ISSN: 2347-4688, Vol. 6, No.(3) 2018, pg. 300-319

Current Agriculture Research Journal

Journal Website: www.agriculturejournal.org

Impacts of Aluminum on Growth and Biochemical Process of Wheat Plants Under Boron Treatments

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Abstract

The objective of the current study was to find out the effect of aluminum on the seedlings pre-treated by two levels of boron concentrations 4µM or 32µM grown in hydroponic solution of Al from 100-500µMAl for 3 days. Data revealed that Al had a negative effect on fresh, dry weight, water content, carbohydrate, protein and amino acids including proline constituents and changes in protein profile were analyzed of fourteen day-old Al-tolerant ('Sakha 93') cultivar of Triticum aestivum. The effect of boron treatment was pronounced at 32µM B level. Pretreatment of 4µM B and exposure to 500 µM Al revealed that insoluble protein increased soluble, total protein and total soluble sugars decreased in comparison to Al treatment only. Levels of amino acids most notably proline, the glutathione forming amino acids cysteine, glycine and glutamic and the branched chain amino acids (BCAAs) leucine, isoleucine and valine were increased under Al stress. The obtained results showed the high resistance of 'Sakha 93' cultivar to aluminum stress. Aluminum detoxification coincides with increased TSS, TP, Pro, BCAAs contents and polypeptides in the root to cope with alleviation of Al-stress; boron may have a role in this concern.

Article History

Received: 1 July 2018
Accepted: 29 September 2018

Keywords

Aluminum,
Amino acids,
Boron,
Protein profile,
Wheat.

Introduction

Wheat (*Triticum aestivum* L.) is a cereal crop globally cultivated for human consumption as a prime source of carbohydrate production, fats, vitamins, minerals and other nutritional constituents. World production of wheat could be rated in the third level after that of maize and rice.[1] There is an increase in acidic soil (pH below 5.0) in wheat production areas worldwide,

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Doi: http://dx.doi.org/10.12944/CARJ.6.3.09

which causes a threat to crop production in these regions.[2] The major growth limiting factor for wheat production on most acid soils is aluminum toxicity.[3]

Aluminum is one of the most abundant elements on the earth, constituting about 8% of soil minerals.[4] The acidification of the ground has increased the level of free Al in soils as well as in lakes and there is a positive correlation between the decrease in pH of the lakes and the increasing level of Al in the water.[4] Natural waters may contain up to 48µM Al.[5] Aluminum (Al^{+3}) is found in approximately 40% of the arable soils of the world and acidic soils favor the dissolution of microscopic quantities of Al^{+3} from metal oxides. Aluminum toxicity is a major factor in limiting growth in plants in most strongly acid soils due to several physiological and biochemical pathways.[6,7]

Plant roots are always exposed to aluminum in some forms, fortunately, most of this aluminum occurs as harmless oxides and aluminnosilicates.[8] Besides the natural occurrence of soil acidity, the extensive use of ammonia and amide containing fertilizers causes further soil acidification and aggravates aluminum toxicity that contributes to an increase in soil acidity and enhanced aluminum solubility in acid sensitive soils at low pH[2]. The use of aluminum tolerant genotypes provides the most effective alternative strategy for production of economically important crops in acid soils.[9,10]

The best approach to this abiotic problem is the improvement of the aluminum tolerance of existing crop varieties so that they may be successfully grown in acidic soils.[11] Wheat genotypes vary widely for aluminum tolerance.[10] Tolerance to aluminum toxicity in wheat is controlled by multiple12 or single dominant genes.[8]

The supply of wheat in Egypt, which represents the basis of the food system, is critical. The degree of self-supply with wheat has shown a tendency to decline within the last 3 decades and reached a very low level at the beginning of the 1990s. Wheat shows a large intraspecific variation in Al resistance13 but establishing the genetic basis for this variation has proved controversial.

Mechanism of Al toxicity and resistance are complex and have not yet been fully characterized.[14,15] Cereal crops exhibit variation in Al tolerance and wheat is considered to be sensitive to Al,[16] followed by triticale and rye. However, in some regions of Egypt, Al availability in soils is very high due to specific geoedaphic characteristics and soils in low pH levels. The cultivars Sakha 93 wheat landrace was recommended by Central Agriculture Research Institute at Dokky Egypt as acclimated to be tolerant to different abiotic stresses including metals when compared with other varieties.

For quite some time, studies on Al toxicity and the detoxification of plants mainly focus on the roots of plants[17] because aluminum contained in soil and available to plant promotes reduction in root growth.[18]

Boron is an essential micronutrient that significantly affects the seed development and quality.[19] A short term B deficiency during microsporogenesis hinders the development of anthers and adversely affects the pollen viability and growth of pollen tube consequently leading to male sterility and poor seed set.[20]

Boron (B) is an essential element required for the normal growth of higher plants and is unique micronutrient as the threshold between deficiency and toxicity is narrow.[21] In some reports, it was suggested that B may alleviate the toxic effects of Al on plant growth and improve the plant performance in acid soils.[22] It has been reported that increased B applications under Al toxicity stimulated the synthesis and accumulation of some antioxidants compounds.[23]

The alleviation of Al toxicity by B is still under debate and least understood in moncot cereals like wheat. So, in the present study was used the highly abiotic tolerant wheat cultivar Sakha 93 to demonstrate whether the increase in the exogenous application of B under Al toxicity conditions and low pH (4.3) may provide an effective strategy for combating the toxicant stress and try attempting to determine how B-Al interaction affects the growth, physiological performance and protein profile.

Material and Methods

Plant Materials and Growth Conditions

The seed of wheat cultivar (Sakha 93) used in this study was obtained from Central Agricultural

Research Institute, El Dokky (Egypt). The seed was surface-sterilized with 10% sodium hypochlorite solution for 10 min, washed several times, treated with acid and alkali and well washed by glass distilled water and imbibed in distilled water for 1 day. Then imbibed 15-20 seeds were planted on to plastic pots filled with about 1 kilo gram sandy soil and grown for 7 days in a growth chamber at 22 ^{0}C with 16 hr light, 14 hr photoperiod, photosynthetic photon flux density 220 ±µ20 µmol photons m^{-2} s^{-1}, temperature 22±1 ◦C/18±1 ◦C day and night and relative humidity 65±2%/75±2% day and night. Seven days old seedlings in the pots were separated into three groups and then treated with boron (boric acid) of concentrations 0, 4 or 32 µM and further placed in a growth chamber under the same conditions previously described. After further 7 days the seedlings from the corresponding concentrations were placed in 2 liters' hydroponic pots and transferred to the growth chamber where they were grown on aerated hydroponic modified Hoagland nutrient solution of the following composition 200 mM $CaSO_4$, 200 mM $CaCl_2$, 100 mM $MgSO_4$, 200 KNO_3, 5 $MnSO_4$, 0.38 $ZnSO_4$, 0.16 $CuSO_4$, 10 Fe-EDTA, 5 NaH_2PO_4, 300 NH_4NO_3 and 0.06 $(NH4)_6Mo_7O_{24}$. Al was supplied as $AlCl_3$ at concentrations 0, 100, 200, 400 or 500 µM Al following in the standard method.[24] The growth solutions were adjusted to pH 4.3±0.1 and again under the same conditions previously described. In order to minimize any metal contamination, ultra-pure water (glass double sterilized water) was used on preparing the nutrient solutions and plastic ware were used for all procedures of solution handling. After 72 hr, the length of the longest root was measured and the plants were transferred to treatment solutions with the same composition as described above (pH 4.3) but supplemented with Al supplied as $AlCl_3$. Control treatments received the nutrient solution without any Al or B supplement. After 72 hr of Al treatment, roots of the wheat cultivar were rinsed thoroughly with distilled water and oven dried for 24 hr at 80 ^{0}C.

Extraction of Soluble Sugars

A known weight of the dry matter was extracted twice with 80% ethanol in a reflux apparatus on a boiling water bath. The two alcoholic extracts and the washings were added together, evaporated to a few ml in an air drying oven at 50 ^{0}C and the residue was taken in water and made up to volume.

Estimation of Reducing Sugars

This fraction was determined by the use of standard method.[25]

Estimation of Total Soluble Sugars (TSS)

This fraction was determined by hydrolyzing an aliquot of the clarified sugar extract with 1.0 N HCl for 30 min and made to volume before neutralization to phenolphthaline end point. The total soluble sugar fraction was determined by the use of standard method.[25]

Extraction of Total (Tp) and Soluble Protein (Sp)

Total proteins were extracted by adding 10ml of 0.5 N NaOH to about 100 mg of the oven-dry plant material and left over night. The extract was completed to 50 ml with distilled water.[26] Soluble proteins were extracted by adding 10 ml of distilled water to about 100 mg of the oven-dry plant material then boiled for 5 min. After cooling, the extract was completed to volume (50 ml) with distilled water.

Estimation of Total and Soluble Protein Content

This was done as the use of standard method.[27]

Analysis of Amino Acids by Amino Acids Analyzer

For the determination of total free individual amino acids, a known dry matter of wheat roots were suspended and extracted in 3% (w/v) 5-sulfosalicylic acid solution and grinded, followed by centrifugation for 10 min at 10,000×g, and the supernatant was hydrolyzed with 6N HCl (10 ml) in a sealed tube at 110 °C in an oven for 24 hours. Acid hydrolysis was carried out according to the standard method.[28]

Proline analysis

Samples of wheat dry roots from control and treatments were used for analysis of proline using the standard methodology.[29] Concentrations of proline in the plant tissue are expressed on a FW basis.

Gel Electrophoresis of Proteins

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) were carried out using the standard discontinuous buffer system[S] and modified standard method.[31] Root segments from seventeen days old were ground with 0.5M Tris HCl pH 6.5. The mixture was then centrifuged at 3000xg for 30 min. All chemical reagents were purchased from e Bio- RAD chemical Co, CA, USA.

Protein Molecular Mass Determination

Isolated proteins were applied to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). To determine the molecular masses using total lab 110 software nonlinear dynamics Newcastle upon tyne, UK to analyze banding pattern, molecular mass and band percentage.

Statistical Analysis

Statistical analysis of the results was carried out according to Duncan's multiple range tests. Data were subjected to a two-way ANOVA and the LSD at $p \leq 0.01$ was determined with standard method.[32]

Results

Fresh and Dry Matter

It was found that the 3 days exposure to a solution with a 500 µM Al only decreased significantly the fresh matter of the examined roots compared to the absolute control value; this reduction reached 34% of the control (without Al and B). Al concentration of 200 and 400µM induced less reduction in root fresh matter, however the lowest Al concentration (100µM Al) cause apparent non-significant change in root fresh matter. Wheat plants treated with 4µM B or 32µMB only, non-significant change was recorded for the fresh matter compared with the absolute control value and decrease in fresh matter, in case of 500µMAl+ 4µM B; on the other hand, in case of 500µM Al+ 32µM B relative increase in root fresh matter by 29% of that 500µM Al-treated only; but the fresh matter values were still lower than those of the control (Table 1 and Figure 1).

Concerning the dry matter, the data presented in table 1 revealed that Al alone of concentrations 200, 400 and 500µM decreased dry matter in the roots of about 20% of the absolute control. No reduction in dry matter could be detected in roots of seedlings treated with the low Al concentration (100µM Al). Plants pretreated with 4µMB or 32µMB only caused a decrease of the dry matter of 10% and an increase of 10% compared with the control, respectively. The interaction between 500µM Al and 4µMB decreased the dry mass significantly by 40% as those of the control value (without Al and B); however (500µM Al+32µM B) decreased the dry matter of about 10% as those of the control value.

Table1: Effect of different treatments on the fresh and dry matters (mg g^{-1} d. m.) in roots of wheat

Treatments	Fresh mass	Dry mass
Control	0.47 ± 0.045a	0.05 ± 0.005a
100 µM Al	0.45 ± 0.043a	0.05 ± 0.005a
200 µM Al	0.40 ± 0.038b	0.04 ± 0.004b
400 µM Al	0.36 ± 0.034c	0.04 ± 0.004b
500 µM Al	0.31 ± 0.029c	0.04 ± 0.004b
Control+4 µM B	0.43 ± 0.041a	0.045 ± 0.003c
100 µM Al+ 4 µM B	0.38 ± 0.036b	0.04 ± 0.004b
200 µM Al+ 4 µM B	0.38 ± 0.036b	0.04 ± 0.004b
400 µM Al+ 4 µM B	0.39 ± 0.037b	0.035 ± 0.005a
500 µM Al+ 4 µM B	0.30 ± 0.028d	0.03 ± 0.003d
Control+32 µM B	0.44 ± 0.042a	0.055 ± 0.005a
100 µM Al+ 32 µM B	0.42 ± 0.040a	0.05 ± 0.005a
200 µM Al+ 32 µM B	0.41 ± 0.039b	0.05 ± 0.005a
400 µM Al+ 32 µM B	0.40 ± 0.038b	0.045 ± 0.004b
500 µM Al+ 32 µM B	0.40 ± 0.038b	0.045 ± 0.004b
P	>0.05	>0.05

Values are means ± SD based on three independent determinations, different letters means significant difference as evaluated by Duncan's' multiple comparison test.

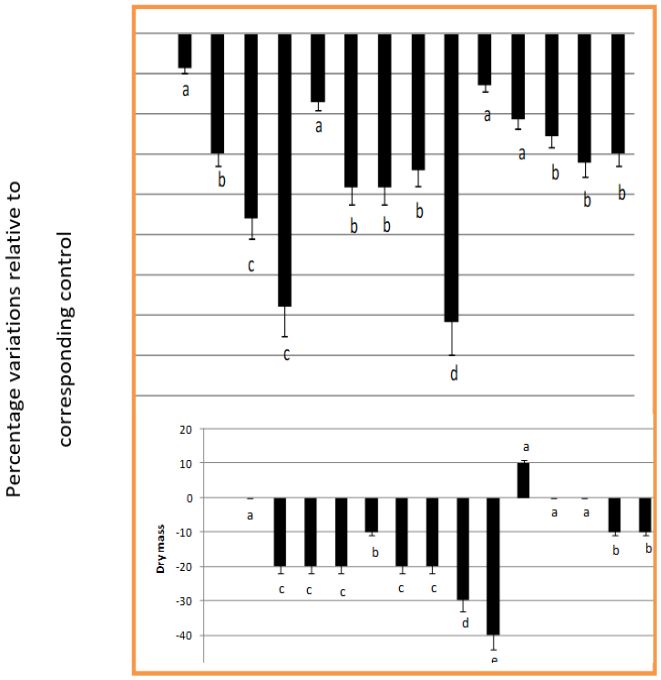

Fig. 1: Variation in percentage ratios of treatments in the growth medium on fresh and dry mass in roots of wheat relative to absolute control. Values are means ± SD based on three independent determinations and bars indicate standard deviations, different letters means significant difference as evaluated by Duncan's' multiple comparison test

Changes in Carbohydrate Constituents

Data presented in table 2 showed the influence of Al and boron interactions on total soluble sugars, reducing sugars and non- reducing sugars of wheat roots. After 72 hr exposure to Al concentrations 100, 200,400 and 500µM, the total soluble sugars increased non-significantly in the lower concentrations of 100 and 200µM Al by about 2.8 and 9.1%of the absolute control value. By increasing Al concentrations in the hydroponic culture solution 400 and 500µM, an increase was recorded of 73.7 and 100.5% of the absolute control value, respectively. A general trend was that reducing sugars represent about 70% of the total soluble sugars and non-reducing sugars represent only 30% of the total soluble sugars at all Al-concentrations (100 ,200 , 400 , 500µM Al). No significant changes in total soluble sugars, reducing and non -reducing sugars were observed in plants pretreated with 4 or 32µM B only.

The interaction between Al and 4µM boron pretreated wheat plants revealed that there was a further decrease in total soluble sugars as compared with the absolute control (without Al and boron), which was more obvious specially at 400 and500µM Al+ 4µM B, while in case of the other two lower concentrations 100µM +4µM B and 200µMAl+4µM B, little changes in total soluble sugars, an increase was detected by about 12 and 13.3% than absolute control, respectively. Total soluble sugars, however, increased by about 21.5 and 41.5% than absolute control at 400 and 500µM Al + 4µM B, respectively; the corresponding values for reducing sugars reached 32.3 and 48% as compared absolute control, respectively.

Application of 32µM boron to Al exposed plants caused an increase of total soluble sugars by about 74.9 and 131.1% than absolute control at 400 and 500µM Al +32µM B, respectively; the corresponding values for reducing sugars reached 95.3 and 174.8%, respectively. Exposure of the wheat seedlings to 500µM Al only led to an increase of total soluble, reducing sugar and non-reducing sugars by 2, 2.2 and 1.6 fold, respectively.

Table 2: Effect of different treatments on the content of reducing sugars, non reducing sugars and total soluble sugars (mg g^{-1} d.wt.) in roots of wheat

Treatments	Reducing Sugars	Non reducing sugar	Total Soluble sugar
Control	12.63 ± 1.28b	8.27 ± 0.84ab	20.90 ± 2.12b
1004µM Al	12.9 ± 1.31b	8.60 ± 0.87ab	21.50 ± 2.18b
200µM Al	14.11 ± 1.43b	8.69 ± 0.88ab	22.80 ± 2.31b
400µM Al	25.41 ± 2.58a	10.89 ± 1.10a	36.30 ± 3.68a
500µM Al	28.33 ± 2.87a	13.57 ± 1.38a	41.90 ± 4.25a
Control+4µM B	14.56 ± 1.48b	6.24 ± 0.63b	20.80 ± 2.11b
100µM Al+ 4µM B	16.45 ± 1.67b	6.88 ± 0.70b	23.41 ± 2.37b
200µM Al+ 4µM B	16.87 ± 1.71b	6.81 ± 0.69b	23.68 ± 2.40b
400µM Al+ 4µM B	19.4 ± 1.97b	7.00 ± 0.71b	26.4 0 ± 2.68b
500µM Al+ 4µM B	21.7 ± 2.20ab	7.87 ± 0.80b	29.57 ± 3.00b
Control+32µM B	14.56 ± 1.48b	6.69 ± 0.68b	20.8 0 ± 2.11b
100µM Al+ 32µM B	16.43 ± 1.67b	6.53 ± 0.66b	23.41 ± 2.37b
200µM Al+ 32µM B	16.87 ± 1.71b	7.3 b ± 0.74b	23.68 ± 2.40b
400µM Al+ 32µM B	19.40 ± 1.97b	7.91 ± 0.80b	26.4 0 ± 2.68b
500µM Al+ 32µM B	21.70 ± 2.20ab	8.87 ± 0.90b	29.57 ± 3.00b
P	0.001*	0.035*	0.016*

Values are means ± SD based on three independent determinations, different letters means significant difference as evaluated by Duncan's' multiple comparison test.
The letters which are the same, indicate that there is no statistically significant different based on mean comparison by Duncan's method at $P < 0.05$.

The data presented in table 3 represent the changes in total, soluble and insoluble proteins in the roots of 14 days old seedlings of wheat in response to treatment with Al only or Al + B for an exposure time of 72 hr. First of all, in the untreated (absolute control) and the Al treated seedling the soluble protein fraction was always much higher than the insoluble fraction particularly in the two higher Al concentrations 400 and 500µM Al alone, where the increase of soluble protein fractions was 2.5 and 2.9 fold of the insoluble fraction, respectively. Total protein content decreased with increasing Al concentration (0, 100, 200, 400, 500µM Al), again the decrease was more pronounced on treatment with the two higher Al concentration 400 and 500 µM Al. The decrease in total protein reached 25.5 and 28.6% as compared to absolute control values, respectively. Total protein content reached only 74.5 and 71.4% of the absolute control value, respectively; soluble protein increased while insoluble fraction decreased with the increase of Al concentration especially at 400, 500µM Al, where the increase was 13.8 and 13.6% and the decrease was 40.8 and 48.4% of the absolute corresponding controls.

Pretreatment with boron only of concentrations 4 and 32µM had non-significant changes in total soluble and insoluble proteins compared with the corresponding absolute control values. Application of 4µMB to the Al concentrations (100, 200, 400, 500µM Al) decreased the total protein and the other two protein fractions, the decrease was particularly at the higher concentration (400µM Al + 4µM B) and (500µM Al + 4µM B), where the reduction in total soluble and insoluble proteins reached (29, 30%), (17.15, 18.5%), (44.6, 45.19%) than absolute control value, respectively. On the other hand, application of 32µMB to different Al concentrations treatment decreased both the total and insoluble protein fraction apparent specially in case of the two higher concentrations 400µM Al + 32µM B and 500µM Al + 32µM B, the decrease in total and insoluble protein was 11 and 48.33% of the corresponding absolute control for 400µM Al + 32µMB treatment and 17.2,

68.3% for 500µM Al + 32µM B, respectively. However, addition of 32µMB to the highest Al concentration 500µM Al increase the soluble protein fraction percentage by 1.2 fold of the absolute control.

It was shown from table 3 that application of 32µMB repressed the percentage values of total protein, induced percentage increased values of soluble protein and induced percentage decreased the values of insoluble protein.

Changes in Proline Contents

Free proline accumulated in wheat roots under different concentrations of Al 0 ,100, 200 ,400 , 500µM (Table 3). Following Al stress, the proline concentration increased significantly in roots. Wheat seedlings pretreated with 4 or 32µM B only induce non-significant change in the proline content compared to the absolute control. However, with increasing Al concentrations of 0, 100, 200, 400, 500µM the proline content increased by1.3-, 1.4- and 1.6- fold of the absolute control at 200, 400 and 500µM Al, respectively, but no change was observed at the lower concentration (100µM Al).

Pretreatment with 4µM B and exposure to different Al concentrations cause no change in the proline content, only in case of 400µM Al+4µM B and 500µM Al+4µM B a slight increase observed about 11.7 and 15.3% of the corresponding absolute control, respectively. On the other hand, interaction of Al with 32µM B pretreated seedlings, increased the proline content of about 1.36, 1.74, 1.8 and 2.01 fold of the absolute control after exposure to 100, 200, 400 and 500µM Al pretreated with 32µM B, which could be related to its role in osmoregulation and membrane stabilization (table 3).

Table 3: Effect of different treatments on the content of soluble protein (mg g^{-1} d.wt) , insoluble protein (mg g^{-1} d.wt.), total protein (mgg^{-1} d.wt.) and proline (µg g-1 f.wt.) in roots of wheat

Treatments	Soluble Protein	Insoluble Protein	Total Protein	Proline
Control	71.75 a ± 7.27a	54.37 ± 5.51a	126.12 ± 12.78a	88.25 ± 8.95c
1004µM Al	67.9 ± 6.88ab	48.91 ± 4.96a	116.81 ± 11.84a	89.05 ± 9.03c
200µM Al	75.48 ± 7.65a	38.48 ± 3.90b	113.96 ± 1.55a	110.16 ± 11.17bc
400µM Al	81.67 ± 8.28a	32.17 ± 3.26b	94.00 ± 9.53ab	119.97 ± 12.16bc
500µM Al	81.54 ± 8.27a	28.05 ± 2.84b	90.01 ± 9.12ab	134.77 ± 13.66b
Control+4µM B	70.31 ± 7.13a	51.29 ± 5.20a	121.6 ± 12.33a	87.15 ± 8.83c
100µM Al+ 4µM B	68.17 ± 6.91ab	46.68 ± 4.73a	114.85 ±11.64a	89.59 ± 9.08c
200µM Al+ 4µM B	63.26 ± 6.41b	38.9 ± 3.94b	102.16 ± 0.36a	91.73 ± 9.30bc
400µM Al+ 4µM B	59.44 ± 6.03b	30.1 ± 3.05b	89.54 ± 9.08b	98.59 ± 9.99bc
500µM Al+ 4µM B	58.48 ± 5.93b	29.8 ± 3.02b	88.28 ± 8.95b	101.79 ± 10.32bc
Control+32µM B	71.61 ± 7.26a	51.19 ±5.19a	122.80± 12.45a	89.46 ± 9.07c
100µM Al+ 32µM B	71.59 ± 7.26a	49.31 ± 5.00a	120.90 ± 12.26a	120.22 ± 12.19b
200µM Al+ 32µM B	80.21 ± 8.13a	38.39 ± 3.89b	118.60 ± 12.02a	153.61a ± 15.57a
400µM Al+ 32µM B	84.16 ± 8.53a	28.09 ± 2.85b	112.25 ± 11.38a	160.23 ± 16.24a
500µM Al+ 32µM B	87.17 ± 8.84a	17.23 ± 1.75bc	104.4 ± 10.58a	177.34 ± 17.98a
P	0.025*	0.001*	0.0036*	0.015*

Values are means ± SD based on three independent determinations, different letters means significant difference as evaluated by Duncan's' multiple comparison test.
The letters, which are the same, indicate that there is no statistically significant different based on mean comparison by Duncan's method at P <0.05.

Interaction Effect of Aluminum and Boron on Amino Acids Content in Wheat

Since metabolite regulation is a major mechanism used to maintain osmotic potential during abiotic stress, a targeted amino acids analyzer was used to measure other individual amino acids. In the current study, 14 primary amino acids were identified in the wheat. It was found that the total amino acids contents changed in roots in the presence of Al only or Al plus B. For example, at the highest Al concentration

500µM treatment, free amino acid pools increased rationally, under stress with 500µMAl plus 4µM B had very little increase of 3 or 4 individual amino acids, if any compared to absolute control values but still lower than the increase in case of Al treatment alone, however stress with 500µM Al + 32µMB caused significant increase in the levels of amino acid contents compared with absolute control (Table 4).

Data presented in table 4 and figure 4 revealed that 32 µM boron, aspartate, glycine and cysteine are the dominant three amino acids and all together represent more than 40% of total amino acids. Alanine, leucine, valine, lysine and serine come next in their contents. Following aluminum stress, aspartic acid, glutamic, glycine, serine, valine, isoleucine and cysteine were the amino acids found to be abundant in the root of wheat, increased by 15, 15, 16, 14, 12, 11, and 11%, respectively of their corresponding absolute control. Leucine followed in their concentration and was found to increase relatively small amounts by about 11% of the control value. Aromatic amino acids tyrosine and phenylalanine increased by about 10% of the corresponding control values. Histidine and lysine slightly increased than the corresponding control value. On the other hand, roots of the wheat seedlings pretreated with 4µMB and cultured in 500µMAl revealed that percentage increase of all amino acids was repressed as compared with their corresponding control.

The majority of amino acids were further increased in seedlings pretreated with 32µM B and cultured in 500µMAl when compared to corresponding control. Most pronounced increase in amino acids was observed for aspartic acid, glutamic, glycine and cysteine reaching of 22, 19, 19 and 24%, respectively as compared with their corresponding control. Also, it is known that the amino acid aspartate, glutamic and cysteine are constituent of the glutathione. The branched chain amino acids serine, valine and leucine showed a similar pattern of increase about 20, 19 and 19%, respectively as compared with their corresponding absolute control (Table 4 and Fig. 2). The aromatic amino acids tyrosine and phenylalanine showed a similar trend of percentage increase of about 17 and 13%, respectively of their corresponding control.

Table 4: Total individual amino acids (mg g-1 d.wt.) profile in wheat under different treatments

Amino acid	Control	500µMAl	500µMAl+4µM B	500µMAl+32µM B
Aspartic	1.78 ± 0.18	2.04 ± 0.21	1.81 ± 0.18	2.18 ± 0.22
Threonine	0.27 ± 0.03	0.29 ± 0.03	0.28 ± 0.03	0.29 ± 0.03
Serine	0.71 ± 0.07	0.82 ± 0.08	0.75 ± 0.08	0.85 ± 0.09
Glutamic acid	0.68 ± 0.07	0.78 ± 0.08	0.72 ± 0.07	0.81 ± 0.08
Glycine	1.76 ± 0.18	1.9 ± 0.19	1.8 ± 0.18	2.1 ± 0.21
Alanine	1.25 ± 0.13	1.35 ± 0.14	1.31 ± 0.13	1.4 ± 0.14
Valine	0.84 ± 0.09	0.94 ± 0.10	0.86 ± 0.09	1 ± 0.10
Isoleucine	0.27 ± 0.03	0.3 ± 0.03	0.28 ± 0.03	0.32 ± 0.03
Leucine	0.92 ± 0.09	1.02 ± 0.10	0.97 ± 0.10	1.09 ± 0.11
Tyrosine	0.29 ± 0.03	0.32 ± 0.03	0.3 ± 0.03	0.34 ± 0.03
Phenyl alanine	0.3 ± 0.03	0.33 ± 0.03	0.31 ± 0.03	0.34 ± 0.03
Histidine	0.59 ± 0.06	0.61 ± 0.06	0.6 ± 0.06	0.63 ± 0.06
Lysine	0.82 ± 0.08	0.87 ± 0.09	0.84 ± 0.09	0.9 ± 0.09
Cysteine	1.65 ± 0.17	1.83 ± 0.19	1.77 ± 0.18	2.05 ± 0.21
Total	12.13 ± 1.23	13.38 ± 1.36	12.49 ± 1.27	14.3 ± 1.45
P	0.001*	0.001*	0.001*	0.001*

Values are mean ± SD of three independent replicates

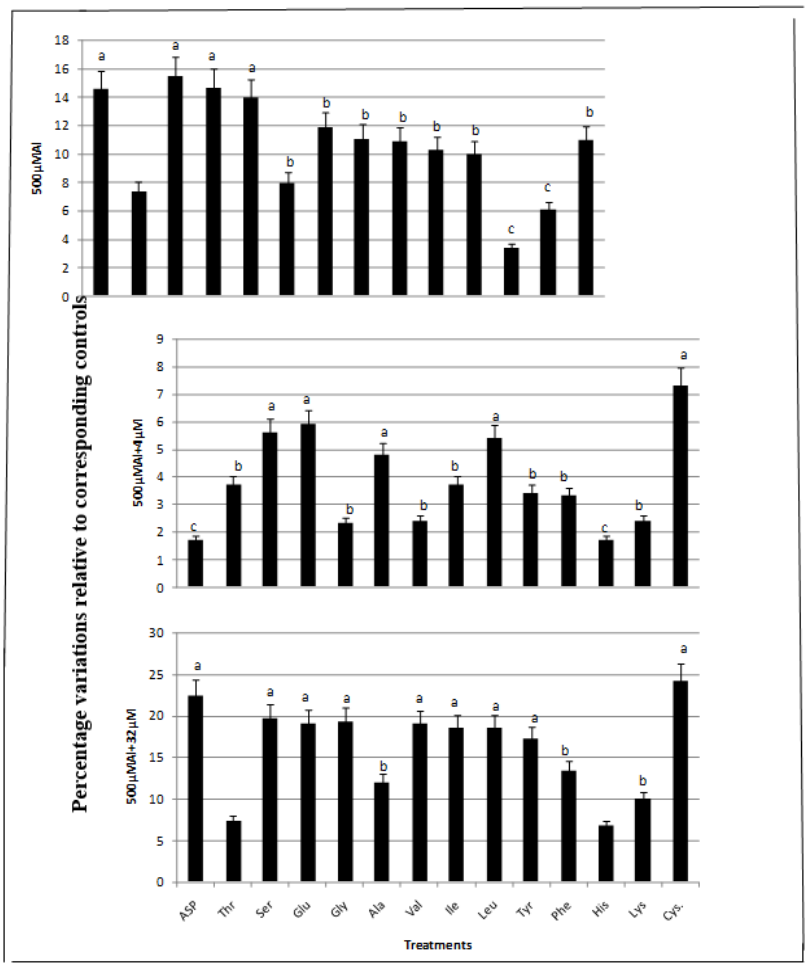

Fig. 2: Variation in percentage values of total individual amino acids profile in wheat under different treatments. Values are mean ±SD of three independent replicates, different letters means significant difference as evaluated by Duncan's' multiple comparison test

Interaction Effect Aluminum and Boron on Protein of Wheat Roots

SDS-PAGE analysis of concentrated root revealed the presence of several peptides, which differed in migration position and band's intensity. The control plants revealed the presence of three peptides with molecular masses ranging from 145 to 20 kDa. The effect of Al treatment was to induce the formation of stress proteins in wheat plants (Table 5). In these plants high and low molecular masses proteins of 136, 43, 35 and 21kDa were synthesized in response to 100µM Al with their band intensities 20, 36, 6, 37, respectively. There were variable peptides with different molecular masses, for example, peptides with molecular masses 125, 36, 20 kDa and 36, 21 KDa were synthesized in response to 200 and 400µM Al, respectively. At 500µM Al, 117, 101, 36, 30, 26 and 21kDa were synthesized, while peptides with 145, 43 and 25kDa were disappeared (Table 5).

Application of 4µMB in control samples revealed the presence of four low molecular masses with 45, 44, 36 and 21kDa with corresponding band intensities 32, 15, 11 and 40, respectively. Several peptides with different band intensities were synthesized after treatment with different concentrations of aluminum (Table 5). Peptides with molecular masses 166, 45, 35 and 21kDa were synthesized in wheat roots with 100 µM Al in seedlings pretreated with 4 µM B. At 200 µM Al, peptides with molecular masses 96, 56,

53, 36, 32, 27 and 21kDa were synthesized, while peptides with 45, 44 and 36 were disappeared. Two low molecular masses peptides of 36 and 21kDa were synthesized in wheat roots in response to 400µM Al pretreated with 4µM B. At 500µM concentration of Al, polypeptides with molecular masses 152, 63, 36, 30, 21kDa were synthesized, while peptides with molecular masses 45 and 44 were disappeared in seedlings pretreated with 4µM B.

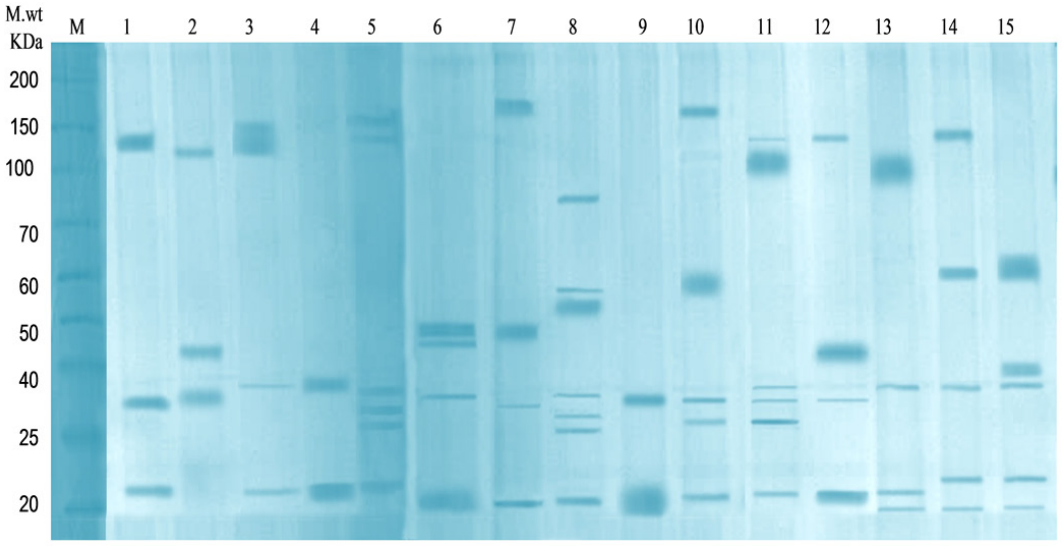

Fig. 3: SDS-PAGE analysis of root separated by gel electrophoresis as resolved by 12.5% stained by comasie brilient blue for scanning the up-down regulated proteins of 17 days old root seedlings of wheat pretreated with 4m and 32m boron then treated with different concentration of aluminum ranging from 0-500M Al. The lanes: M- molecular mass markers are indicated in kDa. Lane 1 represents control, lane 2 of 100M Al, lane 3 of 200M Al, lane 4 of 400M Al, lane 5 of 500M Al, lane 6 of control+4M B, lane 7 of 100M Al+4M B, lane 8 of 200M Al+4M B, lane 9 of 400M Al +M B, lane 10 of 500M Al +4M B, lane 11 of control+32MB, lane 12 of 100M Al +32MB, lane 13 of 200M Al +32M B, lane 14 of 400M Al +32M B and lane 15 of 500M Al +32M B

There were marked differences between migration position and band intensity in response to seedlings pretreated with 32µM B and different concentrations of aluminum. Several other polypeptides enhanced after exposure to 32µM B in water-treated wheat seedlings. Several peptides with different band intensities were synthesized after treatment with different concentrations of aluminum (Fig. 3 and Table 5). The electrophoretic pattern of water and boron treated wheat roots showed number of major six polypeptides of different band's intensity with molecular masses of 121, 107, 36, 30, 26 and 21kDa. Peptides with molecular masses of 125, 46, 36 and 21kDa were synthesized in wheat roots after treatment with 100 µM Al in seedlings pretreated with 32µM B. At 200µM Al, peptides with molecular masses of 103, 38, 21 and 20kDa were synthesized, while peptides with 45, 44 and 36 were disappeared. Low and high molecular masses of peptides 143, 62, 38, 24 and 20kDa were synthesized in response to 400µM Al pretreated with 32 µM B. On the other hand, low molecular masses of peptides 62, 43, 38,24 and 20 kDa and 59, 8,7,14, 11 band intensities were synthesized, respectively with 500µM Al pretreated wheat seedlings (Fig. 3 and Table 5).

Table 5: Molecular weight, band percentage and mobility rate (Rm) of the different types of protein bands of wheat treated with different treatments

M.wt. (kDa)	Control	100µM Al	2ooµM Al	4ooµM Al	5ooµM Al	Rm	M.wt. (kDa)	c+4µ MB	100+4 µMB	200+ 4µMB	400+ 4µMB	500+ 4µ MB	M.wt.(kDa control	contol+ 32µMB	100+ 32µMB	200+ 32µMB	400+ 32µMB	500+ 32µMB
200						0.041	200						200					
150						0.119	166		46.05				150					
145	55.01					0.126	152					26.77	143				26.35	
136		20.38				0.138	145						125		16.34			
125			83.59			0.159	125						121	7.69				
117					20.96	0.212	117						107	59.6				
101					13.96	0.326	101						103			62.21		
100						0.451	100						100					
70							96			17.71			70					
50							63					40.52	62				33.1	59.5
45							56			8.16			50					
44						0.571	53			38.55			46		48.98			
43		35.71					45	32.65	37.01				45					
40						0.663	44	15.52					44					
36			6.14	48.34	10.77	0.715	43						43					33.1
35		6.00					40						38			17.61	18.08	18.01
34	19.19					0.722	36	11.23		6.71	27.00	4.72	36	4.86	4.09			
30					16.85	0.82	35		4.77				35					
26					12.11	0.722	32			7.24			34					
25						0.76	30					13.95	30	4.56				
21	25.8	37.92		51.66	25.34	0.802	27			7.88			26	10.13				
20			10.28				25						24				18.02	18.02
						0.899	21	40.61	12.17	13.75	73.00	14.04	21	13.35	30.59	10.77		
							20						20			9.4	4.45	4.45

Discussion

Soil acidification is becoming a global problem due to detrimental effect of industrial development and incorrect use of acid fertilizer. Thus, considerable effort has been made to cope with the issue in recent years. Since Al stress is a major limitation to plant production on acid soils there is an interest in developing new cultivars with greater degree of Al resistance. Experimental approaches such as the detection of Al in a tolerant cultivar Sakha 93 of wheat recommended by Agricultural Research Institute (ARI) to tolerate abroad biotic and abiotic stresses and presumed to have a high resistance to Al phytotoxicity, which may contribute to clarifying its distribution, physiological and protein alterations and possible amelioration of toxicant.

The current data showed that visual Al toxicity in the root apex of Sakha 93 was only after 3 days treatment and using a high aluminum concentration of 400, 500µM Al, a delayed response to the toxicant, which was observed much probably due to high tolerance of this cultivar grown in hydroponic solution at pH 4.3. In our experiment, the growth parameters of fresh matter and dry matter were reduced on exposure to Al alone or Al+4µMB, however, these Al-toxicity effects were reduced by adequate B supply (32µMB) in presence of Al. Relative water content maintained on treatment Al alone or B alone or Al+B remained almost the same. We speculated that adequate boron exerts an antagoniotic effect on Al uptake and thus leads to alleviate Al toxicity. The reduction in root elongation with increasing concentrations of Al was recorded in another study33. It could comment that B x Al studies in plants with less B requirement is less frequent. [25]

The observed lower values for fresh and dry matters upon Al treatments might be due to aluminum ions were found to affect plasma membrane permeability[34], fluidity[35] and protein-lipid interactions[36]. Therefore, these changes under the prevailing experimental conditions caused by Al resulted in a marked disturbance of plasma membrane function and ion transport as well as reduction of water uptake and consequently reduced fresh mass of wheat. Previous studies have shown that the growth, dry weight and fresh weight of roots and shoots of Cucumber sativus were decreased at 100, 500, 1000 and 2000 mM $Al_{2(}SO_4)_{3.}$[37] Probably, the growth of root cells was affected by aluminum, causing a decrease in cell wall synthesis because aluminum inhibits the secretory function of the Golgi apparatus. [37]

It was proposed that the use of supplemental B could protect against root growth inhibition under Al toxicity.[38] Similarly, it was shown that B can ameliorate Al toxicity in mungbean seedlings along with the improvement of root function.[23] Al was found to cause a decrease in root tip ascorbate concentration in squash, which was parallel to root elongation inhibition. However, boron added to Al toxic medium produced root apices higher in ascorbate concentrations.[24] This may be the case for high B (32 µM) concentrations added to Al stressed seedling. In these cases, B was able to counteract the toxic effects of Al on root elongation. In another study showed that high B additions increased epicotyl length of soyabean[39] and fresh weight under Al stress, which seems to support the previous reports on B amelioration on Al toxicity.[38] Interestingly, relatively low B (4µM) had a decreasing effect on root length in wheat under Al stress. The differential effect of B on root length between Al- stresses undoubtedly complicates the mechanism of B/Al interaction for cell production and elongation. However, it seems to support the conclusion that B has smaller effective concentrations than any other nutrient element40. It is known that though B requirement by plants is relatively small, the range between deficiency and excess is narrow.[41]

The results in table 1 investigate the effect of B on ameliorating the adverse effect of Al treatment on the growth of wheat seedling especially the root. Boron triggered the increase in Ca content in presence of Al as compared with absolute control. Calcium, is an essential plant nutrient, is required for numerous and regulatory functions, act as a counter ion for anion in the cell and an intracellular messenger in the cytosol[42] and aluminum may compete with calcium for membrane binding site.[8] In other study recorded that application of 4 µM B had non-significant effect on Al or Ca content compared to the control without Al and B treatment,[33] whereas application of 32 µM B increased Ca concentration in the root, thus alleviating Al toxicity, which at least in part, may be attributed to less Al absorption because of competition of Ca and /or B with Al in binding to plasma membrane.

Addition of boron (32µM B) may restore the optimal physical properties of the cell wall and membrane, decreasing the possibility of the displacement of Ca from the outer surface of the root-cell plasma membrane under Al stress, resulting in apparent net Ca flux across the membrane and increasing net Ca uptake of wheat. It is therefore, that in the tolerant cultivar sakha 93, the protein structure of the putative Ca channel may be such that it is not accessible to blockage by exogenous Al. Similarly, it was shown that B can ameliorate Al toxicity in mung bean seedlings and that the ameliorative effect of B was suggested to be related to the root function.[23] Boron has positive interactions with N, P, K, Cu, Zn and Fe, whereas, negative interactions with Ca and Mg.[43] Moreover, it is involved in photo-assimilation and assimilates partitioning, and directly or indirectly affects the seed development and seed size.[20]

The interaction of B and Al has not only biochemical but also present chemically and structurally effects. Boron and Al affects the cation exchange properties because although no effect of B on Al toxicity has been detected, it is with corn, which has low cation exchange capacity in roots as well as wheat.[44]

The data in table 2 revealed a pronounced increase in soluble sugars and total soluble sugars (TSS) in the roots of wheat cultivar Sakha 93 with the Al treatments, which might lead that the osmotic potential can be kept low by the increase of soluble sugars in the cell sap, thereby enabling the wheat cultivar to take up water and the root cells could slightly elongated against the rigidity of the cell wall under Al stress, suggesting that Al may affect some sugar-related metabolism in the root cells.[45] Apart from their role in osmotic adjustment, compatible solutes have also osmoprotective functions. Due to their specific hydrophilic structure, they are capable of replacing water on the surfaces of proteins, protein complexes, or membranes, thus preserving their biological functions. Al decreased the hydraulic conductivity and cell wall extensibility in an Al-Sensitive maize variety, but increased the hydraulic conductivity in an Al-resistant variety.[46] Their results indicated that Al affects the water-absorbing ability of the root cells, although the influence of Al on the mechanical properties of cell walls in elongating root tissues might play a prominent role in stress-induced inhibition of root elongation.[47]

Exposure of wheat plants to Al results in a water deficit, in the current study, non-reducing sugars increased in wheat cultivar Sakha 93, which may become important as a replacement for water, providing a hydration shell around proteins.[48] We presumed that the Al-tolerant wheat cultivar Sakha 93 undergoes physiological or biochemical changes that enable it to better cape with the effects of Al toxicity and allow for more response times.

Application of 4 µM B revealed the decrease in TSS of Al- pretreated wheat cultivar, which may be related to the inhibitory effect of Al^{+3} on photosynthesis, photosystem II and synthesis of photosynthetic pigments (data not shown), the destruction of plasma membrane by Al^{+3}, [49] which results in a decreased allocation of photosynthates between shoots and roots.

Application of 32 µM B showed that Al-induced increase in soluble sugars in wheat cultivar, which may be related to less Al-induced inhibition of root elongation and suggested that osmotic potential can be kept low by the increase in the concentration of soluble sugars in the cell sap, thereby enabling the Al-resistant cultivar to take up water and the root cells to elongate against the rigidity of the cell wall under Al stress. The role of boron in addition to the stabilizing effects of membrane is well known. The significant increase of sugars on interaction of high B concentration and Al may provide an initial defense state against water loss from the root of wheat cv. Sakha 93. This total sugar and/or reducing sugar may provide energy during oxidative respiration. Intercalation between phospholipid head groups, and properties to reduce free radical activity by forming long-lived adducts with them are possible protective membrane-stabilizing effects of carbohydrates.[50]

In the present investigation the soluble protein increased while, insoluble and total protein contents decreased significantly in the roots of wheat cv. Sakha 93 under Al-treatment (Table 3). Furthermore, our results revealed that Al exerts a much greater inhibitory effect on the synthesis of insoluble and total proteins and/ or degradation. The decrease in total protein content of wheat cultivar roots under the prevailing environmental conditions may be due to incorporation of Al with higher phytochelatins, which inhibits protein synthesis or the enhancement

of protein degradation. The present study indicated that the increase in soluble protein may be due to the decreased incorporation of amino acids into proteins and increased protein breakdown due to unbalanced N- metabolism. The accumulation of soluble protein under Al-stress may play a role in osmoregulation and serves as available source of carbon and nitrogen. Soluble protein may contribute partially in the building up of the osmotic potential of plants under abiotic stress conditions.

Pretreatment of 4 µM B and exposure to 500 µM Al revealed that insoluble protein increased, soluble and total protein decreased in comparison to Al treatment only, soluble and hence total decreased significantly in Al-treated wheat root seedlings was related to the synergistic effect of boron deficiency (low B concentration) and aluminum toxicity on root elongation. Thereby, the consequences of the decreased carbohydrate constituents, the C-skeleton for amino acid synthesis and reduced amino acid synthesis and hence the protein content.

Addition of 32 µM B showed that Al-induced increase in soluble protein in wheat cultivar, which was related to less Al-induced inhibition of root elongation and suggested that osmotic potential can be kept low by the increase in the concentration of soluble proteins in the cell sap. Thus, enabling the studied Al-resistant cultivar to take up water and root cells to elongate against the rigidity of the cell wall under Al stress.

The current study demonstrated that Al enhanced 14 individual amino acids in addition to proline in root of wheat cultivar Sakha 93 (Table 4) as compared to the absolute control. The Al-induced increase in amino acid content was associated with a decrease in total protein, thus indicating that Al retarded the assembly of amino acids into proteins in roots of wheat seedlings cultivar Sakha 93. Levels of amino acids most notably proline, the glutathione forming amino acids cysteine, glycine and glutamic and the branched chain amino acids (BCAAs) leucine, isoleucine and valine were increased under Al stress. Leucine, isoleucine and valine, the branched chain amino acids (BCAAs), where increased significantly in the tolerant wheat cultivars. Accumulation of BCAAs as well as a number of other amino acids,[51] increased under dehydration stress and was regulated at the transcriptional level. In other study also recorded that increased BCAAs under drought stress in Arabidopsis, but not to the same extent.[52]

Phenylalanine and tyrosine also increased over the course of the experiment in wheat cultivar, these aromatic amino acids are synthesized through the shikimate pathway and serve as precursors for a wide range of secondary metabolites, such as indoleacetate, glycosides, lignin precursors, and terpenoids.[53,54] Following Al stress, conformational changes that lead to protein breakdown by proteases may be induced.[55] This could be the course of the increase in amino acids generally, especially in light of increased leucine amino peptidase seen in the proteomic study of wheat cultivars.[56] The present result demonstrated that an increase in the content of leucine as one of the branched chain amino acids together with isoleucine and valine. Parallel increase in proline was concomitant with the increase in glutamic acid, which is the precursor of proline and glutathione. Al interacts with the uptake of inorganic N as well as with the decline in the rates of nitrogen assimilation followed by decrease in the levels of most amino acids including aspartic acid and a strong increase in proline, which is a stress signal and a stress defense in roots of *Lotus japonicas.*[57] The increase in amino acids than the corresponding control value would probably provide an alternative source of energy in the current study.

Wheat plants pretreated with 4µMB and cultured in 500 µMAl revealed that percentage increase of all amino acids was repressed as compared with their corresponding 500 µMAl alone treated seedlings. The root cells of the wheat plants pretreated with 32 µM B and cultured in 500 µM Al revealed that percentage increase of all amino acids was increased as compared with their absolute controls. Cysteine increased by 24% of the control value, which is required for methionine and glutathione/ phytochelatin synthesis and therefore, is a central metabolite in antioxidant defense and metal sequestration. Increased application of B in the presence of high Al concentrations in the growth medium stimulates GSH biosynthesis, suggesting it could be an effective strategy to combat stress associated with the formation of active-oxygen species (AOS) in sunflower plants.[58] Therefore, it can be concluded that optimal boron concentration is directly involved in up regulation of cysteine in

response to aluminum stress. Moreover, amino acids that are not affected by boron during Al stress in wheat plants seem to be regulated through boron independent pathway. In addition, free amino acids were reported to act as osmolytes in abiotic stress response of many plants such as leucine and valine that are coordinately regulated and induced many-fold during osmotic stress and their accumulation has biological significance under abiotic stress.[57]

In another study found that larger increases in Pro concentrations in the roots of Al-tolerant maize accessions in contrast to the sensitive ones might be involved in conferring tolerance to Al.[59] An increased content of Pro and glycine betaine (GB) were observed in Al treated rice seedlings compared to the levels in untreated seedlings60. The suggestion that compatible solutes contribute to the detoxification of ROS,[61] it was confirmed when an elevated Pro content was found to reduce free-radical levels in response to osmotic stress in tobacco.[62] The functional significance of Al-induced Pro accumulation in the tolerant wheat cultivar possibly lies in its capacity to bind water molecules to itself leading to conformational changes in enzyme molecules through proper thermodynamic interactions between solute-water and protein/amino acids system, which in turn would help in maintaining proper hydration inside the cells.

In the current study wheat plants pretreated with 4 µM B concentration in solutions with Al, the proline content decreased as compared with control value. The decrease in proline may be due to the low (B deficiency) concentration of boron, which is synergistic with Al toxicity to induce inhibitory effect and representing a case of double stress. It is possible that these changes in proline compounds occurred because of an increase in proteolysis combined with a reduction in the protein synthesis. Wheat plants treated with 32 µM B concentration in solutions without Al, the proline content increased significantly as compared with control plants, which has been correlated with stress tolerance. Proline accumulation in plant tissues has been suggested to result from (a) a decrease in proline degradation, (b) an increase in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, and (d) hydrolysis of proteins.[63] The functional significance of Al-induced Pro accumulation in the tolerant wheat cultivar possibly lies in its contribution toward maintenance of high efficient water balance.

There are several efforts to explore the role of Al-induced root polypeptides in mediating Al-tolerance but the role its play in tolerance is still unclear. Several proteins showed change in expression level in response to Al treatment in wheat cultivar Sakha 93 (Fig. 5). Al treatment resulted in up and down regulated proteins in wheat roots with differential expression between control and Al-treated samples. In wheat root plants high and low molecular masses proteins of 136, 43, 35 and 21kDa were synthesized in response to 100µM Al. There were variable peptides with different molecular masses, for example, peptides with molecular masses 125, 36, 20kDa and 36, 21 were synthesized in response to 200 and 400µM Al, respectively. At 500µM Al, 117, 101, 36, 30, 26 and 21kDa were synthesized, while peptides with 145, 43 and 25kDa were disappeared (Table 5). In all Al-treatments, there were more up regulated than down regulated proteins, these polypeptides may have significant role in Al-binding capacity. Treatment with Al leads to the accumulation of several polypeptides 12,23 and 43.5kDa[64] in the root exudates of an Al- resistant cultivar of *Triticum aestivum* these polypeptides may have significant Al-binding capacity. Using of various inhibitors of protein phosphorylation/dephosphorylation[65] and reported that the inhibition of Al-responsive malate efflux in wheat is associated with protein phosphorylation, possibly related to an organic anion-specific channel or its upstream signaling by a K-252a (a broad range inhibitor of protein kinases)-sensitive protein kinase. Using in-gel kinase assay with myelin basic protein (MBP) as an artificial substrate, these authors observed activation of a 48-kDa protein kinase in the root apex treated with 200µM Al. The activity of this kinase was elevated from 0.5 to 5 min after the addition of Al and it diminished after 5 min. This suggested that transient activation of the 48-kDa protein kinase might be involved in the early physiological response to Al. The activity of the 48-kDa kinase was approximately 10-fold higher after the treatment with Al than without Al, and the Al-induced activation was lost within 5 min. Al transiently activates this protein kinase quickly enough to precede the initiation of malate efflux. This

protein kinase phosphorylated MBP, indicating that this kinase may be categorized in the MAP kinase group. Induction of 51-kD protein synthesis after 24 hr exposure to 75μM Al^{3+} was reported in the work[66]. This protein was accumulated in the root tips of Al-tolerant wheat cultivar PT741 and its association with the tonoplast was demonstrated as well.

Two 51 kD proteins were discovered to be induced by aluminum in resistant wheat cultivar cv PT741[67]. The protein sequences were homologous to B subunit of H+-ATP ase and also and subunits of mitochondrial ATP- synthase. Both enzymes were elicited by aluminum in dose-dependent manner only in resistant PT741 but not in the other ones. In other study recorded that acidic and basic proteins were among Al responsive proteins, with molecular weight ranged from 10-40 kDa.[68] Functional analysis of Al responsive proteins in rice and tomato revealed up-regulation of primarily proteins involved in antioxidation and detoxification mechanisms.[69]

Proteins were assigned to molecular functional groups and cellular metabolic pathways using Map Man.[70] Among the proteins whose abundance levels changed significantly were: a number of transcription factors; proteins regulating gene silencing and programmed cell death; proteins in primary and secondary signaling pathways, including phytohormone signaling and proteins for enhancing tolerance to abiotic and biotic stress in Al-treated tomato plants.

Application of 4μMB in control samples revealed the presence of four low molecular masses with 45, 44, 36 and 21kDa with corresponding band intensities 32, 15, 11 and 40, respectively. Several peptides with different band intensities were synthesized after treatment with different concentrations of aluminum (Table 5). Peptides with molecular masses 166, 45, 35 and 21kDa were synthesized in wheat roots after treatment with 100 μM Al in seedlings pretreated with 4 μM B. At 200μMAl, peptides with molecular masses 96, 56, 53, 36, 32, 27, 21kDa were synthesized, while peptides with 45, 44 and 36 were disappeared. Two low molecular masses peptides of 36 and 21kDa were synthesized in wheat roots in response to 400μM Al pretreated with 4μM B. At 500μM concentration of Al, polypeptides with molecular masses 152, 63, 36, 30, 21kDa were synthesized, while peptides with molecular masses 45 and 44 were disappeared in seedlings pretreated with 4μM B. These results indicate that in wheat, certain proteins may be involved in tolerance to Al toxicity and are formed due to interaction between Al and Boron.

The electrophoretic pattern of water and boron treated wheat roots showed number of major six polypeptides of different band's intensity with molecular masses 121, 107, 36, 30, 27 and 21kDa. Peptides with molecular masses 125, 46, 37 and 21kDa were synthesized in wheat roots after treatment with 100 μM Al in seedlings pretreated with 32 μM B. At 200 μMAl, peptides with molecular masses 103, 38, 21 and 20kDa were synthesized, while peptides with 45, 44 and 36 were disappeared. Low and high molecular masses peptides were synthesized in response to 400μM Al pretreated with 32 μM B with 143, 62, 39, 24 and 20kDa. On the other hand, low molecular masses peptides were synthesized in response to 500μM Al pretreated wheat seedlings with 62, 43, 39,22 and 20 kDa with 59, 8,7,14 and 11 band intensities, respectively (Table 5). We speculate that in wheat, certain proteins may be involved in tolerance to Al toxicity and are formed due to interaction between Al and Boron and are involved in Al tolerance.

Conclusion

The current study demonstrate that wheat (*Triticum aestivum*) cultivar Sakha 93 endures high levels of Al concentrations toxicity up till 500μM under low acidic conditions pH 4.3; indicating that this cultivar has an internal adaptive mechanism(s) that minimizes the impact of the toxicant for copying with elevated Al concentrations that can remain protective even under Al stress. It is proposed that a resistance mechanism which is dependent on metabolic integrity exist in roots of this studied wheat cultivar. This study showed that the difference in total soluble sugars, soluble protein, amino acids and proline contents and up and down regulated proteins is due to mechanisms underlying oxidative stress injury and subsequent tolerance to Al-stress. Particular role was for B where B deficiency (4μMB) played a negative (synergestic) with Al inhibiting growth and appearance of wheat seedlings, however adequate B (32μMB) antagonises largely the Al adverse effect on Sakha 93 seedlings to become near of those of the

control and possible alleviation of Al-toxicity in wheat by boron. The possible mechanisms, increasing the accumulating higher levels carbohydrates, soluble proteins, specific amino acids especially proline related to osmoregulation and membrane stabilization. These responses were observed especially in the presence of B concentration more than metabolic requirements (32 µMB).

Acknowledgement

We thank Dr. Said Hafez Professor in Protection and Biomolecular diagnosis Department, City of Scientific Research for his great kind help and facilities in practical of molecular part.

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