EFFECTS OF ALUMINIUM ON SOME BIOCHEMICAL CHARACTERISTICS OF WHEAT (TRITICUM AESTIVUM L.)

A. N. M. ALAMGIR AND SUFIA AKHTER*

Department of Botany, Chittagong University, Chittagong-4331, Bangladesh

Key words: Aluminium stress, HYV wheat, Metabolites, Hematoxylin test, Efficiency ratio

Abstract

Aluminium at 10 ppm increased chl *a* in Aghrani, Gourab, Kanchan and Sourab; chl *b* and carotinoids in Fang-60, Kanchan and Sourab, though at 100 ppm inhibited chl *a* in all nine varieties, chl *b* in Akbar and Protiva and carotinoids in Aghrahani, Gourab, Kanchan, Protiva, and Sonalica. Proline content increased in the seedlings of all varieties and the root had relatively higher proline content than that of shoot. Stimulatory effect of Al^{3+} on proline content was higher in Kanchan. Proline content also increased in Akbar, Gourab and Sonalika. Al^{3+} stress induced increase in protein and reducing sugar contents was more in root than in the shoot. Hamatoxylin test of the root tips also revealed differential responses of the varieties to Al^{3+} stress.

Introduction

Aluminium toxicity is a serious problem for wheat cultivation in the areas of low soil pH (von Uexkull and Mutert 1995). There are about 2.6 billion ha of strongly acid soil with Al^{3+} toxicity in the world (Car *et al.* 1991) and Bangladesh has more than a million ha low pH soil with Al^{3+} toxicity stress. Stress factors are known to affect crop growth, development and yield through their effect on plant metabolic activities (Slaski *et al.* 1996, Bohnert and Shiveleva 1998) and Al^{3+} , by interfering Pi absorption, root elongation and metabolism inhibits crop growth and yield (Tang *et al.* 2002). Liming reduce Al^{3+} toxicity (Foy 1992), but Al^{3+} tolerant plant is effective to resolve the problem (Baligar *et al.* 1993). Aluminium stress tolerant wheat germplasm is still unavailable in Bangladesh. In this study, nine HYV wheat were screened for Al^{3+} tolerance efficiencies on the basis of their performance in some biochemical characteristics.

Materials and Methods

Seeds of nine HYV of wheat (*Triticum aestivum* L.) *viz.* Aghrahani, Akbar, Baw-923, Fang-60, Gourab, Kanchan, Protiva, Sonalika and Sourab were collected from Bangladesh Agricultural Research Institute, Joydevpur, Gajipur. Seeds were germinated in 0.1 mM CaSO₄ and then further grown for ten days hydroponically in 0.1 strength (0.1N) nutrient solution (Arnon and Hoagland 1940) without (control) and with Al³⁺ (treatment) in the open air. Solution was changed after 24h interval. Leaf pigments (Wettstein 1957), protein (Lowry *et al.* 1951), proline (Troll and Lindsley 1954) and sugar (Nelson-Somogyi 1944) were analyzed in ten days old seedlings. Efficiency ratio (ER) was determined by dividing treatment value by that of respective control. Roots of three days old seedlings were used for hematoxylin staining test following Polle *et al.* (1978). In all cases, each set of experiment was replicated thrice.

Results and Discussion

At low concentration of Al^{3+} (10 ppm) chl *a* content increased in Aghrahani, Gourab, Kanchan and Sourab, while it decreased that in other varieties (Fig. 1). Chl *b* in Aghrahani, Akbar and Baw-923 and carotenoids in Gourab, Protiva and Sonalika were decreased following 10 ppm Al^{3+} treatment. At this low stress, the highest contents of chl *a* and *b* were found in Sourab and Fang-60 and the lowest contents were found in Sonalika and Aghrahani, respectively. In all, except Protiva, the concentration of chl *a* content was decreased following 100 ppm Al^{3+}

^{*}Corresponding author. E-mail: sufiacu@yahoo.com

treatment, but chl *b* content was increased in all variety except Akbar and Protiva. At 100 ppm Al^{3+} , the highest content of carotinoids was found in the leaf of Baw-923 and the lowest was in Gourab. The inhibitory effect of Al^{3+} was more in case of chl *a*; whereas, in many cases the Al^{3+}



Fig. 1. Effects of Al³⁺ on the amount of leaf pigments content of ten days old seedlings of wheat.

had stimulatory effect on chl b and carotenoids. Scott *et al.* (1991) reported that aluminium stress decreased leaf pigments (chl *a*, *b*) more in the sensitive wheat cultivars than that in the tolerant ones at all concentrations of Al^{3+} (50 - 400 µM), though in tolerant cultivar, pigment levels reported to increase under low Al^{3+} stress (50 - 100 µM). On the other hand, Albassam (2001)

Table 1. Effect of Al³⁺ on the ER of leaf pigments, root - shoot praline and protein contents of ten days old seedlings of different varieties of wheat.

	Treatments												
Varieties	10 ppm						100 ppm						
	ER of leaf		ER of root- shoot proline		ER of root- shoot protein		ER of leaf			ER of root- shoot proline		ER of root- shoot protein	
	Chl a Chl b Car		RER	SER	RER SER		Chla Chlb Car		RER SER		RER SER		
Aghrahani	1.03	0.66 1.16	1.36	1.28	1.00	0.95	0.59	1.07	0.18	3 33	1.88	1.52	1.23
Aginanani	0.00	0.00 1.10	1.50	1.20	1.00	0.95	0.57	1.57	1.00	2.07	2.15	1.52	0.60
Akbar	0.86	0.96 1.28	1.57	1./6	1.10	0.89	0.77	0.94	1.08	3.07	3.15	1.19	0.60
Baw-923	0.94	0.95 1.03	1.28	1.05	1.95	0.92	0.45	1.97	1.24	1.42	1.58	2.65	1.40
Fang-60	0.76	1.96 1.20	1.05	1.02	0.98	0.92	0.42	2.36	1.57	1.98	2.13	1.04	1.10
Gourab	1.62	1.65 0.33	1.44	1.09	1.34	0.94	0.89	2.18	0.80	3.96	1.45	1.42	1.13
Kanchan	1.24	2.12 1.08	2.68	1.76	1.13	0.74	0.74	1.21	0.73	4.21	3.38	1.26	0.65
Protiva	0.97	1.06 0.90	2.81	1.21	1.23	0.66	0.87	0.84	0.82	1.44	6.18	1.48	0.57
Sonalika	0.90	1.10 0.69	1.49	1.11	0.96	1.33	0.50	1.36	0.23	3.98	1.28	1.67	1.39
Sourab	1.33	1.16 1.57	1.32	1.27	1.23	1.05	0.58	2.58	1.40	2.11	1.34	1.56	0.75

noted the inhibitory effect on leaf pigments in pearl millet. Aluminium stress affected the efficiency ratio (ER) of different leaf pigments (Table 1). Gourab and Protiva had the highest ER for chl a at 10 and 100 ppm Al³⁺, respectively while Fang-60 had the lowest ER at both concentration.

At 10 ppm Al³⁺, the highest ER value for chl *b* was observed in Kanchan and the lowest was recorded in Aghrahani, but at 100 ppm the highest ER for chl *b* was observed in Kanchan and the lowest was recorded in Aghrahani, but at 100 ppm the highest ER for chl *b* was observed in Fang-60 and the lowest was noted in Protiva. For carotinoids, the highest ER at 10 ppm Al³⁺ was observed in Sourab and the lowest was recorded in Gourab. Contrary, at 100 ppm the highest and lowest ER were noted in Fang-60 and Sourab, respectively. High ER for leaf pigments under Al³⁺ stress may be considered as an important factor for the maintenance of proper assimilatory activity under growth limiting environment.

Aluminium at both 10 and 100 ppm increased proline content in the root and shoot of the seedlings of all varieties (Fig. 2). Proline, as an osmoticum, may do some protective roles through osmotic adjustment with chelating function under stress. Role of proline in the adaptive mechanism in a wide variety of species have been known. For example. Handa *et al.* (1986) noted a high correlation between proline level and plant stress tolerance. In control as well as in 10 ppm Al^{3+} , the highest proline content was found in the root of Baw-923 and the shoot of Sonalika, whereas the lowest proline content was found in the root and shoot of Akbar in both the cases. On



Fig. 2. Effects of Al^{3+} toxicity on proline contents in the root (left hand one) and shoot (right hand one) of ten days old seedling of wheat.

the other hand, at 100 ppm Al^{3+} , the highest proline content was found in the root of Sourab and in the shoot of Protiva. The lowest proline was found in the root of Protiva and in the shoot of Akbar at 100 ppm of Al^{3+} . Most of the varieties with Al^{3+} treatment revealed higher proline content in the root than that in the shoot. At 10ppm Al^{3+} , both the root efficiency ratio (RER) and that of the shoot efficiency ratio (SER) for praline were high in Kanchan, but at 100 ppm RER was high in Gourab and Sonalika and SER was high in Protiva and Kanchan (Table 1).

At 10 ppm Al³⁺, the highest protein content was observed in the root and shoot of Fang-60 and Sourab, respectively, whereas the lowest protein was in the root of Aghrahani and in the shoot of Gourab. At 100 ppm Al³⁺, the highest protein content was observed in the root and shoot of Fang-60, Baw-923 and the lowest protein content was in the root and shoot of Gourab and Akbar, respectively (Fig. 3). Stimulatory effect on protein content at 10 ppm Al³⁺ was highest in the root of Baw-923, but the lowest was in Akbar. At 100 ppm Al³⁺, the highest stimulatory effect was

observed in protein content in the shoot of Baw-923 and the lowest stimulatory effect was observed in Fang-60. At 10 ppm Al³⁺, the highest and the lowest root to shoot, protein ratio were found in Fang-60 and Sonalika, respectively but at higher stress such ratio was with BAW-923 (Table 1). Ownby and Hruschka (1991) on examining the root tip protein in Al-tolerant (T \times 84) and Al-sensitive (T×74) wheat cultivars by 2D-PAGE reported that out of 600 proteins, 14 cytoplasmic and eight microsomal proteins were induced or enhanced by Al³⁺ in one or both cultivars, while nine cytoplasmic and 12 microsomal protein were diminished. Among them, 43 proteins were significantly altered by Al³⁺ treatment. Al³⁺ affected the synthetic programme of formation of both cytoplasmic and microsomal proteins, but appeared to cause the greater change in proteins associated with cytoplasm. It was suggested that protein expression in response to AI^{3+} stress may result from its effect on metabolism and may play a role in plant's adaptive response to Al³⁺ stress. Al³⁺ induced polypeptide synthesis was also reported in the root of both sensitive and tolerant cultivars of wheat (Somers et al. 1996). Albassam (2001), however, noted the inhibitory effect of Al^{3+} on the soluble protein contents in pearl millet. A possible mechanism of Al^{3+} resistance offered by protein may be associated with its function as chelator ligands which form stable complex with Al^{3+} and there by reducing the different interfering activity of Al^{3+} in the metabolism of plant (Taylor 1991).





The effect of Al³⁺ stress on sugar content was investigated in three varieties Baw-923, Fang-60 and Kanchan (Table 2). Aluminium stress increased sugar content only in the root of Kanchan and Baw-923. The highest RER and SER for sugar was found in Kanchan and Baw-923, respectively. Lima and Copeland (1990) reported the increasing of reducing sugar content in the

Table 2. Effect of Al^{3+} on sugar content ($\mu g/g \ d. wt.$) in root and shoot of ten days old seedling of wheat \pm standard error.

	Reducing sugar, µg/g d.wt.										
Variety		Control		Treatment							
					1	00 ppm					
	Root	Shoot	R/S	Root	RER	Shoot	SER	R/S			
Baw-923	196.09 ± 7.84	515.63 ± 1.87	0.38	313.77 ± 3.07	1.601	496.04 ± 1.85	0.94	0.65			
Fang-60	258.40 ± 2.46	276.21 ± 1.76	0.94	166.27 ± 2.26	0.64	192.07 ± 1.37	0.69	0.87			
Kanchan	111.35 ± 1.32	300.78 ± 1.09	0.37	545.39 ± 3.28	4.89	211.69 ± 9.21	0.70	2.58			

germinating seeds of tolerant wheat cultivar at low Al^{3+} , but decreased at high Al^{3+} stress; proportionately higher reduction was being in the sensitive cultivar. On the other hand, Scott *et al.* (1991) observed stimulatory effect of Al^{3+} stress on both reducing and non-reducing sugars in the root and shoot of wheat seedlings, with higher stimulatory effect in the Al-tolerant cultivars.

Aluminium stress tolerance seems to be controlled by a complex system; however, some simple methods for screening tolerance in wheat may be useful (Taylor 1991, Tang *et al.* 2002). In the present work, hematoxylin test revealed that root tips of some of the varieties were stained intensively than others, which could be a reflection of sensitivity due to high accumulation Al^{3+} in the region (Fig. 4). The sequence of colour intensity developed due to Al^{3+} sensitivity of different wheat varieties was: Akbar > Fang-60 > Protiva > Kanchan > Sonalika > Aghrahani = Sourab = Gourab > Baw-923. Bona and Varver (1992) evaluated 84 wheat genotypes for Al^{3+} tolerance visually depending on the extent of root tip stains. Root apices of Al-sensitive genotypes were stained more intensely after a short exposure to Al^{3+} (Delhaize *et al.* 1993) and cortical zone of the root apices showed five - tenfold higher accumulation of Al^{3+} than the tolerant genotypes (Archambault *et al.* 1997). In the present work, varieties with high leaf pigments, proline, protein and sugar levels as well as low hematoxylin stain at the root tip under Al^{3+} stress may be considered relatively more tolerant than the others.



Fig. 4. Effects of Al³⁺ on root tip hematoxylin staining of four days old seedlings of different varieties of wheat. 1-Aghrahani, 2-Akbar, 3-Baw-923, 4-Fang-60, 5-Gourab, 6-Kanchan, 7-Protiva, 8-Sonalika and 9-Sourab.

References

- Albassam Badr A. 2001. Growth and nitrate assimilation in pearl millet exposed to aluminium stress. Saudi J. Biol. Sci. 8(2): 105-112.
- Arnon D.I. and D.R. Hoagland. 1940. Crop production in artificial culture solution and in soil with special reference to factors influence in yields and absorption of inorganic nutrient. Soil Sci. **50**: 463-483.

- Archambault D.J., G. Zhang, J.J. Slaski and G.J. Taylor. 1997. Aluminium-induced alterations in lipid composition of microsomal membranes from an aluminium-resistant and an aluminium sensitive cultivar of *Triticum aestivum*. Physiol-Plant. (Denmark) 96(4): 683-691.
- Baligar V.C., T.A. Campbell and R.J. Wright. 1993. Differential responses of alfalfa clones to aluminium toxic-acid soils. J. Plant Nutr. 16: 219-233.
- Bohnert H.J. and E. Shiveleva. 1998. Plant stress adaptations- making metabloic move. Curr. Opin. Plant Biol. 1(3): 267-274.
- Bona L. and B. Varver. 1992. Tolerance of wheat (*Triticum aestivum* L.) genotypes of aluminium toxicity in the seedling stage. Novenytermeles (Hungary) **4**: 381-391.
- Car S.J., G.S.P. Rirchie and W.M. Porter. 1991. A soil test for aluminium toxicity in acidic subsoils of yellow earths in Western Australia. Aust. J. Agric. Res. 42: 875-892.
- Delhaize E, S. Craig, C.D. Beaton, R.J. Bennet, V.C. Jagadish and P.J. Randall. 1993. Aluminium tolerance in wheat (*Triticum aestivum* L.). 1. Uptake and distribution of aluminium in root apics. Plant Physiol. 103: 685-693.
- Foy C.D. 1992. Soil chemical factors limiting plant roots. Adv. Soil. Sci. 19: 97-149.
- Handa S, A. Handa, K. Hasegawa, M. Paul and R.A. Brassan. 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiol. 80: 938-945.
- Lima de and M.L. Copeland. 1990. The effect of aluminium on the germination of wheat seeds. J. Plant Nutr. **13**(12): 1489-1497.
- Lowry O.H., N.J.R. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265-275.
- Nelson-Somogyi N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153**: 375-380.
- Ownby J.D. and W.R. Hruschka. 1991. Quantitative changers in cytoplasmic and microsomal proteins associated with aluminium toxicity in two cultivars of winter wheat. Plant Cell Environ. 14: 303-309.
- Polle E., J.M. Konzak and J.A. Kittrick. 1978. Visual detection of aluminium tolerance in wheat by hematoxylin staining of seedling roots. Crop Sci. 18: 122-130.
- Scott R., J. Hoddinott, G.J. Taylor and K. Briggs. 1991. The influence of aluminium on growth, carbohydrate and organic acid content of an aluminium sensitive cultivar of wheat. Can. J. Bot. 69: 711-716.
- Slaski J.J., G. Zhang, U. Basu, J.L. Stephens and G.L. Taylor. 1996. Aluminium resistance in wheat (*Triticum aestivum*) is associated with rapid Al-induced changes in activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the root apices. Physio. Plant. (Denmark) 98: 477-484.
- Somers D.J., K.G. Briggs and J.P. Gustafson. 1996. Aluminium stress and protein synthesis in near isogenic lines of *Triticum aestivum* differing in aluminium tolerance. Physio. Plant. (Denmark) **97**(4): 694-700.
- Tang Y., D.F. Garvin, L.V. Kochin, M.E. Sorrells and B.F. Carver. 2002. Physiological genetics of aluminium tolerance in wheat cultivar Allas 66. Crop Sci. 42: 1541-1546.
- Taylor G.J. 1991. Current views on aluminium stress response: the physiological basis of tolerance. *In*: Current topics on plant biochemistry and physiology, D.D. Raudal. D.G. Blevins and C.D. Miles (Eds) pp. 57-93. Univ. of Messouri, Columbia.
- Troll W. and J. Lindsley. 1954. A photometric method for the determination of proline. J. Biol. Chem. **215**: 655-661.
- Wettstein D. 1957. Formula of chlorophyll determination. Exp. Cell Res. 3: 427-487.

(Manuscript received on 5 May 2009; revised on 19 January 2010)