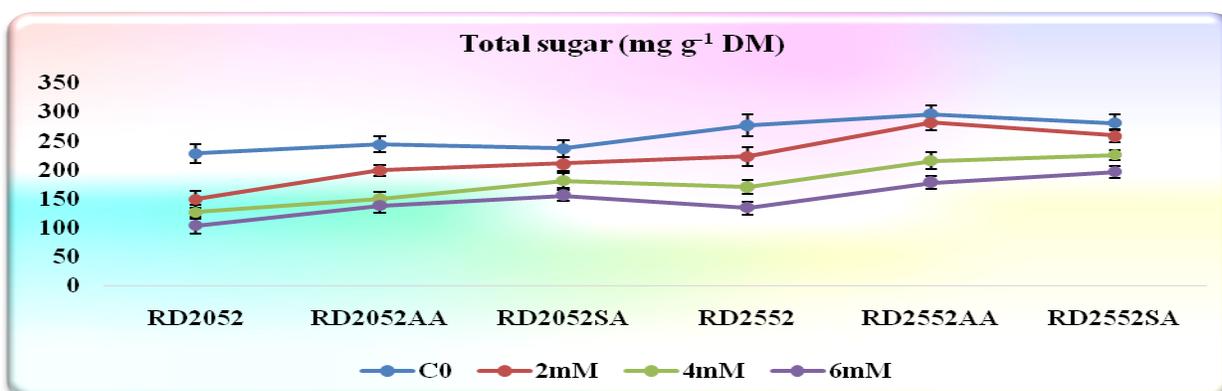
Graph.4 Carotenoid (mg g⁻¹FW) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



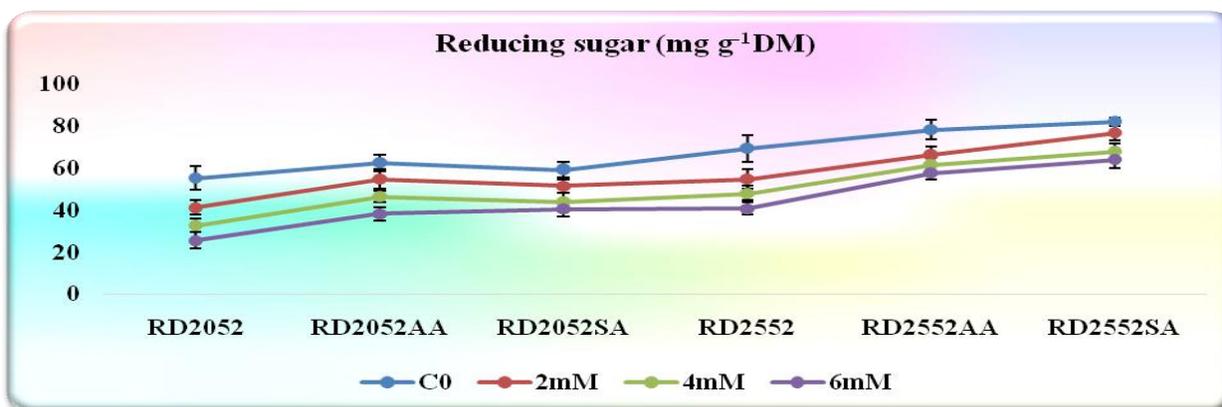
Graph.5 Anthocyanin ($\mu\text{g g}^{-1}\text{FW}$) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



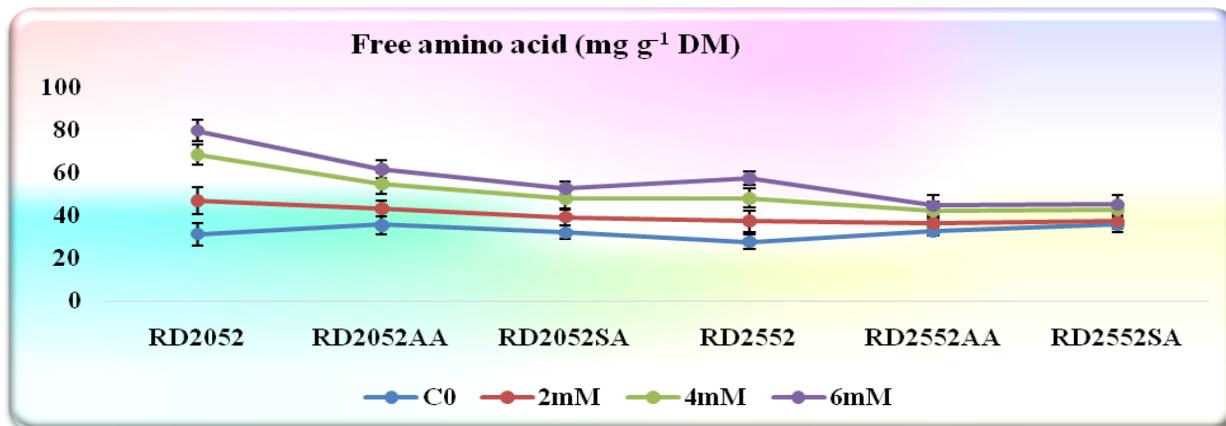
Graph.6 Total sugar ($\text{mg g}^{-1}\text{DM}$) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4.



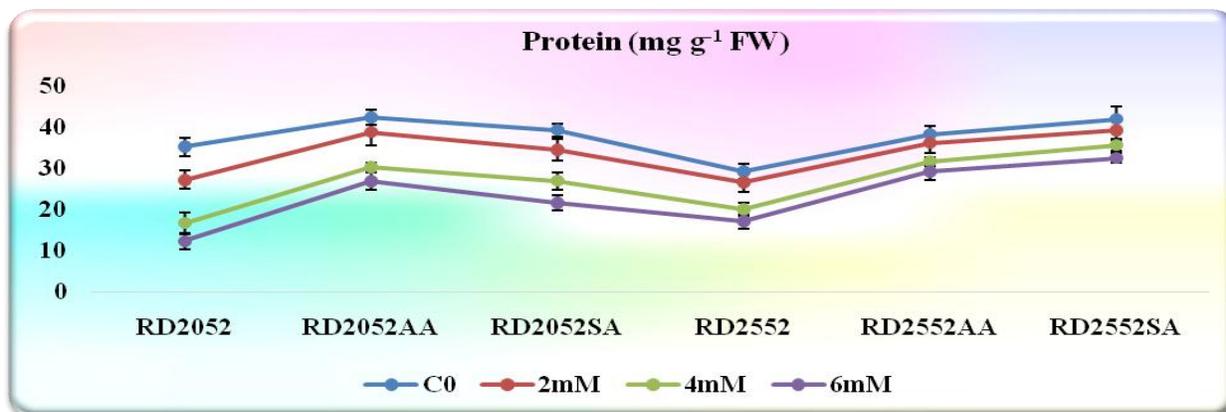
Graph.7 Reducing sugar content ($\text{mg g}^{-1}\text{DM}$) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



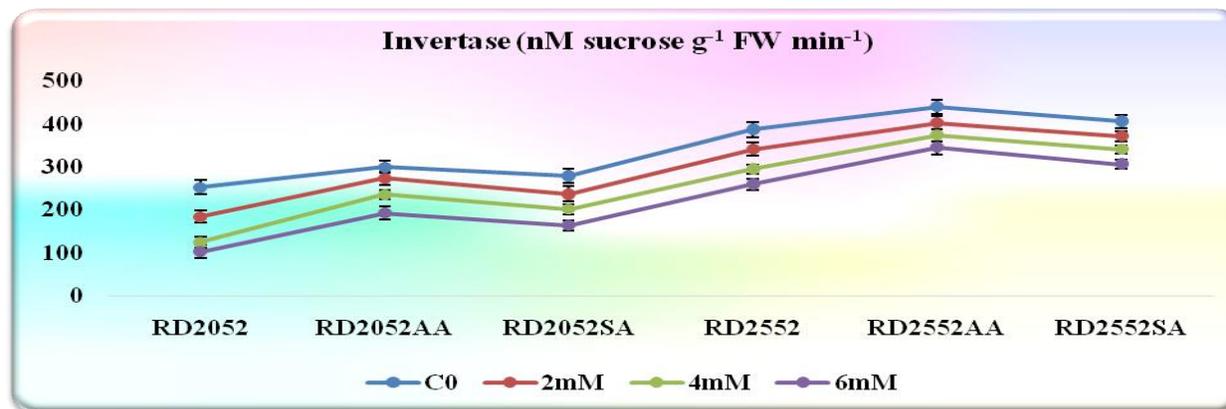
Graph.8 Free amino acid (mg g⁻¹DM) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



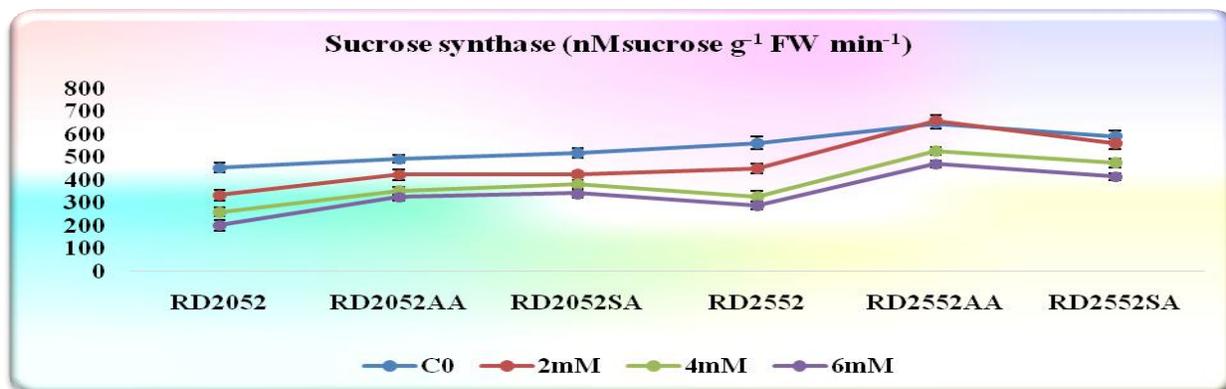
Graph.9 Protein (mg g⁻¹FW) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



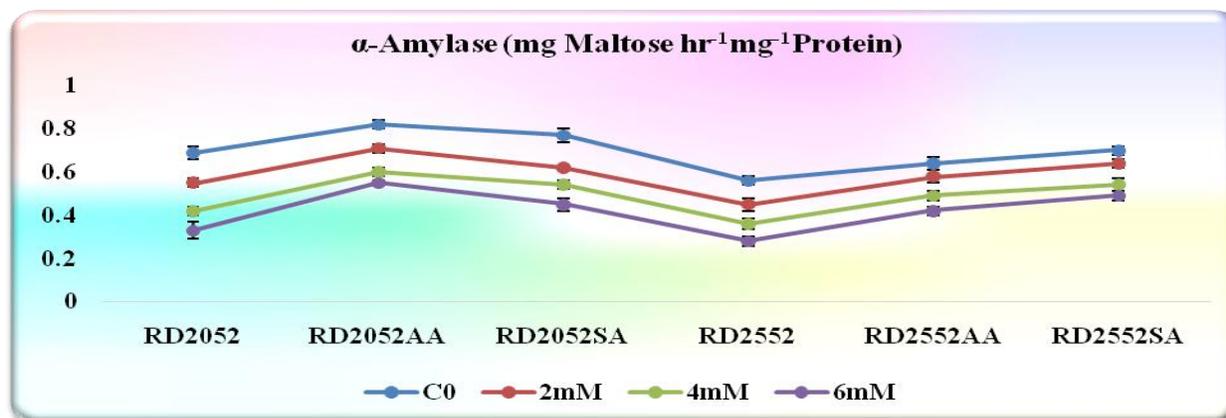
Graph.10 Invertase activity (nM sucrose g⁻¹ FW min⁻¹) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



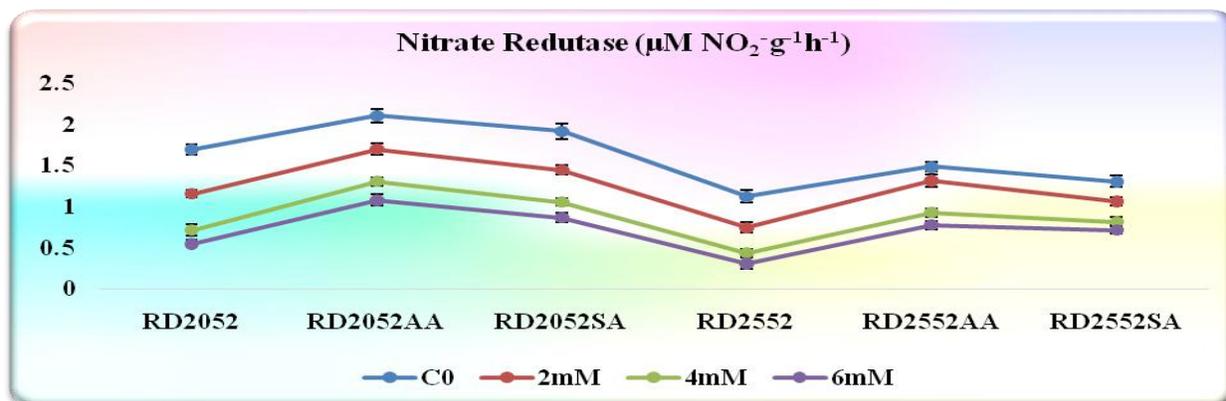
Graph.11 Sucrose synthase (nMsucrose g⁻¹ FW min⁻¹) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



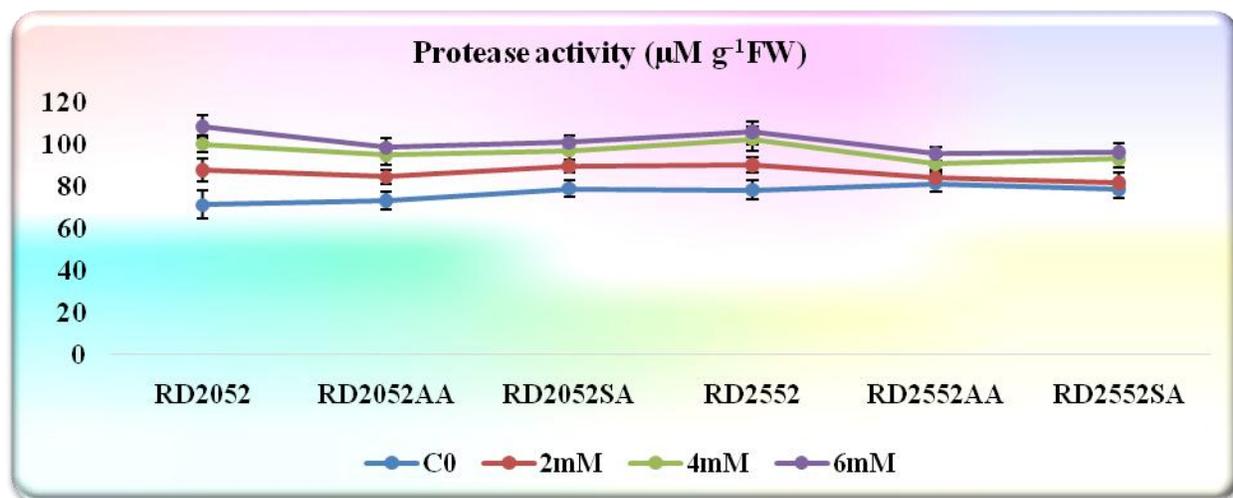
Graph.12 α -Amylase (mg Maltose hr⁻¹mg⁻¹Protein) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



Graph.13 Nitrate Redutase activity (μ MNO₂-g⁻¹h⁻¹) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



Graph.14 Protease activity ($\mu\text{M g}^{-1}\text{FW}$) in RD2052 and RD2552 Barley varieties (unprimed and primed with AA and SA) germinated under different concentrations of Aluminum at pH4



According to the graph no.13 the Nitrate Redutase activity decreased significantly with increased Aluminum concentration. At the highest Al concentration (6mM), minimum Nitrate Redutase activity was observed in susceptible (RD2052) barley variety. NR is the first enzyme in the NO_3 assimilation pathway and probably represents the rate limiting step in this process and generates NO_2 in the cytoplasm of a plant cell, which is translocated into the plastids for further reduction and metabolization (Kaiser *et al.*, 1999; Mazid *et al.*, 2010). Sharma and Dubey (2005) reported that high acidity in the soil can also cause inhibition of nitrate reductase activity. NR primed with AA and SA performed better in comparison to unprimed in both barley varieties.

Protease activity increased with increased Aluminum concentration in both barley seedlings (graph no.14). The present result agreement Muthukumaran and Vijaya (2014) reported that elevated protease activity under aluminum stress points to enhanced proteolysis in two Rice varieties. Hydrolysis of proteins by proteases releases amino acids which can use for storage and/or transport and for osmotic adjustment during stress in

seedlings (Palma *et al.*, 2002). The amino acid pool enlargement in the stressed plants can be attributed to a decreased protein synthesis and enhanced proteolysis (Parida *et al.*, 2004). Proteolysis is also allied to oxidative stress results by ROS (O_2^- , H_2O_2 , and OH^\cdot) whereas oxidative stress can modified the protein which is characterized for the production of carbonyl groups in the molecules (Azmat *et al.*, 2007; Umebese and Motajo, 2008). Protease activity in barley seedlings primed with AA and SA showed constant protease activity in comparison to unprimed barley varieties.

In conclusion, the present study was aimed to mitigate the Al toxic effect with the help of two plant growth regulators viz., AA and SA seed priming. Analysis of all the parameters (Seedlings, photosynthetic, biochemical, enzymes of carbohydrate metabolism and protein metabolism) showed that both plant growth regulator successfully ameliorate Al toxic effect in both barley varieties, and RD2052 performed as susceptible while RD2552act as tolerant Barley species under Al stress.

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