EFFECTS OF ALUMINIUM TOXICITY ON ION TRANSPORT IN *ORYZA SATIVA* L. AND *CICER ARIETINUM* L. IN RELATION TO BIOCHEMICAL AND ANATOMICAL CHANGES

BY

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A

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Dedication

This Ph.D thesis is dedicated to
my late husband
who would be so happy to see
this work completed

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ABSTRACT

Aluminium, at concentrations of 10, 50, 100 and 150 µM, inhibited germination of rice and chickpea seeds. Aluminium stress decreased accumulation of K^+ in the radicle and plumule of germinated rice and chickpea seeds. On the other hand, Cl^- accumulation was increased by a maximum of 2- to 2.4-fold in the radicle and plumule of germinated rice and chickpea seeds following Al treatment. Similarly, Al (10-150 µM) caused a few fold increase in accumulation of Al^{3+} in the radicle and plumule of germinated rice and chickpea seeds. Aluminium-induced decrease in K^+ content with concomitant increase in Cl^- and Al^{3+} content in the radicle and plumule might be correlated with the inhibition of germination.

Aluminium decreased K^+ accumulation in the root and shoot of rice, and the root, stem and leaves of chickpea seedlings grown in solution culture. On the contrary, different concentrations of aluminium caused a few fold increase in Na^+ in rice and chickpea.

Aluminium stress caused a dramatic increase in Cl^- accumulation in different parts of rice and chickpea seedlings. But aluminium toxicity decreased NO_3^- accumulation in rice and chickpea. Al stress decreased phosphate accumulation in rice and chickpea seedlings.

Aluminium toxicity inhibited the accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root and shoot of rice, and the root, stem and leaves of chickpea plants grown in both solution and sand culture.

Application of aluminium caused a dramatic increase in accumulation of Al^{3+} in different parts of rice and chickpea seedlings grown in solution culture.

Exposure of rice and chickpea seedlings to different concentrations of aluminium led to a stimulation of reducing and total sugar in the root, stem and leaves.

Similarly, Al stress increased proline and total amino acid contents in different parts of rice and chickpea seedlings.

Aluminium toxicity caused a dramatic increase in peroxidase and catalase activity in the root and shoot of rice. 150 µM Al caused a 8- to 9-fold increase in peroxidase and catalase activity, respectively, in the root of rice. On the contrary, Al stress decreased superoxide dismutase (SOD) activity in the root and shoot of rice. In chickpea, Al stress caused a few fold increase in peroxidase, catalase and SOD activity in the root and leaves. A dramatic 14.8- and 14.6-fold increase in SOD activity was recorded in the root and leaves of chickpea seedlings respectively. It is interesting to note that there is a generic difference between rice and chickpea with respect to the effect of aluminium stress on SOD activity.

Aluminium toxicity caused a dramatic increase in phenolic compounds in rice and chickpea plants grown in sand culture.

Aluminium stress resulted in a reduction of chlorophyll a, chlorophyll b and carotenoid contents in the leaves of rice and chickpea plants.

Exposure to aluminium decreased primary root length and number of lateral roots in rice and chickpea seedlings grown in rhizobox.

Aluminium toxicity inhibited the root and shoot length of rice and chickpea seedlings grown in solution culture.

Aluminium stress decreased the dry weight of root and shoot of rice and chickpea seedlings. But it increased shoot/root dry weight ratio.

Aluminium toxicity reduced the number of metaxylem vessels in the root of rice. Number of sclerenchyma cells were more in aluminium-treated rice root. Smaller sized vascular bundles were found in the leaf of rice under Al stress. In chickpea, Al decreased the size and number of vessels in the root. Number of palisade parenchyma was reduced in the leaf of chickpea. Al treatment caused closure of stomata both in rice and chickpea leaves.

The effect of aluminium toxicity on ion transport and its correlation with biochemical changes and anatomical structure, and growth is discussed.

CERTIFICATE

This is to certify that the research work embodying this thesis has been carried out under my supervision in the Plant Physiology, Biochemistry and Plant Nutrition laboratory, Department of Botany, University of Dhaka. The research work presented herein is original. This thesis contains no material published elsewhere or extracted from a thesis presented by the candidate for another degree or diploma. No other person's work has been used without due acknowledgement. This thesis has not been submitted for award of any other degree or diploma in any other University.

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This thesis contains no material published elsewhere or extracted from a thesis presented by the candidate for another degree or diploma. No other person's work has been used without acknowledgement. This thesis has not been submitted for the award of any other degree or diploma in any other University.

Rifat Samad

List of publications

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ABBREVIATIONS

BSA	Bovin Serum Albumin
SOD	Superoxide dismutase
Fe-EDTA	Ferric ethylenediaminetetraacetic acid
Na_2-EDTA	Disodium ethylenediaminetetraacetate dihydrate
NBT	Nitroblue tetrazolium chloride
mequiv.	Milliequivalent
µequiv.	Microequivalent

Chapter 1

GENERAL INTRODUCTION

Aluminium (Al) is a light metal that makes up 7% of the earth's crust and the third most abundant element after oxygen and silicon. Plants roots are, therefore, almost always exposed to Al in some form. Fortunately, most of this Al occurs as harmless oxides and aluminosilicate including the feldspars, micas and clay minerals which are the most common primary and secondary minerals in soils (McLean *et al.* 1965). Aluminium oxide, Al_2O_3, occurs as corundum and amery. The hydroxide, $Al(OH)_2$, occurs as gibbsite diaspore (AlOOH) and cryolite is other source of soil aluminium (Hesse 1972). Aluminium occurs in interlayer position in clays often forming complete layers to which the term chlorite is sometimes applied. However, when soil becomes acidic as a result of natural processes or human activities, aluminium is solubolized into toxic trivalent cation Al^{3+} (Ma *et al.* 2001). Aluminium in nutrient solution below pH 5 mainly appears as Al $(H_2O)_6$ which is known as Al^{3+} and is the most toxic form (Vardar and Ünal 2007).

Solubolization of Al-containing minerals is enhanced in acidic environment in many acid soils throughout the world and soluble Al^{3+} is the most growth-limiting factor (Foy 1988), possibly affecting 70% of world's arable land that is potentially usable for food and biomass production (Haug and Caldwell 1985).

Acid soils occupy about 30% of global land surfaces, and predominate in two major regions of the world: humid temperate forests and humid tropics and subtropics (von Uexküll and Mutert 1995). In Bangladesh, aluminium toxicity exists in the soil of some area especially in the soil of Madhupur tract, Chittagong Hill tract and Sytlhet. Besides acid soil covers a small area in coastal area of Bangladesh (Khan *et al.* 2016).

Strategies to maintain production in acidic soils include the application of lime to raise the soil pH and the use of plants that are tolerant of acid soils. Al toxicity has been identified as a problem of acid soils for over 70 years (Delhaize and Ryan 1995).

1.1 Effects of aluminium toxicity on germination of seeds

Germination potential of seeds is an important factor for growing plants in adverse soil condition like aluminium toxicity. There are a few reports on the effect of aluminium stress on germination of seeds.

Al^{3+} decreased seed germination in maize (Nasr 2013). Germination percentage was found to be highly reduced under aluminium stress in chickpea (Singh *et al.* 2012). Al significantly reduced germination of pea (*Pisum sativum* L.) (Singh *et al.* 2011). On the contrary, aluminium toxicity had no effect on germination of seeds of wheat (Jamal *et al.* 2006).

Significant differences of germination ratio of tobacco seeds treated with 50-200 µg $AlCl_3$ were not observed when compared to their control. However germination time was delayed with increasing Al concentrations (Vardar *et al.* 2006).

1.2 Effects of aluminium toxicity on root elongation, root growth and plant growth

1.2.1 Effects of aluminium toxicity on root elongation

The inhibition of root elongation was a general and very sensitive response of several plant species in presence of soluble forms of Al (Kochian 1995, Matsumoto 2000, Rout *et al.* 2001, de Campos *et al.* 2003 and Ma *et al.* 2004). The inhibition of root elongation is the most significant symptom of Al toxicity (Zheng 2010). Absorbed Al inhibited root elongation severely within hours in

higher plants (Vardar and Ünal 2007). Al absorbed in the cell wall reduced cell expansion, thus reducing root elongation (Blamey 2001).

The lowest Al concentration (20 μM) restricted root enlogation in *Allium ursinum*. The growth of new roots was almost equally poor at different Al concentrations (20-70 μM) (Anderson 1993). Al primarily inhibited root elongation and showed different patterns among plant species or different cultivars (Matsumoto 2000).

Percentage of root length was found to be highly reduced under increasing Al^{3+} stress (Singh *et al.* 2012). Al decreased root length in wheat (Hossain *et al.* 2006). The inhibition of root elongation in three varieties of maize (*Zea mays* L. vars Clavito, HS701b and Sikuani) was followed over the first 48 h of Al treatment. Aluminium-induced inhibition of root elongation occurred within 1 h of exposure (Kidd *et al.* 2001). Similarly, root elongation in maize was observed after less than 30 minutes of exposure (Liugany *et al.* 1995).

Root elongation of rye (cv. King) during 24 h period was inhibited by 16.2%, 28.2% and 42.7% by the exposure to 10, 30 and 50 μM Al, respectively, that of wheat was inhibited by 19.6%, 21.4% and 37.7% respectively (Li *et al.* 2000). Root elongation in maize was drastically reduced due to 50 μM Al^{3+} treatment within 45 minutes (Doncheva *et. al.* 2005). Al decreased root elongation in rye (Ma *et al.* 2002). Al-malate, Al-citrate and Al-oxalate complex nullified inhibitory effect of Al on root elongation in corn (Zheng *et al.* 1998).

As an exception, roots were elongated under Al toxicity in peas (Wagatsuma *et al.* 1987). Better root elongation was found in wheat due to aluminium treatment (Aniol 1984).

Al affected root elongation zone, root cap and meristem of maize (Sivaguru and Horst 1998) and wheat (Delhaize and Ryan 1995).

Tepper *et al.* (1989) found that the effect of Al on root elongation was limited to the distal 4 mm of honeylocust and loblolly pine seedling root. Wallace and

Anderson (1984) reported that wheat plants exposed to Al solutions for a short period of time experienced a rapid decrease of root elongation. The rhizosphere Al^{3+} prevailing in acid soil reduced root elongation (Andersson and Brunet 1993, Kinraide 1993, 1997, Liugany *et al.* 1995).

Al inhibited root elongation of soybean, carrot, cabbage and cucumber (Yang and Watts 2005) and corn roots (Lin and Xing 2007). Aluminium reduced elongation of root hairs in *Trifolium repens* (Care 1995). Higher root P caused a higher Al-induced inhibition of the root elongation (Shao 2015).

There were varietal difference in maize and common bean in response to Al toxicity to root elongation as shown in Table 1.

Table 1. Varietal differences in maize and common bean in response to Al toxicity to root elongation.

Crop	Var./Cultvar	Symptoms	References
Maize	Sikuani (tolerant)	No inhibition of root elongation.	Garzon *et al.* (2011)
	Bakero (sensitive)	Significant inhibitation of root elongation.	
	ATP SR Yellow (tolerant)	Higher root elongation.	Gunsé *et al.* (2000)
	H S 70113 (sensitive)	Poor root elongation	
	Cateto (tolerant)	No inhibition of root elongation	Tolra *et al.* (2009).
	HS16 x 36 (sensitive)	Significant inhibition of root elongation.	
Common bean (*Phaseolus vulgaris*)	Preto and Carioca (tolerant)	Slight inhibition of root elongation.	Gunsé *et al.* (2003)
	Condender and Superba (sensitive)	Severe inhibition of root elongation.	
	Adean (tolerant)	Slight reduction in root elongation.	Blair *et al.* (2009)
	Mesoamerican (sensitive)	Higher reduction of root elongation	
	Quimbaya (tolerant)	Severe inhibition of root elongation.	Rangel *et al.* (2009)
	VAX-1 (sensitive)	Severe inhibition of root elongation with no recovery.	

1.2.2 Effects of aluminium toxicity on root growth

Root growth inhibition is the most evident symptom of Al toxicity which can be detected within 30 minutes to 2 hours, even at micromolar concentrations of Al (Barceló and Poschenrieder 2002).

Al toxicity caused inhibition of root growth in a wide range of plant species. Al exposure caused an inhibition of root growth (Silva 2012). Root growth of wheat were inhibited by applying low concentration of Al (Alamgir and Akhter 2009). Al toxicity inhibited plant growth by interfering with the regulatory processes of root growth and development in different plants (Foy 1988, Taylor 1988 and Kochian 1995).

Free aluminium ions were the main factor for inhibiting root growth of acidic soils (Horst 1995, Matsumoto 2000, Kochian *et al.* 2004 and Ma 2007). The addition of Al severely inhibited root biomass of the aluminium sensitive mutants in *Arabidopsis thaliana* even at a low concentration (10 μM) (Bose *et al.* 2010). Al inhibited root growth in cultured tobacco cells (Chang *et al.* 1999) and also in tobacco (Vardar *et al.* 2006). The primary target of Al toxicity was roots of plants where the accumulation of Al inflicts the inhibition of root growth in the space of minutes or hours (Ma *et al.* 2001). Root growth inhibition and changes to the entire root architecture were the primary symptoms of Al toxicity (Delhaize and Ryan 1995 and Kochian *et al.* 2004). Plants establishing under high concentration of soluble Al usually develop shallower root systems in mycorrhizal *Pinus sylvestris* (Ahonen-Jonnarth *et al.* 2000). The most serious negative effect of Al was the reduction in root growth in maize. (Foy 1983, Lidon *et al.* 1998 and Calba *et al.* 1999) and wheat (Tabuchi and Matsumoto 2001). Short exposure of Al (less than 60 minutes) inhibited root growth of wheat (Delhaize and Ryan 1995).

50 μM $AlCl_3$ at pH 4.5 inhibited root growth by 65% in wheat and by 25-50% in oil seed rape and oat. Root growth was hardly affected by the same treatment in

buckwheat and raddish (Zheng *et al.* 1998). On the contrary, low concentration of aluminium led to a stimulation of root growth in tolerant genotypes of *Zea mays* (Clark 1977).

Al^{3+} caused root growth inhibition of different plants viz *Aeschynomene americana* L. (Joint-vetch), *Cajanus cajan* (L.) Millsp. (Pigeonpea), *Calapogonium muconoides* (Calopo), *Canavalia ensiformis* L. (Jackbean), *Crotalaria spectabilis* Roth (Showy Crotalaria), *Desmodium heterocarpon* L. (Ea-Ea), *Mucuna pruriens* L. (Mucuna), *Pueraria phaseoloides* (Roxb.) Benth (Puero), *Sesbania sesban* L. Merr. (Sesbania), *Theobroma cacao* L. (Cacao), *Triticum aestivum* L. (Wheat), and *Vigna unguiculata* L. (Cowpea) (Zobel and Kinraide 2007).

Aluminium inhibited root growth of wild graminae and grasses (Poozesh *et al.* 2007, Poozesh *et al.* 2010). Aluminium was found to inhibit root development (de la Fuente *et al.* 1997). Root growth of maize was inhibited by 2.5 and 20 µM $AlCl_3$ (Akhter *et al.* 2009). Al caused a reduction in root growth of rice cultivars (Meriga *et al.* 2010). The first effect of aluminium toxicity was its negative effect on root growth in sunflower (Arsintescu *et al.* 2001). Al solubilized in acid grounds inhibited root growth (Sasaki *et al.* 1996). Al also inhibited development of root hairs (El-Saht 2001).

1.2.3 Effects of aluminium toxicity on plant growth

Aluminium toxicity is one of the major factor that limits plant growth and development (Mossor-Pietrazewska 2001, Rout *et al.* 2001, Poschenrieder *et al.* 2008 and Stevens *et al.* 2011). Due to Al toxicity poor plant growth was observed in barley (Delhaize *et al.* 2004) and corn, sorghum and in barley (Ligon and Pierre 1932). Shoot biomass decreased following 0.33 µM Al treatment in maize (Lidon *et al.* 2000). Aluminium decreased shoot weight in barley. On the contrary, low concentration of Al (2 and 5 ppm) increased the growth and leaf expansion of *Betula pendula* (Kidd and Proctor 2000).

Al reduced growth of spinach plant (Karimaei and Poozesh 2016). Al inhibited plant growth (Sasaki *et al.* 1996) and leaves developed a red colour indicating a phosphorus deficiency (Rasmussen 1968).

Al caused lesser reduction in the root and shoot length in tolerant variety Sutaksha of rice and higher reduction in the root and shoot length of sensitive variety Vikas (Meriga *et al.* 2010). The changes in the root length and shoot length of *Vigna radiata* showed a gradual decline with the increase in aluminium oxide (Al_2O_3) from 200 to 1000 ppm (Mahapatra *et al.* 2015).

1.3 Effects of aluminium toxicity on the accumulation of ions in plants

1.3.1 Effects of aluminium toxicity on the accumulation of monovalent cations

Aluminum toxicity decreased K^+ content in the root, stem and leaves of tomato (Simon *et al.* 1994a). Al interacted directly with several different plasmamembrane channel proteins blocking the uptake of K^+ in wheat roots (Piňeros and Tester 1997). Al interfered with uptake and transport of K^+ in rice (Silva 2012), wheat (Fleming *et al.* 1974, Johnson and Jackson 1964 and Foy 1992) and in barley (Nichol and Oliveira 1995 and Clarkson and Sanderson 1971).

Al reduced K^+ uptake in cabbage, lettuce and Kikuya grass (Huett and Menary 1980a), cotton (Lance and Pearson 1969) and red spruce (Cumming *et al.* 1985a).

Al-sensitive mutant of *Arabidopsis thaliana* had higher shoot K^+ concentration than any of the other genotypes under Al^{3+} treatment. The higher shoot K^+ concentration in the Al-sensitive mutant of *Arabidopsis thaliana* could be linked to decrease in K^+ efflux or enhanced K^+ influx (Bose *et al.* 2010).

Aluminium outcompeted K^+ from uptake sites in the roots of *Alium ursinum*. High conrentration of Al in Hoagland solution decreased K^+ content in

cotyledons and hypocotyls of common buckwheat (*Fagopyrum esculentum*) (Horbowicz *et al*. 2011). Al ions blocked K^+ uptake in oat (Djuric *et al.* 2011) and in soybean plants (Zheng 2010). Al toxicity reduced K^+ efflux in soybean (Stass and Horst 1995). Al decreased K^+ concentration in barley (Alam and Adams 1980) and maize (Tabuchi *et al.* 2004). Al decreased K^+ content in the root and shoot of rice cultivars (Macêdo and Jan 2008). K^+ uptake was unaffected in the shoot and root of greek melon following Al application (Symeonidis *et al.* 2004). In addition, Al inhibited the K^+ channel in the plasmamembrane in *Zea mays* (Olivetti and Etherton 1991) and in pea (Matsumoto 1991).

Rubidium uptake was only weakly inhibited by 37 or 147 µM Al in nutrient solution (Cumming *et al.* 1985a and b).

On the contrary, Al increased K^+ concentration in S*tylosanthes* (Amaral *et al.* 2013). Al toxicity triggered an increasing accumulation of K^+ in the roots of sorghum (Furlani and Clark 1981) and maize (Lidon *et al.* 2000). Al increased potassium concentration in spinach (Karimaei and Poozesh 2016). Potassium content was increased significantly under Al stress in both root and shoot (Karimaei and Poozesh 2016). Thornton *et al.* (1986) found that low concentration of aluminium increased K^+ content while with the increase in aluminium concentrations, the potassium level was slightly reduced in honeylocust. It was reported that Al resistant genotypes of maize showed higher uptake, influx and transport of potassium than that of the aluminium sensitive genotypes of maize (Mariano and Keltjens 2005). Uptake of K^+ was shown to be less inhibited by Al than Ca and Mg in *Lolium multifolorum* (Rengel and Robinson 1989a). It was shown that in certain cases the uptake of K^+ at low concentration of Al was higher than in solution without Al (Rengel and Robinson 1989b). Some authors observed an increase in K^+ concentration in plants treated by Al (Giannakoula *et al.* 2008, Silva *et al.* 2010) whereas others noted decrease in K^+ concentration (Olivares *et al.* 2009).

At a concentration of 0.33 mM Al, concentration of Na^+ was highest in maize root but Na^+ concentration decreased above 0.33 mM Al (Lidon *et al.* 2000).

1.3.2 Effects of aluminium toxicity on the accumulation of divalent cations

Al decreased Ca and Mg uptake in maize (Mariano and Keltjens 2005), rice plants (Silva 2012), wheat (Foy 1992) and in the root, stem and leaves of tomato (Simon *et al.* 1994a). In wheat roots, Al interacted directly with plasmamembrane channel proteins blocking uptake of Mg and Ca (Piňeros and Tester 1997). Aluminium outcompeted Ca and Mg from uptake sites in the roots of *Allium ursinum* (Anderson 1993). High concentration of Al in Hoagland solution decreased Ca^{2+} and Mg^{2+} contents in cotyledons and hypocotyls of common buckwheat (*Fagopyrum esculentum*) (Horbowicz *et al.* 2011).

With the increase in Al content, Ca^{2+} content was almost constant in *Zea mays* (Bennet *et al.* 1985). Al interfered with the uptake and transport of Ca^{2+} and Mg^{2+} in wheat (Fleming *et al.* 1974, Foy 1984 and 1992) and barley (Nichol and Oliveira 1995).

Ca influx was inhibited by aluminium in wheat root apex (Huang *et al.* 1992a, 1992 b). Al influenced uptake of Ca^{2+} and Mg^{2+} in wheat (Delhaize and Ryan 1995 and Foy *et al.* 1978). Decrease in Ca concentration in soybean shoot and root were associated with Al toxicity (Foy *et al.* 1969).

Ca^{2+} and Mg^{2+} alleviated Al^{3+} stress in different monocotyledonous and dicotyledonous plant species (Keltjens and Tan 1993). Foy and Fleming (1982) showed that Al decreased Ca^{2+} and Mg^{2+} concentrations in wheat. Al reduced Ca and Mg uptake in barley (Clarkson and Sanderson 1971), wheat (Johnson and Jackson 1964) and cotton (Lance and Pearson 1969). Long distance transport of Mg^{2+} in *Arabidopsis* was affected by Al toxicity (Bose *et al.* 2011). Al decreased the ability of Ca and Mg uptake in roots resulting in nutritional deficiency (Silva 2012). Al inhibited Ca and Mg absorption in maize and sorghum (*Sorghum*

bicolar) (Bhalerao and Prabhu 2013, Clark *et al*. 1981, Furlani and Clark 1981), lupin (Alva and Edward 1990), tomato (Simon *et al.* 1994a) and in maize (Lidon *et al.* 1999). Al at a concentration above 330 μM deceased Mg and Ca absorption (Lidon *et al.* 2000). Uptake of Mg was decreased in roots of *Lolium multiflorum* (Rengel and Robinson 1989a, Rengel 1990) and in barley (Alam and Adam 1980).

The ability of Al to reduce Ca uptake in wheat plants was well documented (Huang *et al.* 1992a and b, Rengel 1992). Al reduced the net Ca^{2+} uptake in *Amaranthus* (Rengel and Elliott 1992, Rengel 1994) and *Limnobium stoloniferum* (Jones and Kochian 1995). Al inhibited Ca uptake in maize and snapbean (Barceló *et al.* 1996) in barley (Alam and Adams 1980). On the contrary, Al (50 and 100 μM) increased Ca^{2+} uptake in root apical cells of rye (Ma *et al.* 2002).

Short term studies of divalent cation uptake in presence of Al suggest that Al reduces the uptake and translocation of Ca (Foy and Brown 1963, Johnson and Jackson 1964, Clarkson and Sanderson 1971). Concentration of calcium was drastically reduced in presence of aluminium in spinach (Karimaei and Poozesh 2016). Under aluminium stress calcium content was decreased in both root and shoot of spinach (Karimaei and Poozesh 2016). Al decreased concentration of Mg^{2+} in the root and shoot in rice. Ca content was decreased in the root but that was increased in the shoot of rice following Al treatment (Macêdo and Jan 2008).

Al promoted Ca and Mg accumulation in the shoot in constrast to the root of greek melon (Symeonidis *et al.* 2004). The presence of aluminium inhibited the absorption of Mg^{2+} (Malavolta *et al.* 1997). Several authors showed that, in the presence of Al, the uptake of certain divalent cations particularly Ca and Mg and their accumulation in the root is reduced (Jan 1991, Keltjens 1995, Rengel *et al.* 1995, Olivares *et al.* 2009).

Simon *et al.* (1994a) showed that Al exposure decreased the content of Fe in the root, stem and leaf of tomato. Uptake of Fe was inhibited in wheat (Foy 1992, Foy and Fleming 1982). Toxic Al concentration decreased significantly the concentrations of Fe in sorghum (Clark *et al.* 1981), lupin (Alva and Edwards 1990) and in maize (Lidon *et al.* 1999). Due to 0.33 mM Al^{3+} treatment, Fe concentration was highest in maize root and that was decreased above 0.33 mM Al (Lidon *et al.* 2000). Excess Al induced Fe deficiency symptoms in rice (*Oryza sativa*), sorghum and wheat (Clark *et al.* 1981, Foy and Fleming 1982 and Furlani and Clark 1981) indicating that Al reduced Fe transport. Iron (Fe) concentration, in constrast to Ca, Mg or K^+ was almost four times higher in root and shoot of greek melon under the influence of Aluminium (Symeonidis *et al.* 2004).

1.3.3 Effects of aluminium toxicity on the accumulation of monovalent and divalent anions

1.3.3.1 Effects of aluminium toxicity on the accumulation of monovalent anions

Under aluminium stress condition, uptake and assimilation of nitrate were reduced in *Nostoc linckia* (Husaini and Rai 1992). Al^{3+} interacted directly with several different plasmamembrane channel proteins blocking the uptake of NH_4^+ in wheat roots (Piňeros and Tester 1997). NO_3 uptake by soybean decreased when Al concentration in solution increased from 10 to 50 µM (Rufty *et al.* 1995). Al^{3+} reduced NO_3^- uptake in maize (Calba and Jaillard 1997). In Al-stressed maize and squash roots, acidification capacity and nitrate accumulation were inhibited while the electrolytic conductance was stimulated (Lidon *et al.* 1998, 1999, Ahn *et al.* 2001). Al inhibited NO_3^- uptake in *Glycine max* (Klotz and Horst 1988, Lazof *et al.* 1994), *Triticum aestivum* (Taylor 1991), barley (Nichol *et al.* 1993) and in pine (Lorenc-Plucinska and Ziegler 1996). Al decreased net uptake of NO_3^- in maize (Durieux *et al.* 1993, 1995), soybean (Lazof *et al.* 1994). Toxic Al concentrations decreased significantly the

concentration of nitrogen in sorghum (Clark *et al.* 1981, Furlani and Clark 1981), lupin (Alva and Edwards 1990), tomato (Simon *et al.* 1994a) and in maize (Lidon *et al.* 1999).

There was a decrease in NO_3^- accumulation at 200 μM Al application to cultivar BRS 106 and BRS 4157 of maize plant (de Souza *et al.* 2016). In the presence of aluminium, concentration of NO_3^- was decreased in rice cultivar Fernandes. (Justino *et al.* 2006). Al decreased NO_3^- content in *Urochloa* sp. (Souza *et al.* 2014). Al reduced Cl^- uptake in maize (Calba and Jillard 1997).

1.3.3.2 Effects aluminium toxicity on the accumulation of divalent anions

Aluminium exposure led to a decrease in P accumulation in rice (Silva 2012), *Allium ursinum* (Anderson 1993), wheat (Foy 1996), *Stylosanthes guianensis* and *S. macrocephala* (Machado *et al.* 1992), maize and sorghum (Bhalerao and Prabhu 2013), cacao (Ribeiro *et al.* 2013) and in leaves of physic nut (Steiner *et al.* 2012). Al inhibited uptake of phosphate and its accumulation is leaves (Fageria *et al.* 1988).

Al decreased uptake of P (Foy 1992). Phosphate uptake first inhibited and then increased in corn root following aluminium treatment (Façanha and Okorokova-Façanha 2002). Aluminium ions blocked P uptake in oat (Djuric *et al.* 2011) and in soybean plants (Zheng 2010). Toxic Al concentrations decreased significantly the concentration of P in sorghum (Clark *et al.* 1981, Furlani and Clark 1981), tomato (Simon *et al.* 1994a) and in maize (Lidon *et al.* 1999). Al interfere with uptake and transport of P in wheat (Fleming *et al.* 1974, Foy 1984, 1992) and barley (Nichol and Oliveira 1995). Under aluminium stress condition, uptake and assimilation of phosphate were reduced in *Nostoc linckia* (Husaini and Rai 1992).

Different plant species grown in high levels of Al usually had lower phosphate contents but in maize a clear trend usually could not be found (Rengel 1992, Simon *et al.* 1994a, Lidon *et al.* 2000). A decrease in the uptake and utilization of P is the primary symptom of aluminium toxicity in some susceptible plant species (MacLean and Chiasson 1966, Naidoo *et al.* 1978).

On the contrary, aluminium treatment increased the concentration of ^{32}P in roots of pine over a period of 3 hours and delayed the appearance of ^{32}P in shoot and needles (Cumming *et al.* 1986). Studies with other species showed similar pattern of P transport (Foy and Brown 1963, Clarkson 1966). Aluminium increased P conent in the root but decreased that in the shoot of rice (Macêdo and Jan 2008).

1.3.4 Aluminium concentration in different organs of plant grown in Al containing solution

Exposure of Al increased Al concentration in leaves of buckwheat upto 15000 μg Al g^{-1} dry weight (Ma *et al.* 1998). Al was accumulated in cotyledons, hypocotyl and whole seedlings of common buckwheat following treatment with 10 μM, 100 μM and 1000 μM aluminium concentrations (Horbowicz *et al.* 2011). Accumulation of Al in the root of *Chara corallina* occurred after 30 min to 24 h (Rengel 1996).

Al was accumulated in upper part of tea and buckwheat (Foy *et al.* 1978). Buckwheat accumulated Al in leaves but not in seeds (Shen *et al.* 2006). Al content of oat root were 35.94 to 43.26 ppm (Djuric *et al.* 2011). Al toxicity in sorghum was associated with 640 mg/kg of Al in lower leaves and 1220 mg/kg in upper leaves (Malavolta *et al.* 1979). Concentration of Al was high in the root and generally low in the tops of honeylocust and loblolly pine seedling (Wagatsuma *et al.* 1987). Al decreased Al concentration in wheat (Foy and Fleming 1982). Eighty percent of Al in buckwheat leaves was stored in vacuoles as a 1:3 Al-oxalate complex (Shen *et al.* 2002). Al was detected in the symplasm

of soybean (*Glycine max*) roots after only 30 min of exposure to Al (Lazof *et al.* 1994).

Al accumulated in the apoplasm of epidermal and cortical cells of seedlings of *Brassica oleracea, Lactuca sativa* and *Pennisetum clandestinum* (Huett and Menary 1980b), *Pisum sativum* (Wagatsuma 1984), mycorrhizal *Picea* (Hodson and Wilkins 1991), *Fagus sylvatica* (Hult *et al.* 1992), *Triticum aestivum* L. (Delhaize *et al.* 1993, Ownby 1993), *Sorghum bicolor* L. (Hodson and Sangster 1993) and *Avena sativa* (Marienfeld *et al.* 1995) with endodermis acting as a distinctive barrier.

Roots of buckwheat absorbed Al. Concentration of Al in the xylem sap was 4-fold higher than that in the external solution after 1 h exposure to $AlCl_3$ solution and 10- fold higher after a 2 h exposure (Ma and Hiradate 2000). In wheat and maize, Al was detoxified by forming complexes with organic acid (Ma *et al.* 2001). Al was transported in the xylem sap complexed with citrate in *Melastoma malabathricum* (Watanabe *et al.* 2000).

When exposed for 30 min to 1 mM Al at 1º C, honeylocust and loblolly pine seedlings accumulated 76 and 60%, respectively, of the Al accumulated in four hours. At 22º C the two species accumulated in 4 hours of the Al accumulated in 35 days (Schaedle *et al.* 1986). Wagatsuma (1983) reported that root tissue was nearly saturated with Al after a 30 h exposure.

The root cap of trees, like that of agricultural crops, is highly permeable to Al (Wagatsuma 1984, Rasmussen 1968, Bennet *et al.* 1985). Huett and Menary (1980b) used X-ray analysis of root sections to locate small quantities of Al in the stele of four agricultural species. Wagatsuma (1984) removed the barrier of endodermis in a number of agricultural plants by cutting off the distal 1 cm of all roots of plants grown in Al solution culture to expose the xylem and phloem to the Al solution. The treatment resulted in a two to five-fold increase in the amount of Al in the shoot.

Application of Al increased Al^{3+} contents in the root and shoot of rice (Macêdo and Jan 2008). Shoot/root ratios of Al, which is an indicator of Al translocation from root to shoot, was always lower in Al resistant than that of Al sensitive cultivars of rice (Macêdo and Jan 2008). Application of Al increased accumulation of Al^{3+} in the root of rice (Meriga *et al.* 2010).

Al was accumulated in the root and shoot of four plant species eg. *Allium cepa*, *Zea mays*, *Lepidium sativum* and *Kalanchoe daigremontiana* cultivated in soil and liquid medium contaminated with Al_2O_3. The highest content of aluminium was found in the roots of plants (Asztemborska *et al.* 2015). Al was found to be distributed from the root to the shoot of *Zea mays* (Rasmussen 1968). Exposure to 30 µM $AlCl_3$ increased accumulation of Al in twelve-day-old seedlings of tartary buckwheat (*Fagopyrum tataricum*), wild buckwheat (*F. homotropicum*) and common buckwheat (*F. esculentum*) (Wang *et al.* 2015).

1.4 Effects of aluminium toxicity on biochemical changes in plants

1.4.1 Effects of aluminium toxicity on reducing and total sugar

The concentration of glucose was found to be increased in Al-treated root of *Quercus serrata* (Moriyama *et al.* 2016).

Aluminium-treated cells decreased soluble sugar by one third in tabocco (Abdel-Basset *et al.* 2013). Due to Al treatment (5 µM and 50 µM $AlCl_3$) sugar concentration was increased in one variety but was decreased in other variety of wheat (Tabuchi *et al.* 2004). Al, at a concentration of 1 mM, caused severe reduction in reducing sugars, total soluble carbohydrate and total carbohydrate in root, stem and leaves (Graham 2002). 100 and 200 µM aluminium increased the soluble carbohydrate content in varieties Sirena and Sanbero of sunflower plant (Ziaei *et al.* 2014).

1.4.2 Effects of aluminium toxicity on proline and amino acid content

Theriappan and coworkers (2011) showed that 1000 µM Al caused a 2-fold increase in accumulation of proline in cauliflower (*Brassica oleracea* var. Botrytis) seedlings. The content of proline was found to be induced in geminating seeds of pigeonpea (*Cajanas cajan*) (Bhamburdekar and Chavan 2011). On the contrary, proline was decreased at 50, 100 and 150 µM Al in sorghum plants (da Cruz *et al.* 2011).

As aluminium doses increased from 50 to 200 µM, there was an increase in the concentration of amino acids in the leaves of maize cv. BRS 106 and BRS 4157. (de Souza 2016). Al caused reduction in free amino acids in roots of *Lotus corniculatus* (Balang and Zelinova 2013). It decreased total soluble amino acids in *Sorghum bicolor* plants (da Cruz *et al.* 2011).

1.4.3 Effects of aluminium toxicity on protein content

Al increased protein content in tissues of *Stylosanthes humilis* (Mosquim 1978). On the contrary, it reduced total soluble proteins in sorghum plants (da Cruz *et al.* 2011).

200 µM Al decreased protein content in maize cv. BRS.106 and BRS 4157 (de Souza 2016.). Somers and coworkers (1996) showed that there was a decrease in the content of total soluble proteins in plant subjected to Al-stress.

1.4.4 Effects of aluminium toxicity on the activity of antioxidant enzymes

Aluminium stress caused an increase in superoxide dismutase (SOD) and peroxide (POD) activity in rice (Meriga *et al.* 2010). Aluminium toxicity increased the activities of SOD, POD and glutathione reductase in greengram (*Vigna radiata*). On the contrary, it decreased catalase activity (Panda *et al.* 2003). Al increased acid phosphatase activity in barley roots (Huttová *et al.* 2002).

Al greatly enhanced SOD and catalase activity in rice (Bhoomika *et al.* 2013). It stimulated SOD activity but decreased that of catalase in pearl millet (Suresh Babu *et al*. 2013). The activity of antioxidant enzymes SOD, catalase and POD was increased by Al in two rice cultivars (Ribeiro *et al.* 2012).

1.4.5 Effects of aluminium toxicity on phenols

Release of phenolic compounds was responsible for Al tolerance in many plants. Roots of maize plants exposed to Al exuded about 20-fold more phenolics than organic acid anions (Kidd *et al.* 2001). Al increased total soluble phenols in the shoot of *Matricaria chamomilla* plants (Kováčik *et al.* 2010).

1.4.6 Effects of aluminium toxicity on chlorophyll and carotenoid contents

Aluminium toxicity reduced total chlorophyll content of spinach (Karimaei and Poozesh 2016). Al decreased chlorophyll synthesis by restraining the activity of aminolevulinic acid dehydrogenase responsible for the formation of monopyrrole porphobilonogen (Pereira *et al.* 2006). Increase, decrease or no influence on chlorophyll content in response to Al was reported (Greger *et al.* 1992, Simon *et al.* 1994b, Zavas *et al.* 1996). Cholorophyll a, chlorophyll b, total chlorophyll content was progressively decreased with the increase in Al concentration from 200 to 1000 ppm in *Vigna radiata* (Sabat *et al.* 2016).

Al decreased carotenoid content in varieties Sinera and Sanbero of sunflower plant (Ziaei *et al.* 2014). It also decreased carotenoid content in *Vigna radiata* (Sabat *et al.* 2016).

1.5 Effects of aluminium toxicity on anatomical structures of plants

Heavy Metal stress was found to change the anatomical structure of plants. *Brachiaria decumbens* grown in soil samples from heavy metal contaminated

sites of zinc mining company containing Zn (18 mg/kg), Cd (140 mg/kg), Cu (450 mg/kg) and Pb (410 mg/kg) showed changes in anatomical structure of root and leaf (Forster 1995). In *Brachiaria decumbens* grown in heavy metal contaminated soil, the cell layer of endodermis and exodermis in the root tissue and the cell walls of the xylem and cortical parenchyma were all thickened. In the leaf tissue of *Brachiaria decumbens* grown in heavy metal contaminated soil, adaxial and abaxial epidermis presented increased thickness while the leaf blade presented reduced thickness (Gomes *et al.* 2011). In peanut, 10 to 50 mM Cd decreased the thickness of spongy parenchyma (Shi and Cai 2008).

However, there are only few reports on the changes in anatomical structure of plants caused by Al toxicity. At 75 µM Al, the cortex of root of corn plants presented a higher amount of intercellular spaces, the metaxylem had a larger diameter of oval shaped cell in relation to that of control plant (Batista *et al.* 2012). The protoxylem and metaxylem had a deformed appearance without a developed secondary wall and were small compared to that of the control plants. The region of the pith parenchyma was poorly developed. The most affected part of the root by Al toxicity was the central cylinder region, where cells were darker and difficult to identify (Batista *et al.* 2012). These results disagreed with those of Ouzounidou *et al.* (1995) who reported that cells in the epidermal tissue of corn root were most affected by the action of Al and observed no structural disorder of the hypodermis. A similar result was reported by Budíková *et al.* (1997) who showed that plants grown in 50 µM Al, root epidermis and peripheral cortex layer were more affected than the central cylinder cells.

In the leaf of Al-treated plants few anatomical differences were observed in relation to the control plants. The leaf sheath of the plants grown in solution containing 75 µM Al presented a uniseriate epidermis with thickened secondary walls. On the other hand, the leaf sheath of the plants grown in 300 µM Al had a uniseriate epidermis coated with a thin cuticle layer and the epidermis and cortex cells were less developed. In the vascular bundle, the metaxylem and protoxylem

had no secondary walls and their diameter was much smaller than that of the control plants (Batista *et al.* 2012).

In cotton, at 100 µM Al treatment, thickness of upper epidermis was decreased whereas it was increased significantly in 200 µM Al treatment compared to 100 µM Al. The lower epidermis thickness decreased under both levels of Al. The palisade parenchyma thickness was increased at 100 µM Al treatment but decreased at 200 µM Al. Stomata numbers were significantly reduced in 100 and 200 µM Al-treated plants in both upper and lower epidermis (Özyiğit *et al.* 2013).

Ion transport phenomenon is often different in monocotyledonous and dicotyledonous root systems. For this reason, monocotyledonous rice and dicotyledonous chickpea were chosen as experimental plant material in order to compare the effect of aluminium toxicity in those two root systems.

The aluminium tolerance of plants is commonly evaluated during seedling stage, which might be more critical than their later stages of growth (Merino-Gergichevich *et al.* 2010, Voigt and Mosjidis 2002).

The objective of the research project is to study the effect of aluminium toxicity on

A. (i) the rate of germination and its correlation with Al^{3+}, K^+ and Cl^- in germinating seeds,
   (ii) elongation of roots,
   (iii) growth of plants

B. accumulation of monovalent and divalent cations and anion eg. K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+}, PO_4^{3-} and NO_3^-.

C. Biochemical parameters e.g.
   (i) reducing and total sugar,
   (ii) proline, total amino acid, protein,

(iii) antioxidant enzymes e.g. Peroxidase (POD), Catalase, and Superoxide dismutase (SOD),

(iv) phenolic compounds,

(v) chlorophyll and carotenoids.

D. Changes in anatomical structures of the root, stem and leaf.

E. To establish a correlation between the effect of aluminium toxicity on transport of ions with the changes in biochemical parameters and anatomical structures to understand the mechanism of adaptation of plants in aluminium toxic acid soil.

Chapter 2

MATERIALS AND METHODS

2.1 Plant material

Oryza sativa L. var. BRRI Dhan-53 (Rice, 2n=2x=24) and *Cicer arietinum* L. var. Bari Chhola-7 (Chickpea, 2n=2x=16) were used as experimental plant materials.

The seeds of rice were obtained from Bangladesh Rice Research Institute (BRRI) and that of chickpea were procured from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh.

2.2 Preparation of nutrient solution

Rice and chickpea plants were grown in half-strength Hoagland solution (Hoagland and Arnon 1950). The composition of modified full-strength Hoagland solution was as follows:

KNO_3 0.25 mM, $Ca(NO_3)_2$ 2.5 mM, $MgSO_4$ 1.0 mM, KH_2PO_4 0.25 mM, Fe-EDTA 50 µM, H_3BO_3 23 µM, $MnCl_2$ 4.5 µM, $ZnCl_2$ 0.4 µM, $CuCl_2$ 0.15 µM, Na_2MoO_4 0.05 µM.

2.3 Methods of application of aluminium toxicity

The plants were subjected to different concentrations of aluminium from the initial state of the experiments. The treatments were as follows:

(i) Control (half-strength Hoagland solution)

(ii) Aluminium (Al) concentrations used were 10, 50, 100 and 150 µM made in half-strength Hoagland solution.

The pH of all $AlCl_3$ solutions including control was adjusted to 4.2 with 0.2N H_2SO_4.

2.4 Surface sterilization of seeds

The seeds were surface sterilized to avoid fungal infection by soaking the seeds with 5.25% sodium hypochlorite for three minutes followed by washing 7 to 8 times in running tap water and 4 to 5 times in distilled water. The sterilized seeds submerged in distilled water were aerated for 30 minutes with an air compressor (Rockyvac 320).

2.5 Methods of germination of seeds

Thirty sterilized seeds were placed on Whatman filter paper contained in a petri dish. Three replicates were used for each treatment. Filter papers were soaked with 10, 50, 100 and 150 μM $AlCl_3$ (pH 4.2) and half strength Hoagland solution (pH 4.2) was used as control. The petri dishes were covered by black plastic to avoid the exposure to light. The rice and chickpea seeds were allowed to germinate in dark at $30^oC \pm 1^oC$ and $25^oC \pm 1^oC$, respectively, in an incubator. Seeds were considered to be germinated when radicles and plumules could be clearly distinguished. Germination of seeds was recorded at 48, 72 and 96 h of Al treatment.

2.6 Methods of growing plants in solution culture

Beakers (500 ml capacity) were used for growing plants in solution culture. The seeds were spread over a cotton gauge placed in a lid having twelve holes (1 cm in diameter) and the lid with seeds was placed on a beaker containing 500 ml of distilled water. The beakers were covered by black plastic sheet to avoid the exposure of light to the roots. The beakers containing the seeds were covered by a black polythene sheet for 48 hours to avoid light to facilitate germination of seeds.

After germination, the seedlings were transferred to modified half-strength Hoagland solution (Hoagland and Arnon, 1950) and the beakers with the seedlings were placed in a light bank. Rice seedlings were grown at a day/night temperature of $30^oC \pm 1^oC/25^oC \pm 1^oC$ and day/night length of 14 h/10 h. Chickpea seedlings were grown at a day/night temperature of $25^oC \pm 1^oC/18^oC \pm 1^oC$ and day/night length of 10 h/14 h. Light intensity was 160 μ-einstein $m^{-2}s^{-1}$. The solution was continuously aerated through bubbler with the help of air compressor (Rockyvac 320). The solution was replenished every 48 hours. Thinning of the seedlings was done when they were 7-day-old.

2.7 Methods of application of aluminium treatment in seedlings grown in solution culture

Seven-day-old seedlings were transferred to half strength Hoagland solution (control) and 10, 50, 100 and 150 μM $AlCl_3$ solution made in half strength Hoagland solution. The pH of all solutions including control were adjusted to 4.2 with 0.2N H_2SO_4. The root systems of the seedlings remained completely dipped in solution. The solution was aerated continuously by an air-compressor using 18G × 7.5 needles.

2.8 Collection of samples from seedlings grown in solution culture

The root and shoot of rice, and root, stem and leaves of chickpea seedlings grown in solution culture were collected at 3, 6, 24, 48, 72 and 96 h of aluminium treatment. The root and shoot of rice, and root, stem and leaves of chickpea were separated from the intact seedling. Each root system was washed in two changes of 0.1 mM $CaSO_4$ in a small beaker (50 ml) for two minutes to remove free space ions (Karmoker and van Steveninck 1978). Roots were blotted for removing excess water. The root and shoot of rice, and root, stem and leaves of chickpea seedlings were kept in small paper packets. The samples contained in paper packets were dried in an oven (Memmert 3132, West Germany) at 75^oC for 72 h to a constant weight and transferred to desiccators to

prevent absorption of moisture. Dry weights of the samples were recorded with an electronic balance (FR-200, Japan).

2.9 Methods of growing plants in sand culture

Quartz sand (size 0.1 to 0.5 mm) was soaked in 3% HCl for 24 h. In order to remove HCl, the sand was then washed thoroughly with running tap water and finally the sand was washed 7 to 10 times with distilled water until the pH was neutral (Hewitt 1966). The washed sand was dried in an oven at 110^oC for 96 h.

An earthen pot (15 cm in diameter) was lined with plastic sheet to avoid contact between sand and pot, and a hole was made in the plastic sheet corresponding to the hole at the bottom of the pot for drainage of water and the earthen pot was filled with 5 kg of purified sand.

Thirty five surface sterilized seeds were sown in each pot filled with purified sand. The sand was soaked with distilled water and left for germination by covering the pots with black plastic sheet. The pots were kept in net house of Department of Botany, University of Dhaka, under the normal environmental conditions.

The seeds were germinated within 48 h of sowing. After germination the plastic sheet was removed from the pots. Rice seedlings were grown in summer at a day/night temperature of $30^oC \pm 1^oC/25^oC \pm 1^oC$ and day/night length of 14 h/ 10 h. Chickpea seedlings were grown in winter at a day/night temperature of $25^oC \pm 1^oC/18^oC \pm 1^oC$ and day/night length of 10 h/14 h.

The sand was always moistened with half strength Hoagland solution every 24 h. Thinning of the seedlings were done when they were 7-day old. The seedlings with uneven growth were discarded and sixteen uniform seedlings were selected and allowed to grow in each pot.

2.10 Methods of application of aluminium treatment in plants grown in sand culture

Seven-day-old seedlings grown in sand culture were subjected to half strength Hoagland solution (pH 4.2) which served as control. Similarly, 50, 100 and 150 μM $AlCl_3$ solution made in half strength Hoagland solution (pH 4.2) were applied to each pot containing 7-day-old seedlings which were used as treatments. Later on, half strength Hoagland solution (pH 4.2) was applied to control plants and 50, 100 and 150 μM $AlCl_3$ solution (pH 4.2) were applied to respective Al-treated plants every day up to 28 days.

2.11 Collection of samples from plants grown in sand culture

Root and shoot of rice, and the root, stem and leaves of chickpea plants were collected at 7, 14, 21 and 28-day of aluminium exposure. Roots were washed in two changes of 0.1 mM $CaSO_4$ to remove free space ions. The samples were dried in an oven at 75°C for 72 h. Dry weight of samples were recorded with an electronic balance.

2.12 Methods of extraction and measurement of ions in plant tissue

2.12.1 Extraction of K^+, Na^+, Cl^- and NO_3^-: After recording the dry weight, the samples of the root and shoot of rice, and root, stem and leaves of chickpea were taken into separate test tubes. Ten ml of distilled water was added to each test tube and allowed to stand for 30 minutes and then boiled in a water bath for 30 minutes. The extracts were collected in another set of test tubes. Again, 5 ml of distilled water was added to the residue and again boiled for 15 minutes and the extracts were collected. Finally, 5 ml of distilled water was added to the residue and boiled for further 10 minutes and the extracts were collected. The extract was made up to a final volume of 20 ml with distilled water. The extracts, thus collected, were used for measurement of K^+, Na^+, Cl^- and NO_3^-.

2.12.2 Measurement of K^+ and Na^+: Potassium (K^+) and sodium (Na^+) ions were measured by flame photometer (Jenway, PEP-7, UK) at a wavelength of 767 nm and 589 nm respectively. The concentration of K^+ and Na^+ were calculated using standard curves and expressed as mequiv. g^{-1} dry tissue .

2.12 .3 Measurement of Cl^- : Amount of chloride (Cl^-) ion was measured by standard titrametric method. Sample solution containing Cl^- was titrated with 0.05N $AgNO_3$ solution contained in a micro-burette using 5% potassium dichromate ($K_2Cr_2O_4$) solution as an indicator.

The content of Cl^- was expressed as mequiv. g^{-1} dry tissue.

2.12.4 Measurement of NO_3^- : Nitrate (NO_3^-) was determined following the method of Cataldo *et al.* (1975). 0.8 ml of 5% salicyclic acid dissolved in concentrated H_2SO_4 was added to 0.2 ml extract taken in the test tube. After twenty minutes, 19 ml of 2N NaOH was added and shaken well. A yellow colour appeared. Absorbance was measured with a spectrophotometer (Shimadzu, Model: UV-1800, Japan) at a wavelength of 410 nm after cooling the aliquot at room temperature. The nitrate content was expressed as mequiv. g^{-1} dry tissue.

2.12.5 Extraction of Ca^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+} and PO_4^{3-}: Root and shoot tissue of rice, and root, stem and leaf tissues of chickpea were taken in separate small beakers (50 ml). Four ml of a mixture of nitric acid (HNO_3) and perchloric acid (HCl_3O_4) at a ratio of 4:1 was added to the beaker containing plant tissue and allowed to stand for an hour. The aliquot with samples were then boiled in a hot sand bath kept in a fume hood and the extracts were dried almost to dryness. The aliquot was made up to a final volume of 25 ml with distilled water.

2.12.6 Measurement of Ca^{2+}, Mg^{2+} and Fe^{2+}: The amount of Ca^{2+}, Mg^{2+} and Fe^{2+} in the extract were measured by an atomic absorption spectrophotometer (Perkin-Elmer, Model: A Analyst 200) at wavelengths of 422.67 nm, 285.21 nm and 248.33 nm, respectively, following ASI method. Chemical interference is

common for air acetylene. Addition of releasing agent 1% lanthanum chloride helps to remove the interference.

The contents of Ca^{2+}, Mg^{2+} and Fe^{2+} were expressed as µequiv. g^{-1} dry tissue.

2.12.7 Measurement of Al^{3+}: Al^{3+} was measured by using an atomic absorption spectrophotometer (Shimadzu, Model: AA 7000, Japan). The content of Al^{3+} was expressed in mg. g^{-1} dry tissue.

2.12.8 Measurement of phosphate: Phosphate (PO_4^{3-}) was determined from the aliquot obtained by digesting the plant material according to 2.12.5. Measurement of PO_4^{3-} was done according to the method of Jackson (1967) as described below:

2.12.8.1 Preparation of ammonium molybdate solution (solution A): 24.0 g of ammonium molybdate $(NH_4)_6\ Mo_7O_{24}.\ 4H_2O)$ was dissolved in 500 ml distilled water contained in a beaker. Then, 0.5816 g of potassium antimony tartarate ($C_4\ H_4O_7Ksb$) was dissolved in 100 ml distilled water in another beaker. Two litre of distilled water was taken into a 5 litre volumetric flask and then 300 ml of concentrated H_2SO_4 was added slowly. After the addition of H_2SO_4 was completed, the solution was allowed to be cooled at room temperature. Solution of ammonium molybdate and potassium antimony tartarate was transferred to the flask and made up to a final volume of 5 litre with distilled water and mixed thoroughly. Then it was stored in dark in a refrigerator.

2.12.8.2 Preparation of ammonium molybdate ascorbic acid solution (solution B): 4.1 g of ascorbic acid was dissolved in one litre of solution A and mixed thoroughly. Thus, solution B was prepared as required just before it was needed.

2.12.8.3 Determination of PO_4^{3-}: 10 ml of reagent B was added to 5 ml of extract in a 50 ml volumetric flask and made up to a final volume of 50 ml adding distilled water. Blue colour appeared and the solution was then allowed to stand for 15 minutes. The method was calibrated using a standard phosphate solution.

A blank was prepared with distilled water (45 ml) and 5 ml of reagent B. The absorbance of the aliquot was measured at a wavelength of 440 nm with a spectrophotometer. The content of phosphate was expressed in mequiv. g^{-1} dry tissue.

2.13 Methods of extraction and determination of reducing and total sugar

Reducing and total sugar were determined by Somogyi-Nelson (Nelson 1944 and Somogyi 1952) method and Dubois *et al.* (1956) method respectively.

2.13.1 Extraction of reducing and total sugar: Sugars were extracted by boiling fresh tissue taken in a test tube in 5 ml of 80% ethanol for 5 minutes. The extraction was repeated with two further changes of 5 ml of 80% ethanol. To remove chlorophyll, the shoot extract of rice, and stem and leaf extracts of chickpea were partitioned 3 times with a mixture of petroleum spirit (b.p. 40-60) and 80% alcohol at a ratio of 2:1. To remove ethanol, the extracts of the root and shoot of rice, and root, stem and leaves of chickpea were evaporated to approximately 3 ml in a graduated 50 ml beaker. The root extract was then diluted to 30 ml and the shoot extract of rice and stem and leaf extracts of chickpea were diluted to 50 ml by adding distilled water (Karmoker 1981).

2.13.2 Measurement of reducing sugar: For the measurement of reducing sugar, 2 ml of extract was taken in a test tube and 2 ml of copper reagent was added to each tube. The test tube containing aliquot was heated in a boiling water bath for 10 minutes, by covering with marbles. Then the tubes were placed in ice cold water to bring these to room temperature and 2 ml arsenomolybdate reagent was added to each tube, shaken vigorously immediately after addition. The combined extract was made to a final volume of 20 ml with distilled water. Test tubes with samples were shaken well and allowed to stand for 30 minutes. The optical density was measured at a wavelength of 520 nm using a spectrophotometer. A standard curve of reducing sugar was prepared using glucose following the same

procedure. The concentration of reducing sugar was expressed in μg. g^{-1} fresh tissue.

2.13.3 Measurement of total sugar: In order to measure total sugar, 0.5 ml of extract was taken in a test tube and 0.5 ml of 5% phenol was added to it. 3 ml of concentrated H_2SO_4 was rapidly added directly onto the solution and mixed well. The tubes were allowed to stand in ice cold water for 10 minutes. The optical density was measured at 490 nm using a spectrophotometer. The concentration of total sugar was expressed in μg. g^{-1} fresh tissue.

2.14 Methods of extraction and determination of proline

Determination of proline was done according to the method of Bates *et al.* (1973).

2.14.1 Extraction of proline: 1 g of fresh root and shoot of rice, and root, stem and leaves of chickpea were taken in a clean mortar and homogenized with 5 ml of 0.1 M sulphosalicyclic acid, centrifuged and the supernatant was taken. The volume of the supernatant was adjusted to 5 ml with distilled water.

2.14.2 Determination of proline: Five ml of glacial acetic acid and 5 ml of acetic ninhydrin was added to 2 ml of supernatant and shaken well. After that, the tubes were placed in boiling water bath for one hour. After cooling the tubes, the mixture was extracted with 10 ml toluene in a separating funnel, the upper dark red layer was collected and allowed to stand for some time. Standard curve was prepared using proline following the same procedure. The optical density (OD) was recorded at a wavelength of 520 nm with a spectrophotometer using toluene as a blank. Proline content was expressed in μg. g^{-1} fresh tissue.

2.15 Methods of extraction and determination of total amino acid

For the assay of total amino acid ninhydrin reagent was used following the method of Lee and Takahasi (1966).

2.15.1 Extraction of total amino acid: 1 g of fresh root and shoot of rice, and root, stem and leaves of chickpea were taken in a clean mortar and homogenized with 5 ml of 80% ethanol. It was then centrifuged at 8000 rpm for 15 minutes and the supernatant was taken. The procedure was repeated again with 5 ml of 80% ethanol and the supernatant was collected. The volume of the supernatant was adjusted to 10 ml with distilled water. The supernatant was used for determination of total amino acid.

2.15.2 Determination of total amino acid: Total amino acid in the extract was determined using ninhydrin reagent.

Ninhydrin reagent was prepared by mixing the following constituents A, B, C in the ratio of 5 : 12 : 2.

A = 1% ninhydrin in 0.5 M citrate buffer (pH 5.5)

B = Pure glycerol

C = 0.5 M citrate buffer (pH 5.5)

For reaction, 0.1 ml of aliquot was taken in a test tube and 0.9 ml distilled water was added to it. In each set, 5 ml ninhydrin reagent was added and shaken vigorously. The test tube with the aliquot was placed in boiling water bath for 15 minutes. After cooling, the intensity of colour was measured by spectrophotometer at a wavelength of 570 nm. The standard curve of amino acid was prepared using glycine following the same procedure. Amino acid content was expressed in mg. g^{-1} fresh tissue.

2.16 Methods of extraction and determination of soluble protein

Soluble protein was determined following the method of Lowry *et al.* (1951).

2.16.1 Extraction of soluble protein: After recording the fresh weight, the root and shoot tissue of rice, and the root, stem and leaf tissue of chickpea, were homogenized with chilled 5 ml of 2 mM KH_2PO_4 (pH 7.5) with a mortar and pestle. The homogenate was then centrifuged in refrigerated condition at 3000 rpm for 15 minutes. The supernatant (soluble protein fraction) was collected. The pellet was suspended in further 5 ml of buffer and centrifuged for 5 minutes at 3000 rpm and again the supernatant was collected. The combined extract containing protein was made up to 10 ml with buffer.

2.16.2 Determination of soluble protein: 2 ml of alkaline copper sulphate solution and 0.2 ml of Folin-phenol reagent was added to 0.2 ml of supernatant (extract) in a test tube and mixed well and kept for 30 minutes for development of blue colour. The intensity of blue colour was measured at a wavelength of 550 nm with a spectrophotometer. The standard curve was prepared by using Bovin Serum Albumin (BSA) to determine the protein content of the sample. The protein content was expressed in mg. g^{-1} fresh tissue.

2.17 Methods of extraction and determination of different antioxidant enzymes

Different antioxidant enzymes such as peroxidase (POD), catalase and superoxide dismutase (SOD) activities were determined as described by Zhang *et al.* (1995), Barber (1980) and Zhang *et al.* (2005) respectively. Protein content was determined by Lowry *et al.* (1951) assay using BSA as standard.

2.17.1 Extraction of antioxidant enzymes: For the determination of antioxidant enzyme activity, 0.5 g of plant sample was homogenized in 0.05 M phosphate buffer (pH 7.8). Homogenate was centrifuged at 10,000 rpm for 10 min at 4°C. Supernatant was separated and used for specific enzyme assay.

2.17.2 Determination of the activities of different antioxidant enzymes:

2.17.2.1 Determination of the activity of peroxidase enzyme: The process of determination of peroxidase activity described by Zhang *et al.* (1995) was as follows:

The reaction mixture consisted of 0.1 M phosphate buffer (pH 7.8), 30% H_2O_2 and 0.05 M guaiacol (freshly prepared). 0.2 ml of supernatant was added to the reaction mixture. The change of optical density (O.D) was measured at a wavelength of 470 nm with a spectrophotometer for every 30 seconds for 2 minutes. Peroxidase activity was expressed as µmol. min^{-1} mg^{-1} protein.

2.17.2.2 Determination of the activity of catalase enzyme: The procedure of determination of catalase activity described by Barber (1980) was as follows:

Catalase activity was measured by using assay solution containing 0.05 M phosphate buffer (pH 7.8), 0.1 M H_2O_2 and 0.2 ml extract. Decrease in absorbance of H_2O_2 was recorded within 2 min at 240 nm. One unit of catalase activity was defined as the amount of enzyme required to reduce 1 µmol of H_2O_2 per minute. Catalase activity was expressed as µmol. min^{-1} mg^{-1} protein.

2.17.2.3 Determination of the activity of superoxide dismutase (SOD) enzyme: The SOD activity was determined according to the modified method of Zhang *et al.* (2005) as follows:

The superoxide dismutase activity was determined by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium chloride (NBT). The reagents used for assay include 50 mM phosphate buffer (pH 7.8), 130 mmol/l methionine, 750 µmol/l NBT, 100 µmol/l Na_2-EDTA and 20 µmol/l riboflavin. After adding 1 ml of supernatant (extract) to the reaction mixture, the test tubes were exposed for 10 minutes to fluorescent light (13 W). Then the change in absorbance was followed up to 2 min at a wavelength of 560 nm using a spectrophotometer. A blank reaction was also run using all the components

without protein to get maximum colour. One unit of SOD was defined as the amount of enzyme required to inhibit NBT reduction by 50% under specified conditions of the assay. SOD activity was expressed as unit min^{-1} mg^{-1} protein.

2.18 Methods of extraction and determination of phenolic compounds

The determination of total phenolic- compound was done according to the method of Malik and Singh (1980).

2.18.1 Extraction of phenolic-compounds: One g root or shoot tissue was extracted in 10 ml of 80% ethanol, centrifuged and the supernatant was collected. The extraction procedure was repeated once again. Two supernatants were mixed and then evaporated to almost dryness. The residue was dissolved in 5 ml of distilled water.

2.18.2. Measurement of phenolic-compounds: 0.1 ml of extract was taken in a test tube and made up to a final volume of 3 ml with distilled water. 0.5 ml of folin-phenol reagent was added to it and shaken vigorously. After 3 minutes, 1 ml of 20% sodium carbonate solution was added, shaken and boiled for 1 minute. Then the aliquot was left for some time to cool down. The optical density (O.D.) was recorded at a wavelength of 650 nm by a spectrophotometer. A test tube containing all the reagents minus folin-phenol reagent was used as blank to adjust the absorbance to zero. The phenolic-compounds content was expressed in µg. g^{-1} fresh tissue.

2.19 Methods of extraction and determination of leaf pigments

2.19.1 Extraction of leaf pigments: The leaves of plants grown in sand culture were used to determine leaf pigments. For the extraction of chlorophyll a, b and carotenoid contents, the leaves, 0.25 g of samples (leaving away midrib) were taken in a mortar and grinded finely by a pestle with 25 ml of cold 80% acetone.

Small amount of sodium carbonate (Na_2CO_3) was added to leaf material before grinding to check degradation of pigments during grinding. The homogenate was filtered through Whatman filter paper (number 1) and was made up to 25 ml with cold 80% acetone.

2.19.2 Determination of leaf pigments: The optical density of the extract of leaf pigments was measured at a wavelength of 663, 645 and 440.5 nm using a spectrophotometer against 80% acetone as blank.

Specific absorption co-efficient method of Mckinney (1940) and the formula of Maclachlan and Zalik (1963) were used to determine the amounts of chlorophyll a and b.

The formula used are as follows:

$$C_a = \frac{(12.3D_{663} - 0.86D_{645})\,V}{d.1000.w}$$

$$C_b = \frac{(19.3D_{645} - 3.6D_{663})\,V}{d.1000.w}$$

Where,

C = concentration in mg. g^{-1} fresh weight

a = Chlorophyll a

b = Chlorophyll b

D = Optical density at wave length indicated

V = Final volume of extract

W = fresh weight of leaf material used

d = Length of light path in cm

The amount of carotenoid was determined by the equation of von Wettstein (1957).

The equation is as follows,

$C_c = 4.695\ D_{440.5} - 0.268\ C_{(a+b)}$

Where,

C_c = Concentration of carotenoid in mg. g^{-1} fresh tissue

D = Optical density at wave length indicated

$C_{(a+b)}$ = Chlorophyll a + Chlorophyll b

2.20 Measurement of the root growth of plants using rhizobox

Rhizobox is an excellent instrument for effective study of the rate of elongation of primary roots and to determine the number of lateral roots starting from germination of seeds.

2.20.1 Structure of rhizobox: Rhizobox used in this experiment was a wooden box having length x breadth x depth of 25 x 17 x 5 cm. Lower portion of the rhizobox was perforated for drainage of excess solution.

The wooden box was fitted with a transparent plastic lid so that the elongation of root can be observed and measured. The lid was fitted to the wooden box with screws. There was a slit in front of the box to facilitate growth of stem (Plates 1 and 2).

2.20.2 Techniques for measurement of root growth of seedlings grown in rhizobox: The rhizobox was filled with 2.5 kg purified quartz sand (size 0.1 mm to 0.5 mm). Sterilized rice and chickpea seeds were germinated on moist filter paper placed in petridish at a temperature of 30±1°C and 25±1°C respectively. The sprouting was considered as the zero hour of age of the seedling.

A one-day-old seedling was placed in the rhizobox filled with quartz sand where the radical was in the sand while the plumule was protruding outside through the hole in rhizobox. The sand of three rhizoboxes with the seedlings were

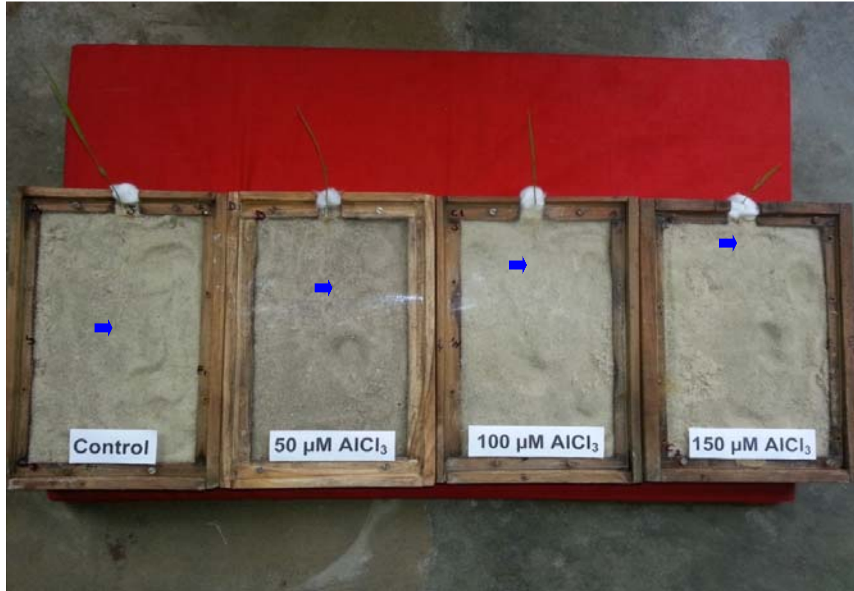


Plate 1. Effects of aluminium toxicity on the root growth of rice seedlings grown in rhizobox.

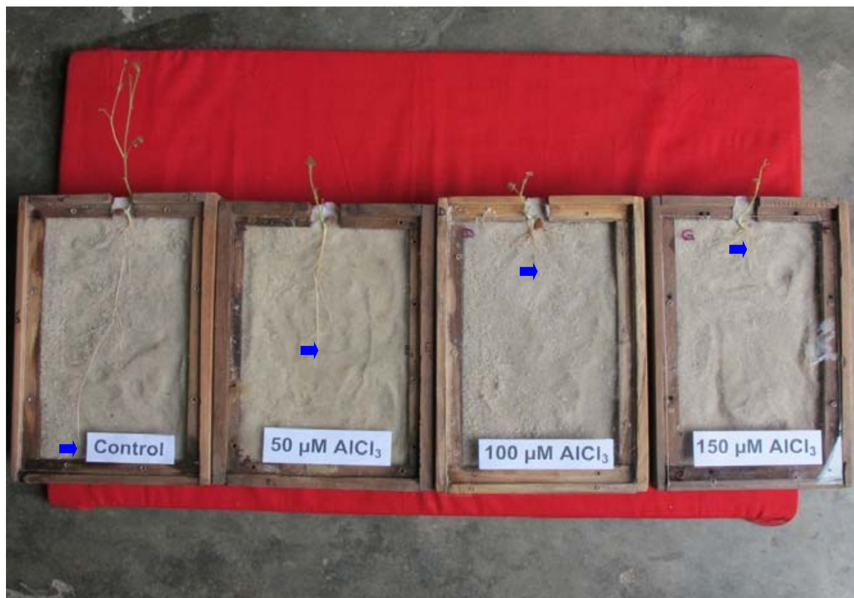


Plate 2. Effects of aluminium toxicity on the root growth of chickpea seedlings grown in rhizobox.

moistened with half strength Hoagland solution, which was used as control and other nine rhizoboxes were moistened with 50, 100 and 150 µM $AlCl_3$ solution made in half strength Hoagland solution. The pH of all solutions including control were adjusted to 4.2 with 0.2 N H_2SO_4. Half strength Hoagland solution (pH 4.2) was supplied to rhizobox containing control seedlings and 50, 100 and 150 µM $AlCl_3$ solution (pH 4.2) were supplied to respective rhizoboxes containing treated seedlings every day. The length of the primary root and the number of lateral roots in control and aluminium stressed seedlings were recorded every day.

2.20.3 Measurement of root length: A transparent sheet was cut having the same size as the plastic lid. The transparent sheet (Canson-250-80g/m^2 210 x 297A-$_4$) was placed on the lid of the rhizobox. The root of the seedling in the rhizobox was traced on the transparent sheet. This was done from the 1st to 5th day of germination. The length of the traced root was measured and recorded. The rate of growth of root from 1 to 5 day of germination was calculated from the data collected from the length of the root traced in the transparent paper.

2.20.4 Measurement of number of lateral roots: The number of the traced lateral roots were counted and recorded. The rate of increased number of lateral root from 1 to 5 day of germination was calculated from the data collected from the number of the root traced in the transparent paper.

2.21 Methods of studying the length of root, shoot and shoot/root length ratio

Seedlings of rice and chickpea were grown in solution culture as described in section 2.6. Seven-day-old seedlings were transferred to half strength Hoagland solution (control) and 10, 50, 100 and 150 µM $AlCl_3$ solution made in half strength Hoagland solution. The pH of all aluminium solutions including control were adjusted to 4.2 with 0.2N H_2SO_4. Root and shoot samples were collected at 3, 6, 24, 48, 72 and 96 h of aluminium treatment according to section 2.8.

Length of root and shoot of the seedlings were measured in cm with a normal scale.

2.22 Methods of studying the growth of plants

The root and shoot dry matter content and shoot/ root dry matter ratio were studied in seedlings grown in solution culture.

Half strength Hoagland solution (pH 4.2) was used as control and 10, 50, 100 and 150 µM $AlCl_3$ (pH 4.2) were used as treatments. After application of $AlCl_3$ treatment, the root and shoot of rice, and root, stem and leaves of chickpea were collected at 3, 6, 24, 48, 72 and 96 h and dried in an oven at 75°C to a constant weight for 72 hours and kept in desiccators to prevent absorptioin of moisture. Dry weight of the tissues were measured separately with an electronic balance. Dry weight was expressed in mg.

2.23 Methods of studying anatomical structures

For studying the anatomical structures, plants were grown in sand culture according to the method described in section 2.9.

2.23.1 Collection of samples: Root, stem and leaf of 5th node from the top (middle portion) of 28-day-old plants were collected and cut into pieces measuring 8 ± 1 mm length.

2.23.2 Fixation of root, stem and leaf sections: Free hand sectioning was done and the sections were stained with saffranin and fixed with glycerine. The sections of the root, stem and leaf were studied with a microscope. Photographs of sections were taken using a camera (Axiocam ERc 5s) at different magnifications (5, 10 and 40X).

2.24 Study of stomata and trichomes

Leaf stoma and trichome on the leaves of 5th node from the top of 28-day-old control and treated plants grown in sand culture were studied. Plants were grown in sand culture according to the method described in section 2.9. Leaves were detached from the plants and petioles were placed in a beaker filled with distilled water. Epidermal peels were taken from lower surface of the leaves (middle portions) and then mounted in 30% glycerine. Photographs of peels showing stomata were taken using a camera (Axiocam ERc 5s) at different magnifications (5, 10 and 40X).

Chapter 3

Effects of aluminium toxicity on germination of seeds and its correlation with K^+, Cl^- and Al^{3+} accumulation in radicle and plumule of rice and chickpea seedlings

3. 1 Introduction

Al toxicity inhibited seed germination in a few plants (Delhaize and Ryan 1995). Aluminium, at a concentration of 500 ppm, had inhibitory effect on wheat seed germination (Alamgir and Akhter 2009). 50 μM Al treatment decreased germination percentage in maize (Gumze *et al.* 2007). Al significantly reduced germination of pea (*Pisum sativum* L.) seeds (Singh *et al.* 2011). There is no report on the mechanism of aluminium-induced inhibition of seed germination. Accumulation of K^+, Cl^- and Al^{3+} in plumule and radicle may have some relation with the inhibition of germination of seeds by aluminium.

3. 2 Materials and Methods

The seeds of rice and chickpea were surface sterilized according to section 2.4. Thirty sterilized seeds were placed on Whatman filter paper contained in a petri dish (11 cm) and were allowed to germinate following the procedure outlined in section 2.5. The germination of seeds was recorded at 48, 72 and 96 h from the date of sowing.

Radicles and plumules of the germinated seeds were separated from cotyledons at 48, 72 and 96 hours from the time of sowing. K^+ and Cl^- were extracted from dry tissue (radicle and plumule) by boiling in a hot water bath following Samad and Karmoker (2013). Al^{3+} was extracted from dry tissue by boiling in a mixture of nitric acid and perchloric acid (4:1) using a hot sand bath. Determination of K^+, Cl^- and Al^{3+} was done following the method described in section 2.12. Al^{3+} was measured using atomic absorption spectrophotometer according to the method described in section 2.12.7.

3. 3 Results

In rice, aluminium at concentrations of 10, 50, 100 and 150 µM, decreased germination of seeds by 12.0, 25.0, 30.0 and 34.0%, respectively, at 48 h of treatment. At 72 h of treatment, aluminium (50-150 µM) inhibited germination of seeds by 13.0 to 28.0%. Aluminium (50-150 µM) decreased germination of rice seeds by 6.0 to 21.0% at 96 h of treatment (Table 1).

In chickpea, aluminium (10-150 µM) inhibited seed germination by 20.0 to 42.0% at 48 h of treatment. At 72 h of treatment, 50-150 µM aluminium decreased germination of chickpea seeds by 10.0 to 21.0%. Aluminium (50-150 µM) inhibited seed germination by 7.0 to 16.0% at 96 h of treatment (Table 2).

In rice, accumulation of K^+ in the radicle was decreased by 7.0% at 10 µM Al treatment and the degree of inhibition increased with the increase in aluminium concentration from 10 to 150 µM. The maximum inhibition was 35.0% at 150 µM Al at 72 h of treatment (Fig. 1a). Similar pattern of inhibition of K^+ accumulation was observed in the plumule of rice following different concentrations of aluminium (10-150 µM) treatment at 72 h of treatment. The degree of inhibition of K^+ accumulation in the plumule increased with the increase in aluminium concentration from 10-150 µM ranging from 6.0 to 25.0% (Fig. 1b).

Similarly, 10-150 µM aluminium inhibited K^+ content in the radicle and the plumule of rice at 96 h of application. In this case also, the degree of inhibition of K^+ accumulation in the radicle and plumule increased with the increase in aluminium concentration from 10-150 µM at 96 h of treatment. The inhibition of K^+ content in the radicle of rice ranged from 9.0 to 38.0% and that of K^+ in the plumule ranged from 9.0 to 28.0% at Al concentration ranging from 10 to 150 µM (Figs. 2a,b).

Table 1. Effects of different concentrations of $AlCl_3$ on germination of seeds of rice. Each value is the mean of three replicates ± standard error.

Duration of treatment (h)	% germination Concentration of $AlCl_3$ (µM)				
	0	10	50	100	150
48	96 ± 0.359	88 ± 0.546	75 ± 0.333	70 ± 0.577	66 ± 0.530
72	100 ± 0.333	95 ± 0.571	87 ± 0.882	79 ± 0.498	72 ± 0.672
96	100 ± 0.333	98 ± 0.333	94 ± 0.571	85 ± 0.667	79 ± 0.571

Table 2. Effects of different concentrations of $AlCl_3$ on germination of seeds of chickpea. Each value is the mean of three replicates ± standard error.

Duration of treatment (h)	% germination Concentration of $AlCl_3$ (µM)				
	0	10	50	100	150
48	94 ± 0.577	80 ± 0.577	72 ± 0.882	66 ± 0.946	58 ± 0.882
72	100 ± 0.333	94 ± 0.495	90 ± 0.651	85 ± 0.333	79 ± 0.577
96	100 ± 0.333	96 ± 0.568	93 ± 0.333	89 ± 0.577	84 ± 0.333

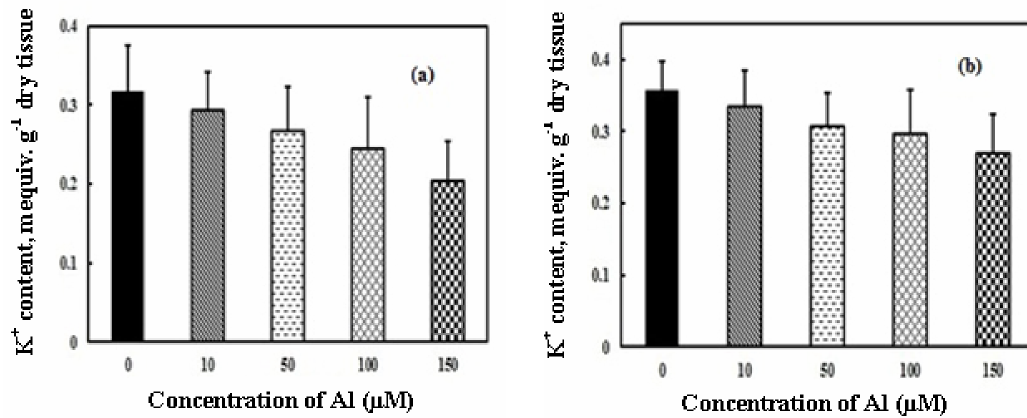


Fig. 1. The effect of different concentrations of aluminium on the accumulation of K^+ in the (a) radicle and (b) plumule of germinating rice seeds at 72 h of treatment. ■ represents control; 10 µM Al, 50 µM Al, 100 µM Al and 150 µM Al. Each value is the mean of three replicates ± standard error.

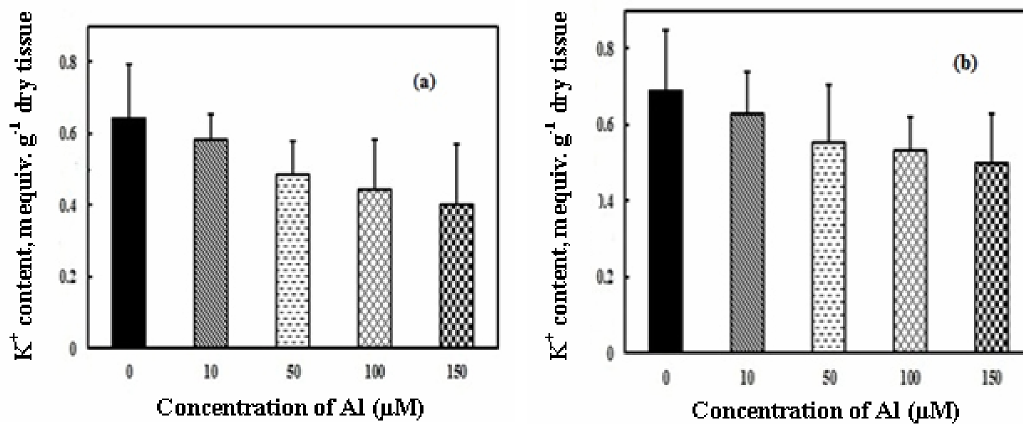


Fig. 2. The effect of different concentrations of aluminium on the accumulation of K^+ in the (a) radicle and (b) plumule of germinating rice seeds at 96 h of treatment. Otherwise as Fig. 1.

In chickpea, accumulation of K^+ was decreased in the radicle by 5.0 to 24.0% following 10 to 150 µM aluminium treatment at 72 h of exposure (Fig. 3a). Similar magnitude of inhibition of K^+ (4.0 to 24.0%) was observed in the plumule of chickpea following Al treatment at 72 h of treatment (Fig. 3b).

Similarly, 10 to 150 µM aluminium inhibited K^+ content in the radicle and plumule of chickpea at 96 h of application. The degree of inhibition increased with the increase in aluminium concentration from 10 to 150 µM at 96 h of treatment. The inhibition of K^+ content in the radicle of chickpea ranged from 3.0 to 27.0% and that of the plumule of chickpea ranged from 6.0 to 29.0% at Al concentration ranging from 10 to 150 µM (Figs. 4a,b).

Aluminium, at concentrations of 10, 50, 100 and 150 µM, increased accumulation of Cl^- by 38.0, 60.0, 77.0 and 96.0%, respectively, in the radicle of rice at 72 h of treatment as compared to control (Fig. 5a). In the plumule of rice, 62.0% to 2.4-fold increase in Cl^- accumulation was observed at 72 h of application of 10-150 µM aluminium (Fig 5b).

Similarly, 10 to 150 µM aluminium caused a 62.0% to 2-fold increase in Cl^- content in the radicle (Fig. 6a) and a 57.0% to 2.3-fold increase in the accumulation of Cl^- in the plumule of rice at 96 h of treatment (Fig 6b).

In chickpea, 10 to 150 µM aluminium caused a 26.0% to 2-fold increase in Cl^- accumulation in the radicle of chickpea at 72 h of treatment (Fig 7a). In the plumule of chickpea seeds, 10-150 µM aluminium caused a 45.0% to 2.7-fold increase in Cl^- accumulation at 72 h of application (Fig. 7b).

A 24 to 83% increase in Cl^- accumulation in the radicle of chickpea was observed at 96 h following 10 and 150 µM aluminium application (Fig. 8a). Similarly, 36.0% to 2.2-fold increase in Cl^- accumulation in the plumule of chickpea was observed at 96 h following 10 to 150 µM aluminium treatment (Fig. 8b).

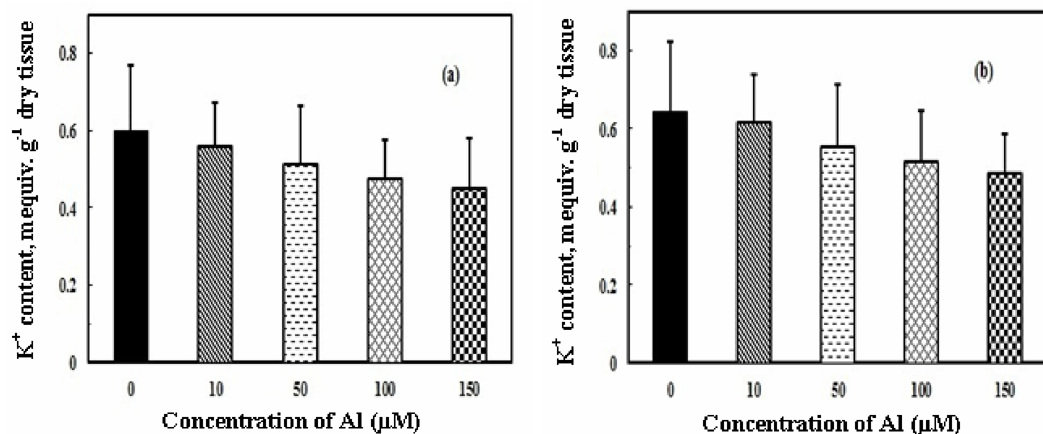


Fig. 3. The effect of different concentrations of aluminium on the accumulation of K^+ in the (a) radicle and (b) plumule of germinating chickpea seeds at 72 h of treatment. Otherwise as Fig. 1.

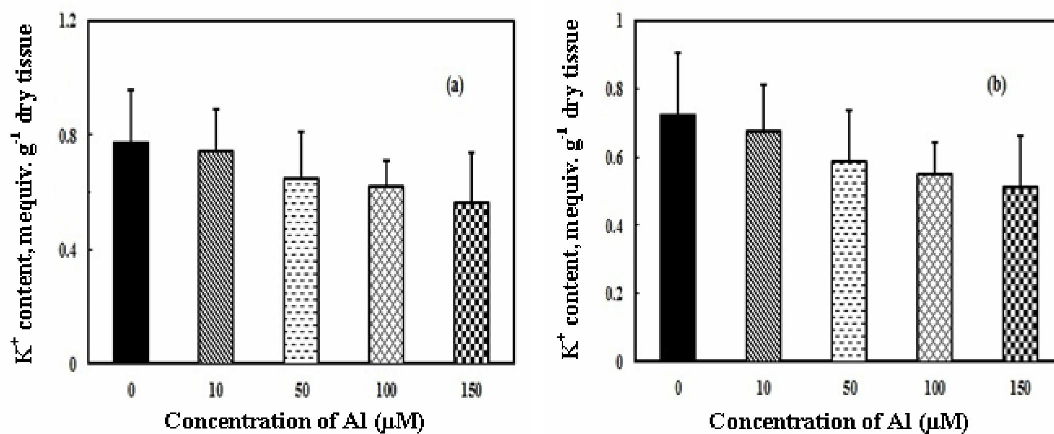


Fig. 4. The effect of different concentrations of aluminium on the accumulation of K^+ in the (a) radicle and (b) plumule of germinating chickpea seeds at 96 h of treatment. Otherwise as Fig. 1.

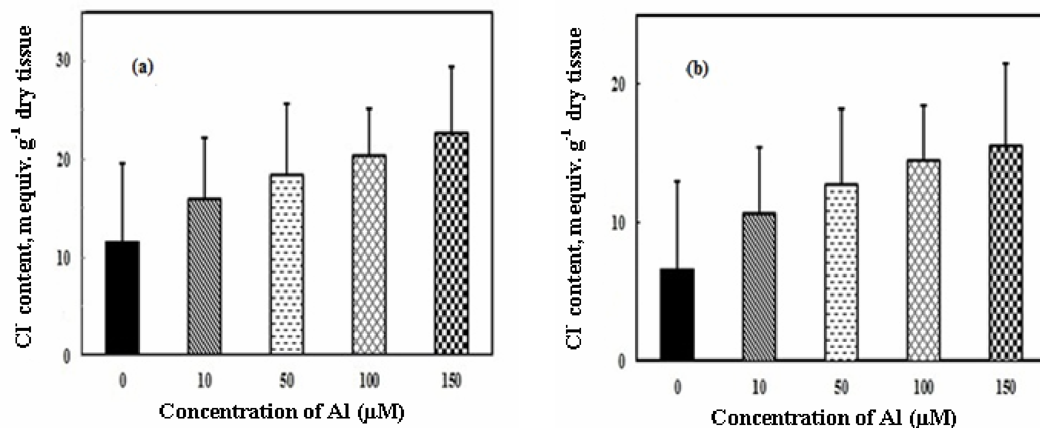


Fig. 5. The effect of different concentrations of aluminium on the accumulation of Cl^- in the (a) radicle and (b) plumule of germinating rice seeds at 72 h of treatment. Otherwise as Fig. 1.

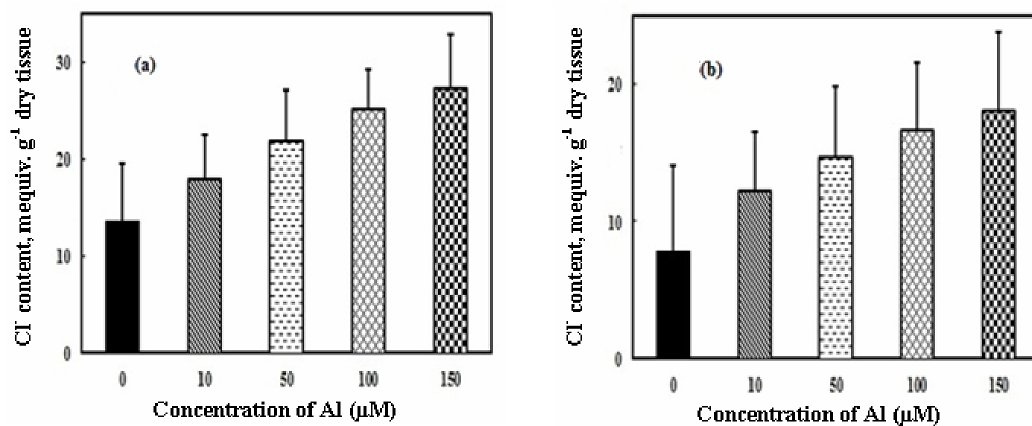


Fig. 6. The effect of different concentrations of aluminium on the accumulation of Cl^- in the (a) radicle and (b) plumule of germinating rice seeds at 96 h of treatment. Otherwise as Fig. 1.

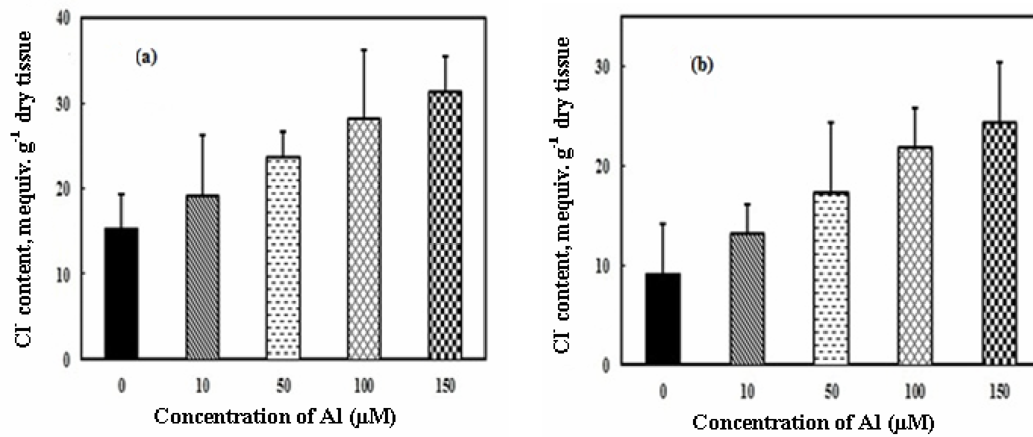


Fig. 7. The effect of different concentrations of aluminium on the accumulation of Cl^- in the (a) radicle and (b) plumule of germinating chickpea seeds at 72 h of treatment. Otherwise as Fig. 1.

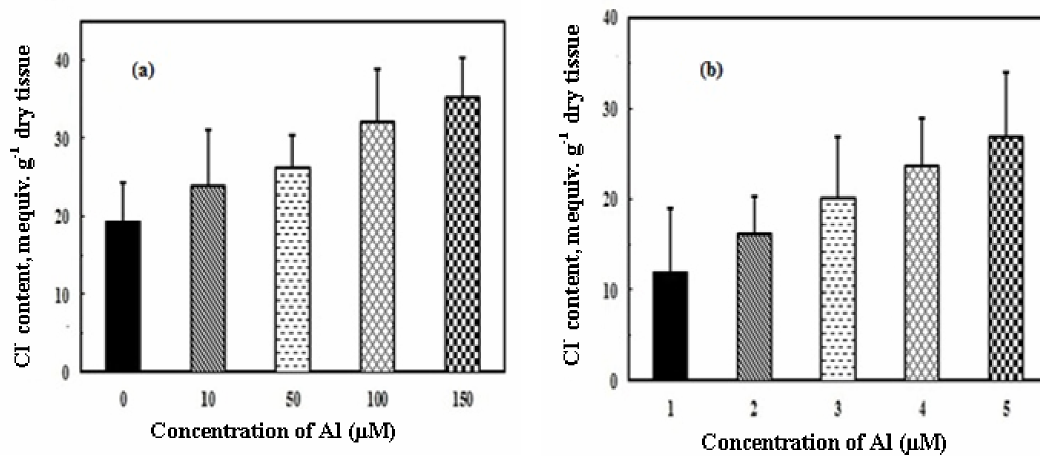


Fig. 8. The effect of different concentrations of aluminium on the accumulation of Cl^- in the (a) radicle and (b) plumule of germinating chickpea seeds at 96 h of treatment. Otherwise as Fig. 1.

At 72 h exposure of 10 and 100 µM Al caused 2.3-fold and 3.8-fold increase in accumulation of Al^{3+}, respectively, in the radicle of rice (Fig. 9a). Similarly, 10 and 100 µM Al caused a 1.6-fold and 2-fold increase in Al content in the plumule, respectively, at 72 h of treatment (Fig. 9b).

A 2.4-fold and 3.6-fold increase in Al^{3+} was recorded in the radicle of rice following 10 and 100 µM aluminium, respectively, at 96 h of treatment (Fig. 10a). In the plumule, 10 and 100 µM Al caused 1.7-fold and 2.4-fold increase in the accumulation of Al^{3+}, respectively, at 96 h of treatment (Fig. 10b).

Application of 10 and 100 µM Al for 72 h caused a 2-fold and 3.4-fold increase in accumulation of Al^{3+}, respectively, in the radicle of chickpea (Fig. 11a). In the plumule, exposure of 10 and 100 µM Al for 72 h resulted in a 2-fold and 4.3-fold increase in Al, respectively (Fig. 11b).

Similarly, a 96 h exposure of 10 and 100 µM aluminium, increased Al^{3+} accumulation by 2-fold and 3.2-fold in the radicle of chickpea (Fig. 12a). In the plumule, 10 and 100 µM aluminium exposure caused a 2.2-fold and 3.1-fold increase in accumulation of Al^{3+}, respectively, in the plumule at 96 h of treatment (Fig. 12b).

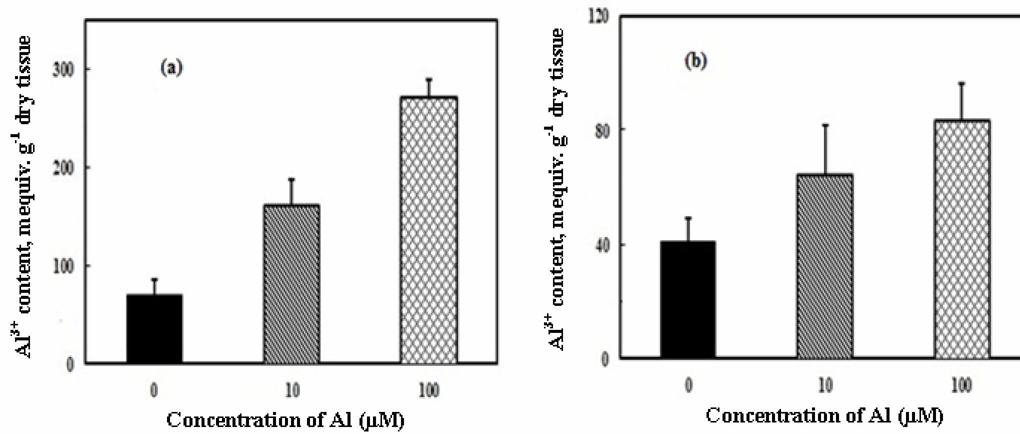


Fig. 9. The effect of different concentrations of aluminium on the accumulation of Al^{3+} in the (a) radicle and (b) plumule of germinating rice seeds at 72 h of treatment. Otherwise as Fig. 1.

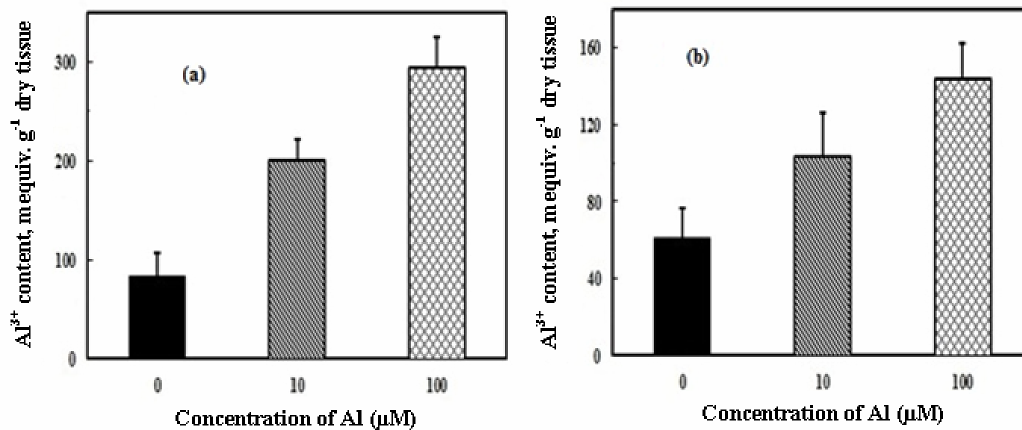


Fig. 10. The effect of different concentrations of aluminium on the accumulation of Al^{3+} in the (a) radicle and (b) plumule of germinating rice seeds at 96 h of treatment. Otherwise as Fig. 1.

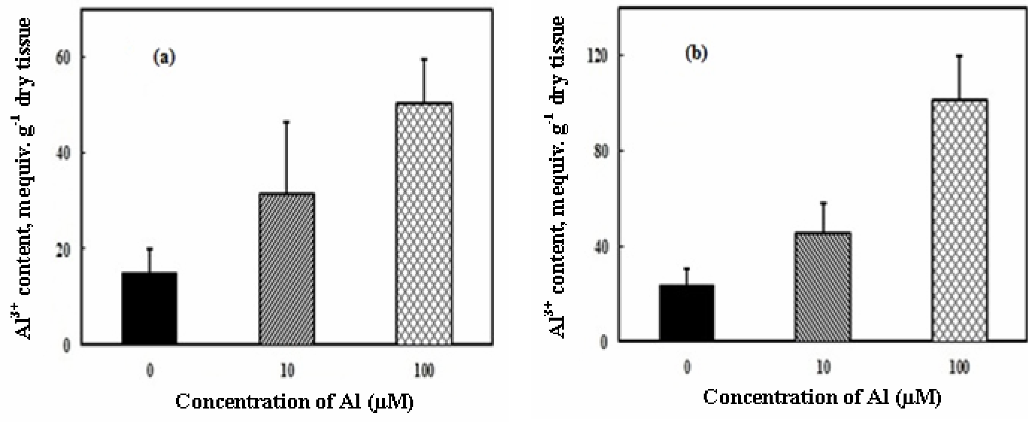


Fig. 11. The effect of different concentrations of aluminium on the accumulation of Al^{3+} in the (a) radicle and (b) plumule of germinating chickpea seeds at 72 h of treatment. Otherwise as Fig. 1.

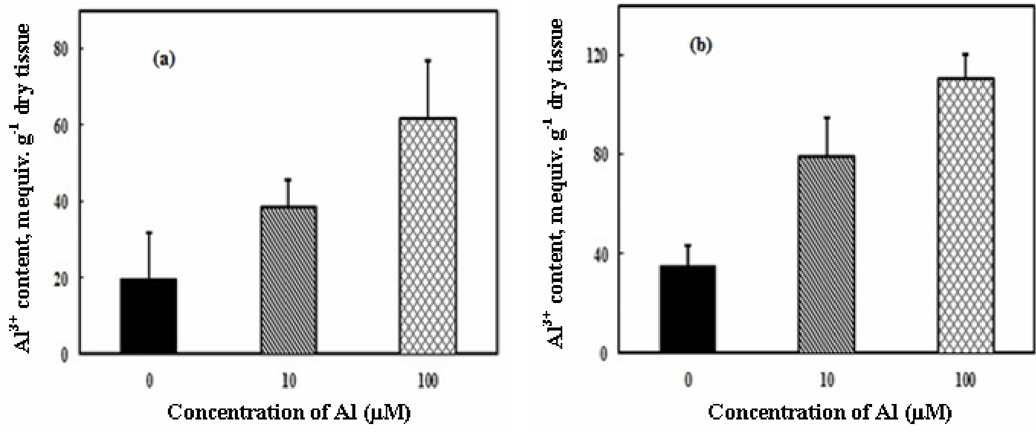


Fig. 12. The effect of different concentrations of aluminium on the accumulation of Al^{3+} in the (a) radicle and (b) plumule of germinating chickpea seeds at 96 h of treatment. Otherwise as Fig. 1.

3. 4 Discussion

Different concentrations of aluminium (10-150 µM) decreased germination of seeds in rice and chickpea (Table 1 and 2). Aluminium-induced seed germination is supported by the work of Nasr (2013) and Alamgir and Akhter (2009) who recorded inhibition of seed germination of maize and wheat seeds following aluminium treatment.

10 to 150 µM aluminium inhibited K^+ content in the radicle and plumule of rice and chickpea (Figs. 1-4). This result is supported by Horbowicz *et al*. (2011) who found that high concentration of Al in Hoagland solution decreased K^+ content in cotyledons and hypocotyls of common buckwheat (*Fagopyrum esculentum* Moench).

Aluminium at concentrations of 10 to 150 µM, increased accumulation of Cl^- in the radicle and plumule of rice and chickpea (Figs. 5-8). The increase in Cl^- accumulation might have detrimental effect on germination.

Al treatment in the germinating seeds of rice and chickpea decreased K^+ accumulation (Figs. 1-4) and increased that of Cl^- (Figs. 5-8) and Al^{3+} (Figs. 9-12) accumulation in both the radicle and plumule. Aluminium toxicity-induced increase in accumulation of Cl^- and Al^{3+} with the concomitant decrease in K^+ accumulation in the radicle and plumule might be responsible for Al-induced inhibition of germination of seeds.

Chapter 4

Effects of aluminium toxicity on the accumulation and distribution of monovalent, divalent and trivalent cations and anions in rice and chickpea seedlings

4a. Effects of aluminium toxicity on the accumulation and distribution of monovalent and divalent cations and anions in rice and chickpea seedlings grown in solution culture

4a.1 Introduction

4a.1.1 Effects of aluminium toxicity on transport of monovalent cations and anions

Al treatments decreased K^+ content in the root but increased that in the stem of *Theobahia* and in the leaves of both genotypes of cacao (Ribeiro *et al.* 2013). Under the influence of Al, two tolerant genotypes of soybean had a higher K^+ uptake than that of susceptible genotypes (Kuswantoro 2014). When Al concentration increased, the concentration of K^+ and Na^+ decreased in the foliage and root of silver birch (*Batula Pendula* Roth) (Bojarczuk *et al.* 2006). Soybean plants subjected to Al solution showed a higher Na^+ uptake in tolerant genotypes than that of susceptible genotypes (Kuswantoro 2014).

Aluminium treatment decreased NO_3^- content in different parts of cacao (Ribeiro *et al.* 2013).

4a.1.2 Effects of aluminium toxicity on the accumulation of divalent, trivalent cations and anions

Aluminium decreased Mg content in the root and leaves of cacao (Ribeiro *et al.* 2013), the leaves and root of physic nut (Steiner *et al.* 2012), the foliage and root of silver birch (Bojarczuk *et al.* 2006) and in rye grains (Rengel and Robinson 1989c).

Aluminium caused calcium deficiency due to its detrimental effect on absorption and translocation of calcium (Long and Foy 1970, Armiger *et al.* 1968, Evan and Kamprath 1970, MacLean and Chiasson 1966). Al decreased calcium content in different plant parts of cacao (Ribeiro *et al.* 2013), the leaves and root of physic nut (Steiner *et al.* 2012), the root of tobacco and in the foliage and root of silver birch (Bojarczuk *et al.* 2006). Al caused a decrease in Mg and Fe content in the root but an increase in those ions in the stem of *Pinus massoniana* (Zhang *et al.* 2014).

When wheat plants were subjected to aluminium solution, aluminium ion (Al^{3+}) was accumulated particularly in the root tips of the main root and lateral root tissue with small quantities in the cortex and epidermal cells in wheat (Fleming and Foy 1968) and in pea (Matsumoto *et al*. 1976). Al^{3+} accumulated preferentially in the root of physic nut, whereas only a small amount was transported to the shoot (Steiner *et al.* 2012). Improved methods for Al^{3+} detection inside cells showed that Al can enter the cytoplasm within a few minutes after exposure to aluminium solution (Lazof *et al.* 1996, Vázquez *et al.* 1999, Taylor *et al.* 2000 and Brauer 2001). Al^{3+} was accumulated in the root and small amount was transported to the above ground organs of *Pinus massoniana* (Zhang *et al.* 2014).

Aluminium decreased phosphorus content in the root and leaves of cacao (Ribeiro *et al.* 2013). Aluminum toxicity decreased phosphorus uptake in barley (MacLean and Chiasson 1966) and in snapbean and cotton roots (Naidoo *et al.* 1978). Higher aluminium activity reduced P concentration in the leaves of phytic nut (Steiner *et al*. 2012).

4a.2 Materials and Methods

Rice and chickpea seedlings were grown in solution culture according to the method described in section 2.6. Seven-day-old seedlings were transferred to half strength Hoagland solution (control) and 10, 50, 100 and 150 μM $AlCl_3$

solution made in half strength Hoagland solution. The pH of all aluminium solutions including control were adjusted to 4.2 with 0.2N H_2SO_4. The root and shoot of rice, and root, stem and leaves of chickpea were collected in triplicate at 3, 6, 24, 48, 72 and 96 h of treatment following the method described in section 2.8. Tissue was digested for the extraction of K^+, Na^+, Cl^- and NO_3^- following the method described in section 2.12.1. K^+ and Na^+ were measured by flame photometer (Jenway, PEP-7, UK) at a wavelength of 767 nm and 589 nm, respectively, according to the method outlined in section 2.12.2. Amount of Cl^- was measured by standard titrametric method following the method described in section 2.12.3. NO_3^- was determined following the method of Cataldo *et al.* (1975) as outlined in section 2.12.4.

Ca^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+} and PO_4^{3-} were extracted from samples following the method as described in section 2.12.5. Amount of Ca^{2+}, Mg^{2+} and Fe^{2+} in the extract were determined by an atomic absorption spectrophotometer (Perkin-Elmer, Model: AAnalyst 200) according to method outlined in section 2.12.6.

Al^{3+} content was measured by using an atomic absorption spectrophotometer following the method described in section 2.12.7.

Phosphate was measured by Vanadomolybdate method (Jackson 1967) using the method outlined in section 2.12.8.

4a.3 Results

4a.3.1 Effects of aluminium toxicity on the accumulation and distribution of K^+, Na^+, Cl^- and NO_3^- in rice and chickpea seedlings grown in solution culture

Effects of aluminium toxicity on the accumulation and distribution of K^+ in rice and chickpea seedlings grown in solution culture: Al (10-150 µM) decreased K^+ accumulation in the root of rice except an initial stimulation. 10 µM Al caused a 8.0 to 20.0% inhibition of K^+ accumulation the root from 24 to 96 h of treatment.

The inhibitory effect increased with the increase in aluminium concentration. Maximum decrease in K^+ accumulation in the root was caused by 150 µM Al which ranged from 18.0 to 39.0% at 24 to 96 h of application (Fig. 13a).

In the shoot of rice, 10 and 50 µM Al caused a 13.0 to 27.0% and 17.0 to 32.0% inhibition of K^+ content, respectively, from 24 to 96 h of treatment. Similarly, 150 µM Al resulted in 13.0 to 48.0% decrease in K^+ accumulation in the shoot from 6 to 96 h of application (Fig. 13b).

In chickpea seedlings, 10 µM Al decreased K^+ content in the root from 8.0 to 24.0% from 3 to 96 h of treatment. Al-induced inhibition of K^+ accumulation in the root increased with the increase in concentration of Al concentration. 150 µM Al caused the maximum inhibition of K^+ in the root from 32.0 to 61.0% from 3 to 96 h of application (Fig. 14a).

In the stem of chickpea, 10 to 150 µM Al inhibited K^+ content from 3 to 96 h of treatment. The maximum inhibition of K^+ accumulation in the stem occurred at 150 µM Al treatment which ranged from 25.0 to 44.0% from 3 to 96 h of exposure (Fig. 14b).

10 µM Al decreased K^+ accumulation in the leaf of chickpea from 7.0 to 15.0% from 3 to 96 h of application. At a concentration of 150 µM, Al inhibited K^+ content in the leaves by 17.0 to 28.0% from 3 to 96 h of treatment (Fig. 14c).

Effects of aluminium toxicity on the accumulation and distribution of Na^+ in rice and chickpea seedlings: Aluminium, at a concentration of 10 µM, increased the accumulation of Na^+ from 28.0 to 37.0% in the root of rice from 3 to 96 h to treatment. The stimulation of Na^+ accumulation increased with the increase in Al concentration from 10 to 150 µM. The highest stimulation of Na^+ accumulation in the root occurred at 150 µM Al application which ranged from 2.1- to 2.2-fold from 3 to 96 h of treatment (Fig. 15a).

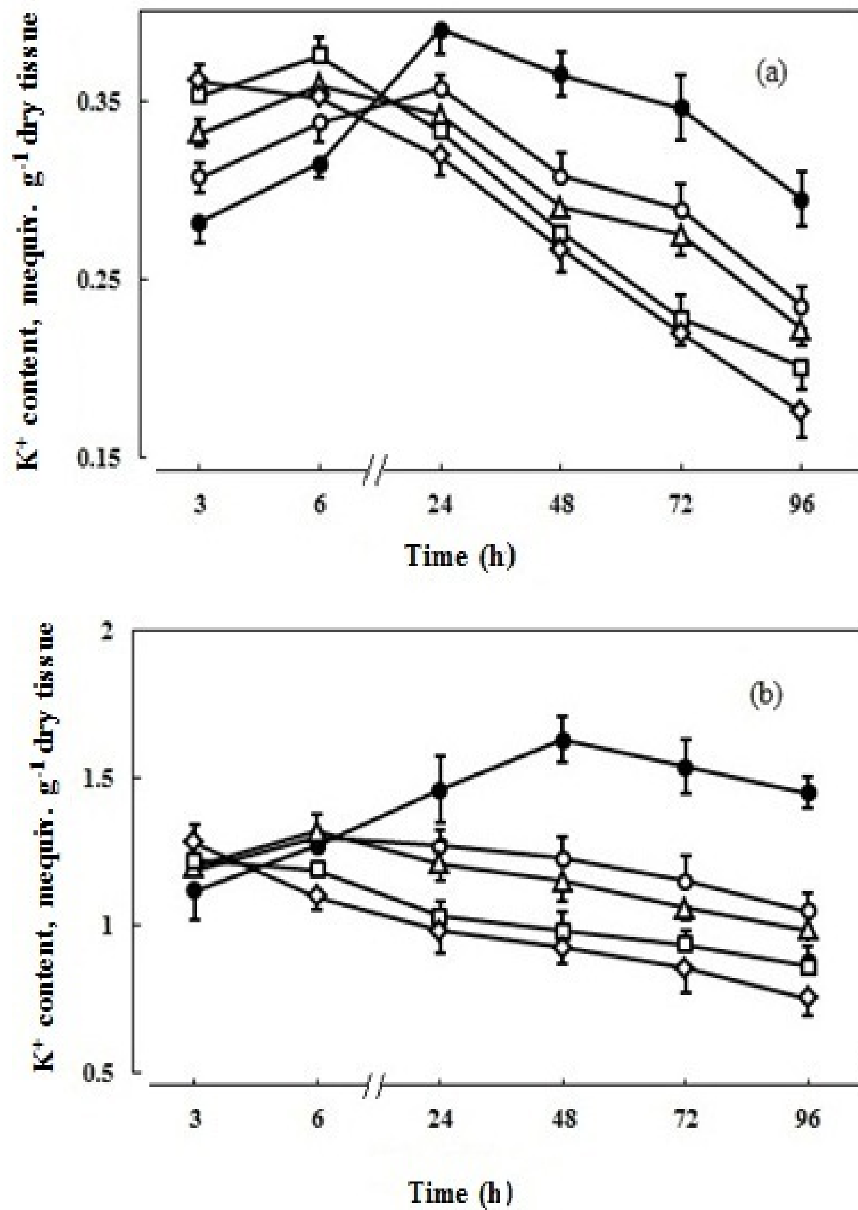


Fig. 13. The effect of different concentrations of aluminium on the accumulation of K^+ in the (a) root and (b) shoot of rice seedlings grown in solution culture. ● represents control; ○ 10 µM Al; Δ 50µM Al; □ 100µM Al; ◊ 150µM Al. Each value is the mean of three replicates ± standard error.

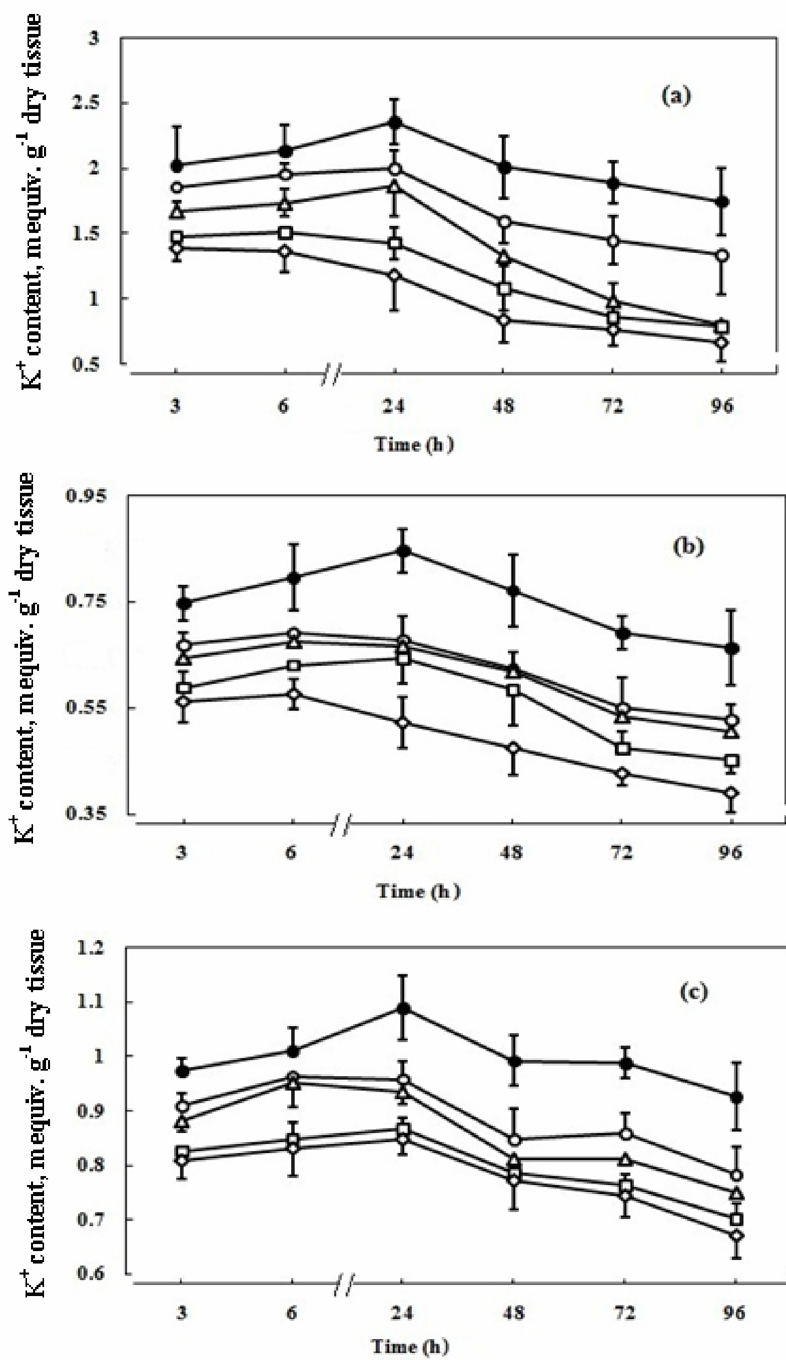


Fig. 14. The effect of different concentrations of aluminium on the accumulation of K^+ in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

50 µM Al caused a 42.0 to 45.0% stimulation of the accumulation of Na^+ in the shoot of rice from 3 to 96 h of application. A 53.0 to 55.0% increase in Na^+ accumulation in the shoot was observed following 50 µM Al treatment from 3 to 96 h of treatment (Fig. 15b).

In chickpea seedlings, 10 µM Al increased Na^+ accumulation in the root from 9.0 to 16.0% from 3 to 96 h of treatment. 100 µM Al increased accumulation of Na^+ in the root by 30.0 to 41.0% over a period of 3 to 96 h of application. A 40.0 to 50.0% stimulation of Na^+ accumulation in the root was recorded at 150 µM Al treatment from 3 to 96 h of exposure (Fig. 16a).

In the stem of chickpea, Al (10-150 µM) increased Na^+ accumulation from 3 to 96 h of treatment. 100 µM Al increased the accumulation of Na^+ by 23.0 to 29.0% from 3 to 96 h of exposure. Al at a concentration of 150 µM caused the maximum of 37.0 to 43.0% increase in Na^+ accumulation in the stem from 3 to 96 h of application (Fig. 16b).

In the leaves of chickpea, all the concentrations of aluminium used increased Na^+ accumulation. 100 and 150 µM Al caused a 36.0 to 52.0% and 53.0 to 72.0% increase in Na^+ accumulation in the leaves, respectively, from 3 to 96 h of treatment (Fig. 16c).

Effects of aluminium toxicity on the accumulation and distribution of Cl^- in rice and chickpea seedlings grown in solution culture: Al (10-150 µM) increased Cl^- accumulation in the root of rice seedlings. 10 µM Al increased Cl^- accumulation in the root by 19.0 to 58.0% from 3 to 96 h of treatment. The stimulatory effect of Al on Cl^- accumulation in the root increased with the increase in the concentration of Al. The highest stimulation in Cl^- accumulation in the root occurred at 150 µM Al which ranged from 61.0% to 2.2-fold from 3 to 96 h of application (Fig. 17a).

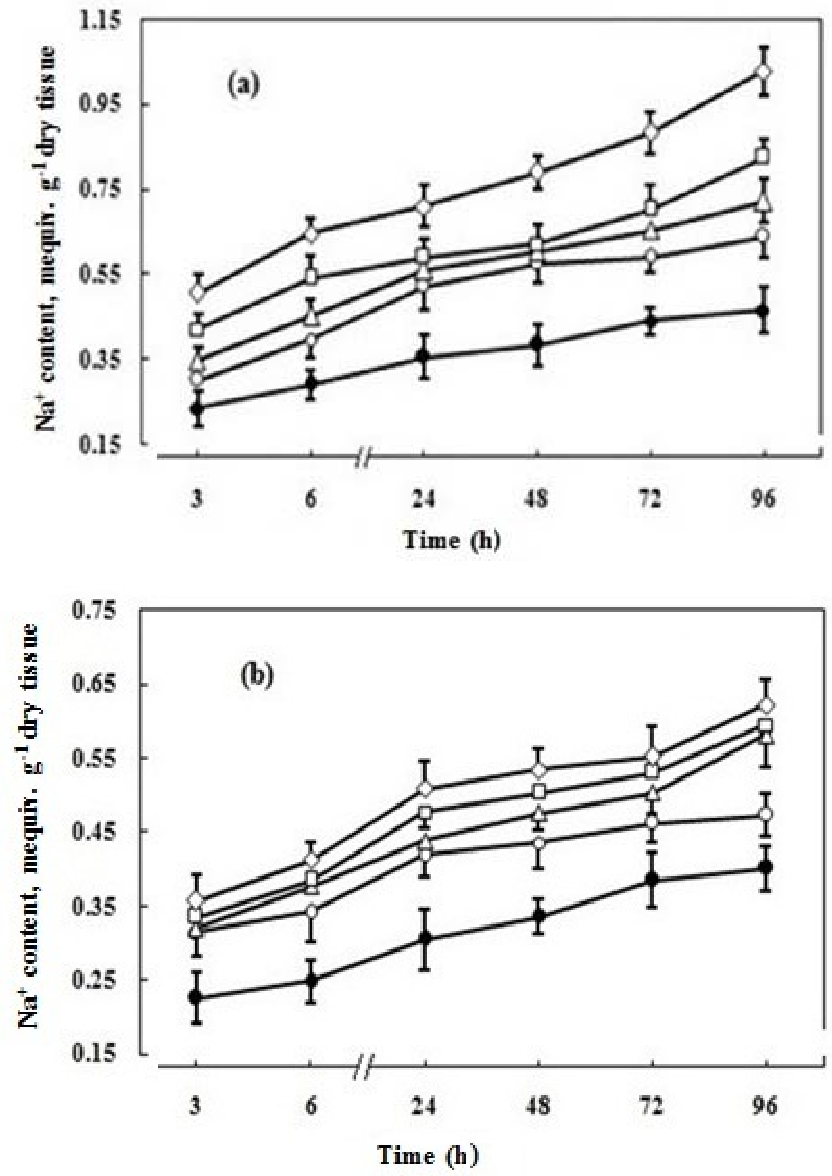


Fig. 15. The effect of different concentrations of aluminium on the accumulation of Na^+ in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

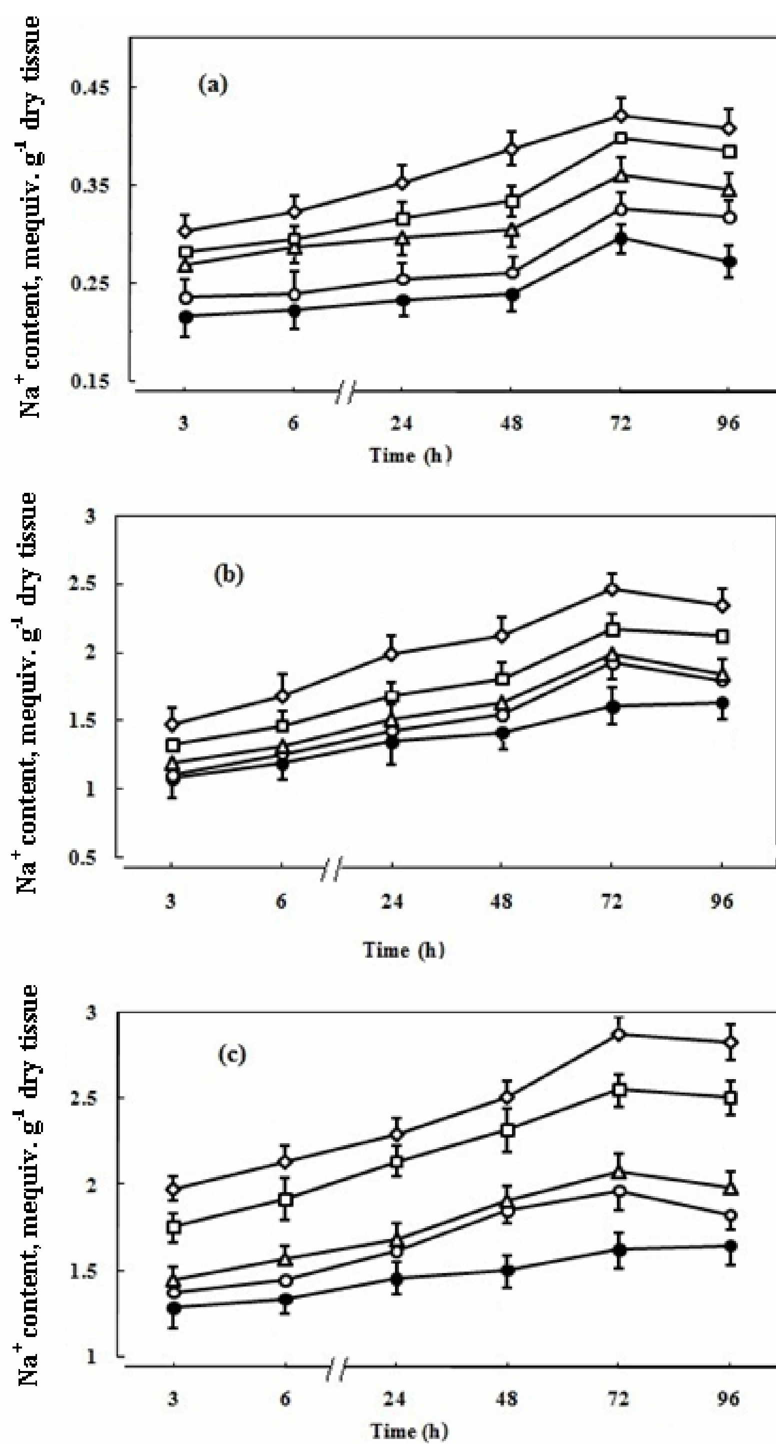


Fig. 16. The effect of different concentrations of aluminium on the accumulation of Na^+ in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

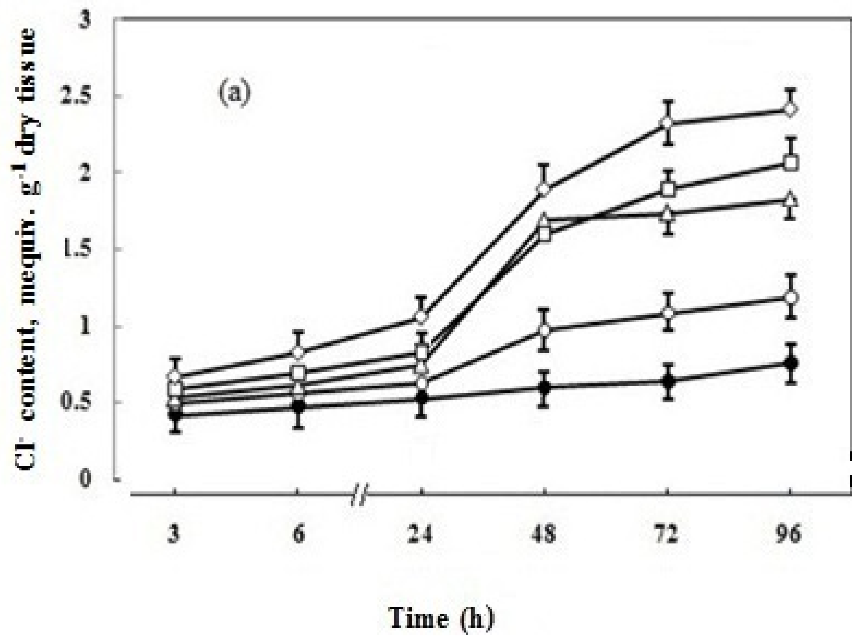
Al (10 μM) increased Cl^- accumulation by 24.0 to 78.0% in the shoot of rice from 3 to 96 h of treatment. 10 and 150 μM Al caused a 2- to 2.5-fold and 2.7- to 3.4-fold increase in Cl^- accumulation in the shoot, respectively, from 3 to 72 h of application. This stimulatory effect was sustained up to 96 h of treatment (Fig. 17b).

In the root of chickpea seedlings, 10 μM Al caused a 19.0 to 27.0% increase in Cl^- accumulation from 3 to 96 h of exposure. Similarly, 100 μM Al increased Cl^- accumulation in the root by 66.0 to 80.0% from 3 to 96 h of application. The maximum inhibition of Cl^- ranging from 85.0% to 2-fold was observed in the root of chickpea from 3 to 96 h Al treatment (Fig. 18a).

Al (50 μM) resulted in a 23.0 to 28.0% increase in Cl^- accumulation in the stem of chickpea from 3 to 96 h of treatment. A 36.0 to 49.0% increase in the accumulation of Cl^- in the stem was observed following 150 μM Al application from 3 to 96 h of application (Fig. 18b).

In the leaves of chickpea, 10 μM Al caused a 10.0 to 25.0% increase in Cl^- accumulation from 3 to 96 h of treatment. 100 μM Al increased Cl^- accumulation in the leaves from 51.0 to 67.0% from 3 to 72 h of exposure. Al, at a concentration of 150 μM, caused a 74.0 to 81.0% increase in Cl^- accumulation in the leaves from 3 to 96 h of treatment (Fig. 18c).

Effects of aluminium toxicity on the accumulation and distribution of NO_3^- in rice and chickpea seedlings grown in solution culture: Al (10 -150 μM) decreased NO_3^- accumulation in the root of rice except an initial stimulation. 10 μM Al inhibited the accumulation of NO_3^- by 14.6 to 25.5% in the root from 48 to 96 h of treatment. A maximum inhibition of 34.0 to 82.8% in NO_3^- accumulation in the root occurred at 150 μM Al treatment (Fig. 19a).



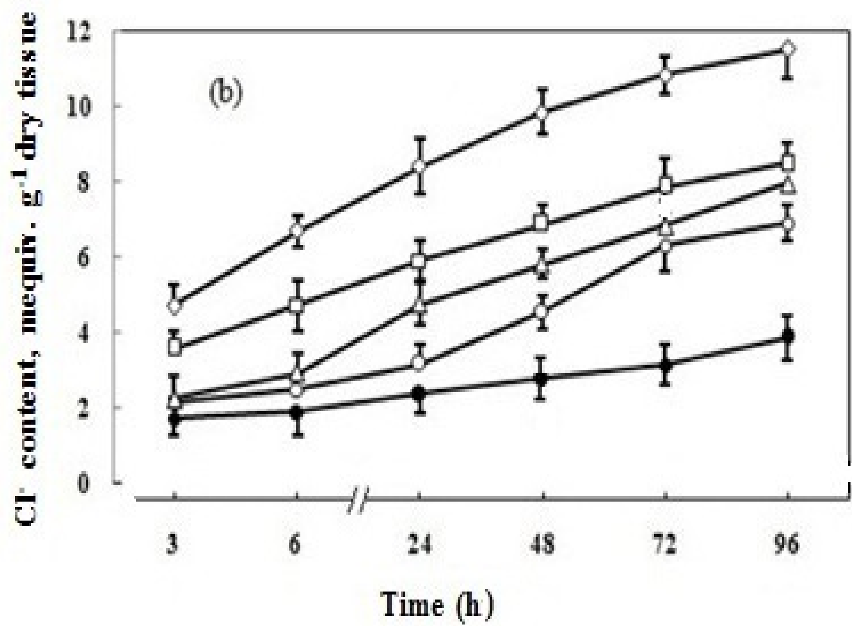


Fig. 17. The effect of different concentrations of aluminium on the accumulation of Cl^- in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

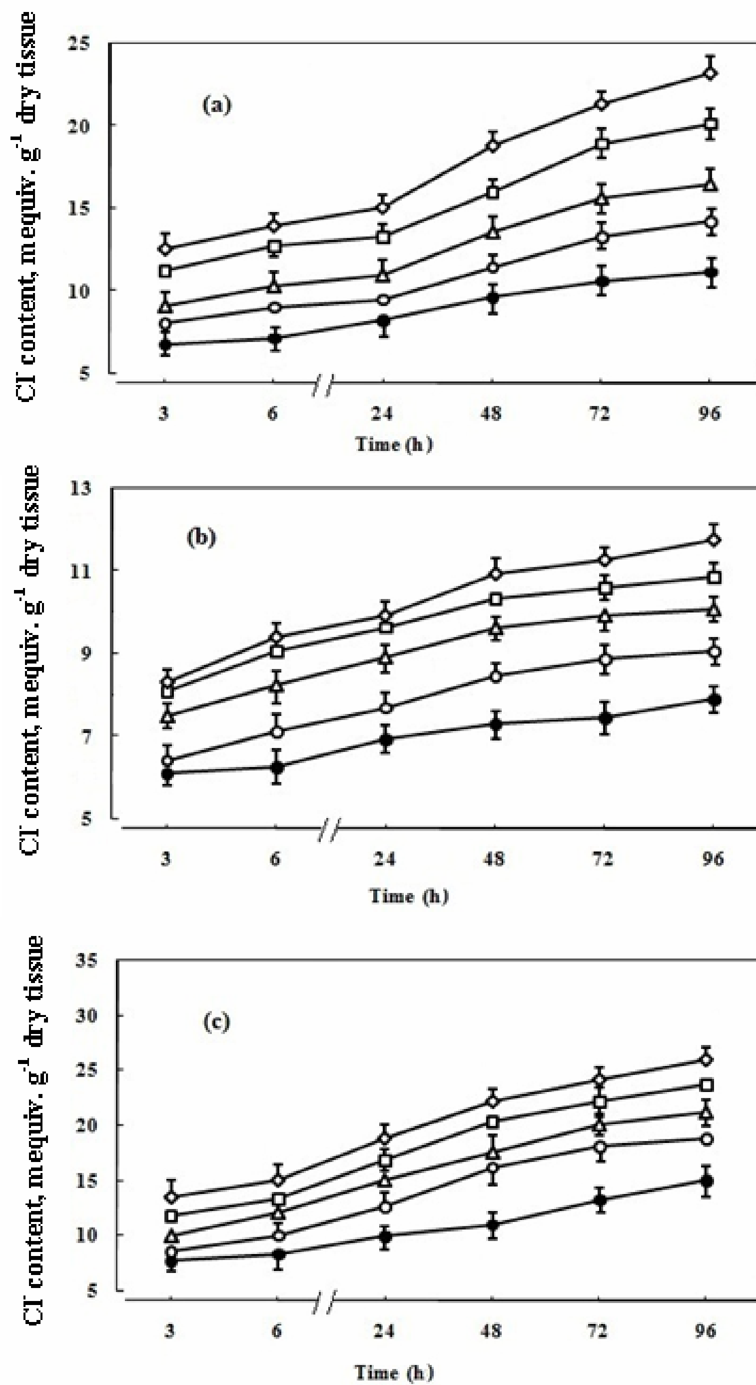


Fig. 18. The effect of different concentrations of aluminium on the accumulation of Cl^- in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

Similarly, different concentrations of Al (10-150 µM) inhibited NO_3^- content in shoot of rice except an initial stimulation. 50 µM Al decreased NO_3 accumulation in the shoot of rice by 17.0 to 5.7% from 24 to 96 h of treatment. 150 µM Al inhibited the accumulation of NO_3^- in the shoot of rice by 11.7 to 79.8% from 6 to 96 h of application except an increase of that at 3 h of exposure (Fig. 19b).

In chickpea seedlings, 10 µM Al inhibited NO_3^- accumulation in the root by 21.7 to 50.0% from 48 to 96 h of application. Accumulation of NO_3^- in the root was decreased by 27.0 to 61.0% at 48 to 96 h of treatment following exposure to 150 µM Al (Fig. 20a).

Al (10 µM) decreased the accumulation of NO_3^- in the stem of chickpea by 7.5 to 57.0% from 3 to 96 h of application. A 26.6 to 69.0% inhibition of NO_3^- accumulation was recorded following 100 µM Al treatment at a time period of 3 to 96 h. 150 µM Al inhibited NO_3^- accumulation in the stem by 28.8 to 76.5% from 3 to 96 h of exposure (Fig. 20b).

In the leaves of chickpea, all the concentrations of Al (10-150 µM) decreased NO_3^- accumulation. The inhibiting effect of Al increased with the increase in Al concentration. The highest inhibition of NO_3^- in the leaves was exerted by 150 µM Al where a 37.0 to 77.9% inhibition was recorded from 3 to 96 h of treatment (Fig. 20c).

4a.3.2 Effects of aluminium toxicity on the accumulation and distribution of Ca^{2+}, Mg^{2+}, Fe^{2+} in rice and chickpea seedlings grown in solution culture

Effects of aluminium toxicity on the accumulation and distribution of Ca^{2+} in rice and chickpea seedlings grown in solution culture: Aluminium at a concentration of 10 and 50 µM decreased Ca^{2+} accumulation in the root of rice seedlings by 27.0 to 43.0% and 40.0 to 48.0%, respectively, from 3 to 96 h of

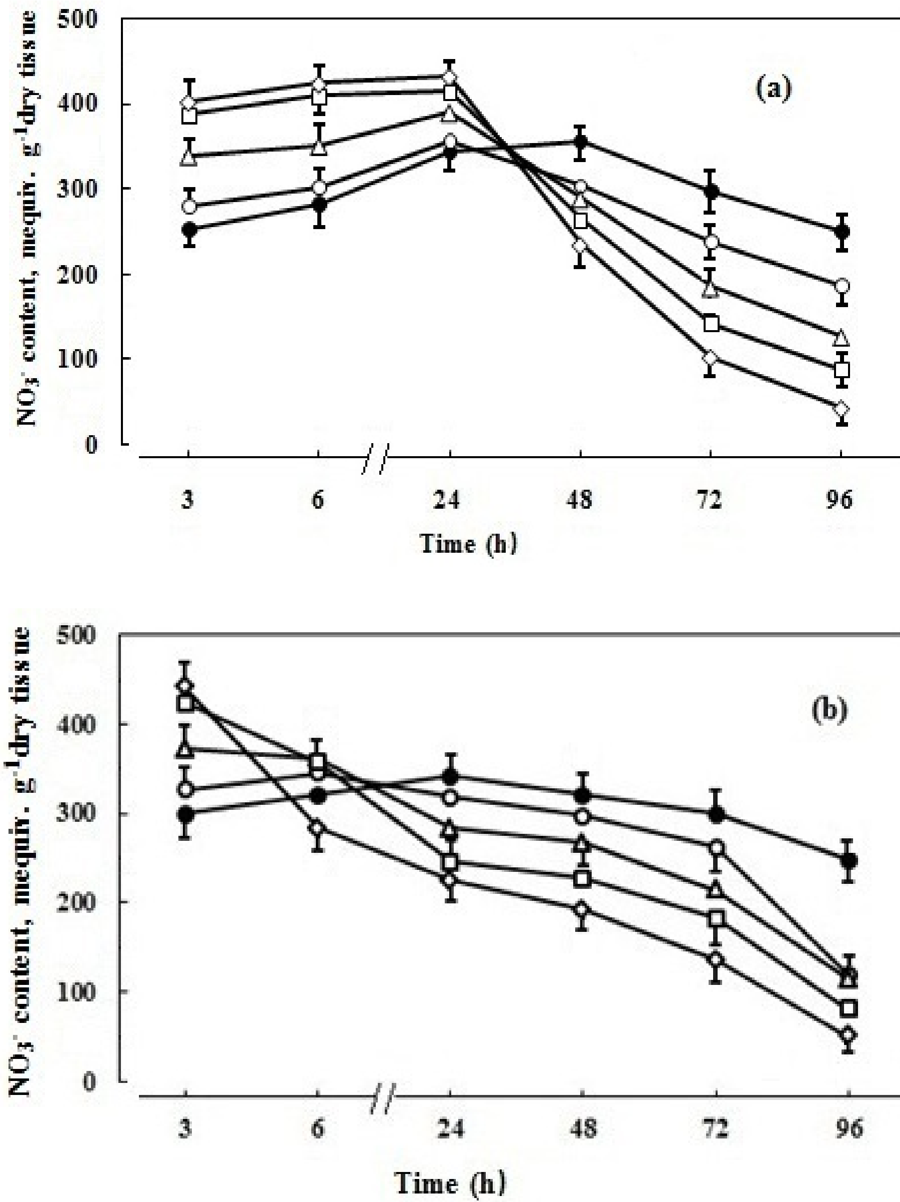


Fig. 19. The effect of different concentrations of aluminium on the accumulation of NO_3^- in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

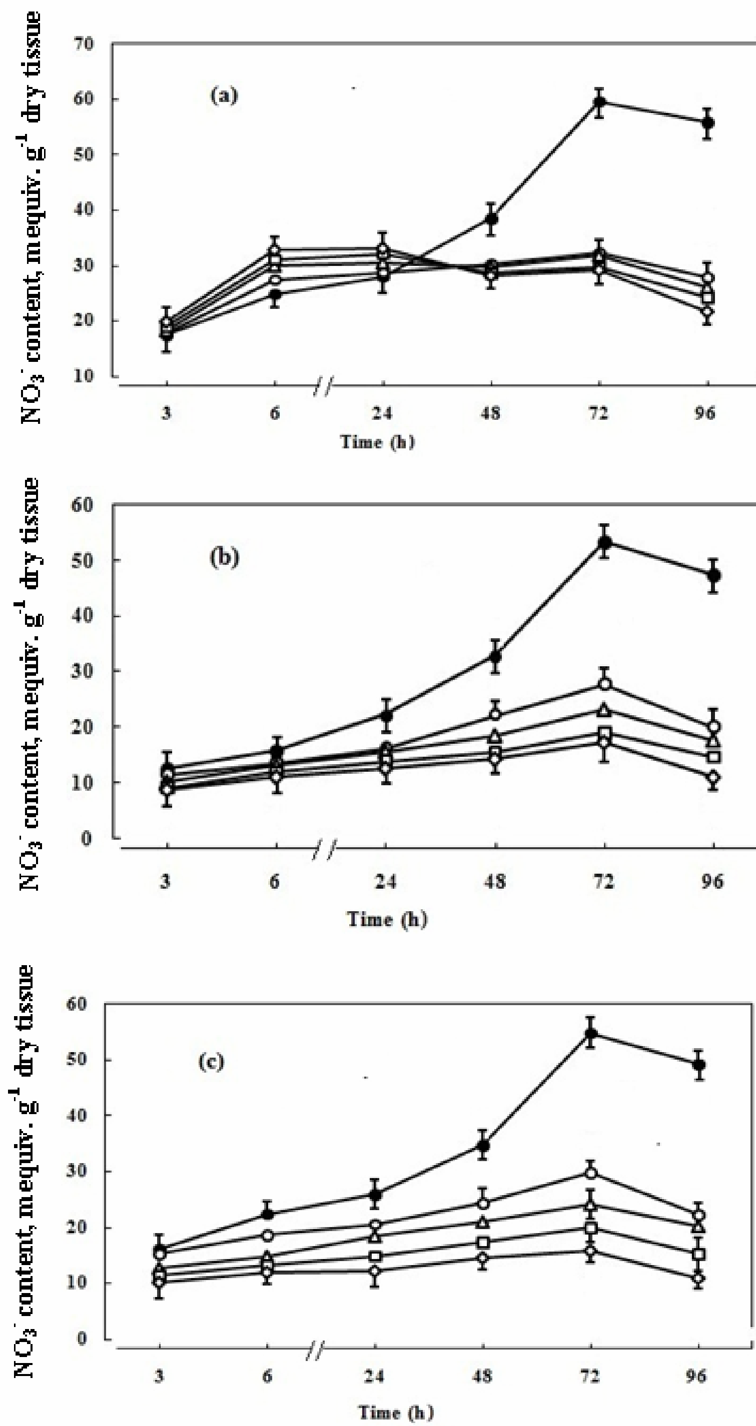


Fig. 20. The effect of different concentrations of aluminium on the accumulation of NO_3^- in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

treatment. The highest inhibition of Ca^{2+} accumulation in the root was observed at 150 µM Al treatment which ranged from 54.0 to 65.7% from 3 to 96 h of application (Fig. 21a).

Al (10 -150 µM) inhibited Ca^{2+} accumulation in the shoot of rice at 3 to 96 h of treatment. The inhibitory effect of Al on Ca^{2+} accumulation increased with the increase in Al concentration from 10 to 150 µM. Al (10 µM) caused a 27.5 to 47.9% inhibition of Ca^{2+} uptake in the shoot from 3 to 96 h of exposure. 150 µM Al decreased Ca^{2+} accumulation in the shoot of rice from 68.9 to 82.0% at a time period of 3 to 96 h (Fig. 21b).

In chickpea seedlings, 10 µM Al caused a 7.0 to 43.0% inhibition of Ca^{2+} accumulation in the root from 3 to 96 h of treatment. Inhibitory effect of Al on Ca^{2+} accumulation was increased with the increase in Al concentrations leading to a maximum inhibition of 23.7 to 69.0% at 150 µM Al application (Fig. 22a).

Accumulation of Ca^{2+} in stem of chickpea was decreased by 5.0 to 18.5% at 10 µM Al treatment from 3 to 96 h of application. Al (150 µM) caused the highest inhibition of Ca^{2+} accumulation in the stem of chickpea ranging from 24.7 to 58.0% from 3 to 96 h of exposure (Fig. 22b).

Similar pattern of Al-induced inhibition of Ca^{2+} accumulation in the leaves of chickpea was recorded. In the leaves, 10 µM Al decreased Ca^{2+} accumulation by 8.9 to 33.0%. Similarly 50 and 100 µM Al inhibited Ca^{2+} accumulation in the leaf by 17.7 to 44.6% and 28.0 to 49.0%, respectively, from 3 to 96 h of treatment. Exposure of chickpea seedlings to 150 µM Al resulted in the maximum 36.0 to 57.8% inhibition of Ca^{2+} content in the leaves from 3 to 96 h of application (Fig. 22c).

Effects of aluminium toxicity on accumulation and distribution of Mg^{2+} in rice and chickpea seedlings grown in solution culture: Exposure of roots of rice seedlings to 10 µM Al caused a 18.0 to 30.0% inhibition of Mg^{2+} content from

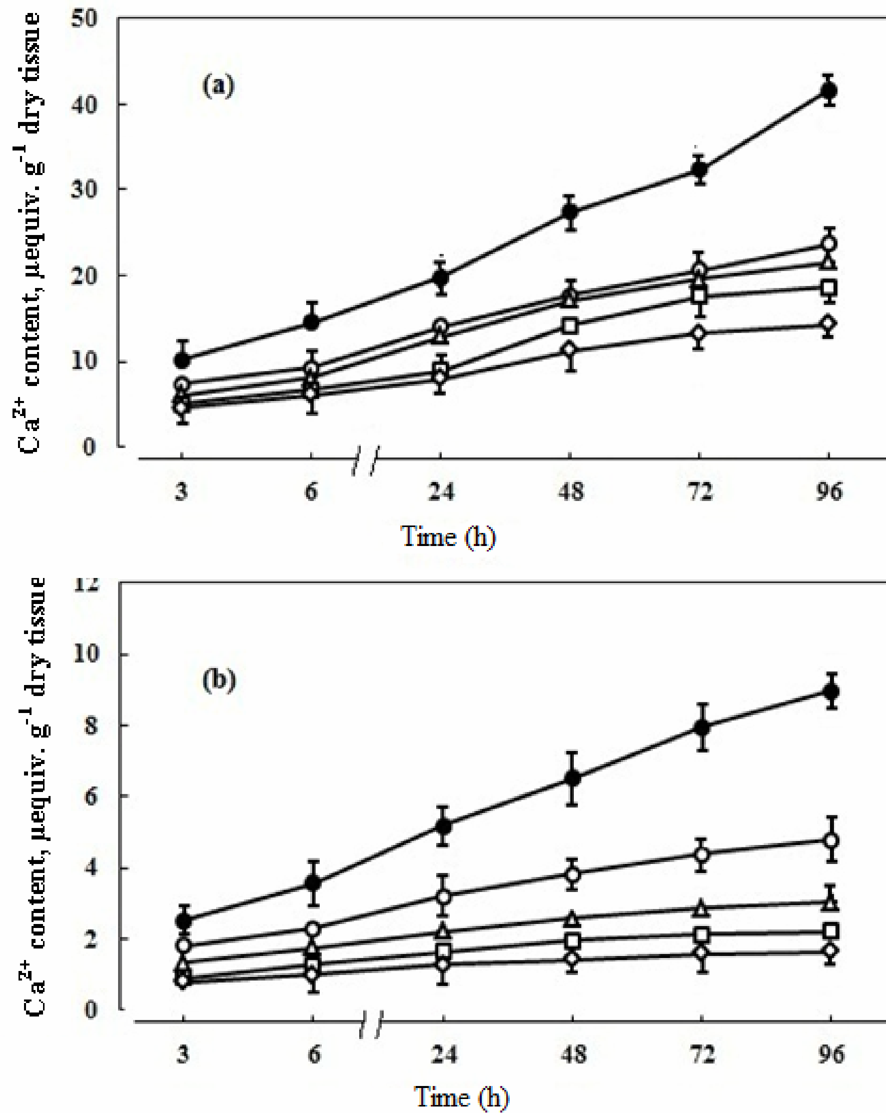


Fig. 21. The effect of different concentrations of aluminium on the accumulation of Ca^{2+} in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

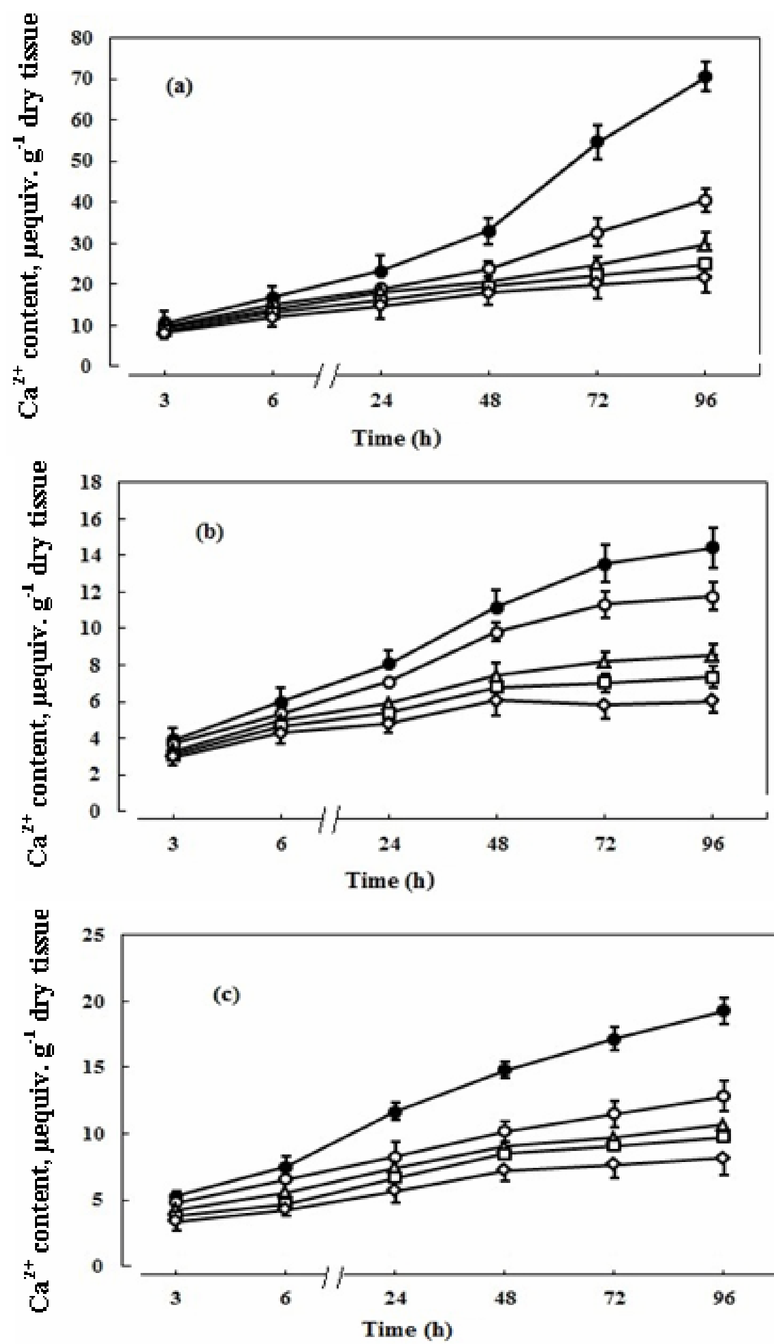


Fig. 22. The effect of different concentrations of aluminium on the accumulation of Ca^{2+} in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

3 to 96 h of treatment. The degree of inhibition of Mg^{2+} accumulation in the root increased with the increase in concentration of Al from 10 to 150 μM. At 150 μM Al, the highest decrease in Mg^{2+} accumulation in the root was observed which ranged from 38.8 to 67.0% from 3 to 96 h of application (Fig. 23a).

Similarly, 10 to 50 μM Al caused 18.0 to 36.0% and 30.0 to 45.0% reduction in Mg^{2+} accumulation, respectively, in the shoot of rice from 3 to 96 h of treatment. Al, at a concentration of 100 and 150 μM, resulted in a 44.6 to 59.0% and 57.8 to 69% inhibition of Mg^{2+} content in the shoot, respectively, from 3 to 96 h of application (Fig. 23b).

In the root of chickpea seedlings, the reduction in Mg^{2+} accumulation gradually increased with the increase in Al concentration from 10 to 150 μM. The lowest inhibition of Mg^{2+} was obtained following 10 μM Al treatment which ranged from 5.9 to 27.0% from 3 to 96 h of exposure. The highest inhibition of Mg^{2+} accumulation in the root was recorded at 150 μM Al treatment ranging from 25.7 to 53.0% from 3 to 96 h of application (Fig. 24a).

In the stem of chickpea, 10 μM Al decreased Mg^{2+} content from 14.6 to 31.7% from 3 to 96 h of treatment. The inhibition of Mg^{2+} accumulation in the stem increased further with the increase in Al concentration from 100 to 150 μM Al. The maximum inhibition of Mg^{2+} accumulation in the stem (35.0 to 61.0%) was recorded following 150 μM Al application from 3 to 96 h of treatment (Fig. 24b).

Al (10 μM) decreased Mg^{2+} content in the leaves of chickpea from 10.0 to 33.8% at 3 to 96 h of exposure. Inhibition of accumulation of Mg^{2+} in the leaves gradually increased from 16.0 to 45.0% and 25.7 to 51.6% when chickpea seedlings were exposed to 50 and 100 μM Al, respectively, from 3 to 96 h of application. The inhibition of Mg^{2+} accumulation in the leaves reached the highest value of 34.6 to 59.0% at 150 μM Al treatment (Fig. 24c).

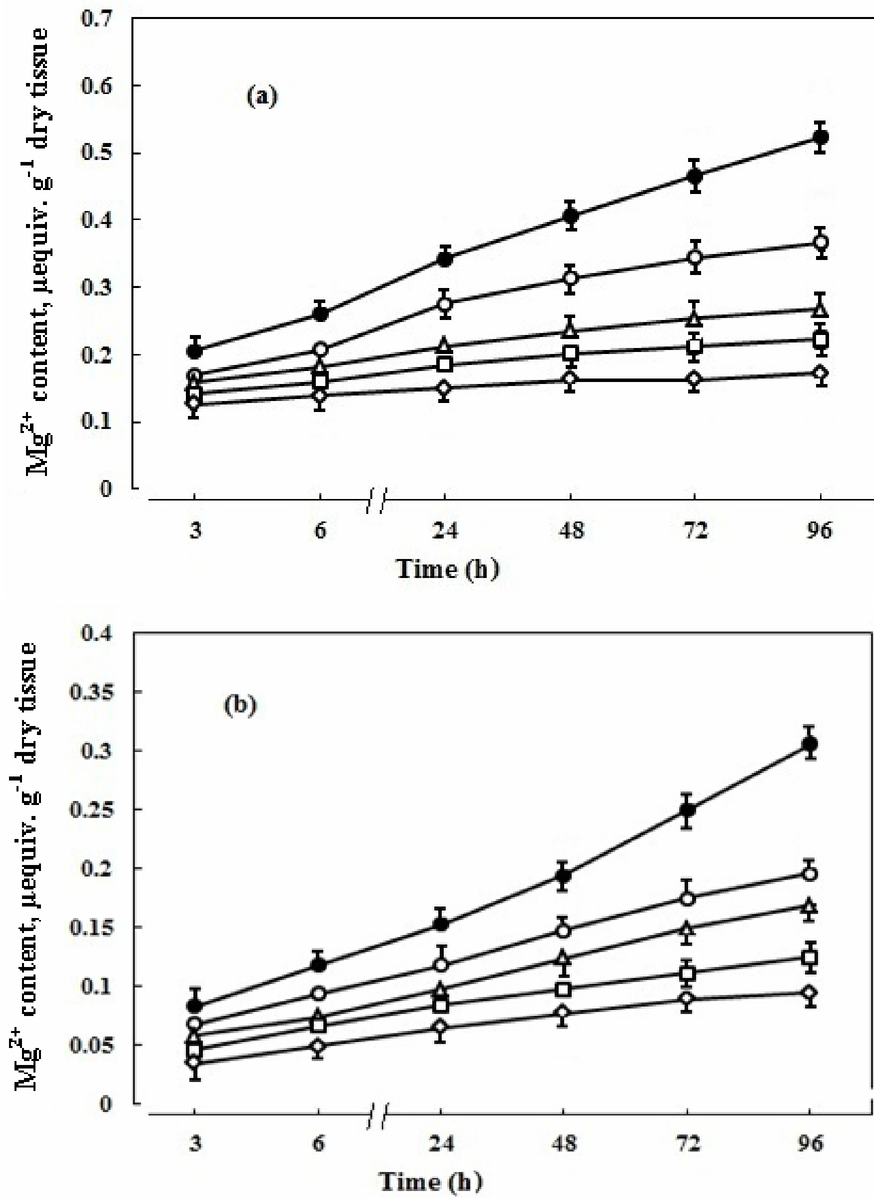


Fig. 23. The effect of different concentrations of aluminium on the accumulation of Mg^{2+} in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

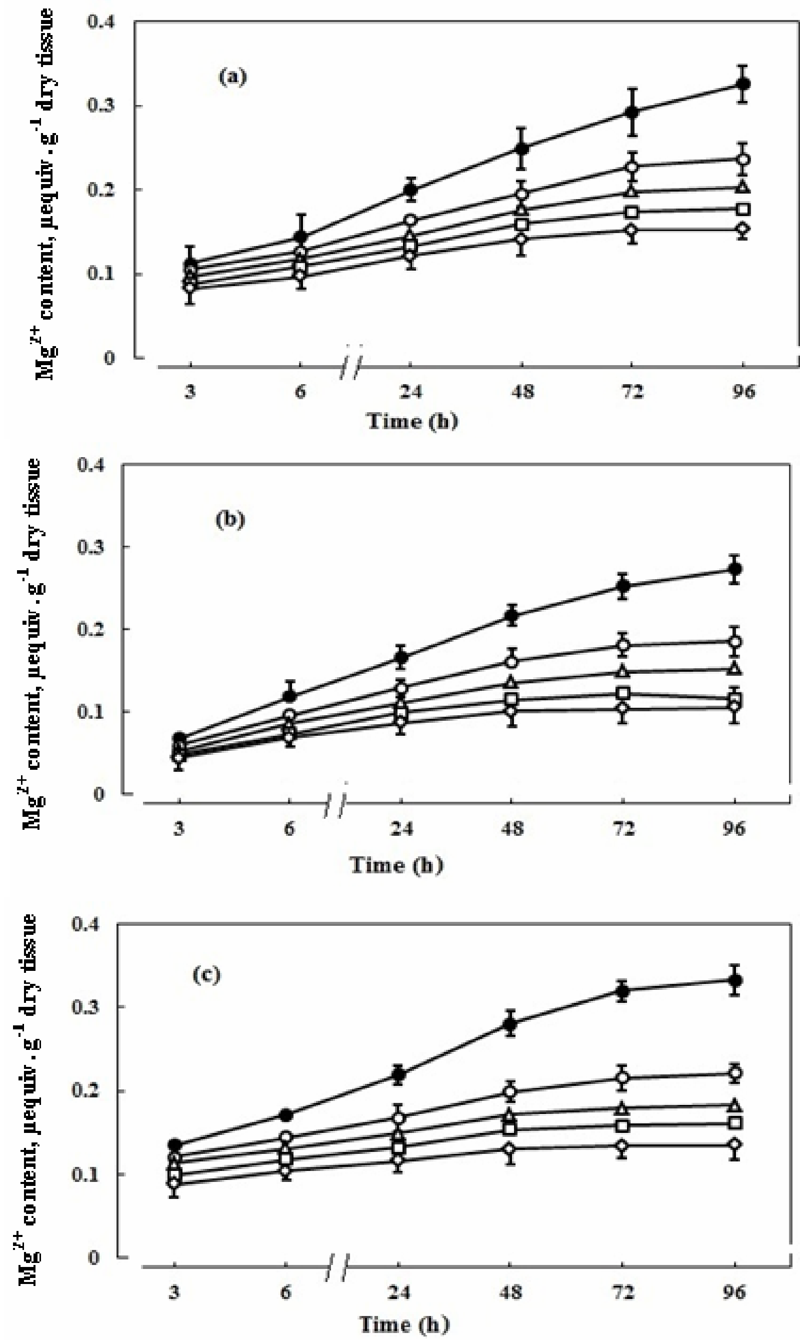


Fig. 24. The effect of different concentrations of aluminium on the accumulation of Mg^{2+} in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

Effects of aluminium toxicity on the accumulation and distribution of Fe^{2+} in rice and chickpea seedlings grown in solution culture: Al (10-150 µM) decreased Fe^{2+} accumulation in the root of rice except an initial stimulation. In the root, 50 µM Al decreased Fe^{2+} content by 11.0 to 20.9% from 48 to 96 h of treatment. A 16.0 to 31.6% and 23.6 to 43.0% reduction in Fe^{2+} accumulation was recorded at 100 and 150 µM Al treatment, respectively, from 48 to 96 h of exposure (Fig. 25a).

In the shoot of rice, Fe^{2+} accumulation was also decreased by Al (10-150 µM) except an initial stimulation. At a concentration of 10 and 50 µM, Al caused a slight inhibition of Fe^{2+} accumulation in the shoot of rice. 100 and 150 µM Al decreased Fe^{2+} content in the shoot by 9.9 to 13.0% and 12.0 to 16.5%, respectively, from 48 to 96 h of treatment (Fig. 25b).

In chickpea seedlings, 50 µM Al increased Fe^{2+} accumulation in the root by 14.0 to 12.0% from 3 to 24 h leading to a decrease in Fe^{2+} content (5.0-16.7%) from 48 to 96 h of treatment. 150 µM Al caused a 31.8 to 19.7% stimulation of Fe^{2+} content from 3 to 24 h followed by a decrease by 9.0 to 32.0% from 48 to 96 h of application (Fig. 26a).

Al (10 to 150 µM) exerted stimulation of Fe^{2+} accumulation in the stem of chickpea seedlings from 3 to 48 h of treatment leading to a decrease of that from 72 to 96 h of treatment. 50, 100 and 150 µM Al decreased Fe^{2+} accumulation in the stem by 8.0 to 11.5%, 11.7 to 16.7% and 16.8 to 24.0%, respectively, from 72 to 96 h of application (Fig. 26b).

In the leaves of chickpea, 10 µM Al decreased Fe^{2+} accumulation by 6.0 to 16.0% from 24 to 96 h of treatment after an initial stimulation. The inhibitory effect increased with the increase in Al concentration from 50 to 150 µM from 24 to 96 h of application. 150 µM Al resulted in a maximum inhibition of Fe^{2+} accumulation in the leaves by 16.5 to 36.0% from 24 to 96 h of exposure except an initial increase by 21.0 and 14.0% at 3 and 6 h of treatment (Fig. 26c).

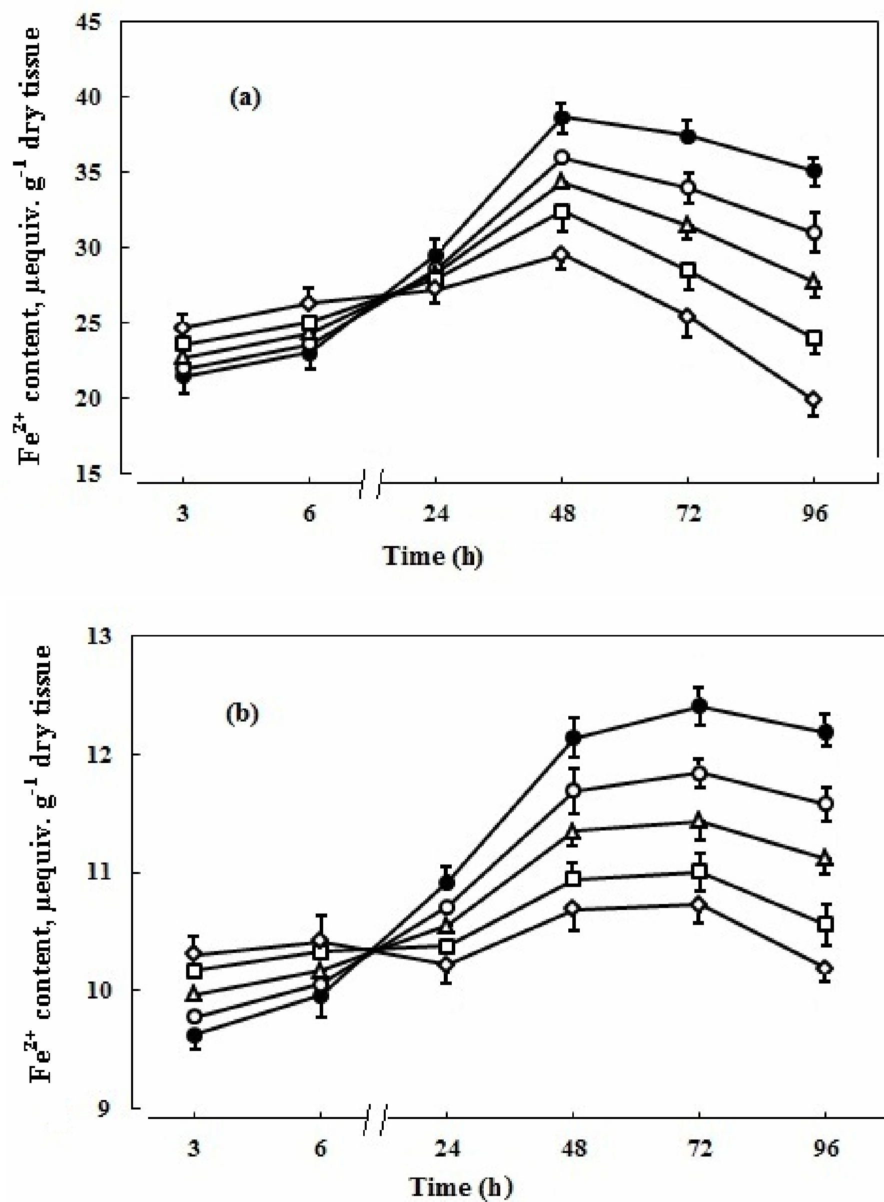


Fig. 25. The effect of different concentrations of aluminium on the accumulation of Fe^{2+} in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

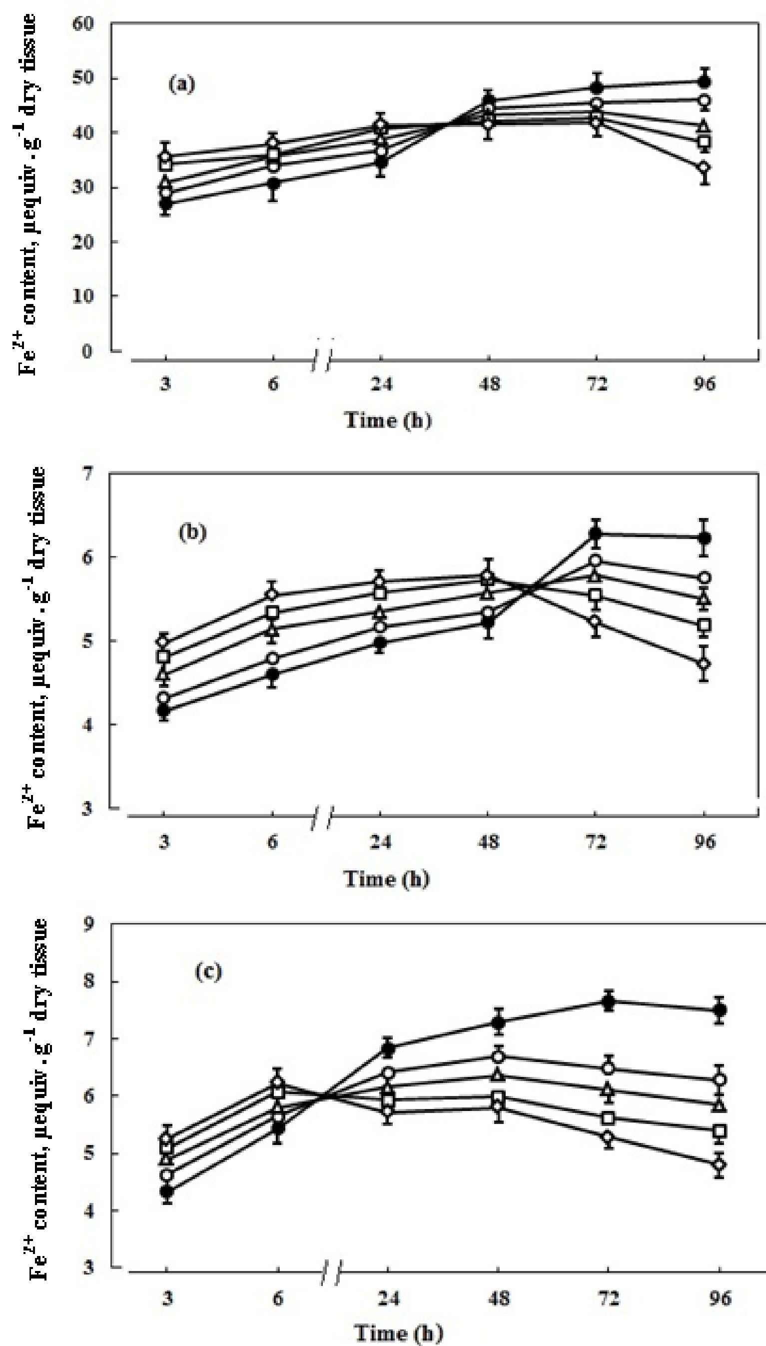


Fig. 26. The effect of different concentrations of aluminium on the accumulation of Fe^{2+} in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

4a.3.3 Effects of aluminium application on the accumulation and distribution of Al^{3+} in rice and chickpea seedlings grown in solution culture

Al (10 μM) increased the accumulation Al^{3+} in the root of rice seedlings by 41.0% to 3-fold from 3 to 96 h of treatment. 50 μM Al increased Al^{3+} content in the root by 2- to 3.5-fold from 3 to 96 h of exposure. Al (150 μM) caused the maximum increase in Al^{3+} in the root ranging from 3- to 4.6-fold at 3 to 96 h of application (Fig. 27a).

In the shoot of rice seedlings, 10 μM Al caused a 1.8- to 2-fold increase in Al^{3+} content from 3 to 72 h of application and the stimulatory effect was sustained up to 96 h of treatment. 50 and 100 μM Al increased Al^{3+} accumulation in the shoot by 47.6% to 2.8-fold and 2- to 3.3-fold, respectively, from 3 to 48 h of treatment and the stimulatory effect was sustained up to 96 h of application. The maximum accumulation of Al^{3+} in the shoot was recorded following 150 μM Al treatment which amounted to 2.7- to 3.5-fold from 3 to 48 h of treatment and the stimulatory effect was maintained up to 96 h of exposure (Fig. 27b).

In chickpea seedlings, 10 μM Al increased the accumulation of Al^{3+} in the root by 12.8% to 2.1-fold from 3 to 96 h of treatment. Al (50 μM) caused 2.1- to 2.4-fold increase in Al^{3+} accumulation in the root from 24 to 96 h of application. 100 and 150 μM Al increased the accumulation of Al^{3+} in the root of chickpea by 2.2- to 3-fold and 2.4- to 3.3-fold, respectively, from 3 to 96 h of treatment (Fig. 28a).

In the stem of chickpea, 10 μM Al increased the accumulation of Al^{3+} by 10.7% to 76.9% from 3 to 96 h of application. 50 and 100 μM Al caused a 46.6% to 2.2-fold and 84.7% to 2.7-fold increase in Al^{3+} content in the stem, respectively, from 3 to 96 h of treatment. The maximum 2.1- to 3-fold stimulation of Al^{3+} accumulation was observed in the stem of chickpea seedlings exposed to 150 μM Al from 3 to 96 h (Fig 28b).

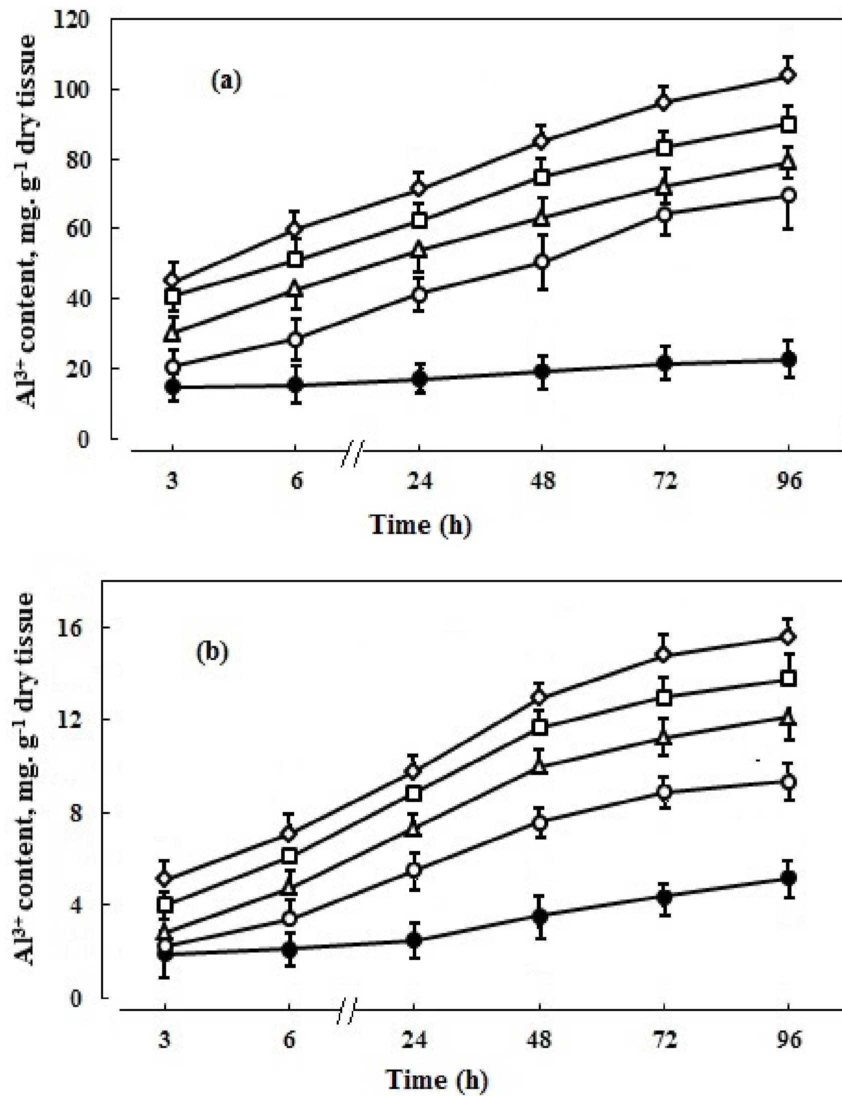


Fig. 27. The effect of different concentrations of aluminium on the accumulation of Al^{3+} in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

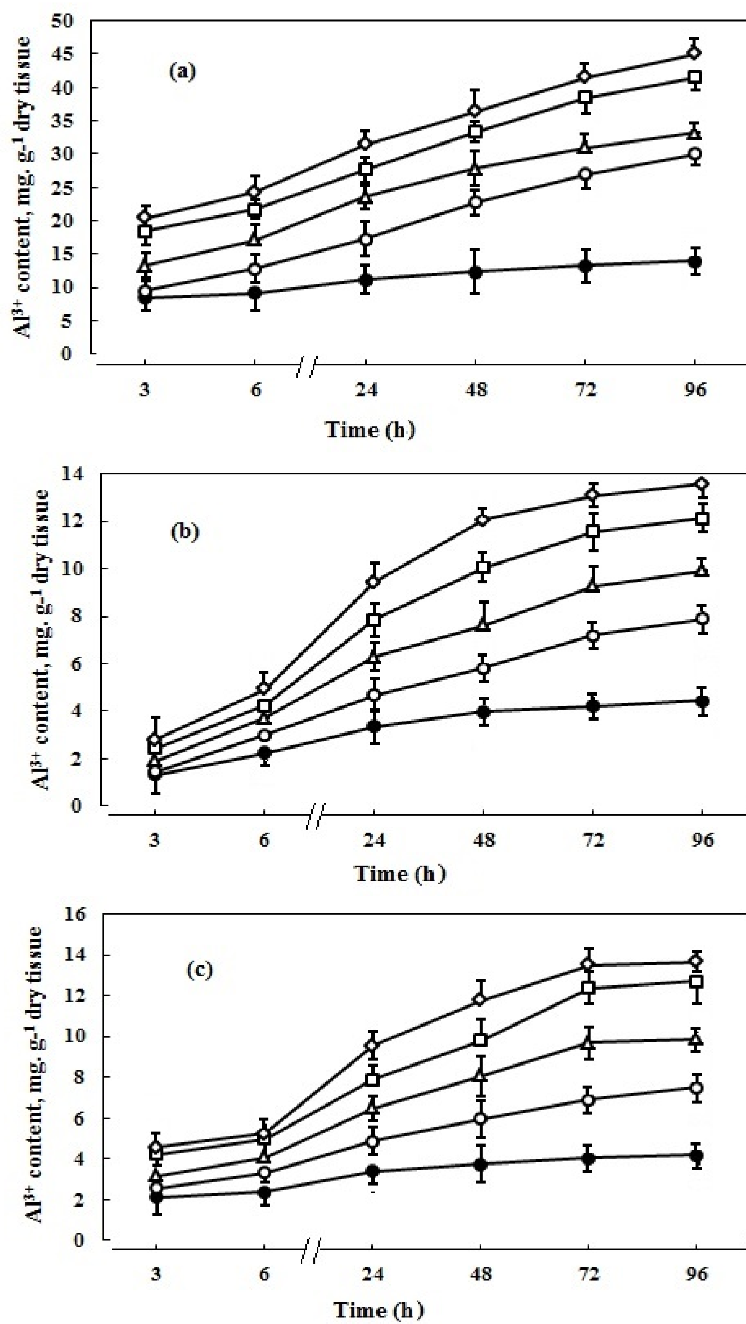


Fig. 28. The effect of different concentrations of aluminium on the accumulation of Al^{3+} in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

Al (10 μM) increased the accumulation of Al^{3+} in the leaves of chickpea by 10.6 to 76.8% from 3 to 96 h of treatment. The accumulation of Al^{3+} in the leaves increased with the increase in aluminium concentration from 50 to 150 μM. A maximum of 2.2- to 3.3-fold increase in Al^{3+} in the leaves was recorded in the leaves of chickpea seedlings exposed to 150 μM Al from 3 to 96 h of application (Fig. 28c).

4a.3.4 Effects of aluminium toxicity on the accumulation and distribution of phosphate in rice and chickpea seedlings grown in solution culture

At a concentration of 10 μM, aluminium inhibited accumulation of phosphate from 4 to 19% in the root of rice seedlings from 3 to 96 h of treatment. The degree of Al-induced inhibition of phosphate increased with the increase in concentration of aluminium from 10 to 150 μM. A maximum of 20.0 to 57.0% inhibition of phosphate accumulation in the root of rice was recorded under the influence of 150 μM Al from 3 to 96 h of application (Fig. 29a).

In the shoot of rice seedlings, intensity of inhibition of phosphate accumulation increased with the increase in Al concentration from 10 to 100 μM. At a concentration of 50 μM, aluminium decreased phosphate accumulation in the shoot by 8.0 to 22.0% from 3 to 96 h of treatment whereas 150 μM Al caused a 17.0 to 39.0% inhibition of phosphate accumulation in the shoot following 3 to 96 h of application (Fig. 29b).

In the root of chickpea seedlings, 10 to 150 μM Al treatment caused an increase in phosphate accumulation from 3 to 24 h followed by an inhibition of that from 48 to 96 h of treatment. For example, 50 μM Al caused a 11.0 to 7.5% increase in phosphate accumulation from 3 to 24 h of treatment and it gradually decreased that from 12.0 to 26.0% from 48 to 96 h of application. 150 μM Al increased phosphate accumulation in the root from 33.0 to 22.6% from 3 to 24 h but it decreased that from 29.0 to 53.7% from 48 to 96 h of exposure (Fig. 30a).

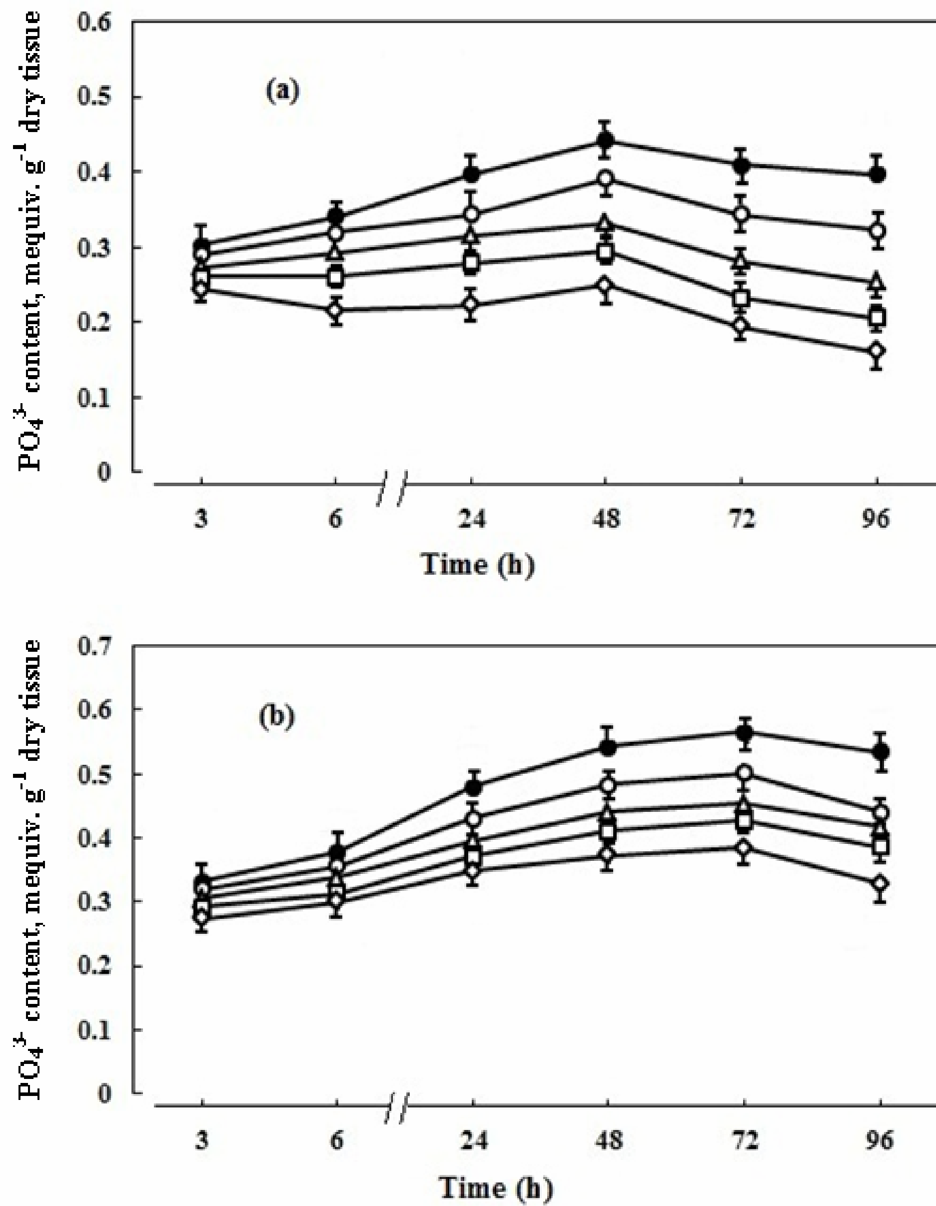


Fig. 29. The effect of different concentrations of aluminium on the accumulation of PO_4^{3-} in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

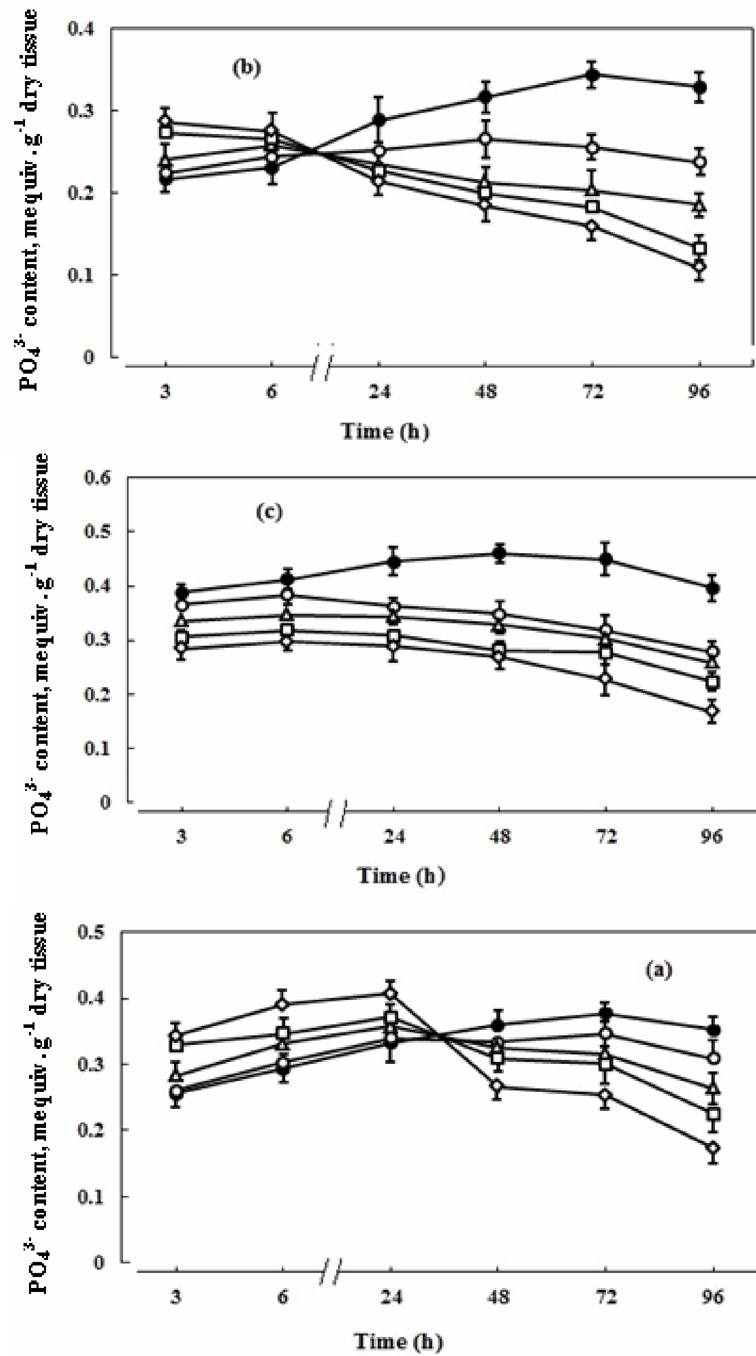


Fig. 30. The effect of different concentrations of aluminium on the accumulation of PO_4^{3-} in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

Al (10 μM) inhibited phosphate accumulation in the stem of chickpea seedlings from 12.0 to 27.6% from 24 to 96 h of treatment except an initial stimulation at 3 to 6 h of exposure. This trend of effect exerted by Al was maintained following 50, 100 and 150 μM Al application. 150 μM Al caused a 32.0 to 19.0% stimulation of phosphate uptake in the stem at 3 to 6 h of treatment followed by 26.0 to 66.8% inhibition of that in the stem of chickpea from 24 to 96 h of application (Fig. 30b).

In the leaves of chickpea seedlings, Al (10 μM) caused a 6.0 to 29.5% inhibition of phosphate in the leaves from 3 to 96 h of treatment. Inhibition of phosphate accumulation increased with the increase in concentration of Al from 10 to 150 μM. The maximum inhibition of phosphate was observed in the leaves of chickpea seedlings exposed to 150 μM Al which ranged from 26.8 to 57.5% from 3 to 96 h of application (Fig. 30c).

4a.4 Discussion

Effects of aluminium toxicity on the accumulation and distribution of K^+ and Na^+ in rice and chickpea seedlings grown in solution culture: Different concentrations of aluminium (10-50 μM) decreased the accumulation of K^+ in the root and shoot of rice (Fig. 13), and the root, stem and the leaves of chickpea (Fig. 14). Similarly, Al decreased K^+ accumulation in maize and sorghum (Bhalerao and Prabhu 2013). On the contrary, Al increased K^+ content in *Stylosanthes* (Amaral *et al.* 2013).

Aluminium, at concentrations of 10 to 150 μM, caused an increase in Na^+ accumulation in the root and shoot of rice (Fig. 15), and the root, stem and leaves of chickpea (Fig. 16). Earlier, it was found by Lidon and coworkers (2000) that 0.33 μM Al increased Na^+ content in the root of maize. Uptake of K^+ was decreased by aluminium treatment but that of Na^+ was increased in rice and chickpea (Figs. 13-16). Therefore, it appears that Al alters the K^+/Na selectivity.

High concentration of Na^+ and low level of K^+ may disrupt metabolic functions of rice and chickpea.

Effects of aluminium toxicity on the accumulation and distribution of Cl^- and NO_3^- in rice and chickpea seedlings grown in solution culture: Different concentrations of Al (10-150 µM) increased Cl^- accumulation in the root and shoot of rice (Fig. 17), and the root, stem and leaves of chickpea (Fig. 18). Al-induced dramatic stimulation of Cl^- in rice and chickpea (Figs. 17 and 18) might be toxic for the plant. On the contrary, Al decreased accumulation of Cl^- in maize (Calba and Jillard 1997).

Al (10-150 µM) inhibited NO_3^- accumulation in different organs of rice and chickpea (Figs. 19 and 20). Similar Al-induced inhibition of NO_3^- was found in sorghum (Keltjens and van Ulden 1987, Keltjens 1988). Similarly, Al increased NO_3^- accumulation in *Stylosanthes guianensis* and *S. macrocephala* (Cordeiro 1981 and Amaral *et al.* 2000) and *S. humilis* (Mosquim 1978).

Effects of aluminium toxicity on the accumulation and distribution of Ca^{2+}, Mg^{2+} and Fe^{2+} in rice and chickpea seedlings grown in solution culture: Al (10-150 µM) decreased the accumulation of Ca^{2+} in the root and shoot of rice (Fig. 21), and the root, stem and leaves of chickpea (Fig. 22). This result is in agreement with the work of Zheng *et al.* (2005) who found that Ca^{2+} accumulation decreased progressively in the root of buckwheat with the increase in Al concentrations. Besides, Ca^{2+} uptake in barley was also inhibited by Al (Nichol and Oliveira 1995).

Different concentrations of Al (10-150 µM) decreased Mg^{2+} accumulation in the root and shoot of rice (Fig. 23), and the root, stem and leaves of chickpea (Fig. 24). Similarly, Al decreased Mg^{2+} accumulation in cabbage, lettuce and kikuya grass (Huett and Menary 1980a) and red spruce (Cumming *et al.* 1985b).

Al toxicity decreased Fe^{2+} accumulation in the root and shoot of rice (Fig. 25), and the root, stem and leaves of chickpea (Fig. 26). This result is supported by Simon and coworkers (1994a) who found that Al decreased Fe^{2+} content in the root, stem and leaves of tomato.

Effects of aluminium application on the accumulation and distribution of Al^{3+} in rice and chickpea seedlings grown in solution culture: Al (10-150 µM) caused a 2- to 4.6-fold increase in Al content in the root and shoot of rice and chickpea seedlings (Fig. 27 and 28). Similarly, Al application caused a 3-fold increase in Al^{3+} in the root of maize (Lidon *et al.* 2000). Application of Al increased accumulation of Al^{3+} in seedlings of tertiary buckwheat (Wang *et al.* 2015).

Effects of aluminium toxicity on the accumulation and distribution of phosphate in rice and chickpea seedlings grown in solution culture: At a concentration of 10-150 µM, aluminium decreased phosphate accumulation in the root and shoot of rice (Fig. 29). In chickpea, aluminium decreased phosphate content in the root, stem and leaves after an initial promotion (Fig. 30). Similarly Al decreased phosphate accumulation in wheat (Foy and Flemming 1982) and in lupin (Alva and Edwards 1990). Al inhibited the concentration of P in barley shoot but it had no effect on that of the root (Alam and Adams 1980).

4b Effects of aluminium toxicity on the accumulation and distribution of monovalent and divalent cations and Cl^- in rice and chickpea plants grown in sand culture

4b.1 Introduction

The aim of this experiment was to compare the effect of aluminium toxicity on the accumulation and distribution of ions in rice and chickpea plants grown in sand culture in natural environmental conditions with that of these plants grown in solution culture in light bank under controlled conditions.

Al toxicity decreased K^+ content in *Zea mays* (Bennet *et al.* 1985). Al reduced the absorption of K^+ in four species of coffee (Malavolta *et al.* 1997).

Al inhibited Ca^{2+} uptake in cultured tobacco cells (Chang *et al.* 1999 and Jones *et al.* 1998). Ca^{2+} uptake was inhibited by aluminium in root apex of wheat (Huang *et al.* 1992a and b) and in barley (Nichol and Oliveira 1995). High aluminium concentration decreased Mg^{2+} accumulation in sorghum (Ohki 1987).

Accumulation of Fe^{2+} was decreased by Al in sorghum (Furlani and Clark 1981) and in maize (Lidon *et al.* 2000).

4b.2 Materials and Methods

Rice and chickpea plants were grown in sand culture following the method described in section 2.9. Seven-day-old seedlings grown in sand culture were subjected to half strength Hoagland solution (pH 4.2) (control) and 50, 100 and 150 µM $AlCl_3$ made in half strength Hoagland solution (pH 4.2) according to the method described in section 2.10.

The root and shoot of rice, and root, stem and leaves of chickpea were collected in triplicate according to the technique described in section 2.11.

Extraction of K^+, Na^+ and Cl^- in the tissue samples was carried out following the method described in section 2.12.1.

K^+, Na^+ and Cl^- were measured according to method outlined in 2.12.2 to 2.12.4.

Extraction of Ca^{2+}, Mg^{2+} and Fe^{2+} were extracted by digesting the tissue following the method described in section 2.12.5. Ca^{2+}, Mg^{2+} and Fe^{2+} were measured according to the method outlined in sections 2.12.6 to 2.12.8.

4b.3 Results

Effects of aluminium toxicity on the accumulation and distribution of K^+ in rice and chickpea plants grown in sand culture: Al (50 µM) decreased K^+ accumulation in the root by 7.8 to 27.8% from 7 to 28 day of treatment. A 15.5 to 36.0% reduction in K^+ content in the root by 100 µM Al treatment was observed from 7 to 28 day of application. 150µM Al caused a maximum inhibition of K^+ accumulation in the root ranging from 25.5 to 49.0% from 7 to 28 day of treatment (Fig. 31a).

In shoot of rice plants, 50 µM Al inhibited K^+ content of the shoot from 13.0 to 30.6% from 7 to 28 day of treatment. The reduction in K^+ accumulation was higher with the increase in Al concentrations. 100 and 150 µM Al decreased the accumulation of K^+ in the shoot by 21.0 to 44.8% and 33 to 55.5%, respectively, from 7 to 28 day of exposure (Fig. 31 b).

Al (50 µM) decreased K^+ accumulation in the root of chickpea plants by 15.9 to 61.4% from 7 to 28 day of treatment. 100 and 150 µM Al inhibited K^+ content in the root of chickpea by 19.3 to 75.0% and 23.9 to 84.0%, respectively, from 7 to 28 day of application (Fig. 32a).

In the stem of chickpea, 50 µM Al decreased K^+ accumulation by 9.7 to 25.9% from 7 to 28 day of treatment. Al, at a concentration of 100 and 150 µM decreased K^+ content in the stem by 20.3 to 38.2% and 13.2 to 54.4%, respectively, from 7 to 28 day of exposure (Fig. 32b).

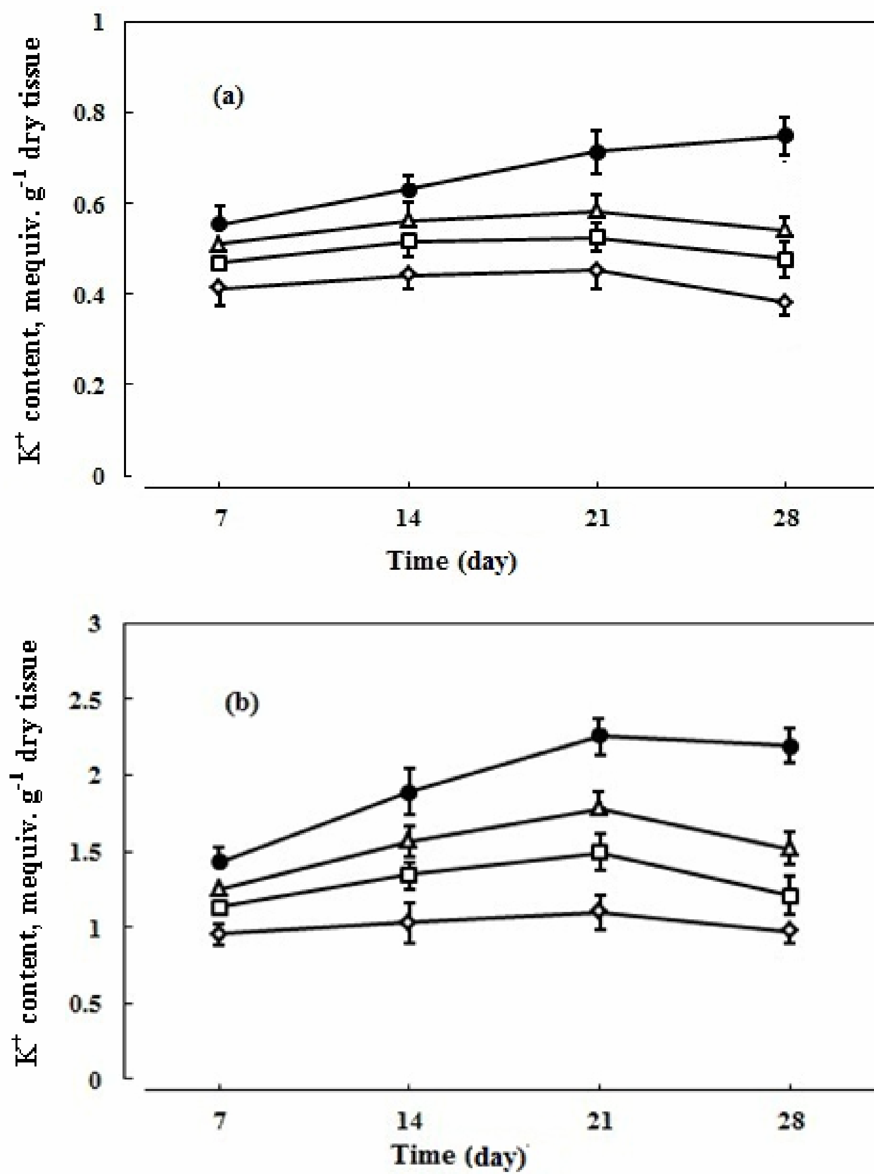


Fig. 31. The effect of different concentrations of aluminium on the accumulation of K^+ in the (a) root and (b) shoot of rice plants grown in sand culture. ● represents control; Δ 50 µM Al; □ 100 µM Al; ◊ 150 µM Al. Each value is the mean of three replicates ± standard error.

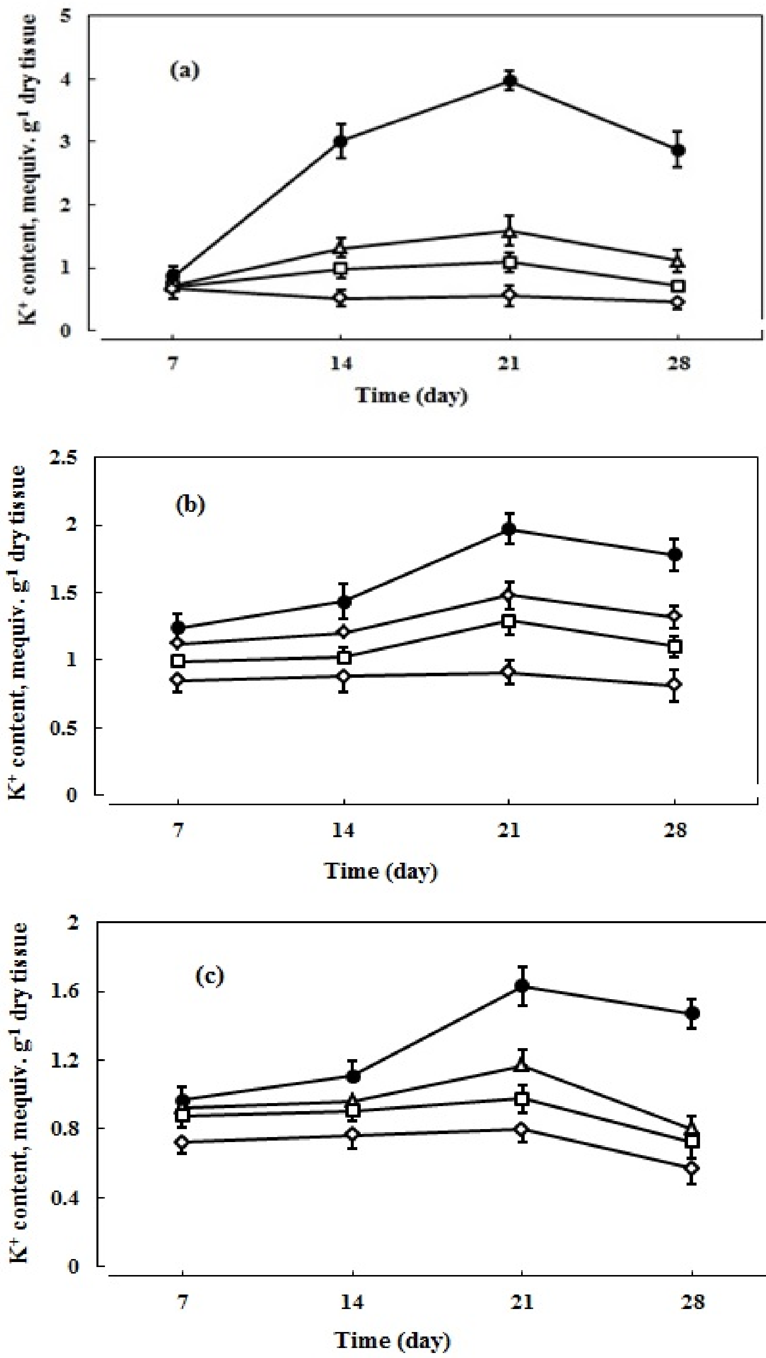


Fig. 32. The effect of different concentrations of aluminium on the accumulation of K^+ in the (a) root, (b) stem and (c) leaves of chickpea plants grown in sand culture. Otherwise as Fig. 31.

Al (50 μM) inhibited K^+ content in the leaves of chickpea by 13.3 to 45.58% from 14 to 28 day of application. Accumulation of K^+ in the leaves decreased with the increase in Al concentration from 100 to 150 μM. A maximum of 25.3 to 61.2% inhibition of K^+ in the leaves was caused by 150 μM Al from 7 to 28 day of treatment (Fig. 32c).

Effects of aluminium toxicity on the accumulation and distribution of Na^+ in rice and chickpea plants grown in sand culture: Al, at concentration of 50 μM, increased Na^+ content in the root of rice by 63.7 to 2-fold at 7 to 28 day of treatment. 100 μM Al caused a 2.7- to 3.1-fold increase in Na^+ accumulation in the root. A dramatic 3.2- to 3.7-fold stimulation of Na^+ accumulation was recorded following 150 μM Al treatment from 7 to 28 day of treatment (Fig. 33 a).

In the shoot of rice, 50 μM Al increased Na^+ content by 46.0 to 66.6% from 7 to 28 day of exposure. 100 μM Al caused 72.0% to 2.2-fold stimulation of Na^+ accumulation in the shoot from 7 to 28 day of application. Maximum increase in Na^+ accumulation in the shoot was observed at 150 μM Al ranging from 97.6% to 2.3-fold from 7 to 28 day of treatment (Fig. 33 b).

In chickpea plants, 50 μM Al increased accumulation of Na^+ in the root by 46.9 to 70.3% from 7 to 28 day of treatment. 100 μM Al increased Na^+ content in the root by 70.8% to 2.3-fold from 7 to 24 day of application. 150 μM Al caused the maximum 2- to 2.4-fold stimulation of Na^+ accumulation in the root from 7 to 28 day of exposure (Fig. 34a).

Al, at concentrations of 50 and 100 μM, caused 31.0 to 64.4% and 66.2 to 94.9% increase in Na^+ content, respectively, in the stem of chickpea from 7 to 28 day of application. 150 μM Al caused the maximum stimulation of Na^+ accumulation in the stem by 85.9% to 2.3-fold from 7 to 28 day of treatment (Fig. 34b).

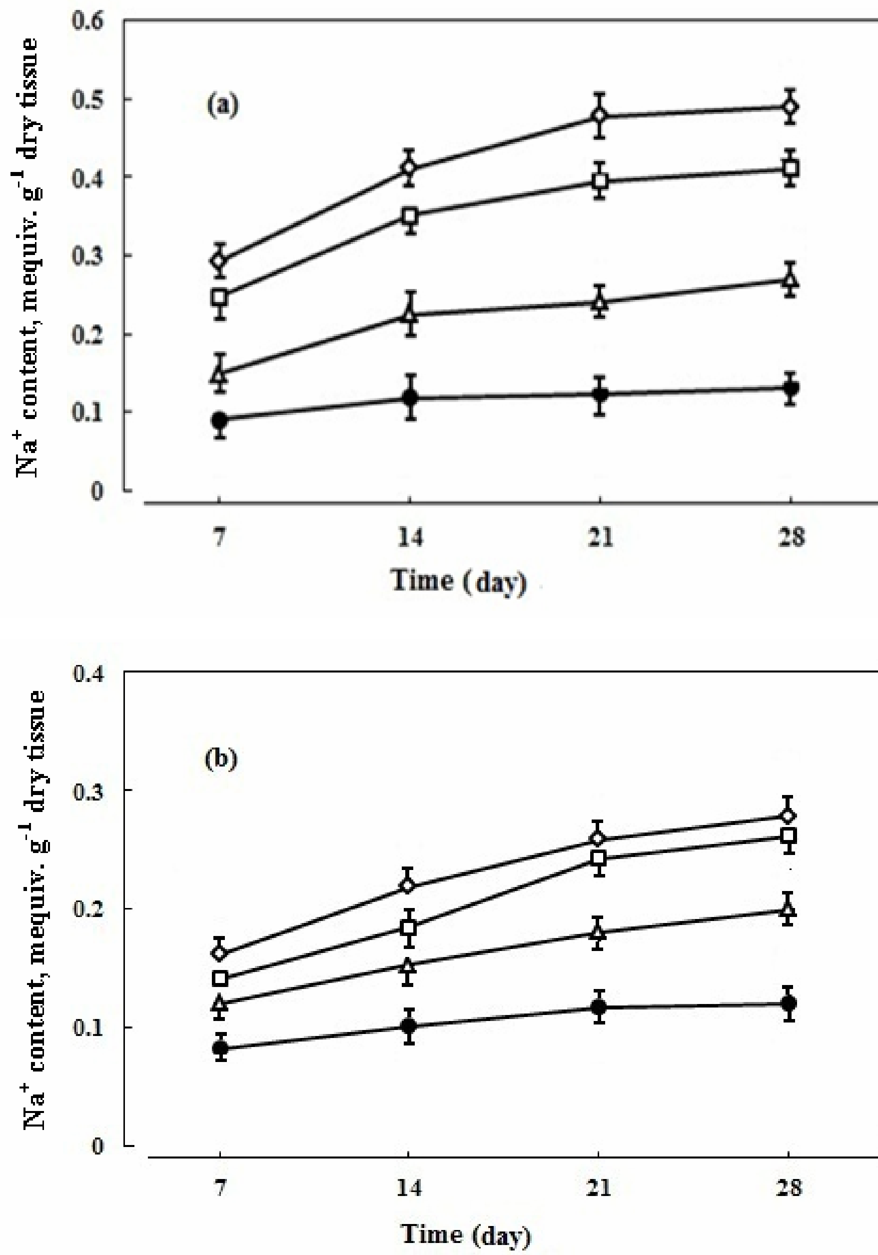


Fig. 33. The effect of different concentrations of aluminium on the accumulation of Na^+ in the (a) root and (b) shoot of rice plants grown in sand culture. Otherwise as Fig. 31.

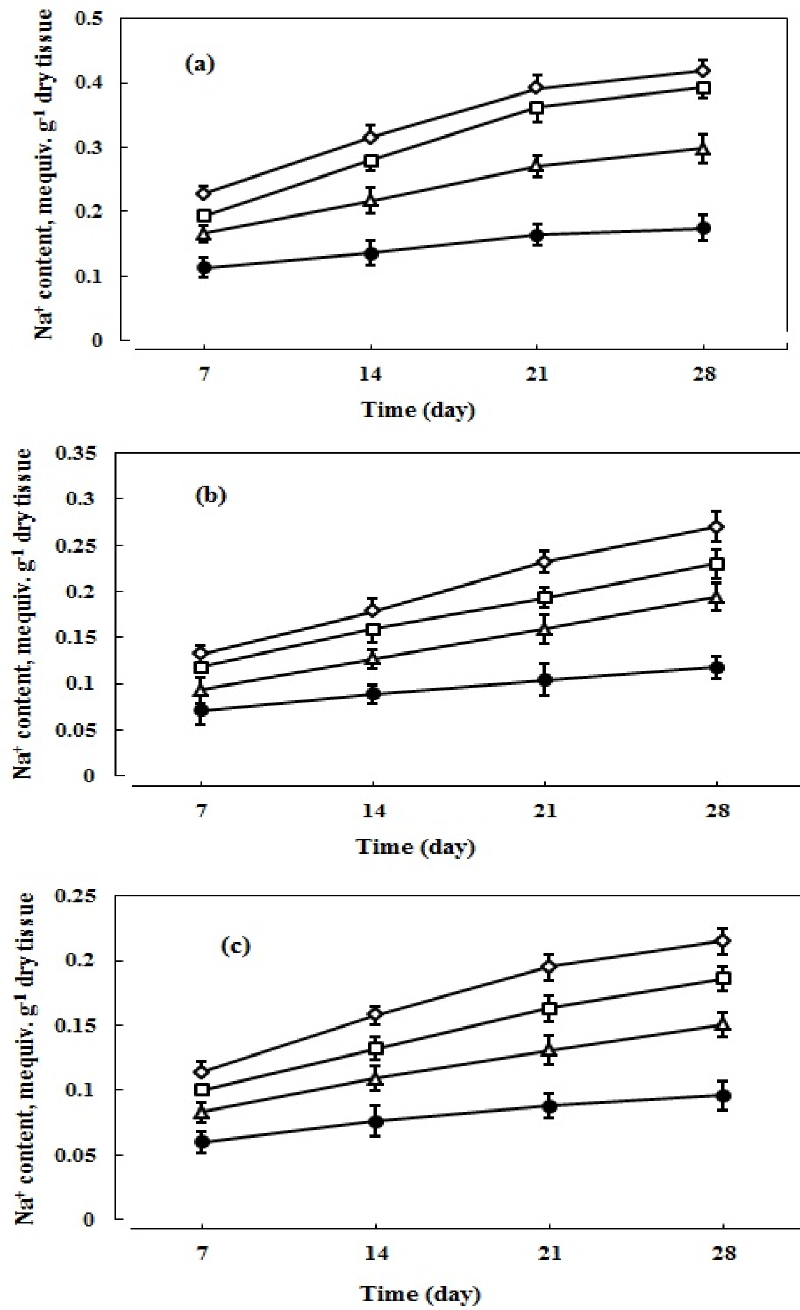


Fig. 34. The effect of different concentrations of aluminium on the accumulation of Na^+ in the (a) root, (b) stem and (c) leaves of chickpea plants grown in sand culture. Otherwise as Fig. 31.

In the leaves of chickpea, 50 μM Al increased Na^+ accumulation by 38.3 to 57.3% from 7 to 28 day of application. 100 and 150 μM Al enhanced Na^+ uptake by 66.7 to 93.8% and 90.0% to 2-fold, respectively, from 7 to 28 day of treatment (Fig. 34c).

Effects of aluminium toxicity on the accumulation and distribution of Cl^- in rice and chickpea plants grown in sand culture: Al (50 μM) increased Cl^- accumulation in the root of rice plants from 31.5 to 49.0% from 7 to 28 day of treatment. 100 μM Al caused a 58.0 to 74.0% increase in Cl^- content in the root from 7 to 28 day of application. A maximum 80.8 to 87.0% stimulation of Cl^- occurred in the root following 150 μM Al treatment from 7 to 28 day of application (Fig. 35 a).

In the shoot of rice plants, 50 μM Al increased Cl^- accumulation by 38.7% to 2-fold from 7 to 28 day of treatment. 100 μM Al caused a 64.9% to 2.1-fold stimulation of Cl^- content from 7 to 28 day of application. Similarly, 150 μM Al caused the highest 82.0% to 2.3-fold increase in Cl^- accumulation in the shoot from 7 to 28 day of treatment (Fig. 35b).

Different concentrations of Al (50, 100 and 150 μM) increased the accumulation of Cl^- in the root of chickpea plants. 50 μM Al increased Cl^- accumulation in the root by 21.8 to 96.8% from 7 to 28 day of treatment. Al, at a concentration of 100 μM, increased Cl^- accumulation in the root by 2.1-, 2.2- and 2.3-fold at 14, 21 and 28 day of application respectively. 150 μM Al caused the maximum accumulation of 2- to 2.7-fold in the root of chickpea from 7 to 28 day of treatment (Fig. 36a).

Al, at a concentration of 50 μM, increased Cl^- accumulation in the stem by 22.5 to 48.3% from 7 to 28 day of treatment. 100 and 150 μM Al caused a 52.8 to 78.0% and 86.0% to 2-fold increase in Cl^- accumulation in the stem, respectively, from 7 to 28 day of application (Fig. 36b).

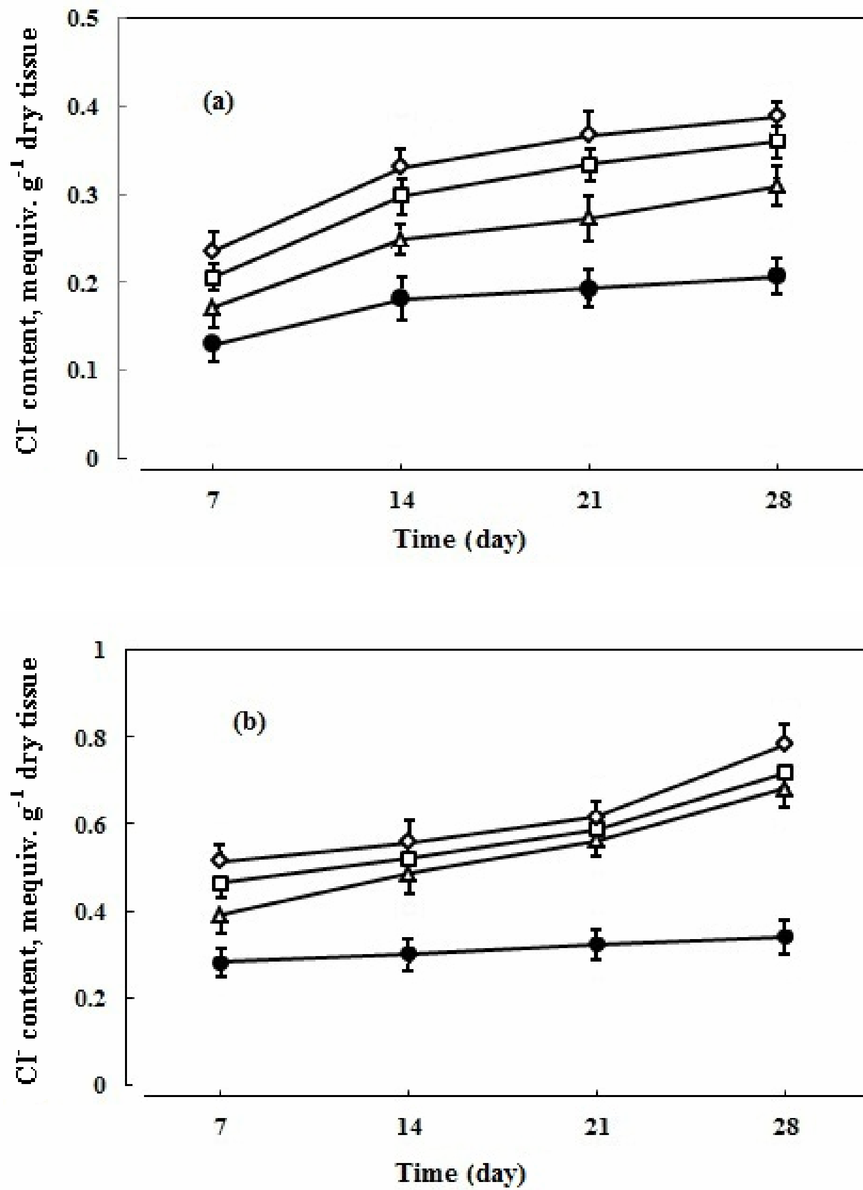


Fig. 35. The effect of different concentrations of aluminium on the accumulation of Cl^- in the (a) root and (b) shoot of rice plants grown in sand culture. Otherwise as Fig. 31.

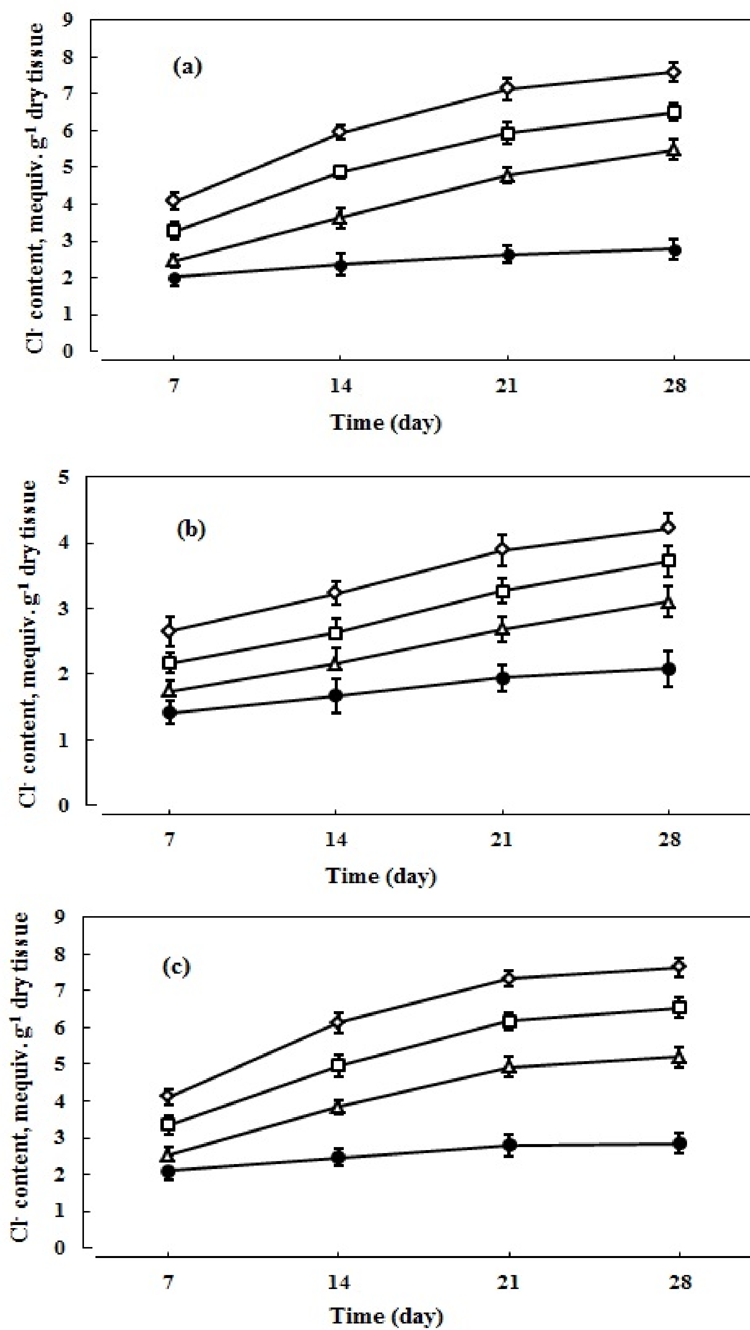


Fig. 36. The effect of different concentrations of aluminium on the accumulation of Cl^- in the (a) root, (b) stem and (c) leaves of chickpea plants grown in sand culture. Otherwise as Fig. 31.

Al (50 μM) increased accumulation of Cl^- in the leaves from 20.6 to 82.1% from 7 to 28 day of treatment. 100 and 150 μM Al caused a 2- to 2.3-fold and 2.5- to 2.7-fold increase in Cl^- accumulation in the leaves, respectively, from 14 to 28 day of application (Fig. 36c).

Effects of aluminium toxicity on Ca^{2+} accumulation in the root of rice and chickpea plants grown in sand culture: 50 μM Al progressively decreased accumulation of Ca^{2+} in the root of rice from 7 to 28 day of treatment (Fig. 37a). A 35.0 to 70.0% inhibition of Ca^{2+} content was observed in the root of rice exposed to 100 μM Al from 7 to 28 day of exposure. 150 μM Al caused a 61.0 to 74.0% reduction in Ca^{2+} content in the root from 7 to 28 day of application (Fig. 37a).

In the shoot of rice plants, 50 μM Al decreased Ca^{2+} content by 15.8 to 41.9% from 7 to 28 day of treatment. At a concentration of 100 μM Al, a 29.5 to 52.9% inhibition of Ca^{2+} content in the shoot was observed from 7 to 28 day of application. The maximum inhibition of Ca^{2+} accumulation in the shoot ranging from 34.0 to 60.0% was exerted by 150 μM Al from 7 to 28 day of treatment (Fig. 37b).

In chickpea plants, 50 μM Al decreased Ca^{2+} accumulation in the root by 28.5 to 51.5% from 7 to 28 day of treatment. 100 and 150 μM Al resulted in an inhibition of Ca^{2+} accumulation by 35.8 to 60.2% and 57.0 to 66.8% in the root, respectively, from 7 to 28 day of application (Fig. 38a).

In the stem of chickpea, 50, 100 and 150 μM Al caused an inhibition of Ca^{2+} accumulation by 33.0 to 52.8%, 53.9 to 40.0% and 61.5 to 70.4%, respectively, from 7 to 28 day of application (Fig. 38b).

Al (50 μM) decreased Ca^{2+} accumulation by 29.0 to 51.0% in the leaves from 7 to 28 day of treatment. 100 and 150 μM Al caused a 39.4 to 60.3% and 64.6 to 77% inhibition of Ca^{2+} in the leaves from 7 to 28 day of application (Fig. 38c).

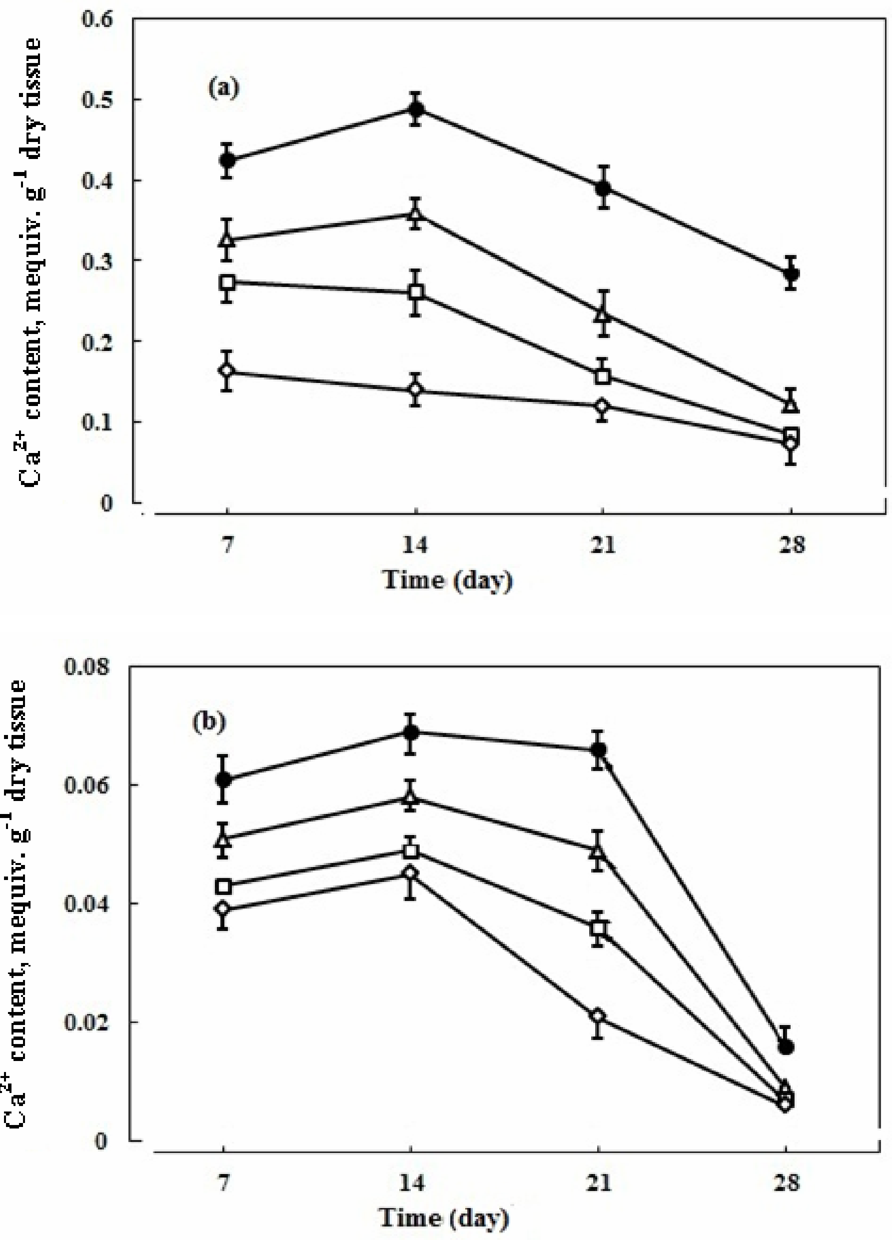


Fig. 37. The effect of different concentrations of aluminium on the accumulation of Ca^{2+} in the (a) root and (b) shoot of rice plants grown in sand culture. Otherwise as Fig. 31.

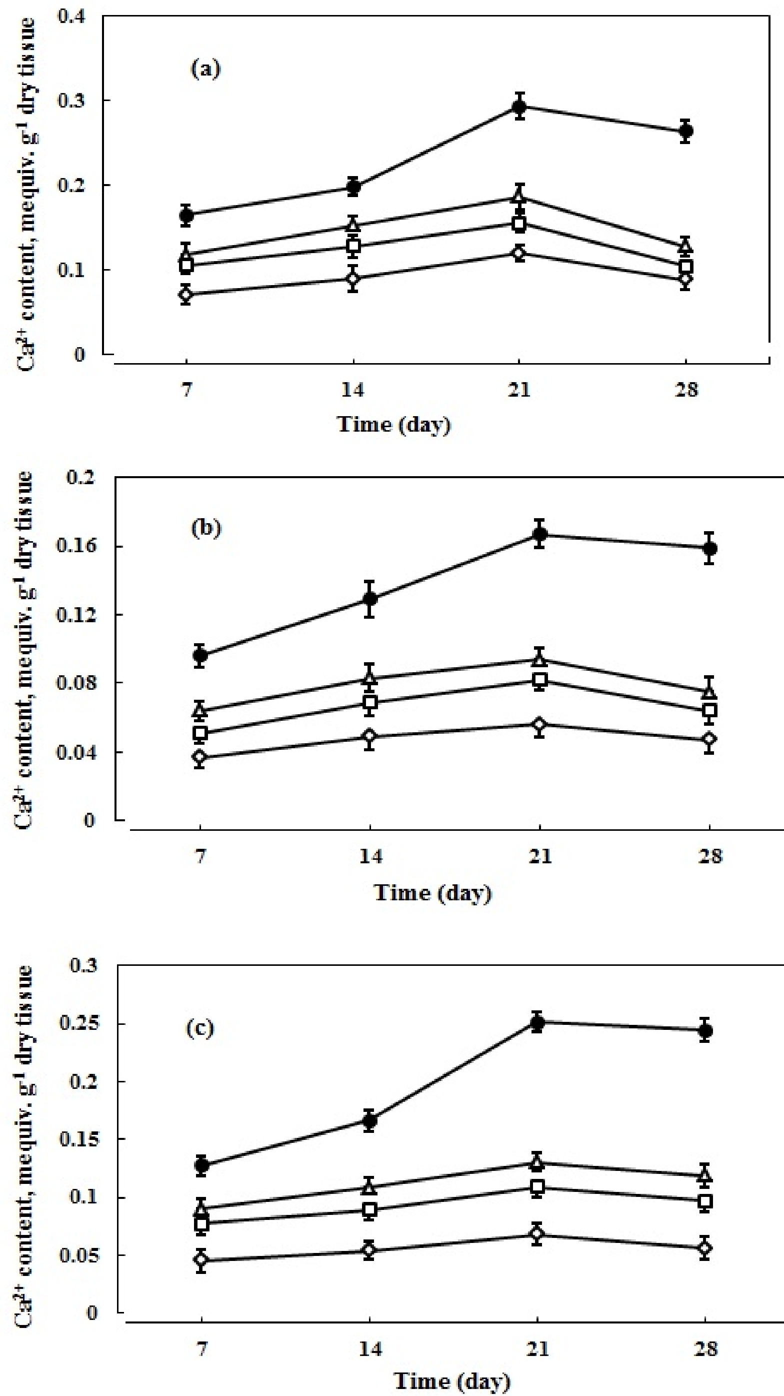


Fig. 38. The effect of different concentrations of aluminium on the accumulation of Ca^{2+} in the (a) root, (b) stem and (c) leaves of chickpea plants grown in sand culture. Otherwise as Fig. 31.

Effects of aluminium toxicity on accumulation and distribution of Mg^{2+} in rice and chickpea plants grown in sand culture: Al, at a concentration of 50 µM, decreased Mg^{2+} accumulation in the root of rice by 28.0 to 56.7% from 7 to 28 day of treatment. Similarly 100 µM Al inhibited Mg^{2+} content in the root by 39.8 to 66.0% from 7 to 28 day of application. 150 µM Al caused the highest 52.0 to 75.0% inhibition of Mg^{2+} accumulation in the root from 7 to 28 day of treatment (Fig. 39a).

In the shoot of rice, 50 µM Al inhibited Mg^{2+} accumulation by 21.0 to 52.9% from 7 to 28 days of treatment. The inhibitory effect increased with the increase in Al concentration from 100 to 150 µM. For example, 100 µM Al resulted in 32.8 to 59.9% inhibition of Mg^{2+} accumulation in the shoot from 7 to 28 day of exposure. The degree of inhibition was even more at a concentration of 150 µM Al where a 42.7 to 77.0% inhibition of Mg^{2+} accumulation was recorded from 7 to 28 day of application (Fig. 39b).

In chickpea plants, 50 µM Al decreased the accumulation of Mg^{2+} in the root by 19.3 to 47.9% from 7 to 28 day of treatment. Al, at concentrations of 100 and 150 µM, inhibited Mg^{2+} content by 63.7 to 72.8% and 44.4 to 88.8% in the root, respectively, from 7 to 24 day of application (Fig. 40a).

Al (50 µM) inhibited Mg^{2+} accumulation by 18.0 to 47.8% in the stem of chickpea from 7 to 28 day of treatment. 100 and 150 µM Al decreased the accumulation of Mg^{2+} by 28.8 to 67.0% and 36.0 to 85.0% in the stem, respectively, from 7 to 28 day of application (Fig. 40b).

Al, at concentrations of 50, 100 and 150 µM, inhibited the accumulation of Mg^{2+} by 22.0 to 42.8%, 37.7 to 60.6% and 47.7 to 67.6% in the leaves, respectively, from 7 to 28 day of treatment (Fig. 40c).

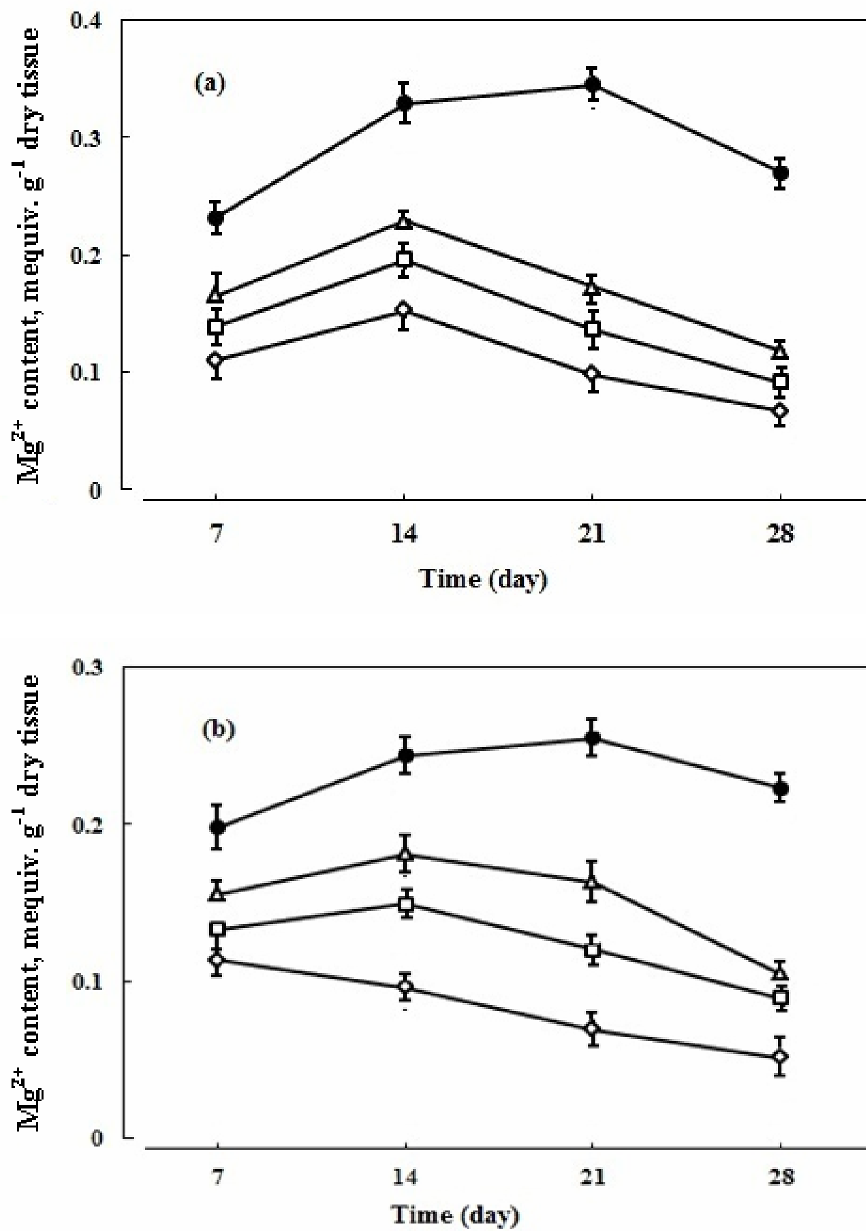


Fig. 39. The effect of different concentrations of aluminium on the accumulation of Mg^{2+} in the (a) root and (b) shoot of rice plants grown in sand culture. Otherwise as Fig. 31.

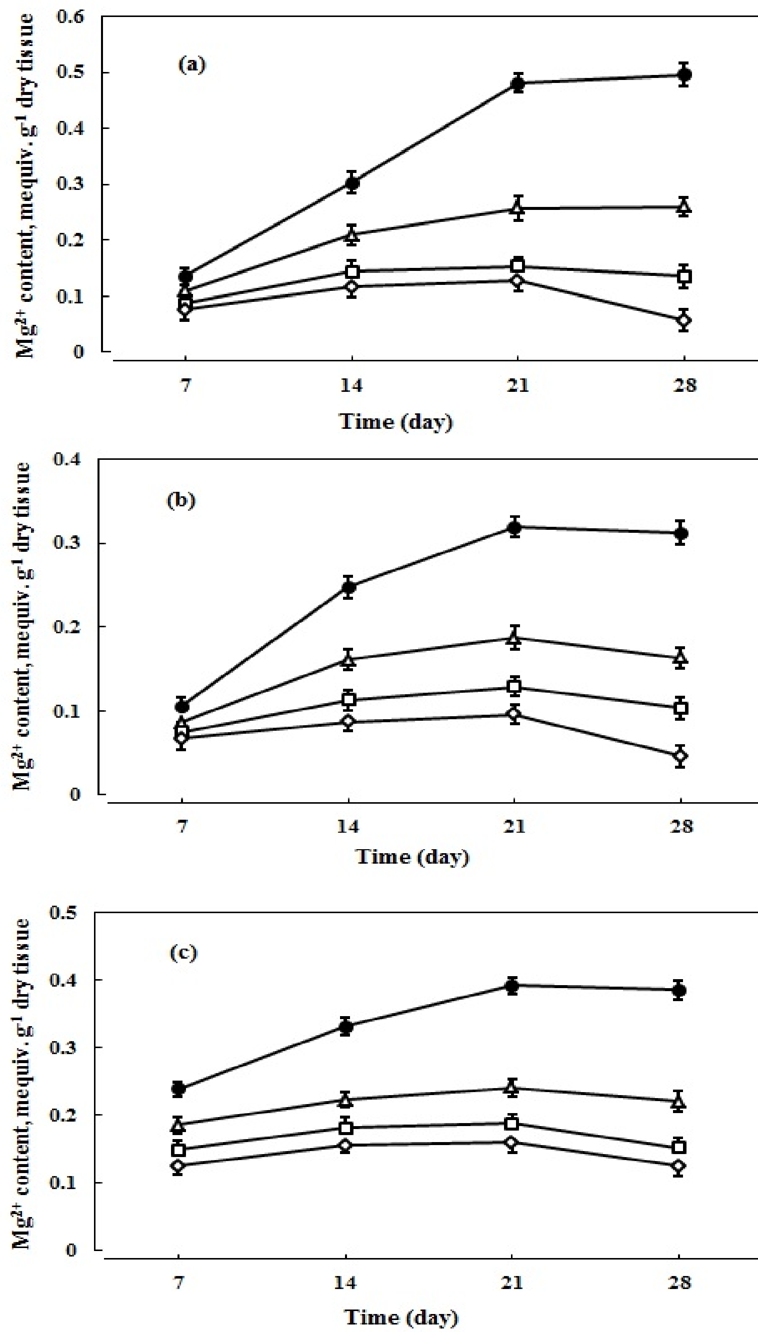


Fig. 40. The effect of different concentrations of aluminium on the accumulation of Mg^{2+} in the (a) root, (b) stem and (c) leaves of chickpea plants grown in sand culture. Otherwise as Fig. 31.

Effects of aluminium toxicity on the accumulation and distribution of Fe^{2+} in rice and chickpea plants grown in sand culture: Accumulation of Fe^{2+} in the root of rice progressively decreased from 22.0 to 41.7% when subjected to 50 µM Al from 7 to 28 day of treatment. 100 µM Al caused a 35.0 to 71.6% inhibition of Fe^{2+} in the root from 7 to 28 day of exposure. Maximum inhibition of Fe^{2+} accumulation was recorded in the root of rice seedling grown in 150 µM Al which ranged from 55.5 to 85.9% from 7 to 28 day of application (Fig. 41a).

In shoot of rice plants, 50 µM Al caused 30.0 to 61.8% reduction of Fe^{2+} content from 7 to 28 day of treatment. A 50.0 to 82.7% and 63.8 to 91.5% inhibition of Fe^{2+} in the shoot was recorded following 100 and 150 µM Al treatment, respectively, from 7 to 28 day of application (Fig. 41b).

In chickpea plants, 50 µM Al decreased the accumulation of Fe^{2+} in the root by 28.6 to 45.6% from 7 to 28 day of application. 100 and 150 µM Al caused 42.9 to 67.0% and 54.8 to 74.7%, respectively, in the root from 7 to 28 day of treatment (Fig. 42a).

In the stem of chickpea, 50, 100 and 150 µM Al caused 35.0 to 50.7%, 46.0 to 67.6% and 59.5 to 76.0% inhibition in the accumulation of Fe^{2+}, respectively, from 7 to 28 day of application (Fig. 42b).

Al, at a concentration of 50 µM, decreased Fe^{2+} accumulation in the leaves by 16.0 to 43.6% from 7 to 28 day of treatment. 100 and 150 µM Al inhibited the accumulation of Fe^{2+} in the leaves by 40.0 to 63.6% and 56.0 to 78.0%, respectively, from 7 to 28 day of exposure (Fig. 42c).

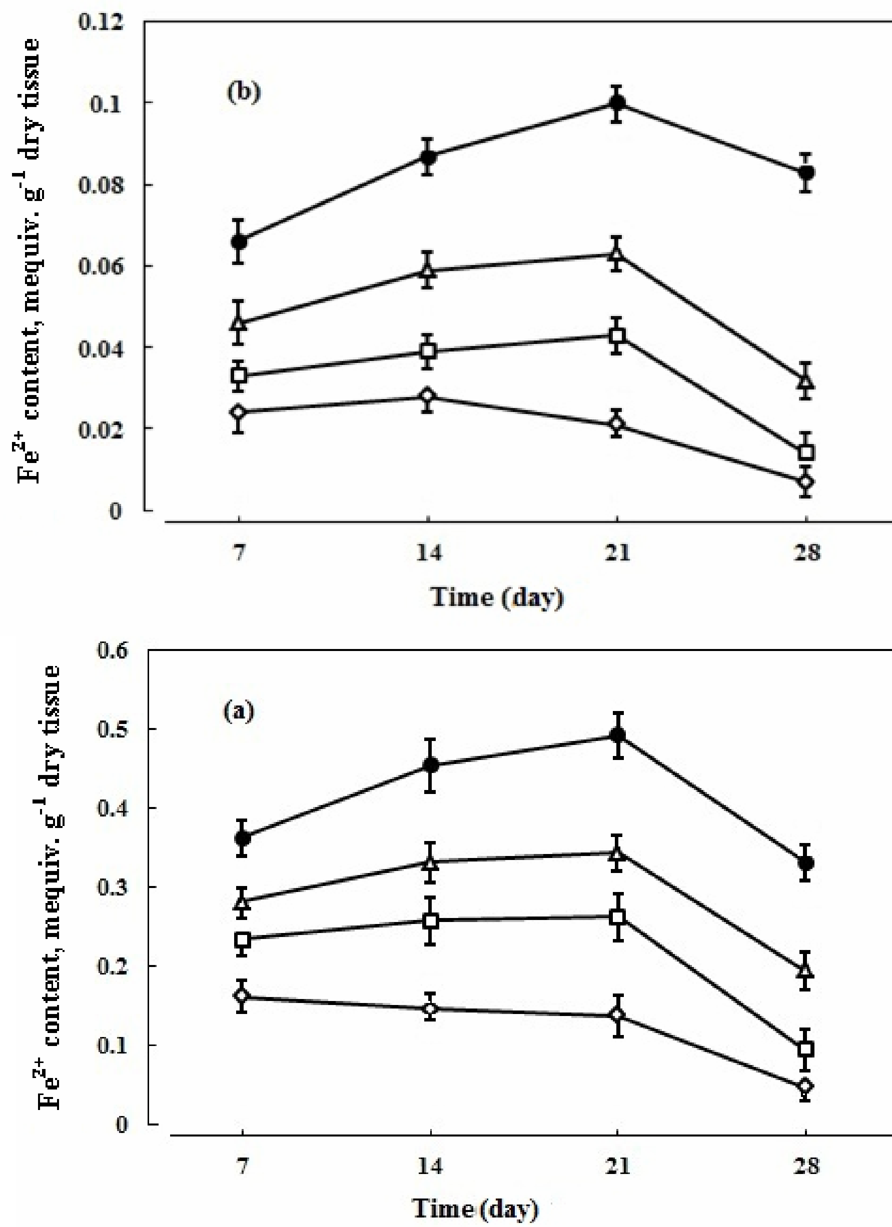


Fig. 41. The effect of different concentrations of aluminium on the accumulation of Fe^{2+} in the (a) root and (b) shoot of rice plants grown in sand culture. Otherwise as Fig. 31.

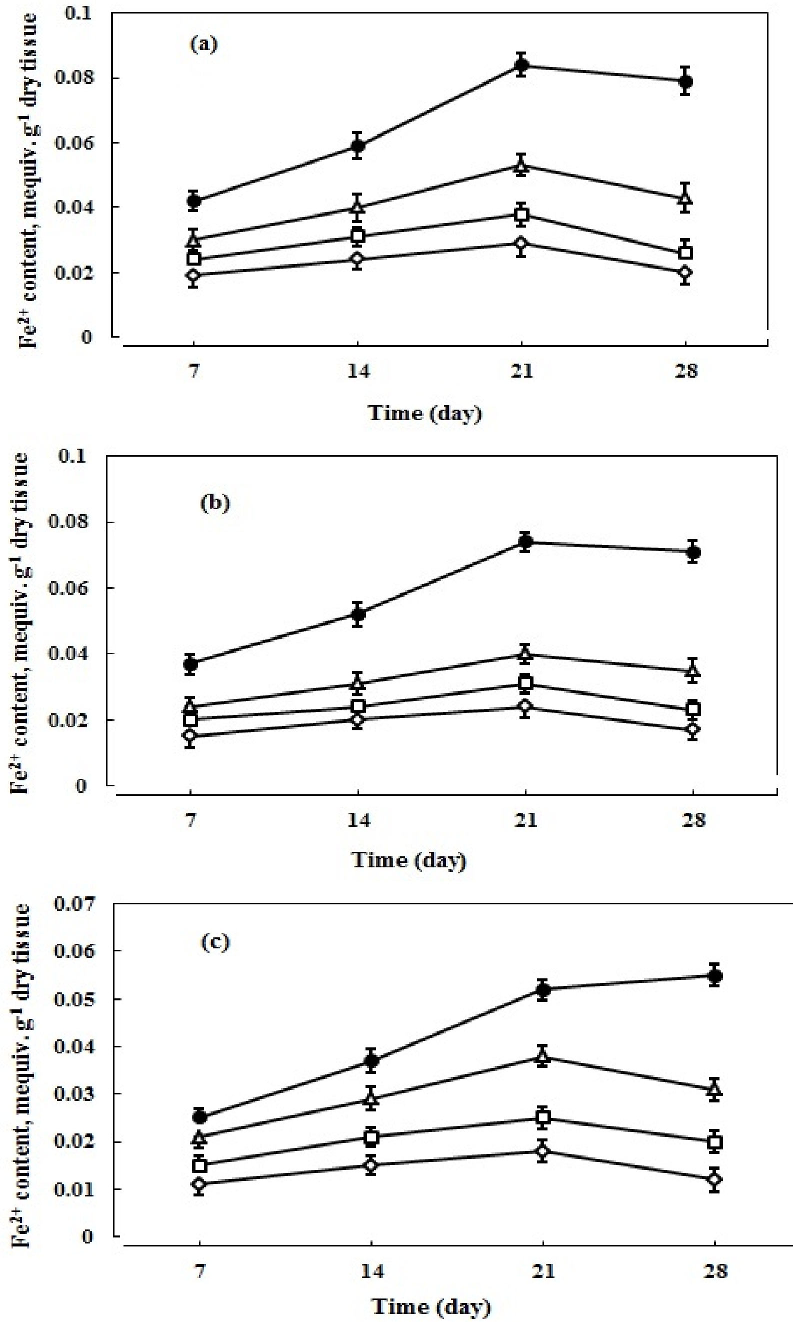


Fig. 42. The effect of different concentrations of aluminium on the accumulation of Fe^{2+} in the (a) root, (b) stem and (c) leaves of chickpea plants grown in sand culture. Otherwise as Fig. 31.

4b.4 Discussion

Aluminium toxicity decreased the accumulation of K^+ and increased that in Na^+ and Cl^- in both rice and chickpea plants grown in sand culture (Figs. 31 to 36). Al-toxicity-induced decrease in K^+ uptake with concomitant increase in that of Na^+ indicates that Al stress alters K^+/Na^+ selectivity.

Aluminium stress decreased Ca^{2+}, Mg^{2+} and Fe^{2+} accumulation in rice and chickpea plants grown in sand culture (Figs. 37 to 42). Similarly, Al stress decreased the accumulation of Ca and Mg in wheat (Foy 1996) and Fe in sorghum (Clark *et al.* 1981).

4c. Reconcilliation of the results of the effect of aluminium toxicity on ion transport in plants grown in solution culture with that in plants grown in sand culture

Results on the effect of aluminium toxicity on K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{2+} and Cl^- in rice and chickpea seedlings grown in solution culture under controlled environmental conditions (section 4a) may be reconciled with that in plants grown in sand culture under natural environmental conditions (section 4b). For example, aluminium toxicity decreased the accumulation of K^+ in the root of rice seedlings grown in solution culture (Fig. 13a) while it caused a decrease in accumulation of K^+ in the root of rice plants grown in sand culture (Fig. 31a). Aluminium toxicity inhibited K^+ accumulation in the shoot of rice seedling grown in solution culture (Fig. 13b) while it decreased that of K^+ in the shoot of rice plants grown in sand culture (Fig. 31b).

Aluminium toxicity increased Na^+ content in the root of rice seedlings grown in solution culture (Fig. 15a) while it increased Na^+ content in the root in rice plants grown in sand culture (Fig. 33a). Aluminium toxicity increased Na^+ accumulation in the shoot of rice seedlings grown in solution culture (Fig. 15b) while it increased Na^+ content in the shoot of rice plants grown in sand culture (Fig. 33b).

Aluminium toxicity increased Cl^- content in the root of rice seedlings grown in solution culture (Fig. 17a) while it increased Cl^- content in the root of rice plants grown in sand culture (Fig. 35a). Al toxicity increased Cl^- accumulation in the shoot of rice seedlings grown in solution culture (Fig. 17b) while it increased Cl^- content in shoot of rice plants grown in sand culture (Fig. 35b).

Aluminium toxicity caused a decrease in Ca^{2+} accumulation in the root of rice seedlings grown in solution culture (Fig. 21a) while it inhibited Ca^{2+} content in the root of rice plants grown in sand culture (Fig. 37a). Al toxicity inhibited Ca^{2+} content in the shoot of rice seedlings grown in solution culture (Fig 21b) while in deceased Ca^{2+} accumulation in the shoot of rice plants grown in sand culture (Fig. 37b).

Aluminium toxicity inhibited the accumulation of Mg^{2+} in the root of rice seedlings grown in solution culture (Fig. 23a) while it decreased Mg^{2+} content in the root of rice plants grown in sand culture (Fig. 39a). Aluminium toxicity decreased Mg^{2+} content in the shoot of rice seedlings grown in solution culture (Fig. 23b) while it inhibited Mg^{2+} accumulation in the shoot of rice plants grown in sand culture (Fig. 39b).

Aluminium toxicity decreased the accumulation of Fe^{2+} in the root of rice seedlings grown in solution culture (Fig. 25a) while it inhibited Fe^{2+} content in the root of rice plants grown in sand culture (Fig. 41a). Al toxicity decreased Fe^{2+} content in the shoot of rice seedlings grown in solution culture (Fig. 25b) while it inhibited Fe^{2+} accumulation in the shoot of rice plants grown in sand culture (Fig. 41b).

Similar aluminium toxicity-induced inhibition of K^+, Ca^{2+}, Mg^{2+} and Fe^{2+} and stimulation of Na^+ and Cl^- transport was also observed in the root and shoot of aluminium-stressed chickpea plants grown both in solution (Fig. 14, 16, 18, 22, 24 and 26) and sand culture (Fig. 32, 34, 36, 38, 40 and 42).

Therefore, it may be concluded from the comparison outlined above that the results obtained in the laboratory on the effect of aluminium toxicity on the transport of K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+} and Fe^{2+} in rice and chickpea seedlings grown in solution culture under controlled environment might be reconciled with that in plants grown in sand culture under natural environmental conditions.

Chapter 5

Effects of aluminium toxicity on biochemical changes in rice and chickpea seedlings

5.1 Introduction

5.1.1 Effects of aluminium toxicity on reducing sugar, total sugar, protein, proline and total amino acid contents: The concentration of glucose was found to increase in Al treated root of *Quercus serrata* (Moriyama *et al.* 2016). Al increased soluble sugar in leaves of bean (Khavarinejad *et al.* 2010). Soluble protein was not affected in root and shoot of *Matricaria chamomilla* following Al application (Kováčik *et al.* 2010).

Aluminium toxicity increased proline content in sunflower (Ziaei *et al.* 2014). The proline content was found to be induced by aluminium particularly at 48, 72 and 120 h of seed germination in *Pigeon pea* (Bhamburdekar and Chavan 2011).

Al reduced total soluble protein content in sorghum (da Cruz *et al.* 2011).

5.1.2 Effects of aluminium toxicity on antioxidant enzymes, phenolic compounds, chlorophyll and carotenoid contents: Al stimulated the activity of SOD and Catalase in wheat cultivars (Nasr *et al.* 2011). SOD activity in roots of *Arabidopsis* was enhanced by Al treatment (Richards *et al.* 1998).

Roots of maize exposed to Al exuded 20-fold more phenolics than organic anions (Kidd *et al.* 2001). Al increased total soluble phenols in the shoot of *Matricaria chamomilla* plants (Kováčik *et al.* 2010).

Chlorophyll content of leaves of green melon was decreased following 74-296 μM Al treatment (Symeonidis *et al.* 2004).

Al decreased carotenoid content in sunflower (Ziaei *et al.* 2014).

5.2 Materials and Methods

5.2a Methods of growing plants and extraction and determination of reducing sugar, total sugar, proline, total amino acid, protein and enzymes in rice and chickpea seedlings grown in solution culture

5.2a.1 Methods of growing plants for determination of reducing sugar, total sugar, proline, total amino acid, protein and enzymes: Rice and chickpea seedlings were grown in solution culture according to the method described in section 2.6. Aluminium treatments (10, 50, 100 and 150 µM) (pH 4.2) were applied to 7-day-old seedlings. Half-strength Hoagland solution (pH 4.2) was used as control. Samples were collected after 3, 6, 24, 48, 72 and 96 h of treatment. The root and shoot of rice, and root, stem and leaves of chickpea were separated and fresh weights were recorded before the extraction of tissue.

5.2a.2 Methods of extraction and determination of reducing sugar, total sugar, proline, total amino acid, protein and enzymes: Reducing and total sugar, proline, total amino acid, protein, antioxidant enzymes were extracted and determined in the root, stem and leaves tissue collected from seedlings grown in solution culture according to the following methods:

5.2a.2.1 Extraction and determination of reducing and total sugar: Reducing and total sugar were extracted from the fresh samples with alcohol following the procedure outlined in section 2.13. Reducing and total sugar were determined by Somogyi-Nelson (Nelson 1944 and Somogyi 1952) method (section 2.13.2) and Dubois *et al.* (1956) method (section 2.13.3) respectively.

5.2a.2.2 Extraction and determination of proline: Extraction of proline from fresh plant tissue was carried out according to the process outlined in section 2.14.1. Determination of proline was done according to the method of Bates *et al.* (1973) as described in section 2.14.2.

5.2a.2.3 Extraction and determination of total amino acid: Extraction of total amino acid from fresh plant tissue was done with 80% ethanol according to the method described in section 2.15.1. For the assay of total amino acid, ninhydrin reagent was used following the method of Lee and Takahasi (1966) according to section 2.15.2.

5.2a.2.4 Extraction and determination of protein: Extraction of soluble protein was done from fresh plant tissue according to the method outlined in section 2.16.1.

Protein was determined by the method of Lowry *et al.* (1951) as described in section 2.16.2.

5.2a.2.5 Extraction and determination of antioxidant enzymes: Extraction of different antioxidant enzymes was done according to the method described in section 2.17.1.

Different antioxidant enzymes such as peroxidase (POD), catalase and superoxide dismutase (SOD) activities were assayed as described by Zhang *et al.* (1995), Barber (1980) and Zhang *et al.* (2005) respectively according to section 2.17.2.

5.2b Methods of growing plants and extraction and determination of phenolic compounds, chlorophyll a, chlorophyll b and carotenoid contents in rice and chickpea plants grown in sand culture

5.2b.1 Methods of growing plants for determination of phenolic compounds, chlorophyll a, chlorophyll b and carotenoid contents: Plants were grown in sand culture in natural environmental conditions according to section 2.9. Aluminium stress was applied to 7-days-old plants according to the method as described in section 2.10. Samples were collected at 7, 14, 21 and 28 day of treatment. The root and shoot of rice and the root, stem and leaves of chickpea were separated and the fresh weight of the samples were recorded.

5.2b.2 Methods of extraction and determination of phenolic compounds, chlorophyll a, chlorophyll b and carotenoid contents: Phenolic compounds, chlorophyll a, chlorophyll b and carotenoids were extracted and determined in the root, stem and leaves tissue collected from plants grown in sand culture according to the following methods:

5.2b.2.1 Extraction and determination of phenolic compounds: Phenolic compounds were extracted in 80% ethanol according to the method described in section 2.18.1. Phenolic compounds were determined by the method of Malik and Singh (1980) as outlined in section 2.18.2.

5.2b.2.2 Extraction and determination of leaf pigments: Chlorophyll a, b and carotenoid contents in the leaves were extracted of 25 ml cold 80% acetone according to the method described in section 2.19.1.

Specific absorption co-efficient method of Mckinney (1940) and the formula of Maclachlan and Zalik (1963) were used to determine the amounts of chlorophyll a and b. Formulae used was outlined in section 2.19.2.

The amount of carotenoids was determined by the equation of von Wettstein (1957). The equation was outlined in section 2.19.2.

5.3 Results

5.3a Effects of aluminium toxicity on reducing and total sugar, proline and total amino acid and protein contents in rice and chickpea seedlings grown in solution culture

Effects of aluminium toxicity on the accumulation of reducing sugar in rice and chickpea seedlings grown in solution culture: Al (10 to 150 μM) increased reducing sugar content in the root of rice from 48 to 96 h of treatment except an initial inhibition. The degree of stimulation increased with the increase in Al concentration from 10 to 150 μM. The maximum stimulation of reducing sugar

accumulation in the root of rice ranged from 71.0% to 2.6-fold following 150 μM Al application over a period of 48 to 96 h of exposure (Fig. 43a).

In the shoot of rice, Al (10-150 μM) increased the reducing sugar content except an initial inhibition (Fig. 43b). 150 μM Al increased reducing sugar content in the shoot by 69.0 and 67.9% at 72 and 96 h of treatment, respectively, except a decrease in that by 29.0 to 46.8% from 6 to 48 h of treatment (Fig. 43b).

Al, at concentrations of 10, 50, 100 and 150 μM, increased reducing sugar content in the root of chickpea from 24 to 96 h of treatment. The stimulatory effects on reducing sugar content in the root was increased with increase in concentration of Al from 10 to 150 μM. Maximum stimulation of reducing sugar content was observed at 150 μM Al ranging from 57.8% to 2-fold from 24 to 96 h of treatment (Fig. 44a).

In the stem of chickpea, Al (10-150 μM) increased reducing sugar content from 48 to 96 h of treatment except an initial inhibition of that from 3 to 24 h of treatment. Al increased reducing sugar content from 23.5 to 46.7% from 48 to 96 h of treatment. Al (150 μM) caused a maximum of 55.0 to 93.0% stimulation of reducing sugar in the stem from 48 to 96 h of application (Fig. 44b).

In the leaves of chickpea, 10 μM Al increased reducing sugar content by 18.9 and 22.0% at 72 and 96 h of treatment, respectively, except a slight inhibition of that at 3 to 48 h of treatment. Similarly, a stimulation of reducing sugar content in the leaves was observed at 50, 100 and 150 μM Al treatment from 72 to 96 h of treatment except an inhibition of that from 3 to 48 h of application. 150 μM Al application resulted in a 38.0 to 41.0% inhibition of reducing sugar content in the leaves from 3 to 48 h but it increased that by 48.9 and 46.0% at 72 and 96 h of treatment respectively (Fig. 44c).

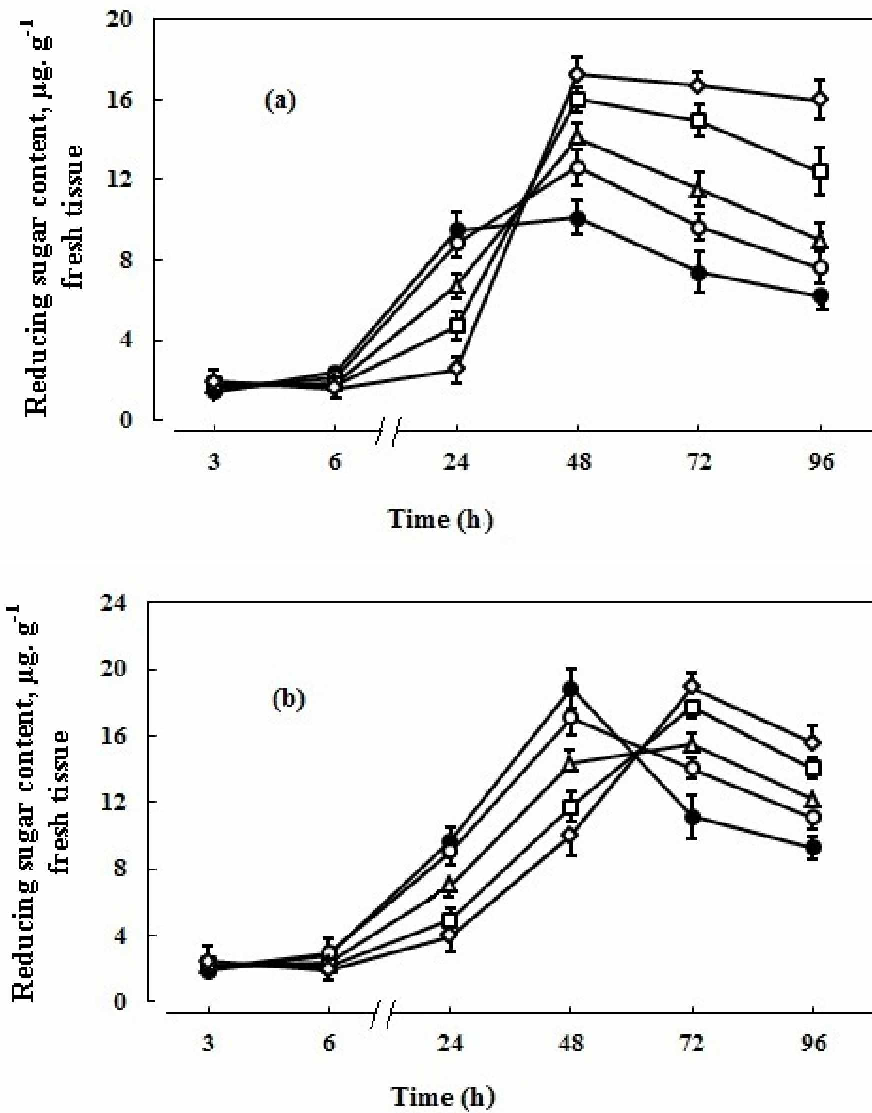


Fig. 43. The effect of different concentrations of aluminium on the accumulation of reducing sugar in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

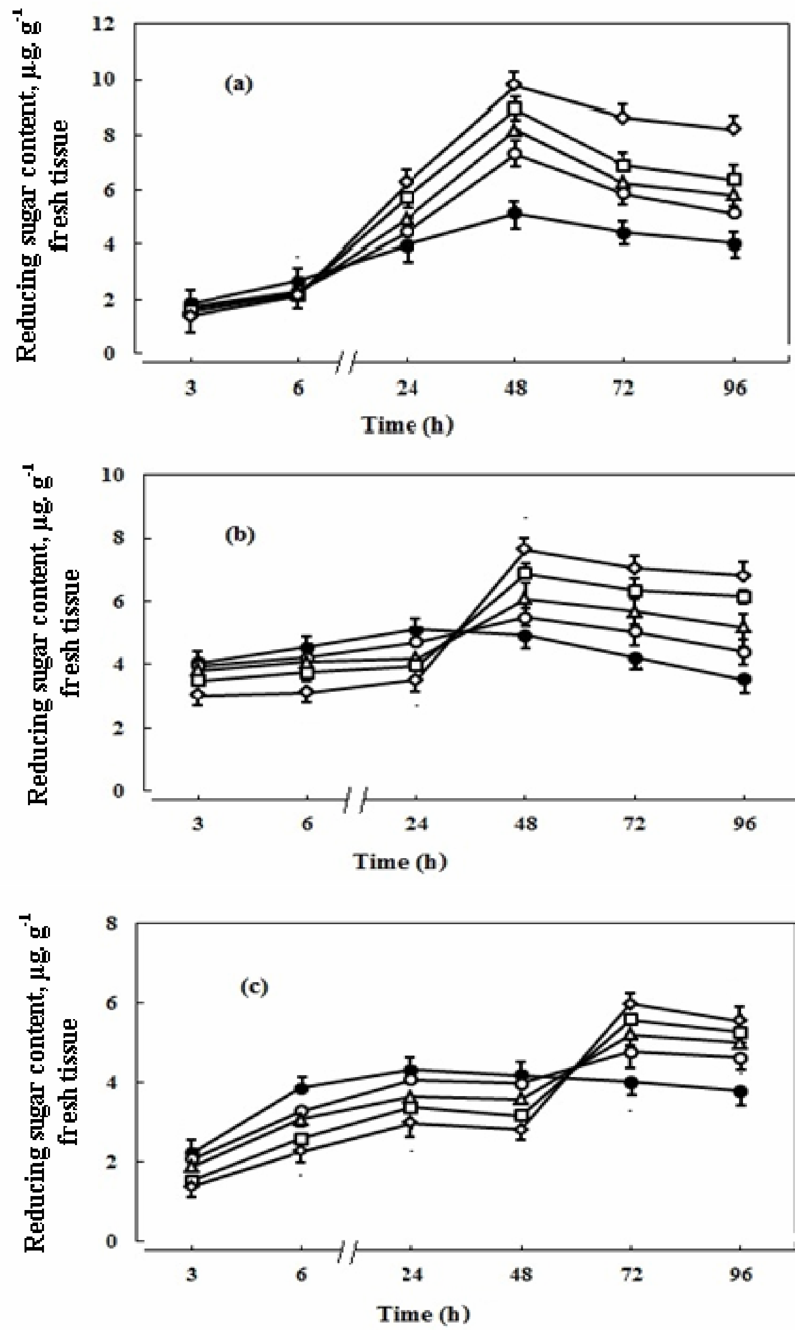


Fig. 44. The effect of different concentrations of aluminium on the accumulation of reducing sugar in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

Effects of aluminium toxicity on total sugar accumulation in rice and chickpea seedlings grown in solution culture: Al (10 to 150 µM) caused an increase in total sugar content in the root of rice from 24 to 96 h of treatment (Fig. 45a). The stimulatory effect increased with the increase in concentration of Al. At a concentration of 10 µM, aluminium increased total sugar in the root from 3.7 to 30.0% from 24 to 96 h of treatment. Stimulatory effect was the highest (28.7 to 86.0%) in root of rice seedlings exposed to 150 µM Al (Fig. 45a).

Al concentration as low as 10 µM, increased total sugar content in the shoot of rice by 11.8 to 21.0% from 24 to 96 h of treatment. The stimulation of total sugar content in the shoot gradually increased with the increase in Al concentration from 50 to 150 µM which ranged from 67.8 to 98.9% from 24 to 96 h of application (Fig. 45b).

In the root of chickpea seedlings, Al increased total sugar content with the increase in Al concentration from 10 to 150 µM from 24 to 96 h of treatment except a slight initial inhibition of that from 3 to 6 h of treatment (Fig. 46a). Low concentration of Al (10 µM) increased total sugar content of the root from 6 to 22% from 24 to 96 h of exposure. A 21.9 to 49.8% and 58.0 to 83.8% increase in total sugar content of the root was observed following 50 and 100 µM Al application respectively. 150 µM Al caused a maximum of 48.6% to 2-fold stimulation of total sugar content in the root from 24 to 96 h of application (Fig. 46a).

Al (10-150 µM) increased total sugar content in stem of chickpea from 24 to 96 h of treatment (Fig. 46b). The stimulatory effect of total sugar content increased with the increase in Al concentrations. A maximum of 25.0 to 66.0% stimulation of total sugar content was recorded in the stem of chickpea seedlings exposed to 150 µM Al (Fig. 46b).

In the leaves of chickpea seedlings, lowest concentration of Al (10 µM) increased total sugar content of root from 9.7 to 13.8% from 24 to 96 h of treatment (Fig. 46c). A 59.0% to 2.2-fold and 97.0% to 2.9-fold increase in total sugar content in the leaves of chickpea was recorded at 100 and 150 µM Al treatment respectively (Fig. 46c).

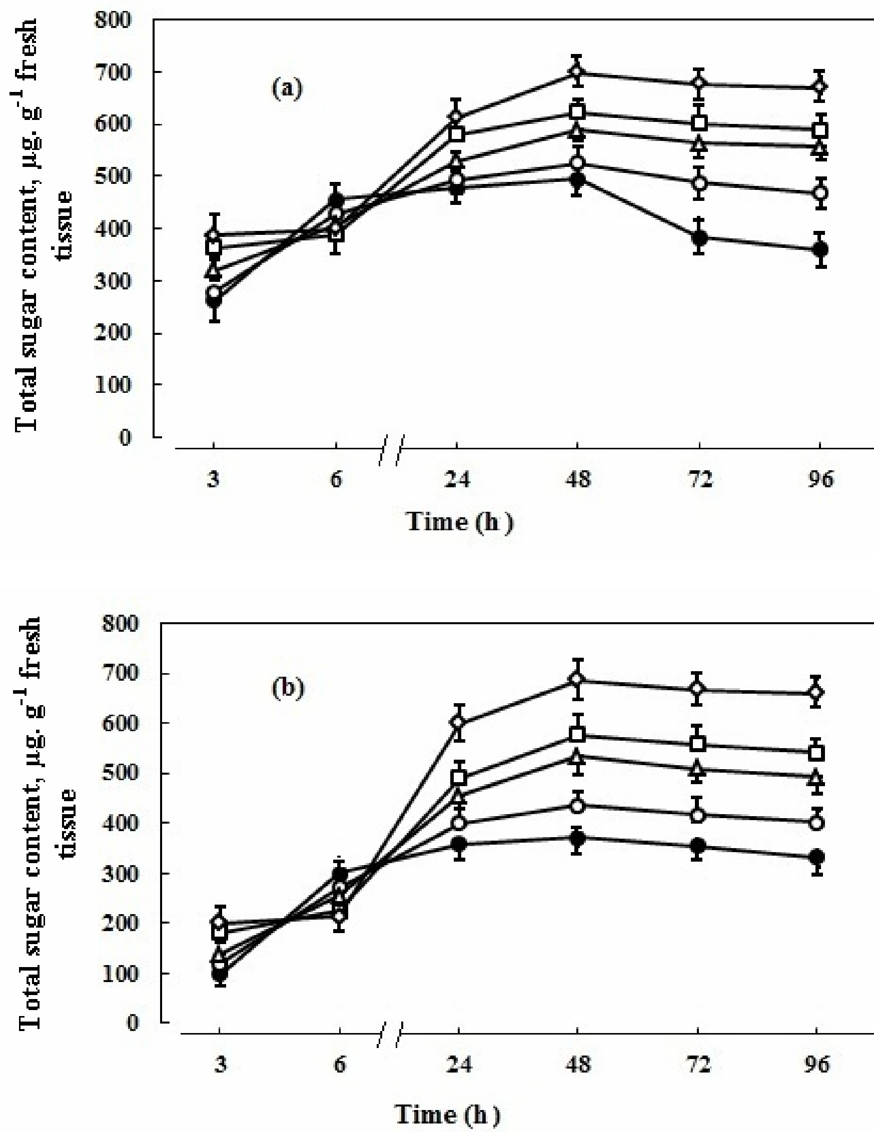


Fig. 45. The effect of different concentrations of aluminium on the accumulation of total sugar in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

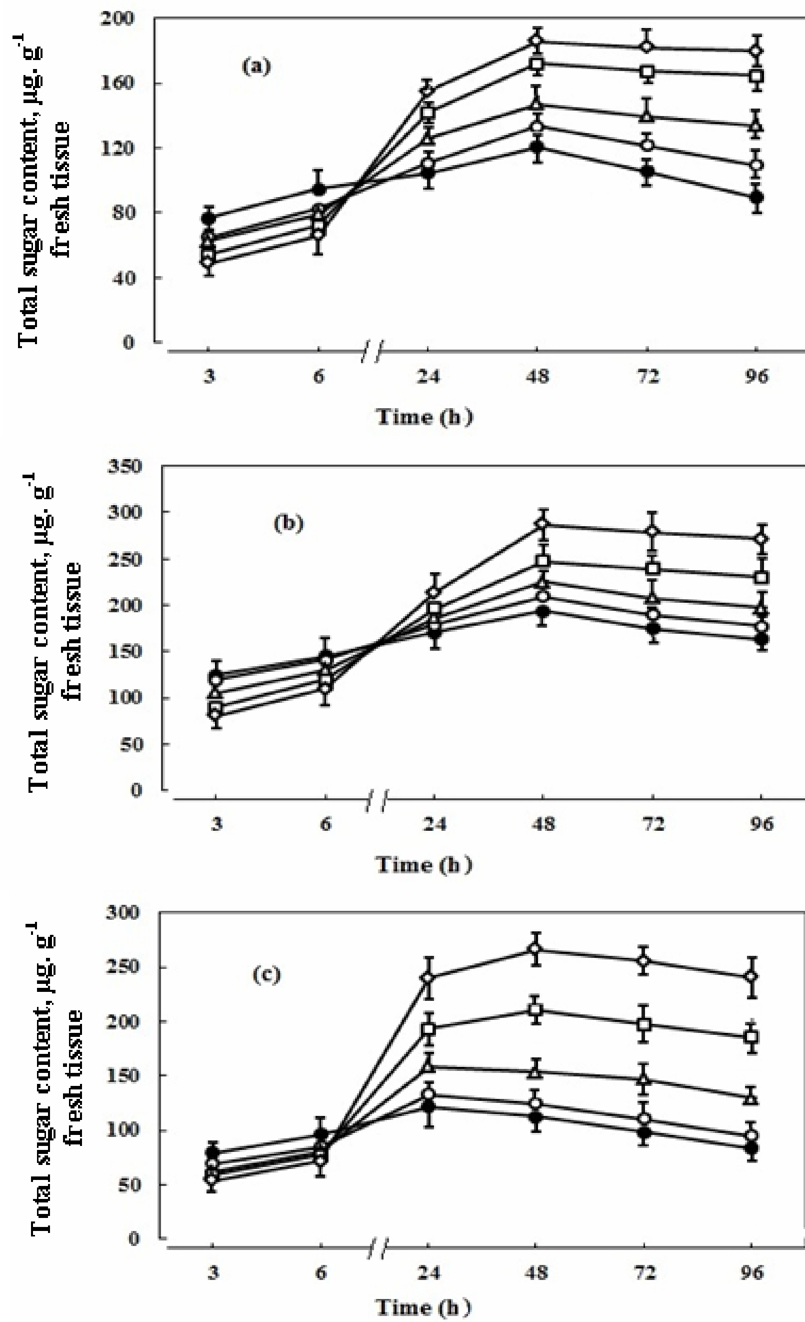


Fig. 46. The effect of different concentrations of aluminium on the accumulation of total sugar in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

Effects of aluminium toxicity on proline content in rice and chickpea seedlings grown in solution culture: Proline content in the root of rice seedlings was increased in all the concentrations of Al (10-150 µM) used. The rate of stimulation increased with the increase in concentration of Al from 10 to 150 µM. At a concentration of 150 µM, Al caused a maximum of 13.0 to 60.7% increase in proline content in the root from 3 to 96 h of treatment (Fig. 47a).

Aluminium (10-150 µM) also increased proline content in the shoot of rice but the rate of stimulation was lesser in the shoot than that of the root. 150 µM Al caused a 4.0 to 11.0% increase in proline content in the shoot from 3 to 96 h of treatment (Fig. 47b).

10 µM Al increased proline content in the root of chickpea by 11.5 to 66.9% from 24 to 96 h of treatment. The stimulation of proline level increased with the increase in Al concentration. A maximum increase of 27% to 2.7-fold increase in proline content was observed at 150 µM Al application over a period of 24 to 96 h (Fig. 48a).

Al (10-150 µM) resulted in a stimulation of proline level in the stem of chickpea seedlings. 10 and 150 µM Al increased proline content by 8.0 to 60.0% and 59.0% to 2.6-fold, respectively, from 24 to 96 h of treatment (Fig. 48b).

In the leaves of chickpea, Al-induced stimulation of proline level was initiated at 3 h of application (Fig. 48c). It is interesting to note that, Al-induced stimulation of proline level in the root and stem started at 24 h of treatment (Fig. 48a and 48b). In the leaf, 10 µM Al caused a 29.0 to 26.0% increase in proline level from 3 to 96 h of treatment (Fig. 48c). The stimulation of proline accumulation in the leaves increased with the increase in concentration of Al (10-150 µM). A maximum of 82.0 to 90.0% increase in proline content in the leaves of chickpea seedlings subjected to 150 µM Al was recorded from 3 to 96 h of treatment (Fig. 48c).

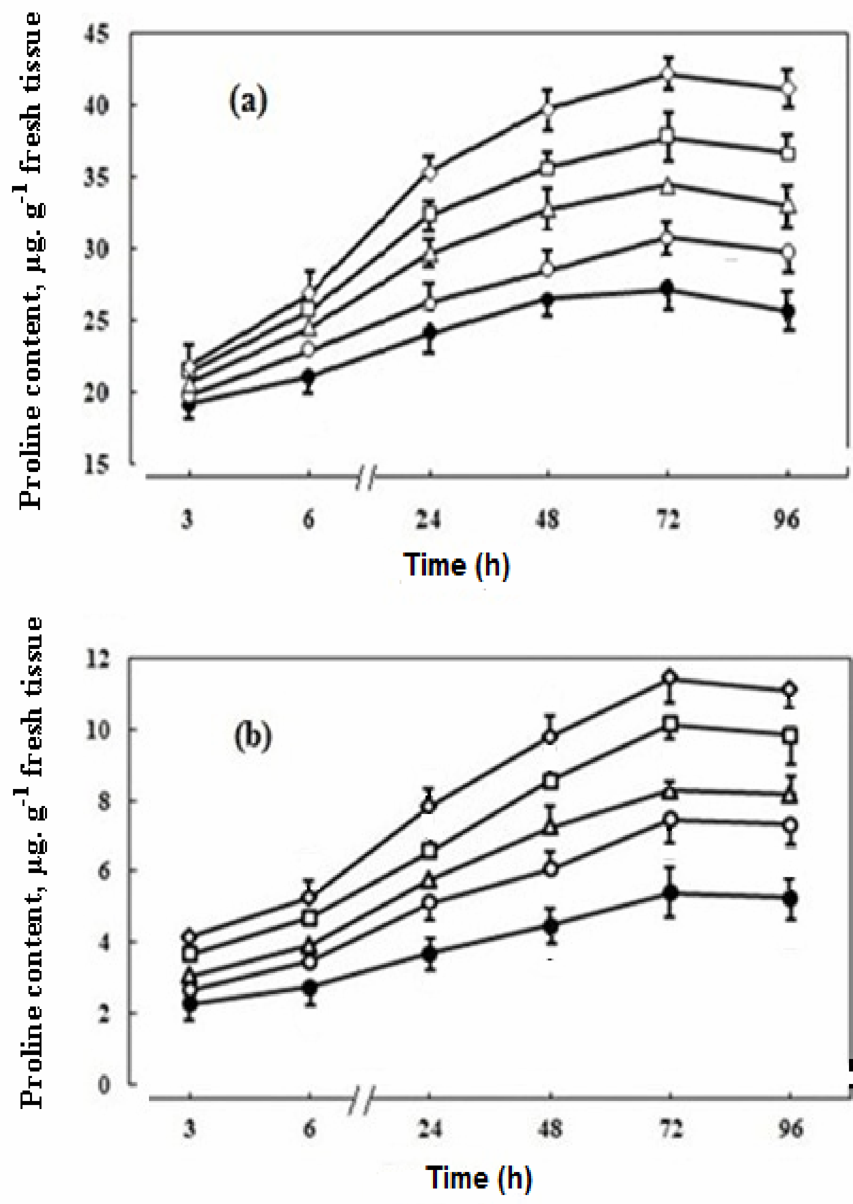


Fig. 47. The effect of different concentrations of aluminium on the accumulation of proline in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

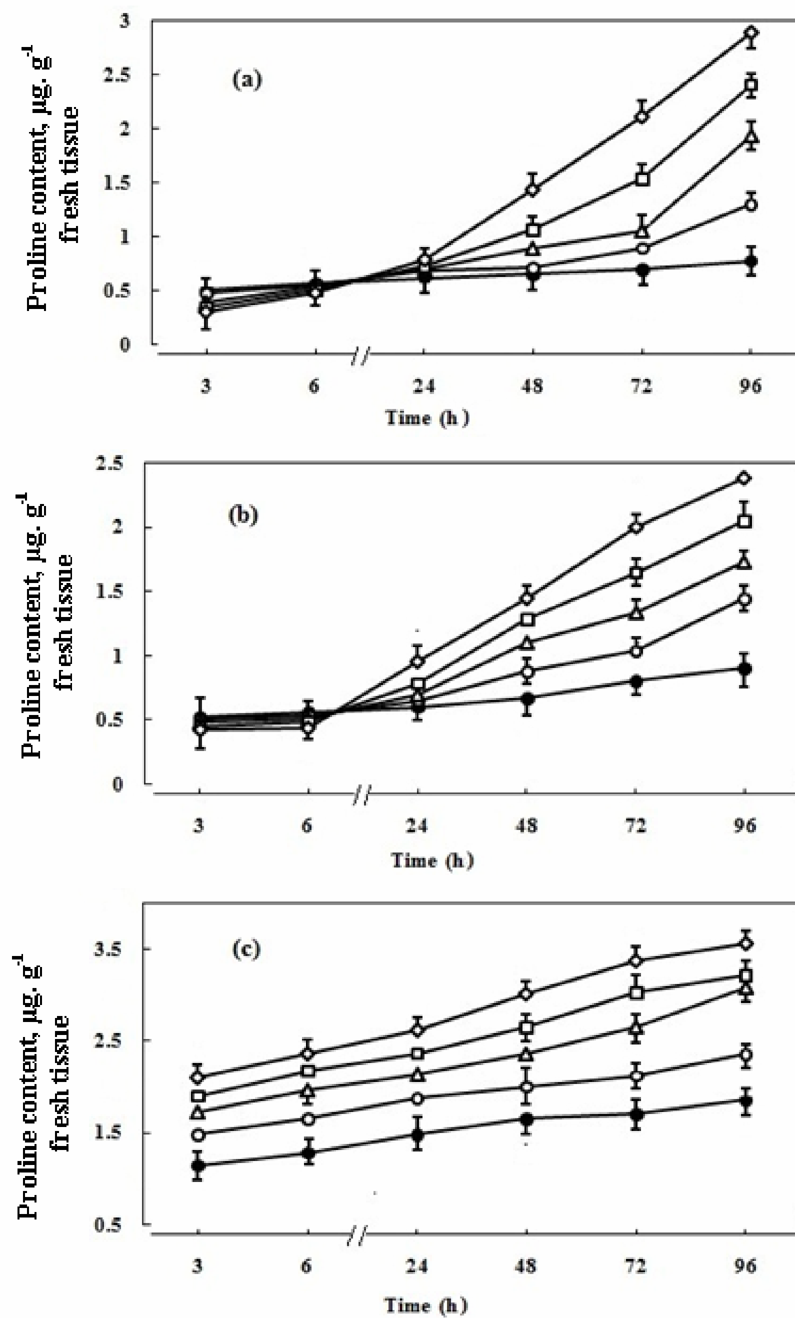


Fig. 48. The effect of different concentrations of aluminium on the accumulation of proline in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

Effects of aluminium toxicity on total amino acid in rice and chickpea seedlings grown in solution culture: Total amino acid was increased in the root of rice seedlings in all the concentrations of Al (10-150 µM) used. 50 µM Al caused a 2-fold increase in total amino acid in the root at 3 h of treatment and the stimulatory effect declined from 6 to 96 h of treatment. Exposure of rice seedlings to 150 µM Al resulted in a 3.5- to 2.3-fold increase in total amino acid in the root from 3 to 72 h of treatment (Fig. 49a).

In the shoot of rice seedlings, 10 µM Al increased total amino acid content by 21.0 to 50% from 3 to 72 h of treatment. 50 and 100 µM Al caused a 94.0% to 2-fold and 2.7- to 2.1-fold increase in total amino acid, respectively, in the shoot from 3 to 48 h of treatment. A maximum of 3.6-fold increase in total amino acid in the shoot was observed at 3 h following 150 µM Al treatment and this high stimulation was sustained up to 96 h of exposure (Fig. 49b).

In the root of chickpea seedlings, 10 µM Al increased total amino acid content from 8.5 to 13.7% from 3 to 96 h of treatment. The stimulatory effect of Al on total amino acid content in the root increased with the increase in Al concentration from 10 to 150 µM Al. Application of 100 µM Al caused a 28% to 2.4-fold increase in total amino acid content in the root of chickpea over a period of 3 to 96 h. Highest stimulation of total amino acid content was exerted by 150 µM Al which ranged from 3.3- to 3-fold from 24 to 96 h of application (Fig. 50a).

Al (10 µM) increased total amino acid content in the stem of chickpea from 8.0 to 19.5% from 3 to 48 h of treatment and the stimulatory effect was sustained up to 96 h of exposure. Similar trend of stimulation of total amino acid content was shown by 50, 100 and 150 µM Al treatment. Maximum stimulation of total amino acid content in the stem of chickpea was caused by 150 µM Al which ranged from 43.0 to 50.6% from 3 to 48 h of treatment and the stimulatory effect was maintained up to 96 h of application (Fig. 50b).

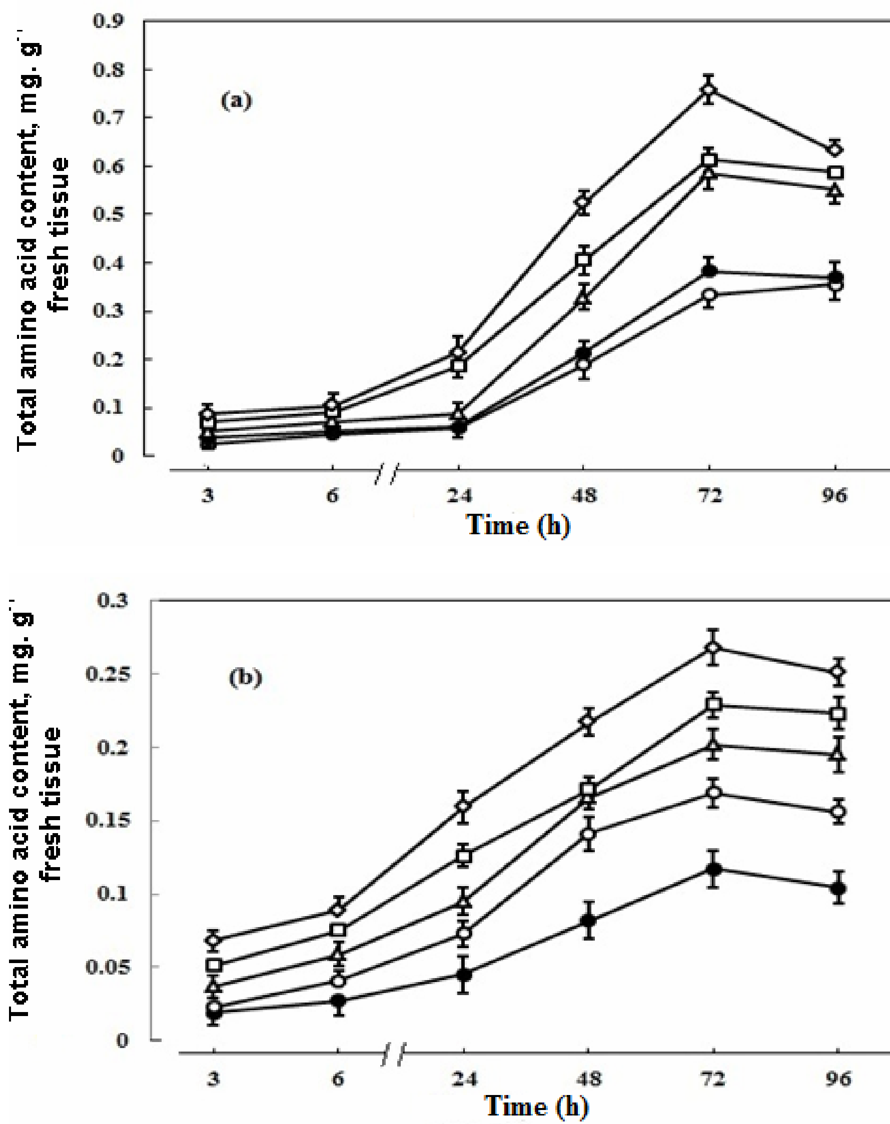


Fig. 49. The effect of different concentrations of aluminium on total amino acid content in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

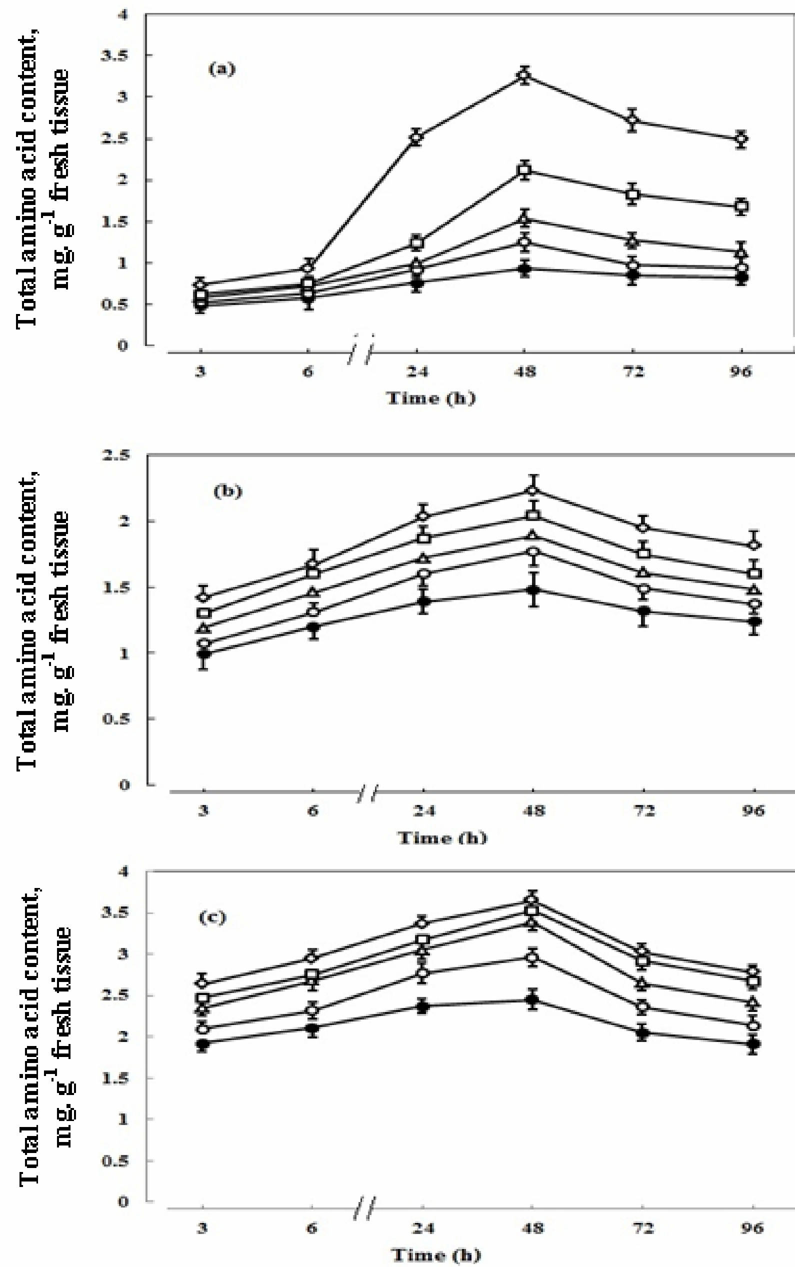


Fig. 50. The effect of different concentrations of aluminium on the total amino acid content in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

In the leaves of chickpea, 10-150 μM Al increased total amino acid content from 3 to 96 h of treatment. The stimulatory effect on total amino acid increased with the increase in concentration of Al from 10 to 150 μM. At concentrations of 100 and 150 μM Al, a 28.7 to 44.0% and 37.5 to 49% increase in total amino acid content was observed, respectively and the stimulatory effect was sustained up to 96 h of exposure (Fig. 50c).

Effects of aluminium toxicity on protein content in rice and chickpea seedlings grown in solution culture: Aluminium concentrations of 10 to 150 μM, increased protein content in the root of rice seedlings at 3 and 6 h of treatment and then gradually declined leading to an inhibition of protein content from 48 to 96 h of application (Fig. 51a). 100 μM Al increased protein content in the root by 44.0 and 38.0% at 3 and 6 h of treatment, respectively, and then it decreased that from 28.0 to 33.0% at 48 to 96 h of exposure. 150 μM Al decreased protein content by 23.0 to 42.5% from 48 to 96 h of treatment after an initial increase of that at 3 and 6 h (Fig. 51a).

In the shoot of rice seedlings, all the different concentrations of Al (10-150 μM) decreased protein content at 72 to 96 h of treatment except an stimulation of that from 3 to 48 h of treatment (Fig. 51b). 150 μM Al increased protein content in the shoot by 28.6 to 17.0% over a period of 3 to 48 h leading to an inhibition of that by 27.0 and 32.5% at 72 and 96 h of application respectively (Fig.51b).

In chickpea seedlings, Al (10-150 μM) had no significant effect on protein content of the root from 3 to 24 h of treatment but caused a significant reduction in protein content from 48 to 96 h of exposure (Fig. 52a). 10 μM Al decreased protein content in the shoot by 13.0 to 22.0% from 48 to 96 h of application. The highest inhibition of protein was observed at 150 μM Al treatment where a 24.0 to 42.7% inhibition was observed from 48 to 96 h of treatment (Fig. 52a).

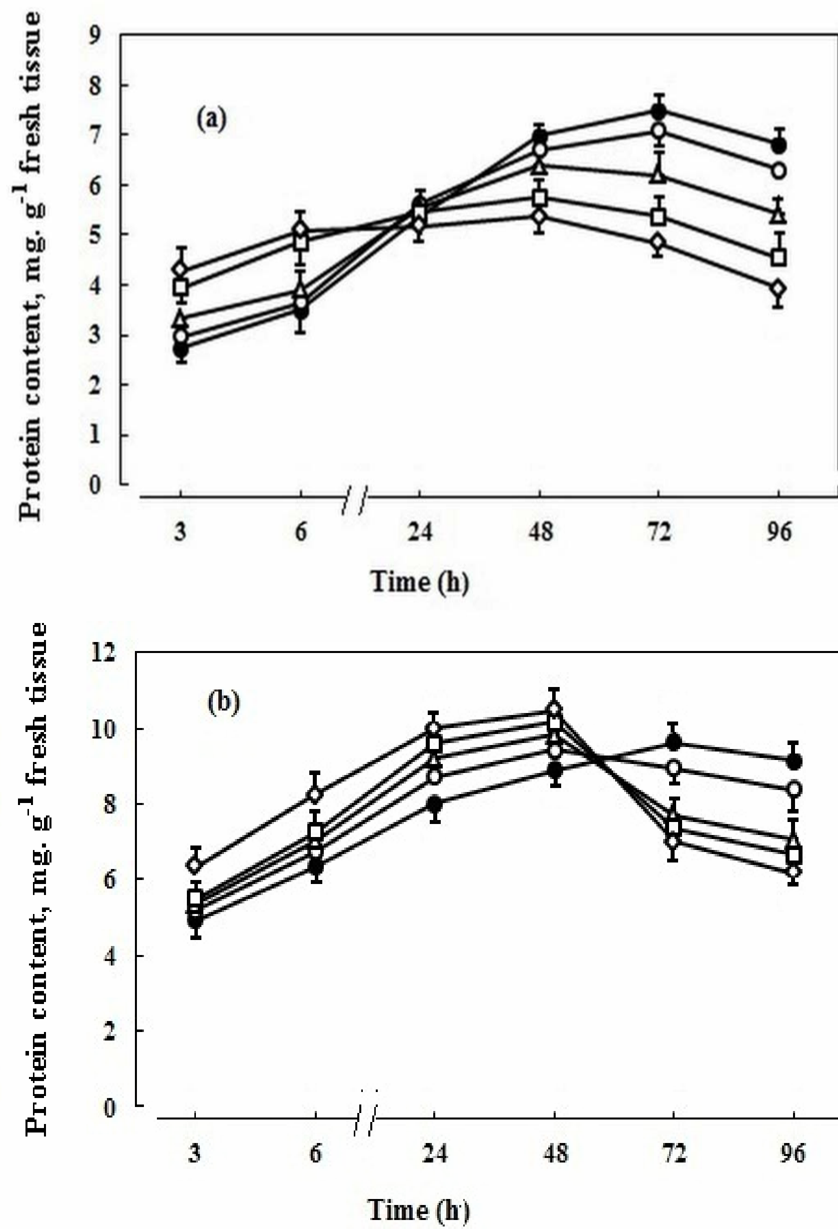


Fig. 51. The effect of different concentrations of aluminium on the protein content in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 13.

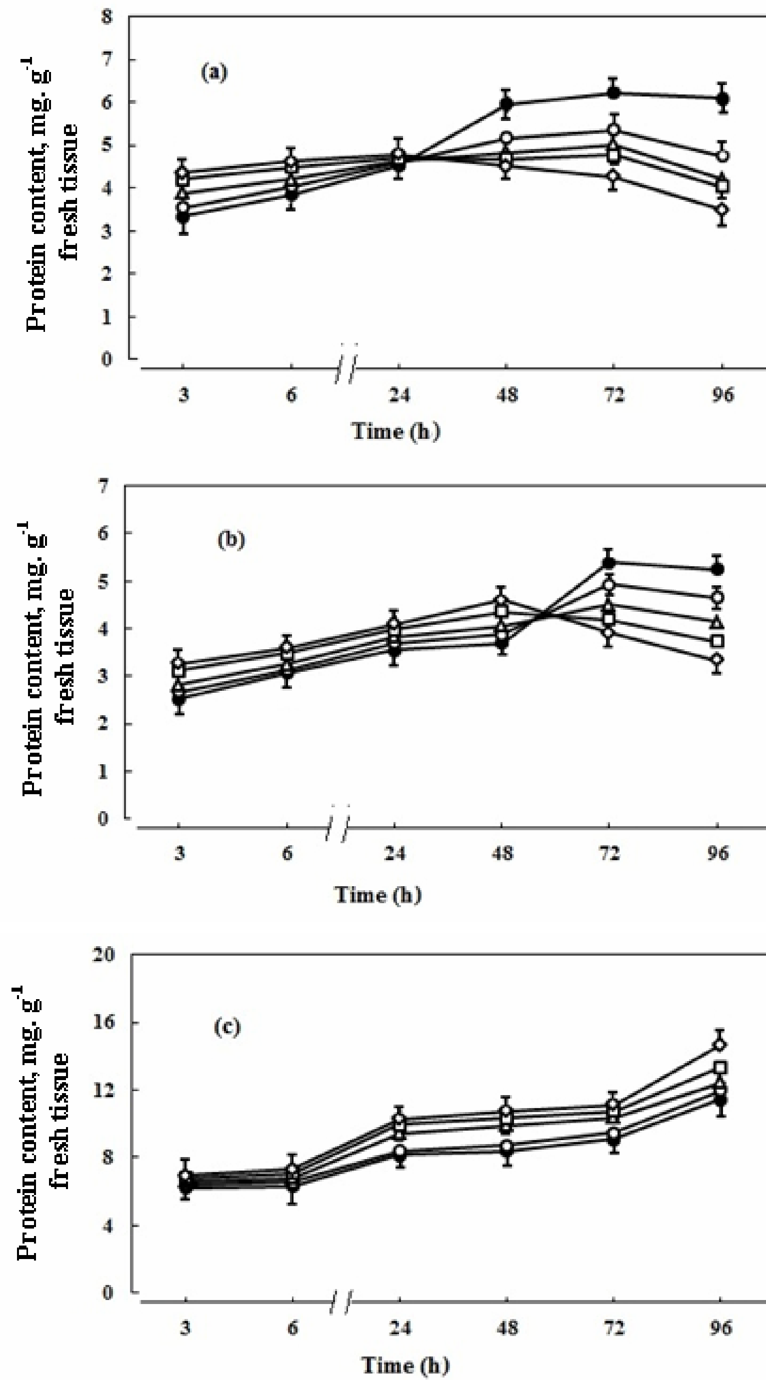


Fig. 52. The effect of different concentrations of aluminium on the protein content in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 13.

In the stem of chickpea seedlings, 10-150 μM Al increased protein content from 3 to 48 h followed by an inhibition of that from 72 to 96 h of treatment. 100 μM Al-induced stimulation of protein content gradually declined from 23 to 17.6% from 3 to 48 h of application leading to an inhibition of 22.0 to 28.8% over a period of 72 to 96 h. A 29.0 to 24.0% stimulation of protein content in the stem was observed at 150 μM Al treatment from 3 to 48 h of treatment. But 150 μM Al decreased protein content in the stem by 27.5 and 36.5% at 72 and 96 h of exposure respectively (Fig. 52b).

Al (10-150 μM) increased protein content in the leaves of chickpea seedlings. 100 μM Al caused a 9.5 to 22.9% increase in protein content in leaf from 3 to 48 h of exposure and the stimulatory effect was sustained up to 96 h of treatment (Fig. 52c). An 11.0 to 27.9% increase in protein content was observed in the leaves of chickpea exposed to 150 μM Al from 3 to 96 h (Fig. 52c).

5.3b Effects of aluminium toxicity on the activities of antioxidant enzymes in rice and chickpea seedlings grown in solution culture

Effects of aluminium toxicity on peroxidase, catalase and superoxide dismutase activities in rice seedlings: Peroxidase activity was increased in the root of rice by 4- to 7.9-fold following 96 h of exposure to 10 to 150 μM Al (Fig. 53a).

In the shoot of rice, a 1.8- to 2.9-fold stimulation of peroxidase activity was recorded at 10 to 150 μM Al application respectively (Fig. 53 b).

Similarly, catalase activity in the root of rice was stimulated by Al stress. 50, 100 and 150 μM Al caused a 2.8-, 5- and 9-fold increase in catalase activity in the root, respectively, at 96 h of exposure (Fig. 54a).

In the shoot of rice, 10 to 150 μM Al increased the activity of catalase by 38.4% to 3.5-fold at 96 h of treatment (Fig. 54b).

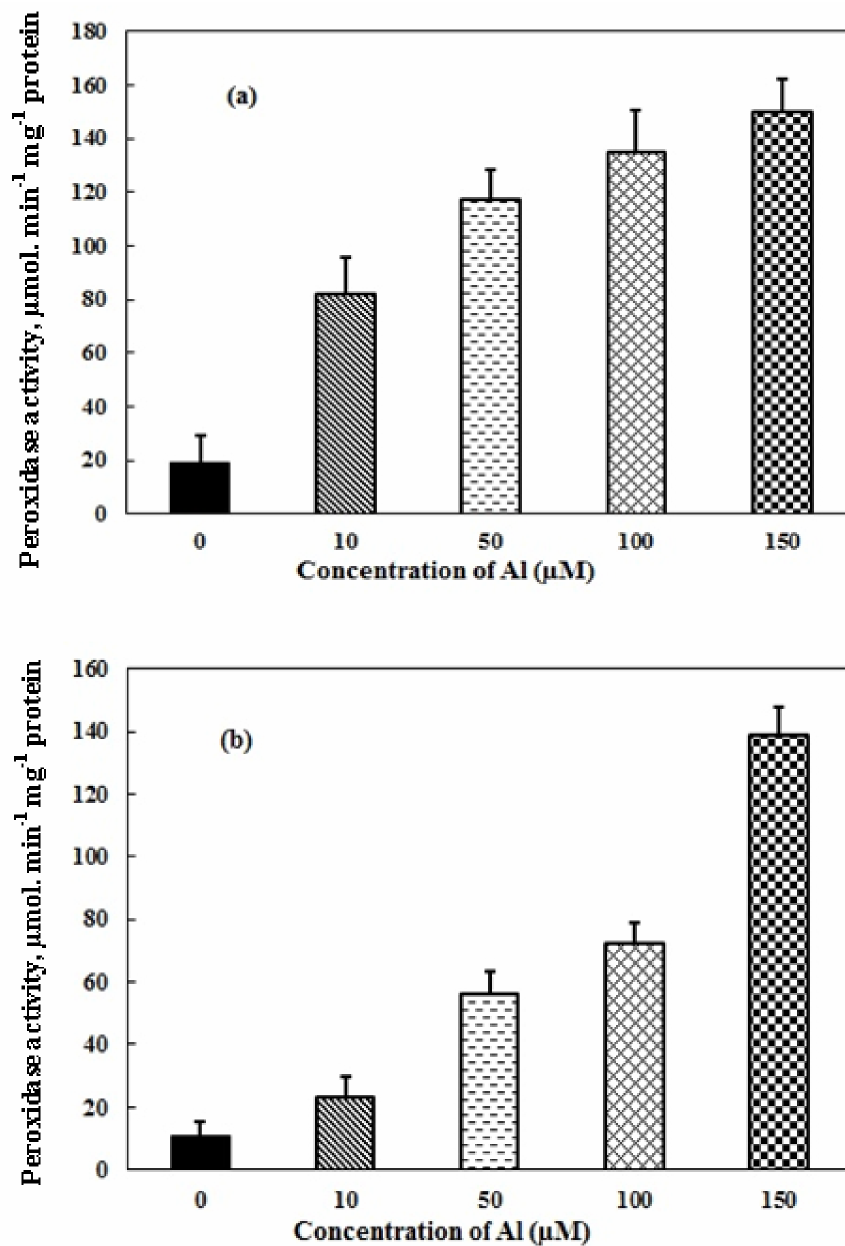


Fig. 53. The effect of different concentrations of aluminium on the activity of peroxidase in the (a) root and (b) shoot of rice seedlings grown in solution culture at 96 h of treatment. ■ represents control; 10 µM Al, 50 µM Al, 100 µM Al and 150 µM Al. Each value is the mean of three replicates ± standard error.

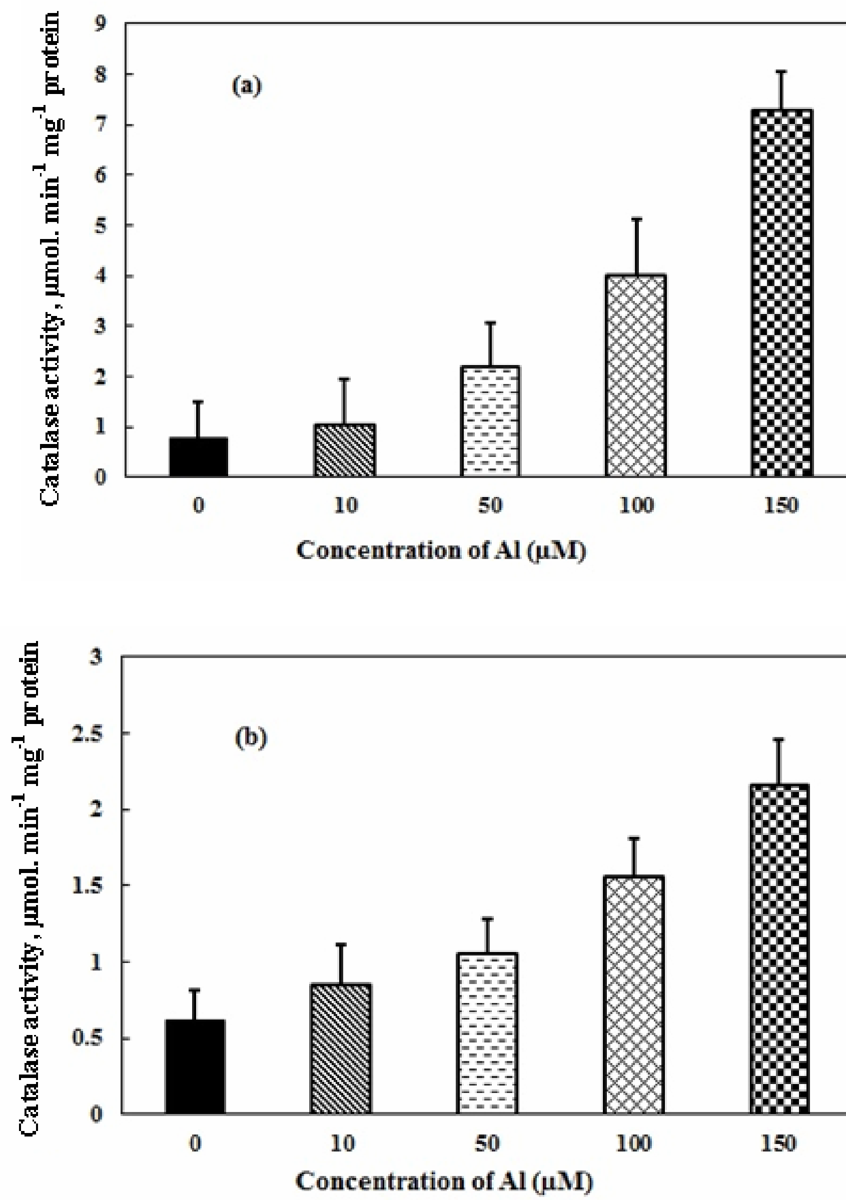


Fig. 54. The effect of different concentrations of aluminium on the activity of catalase in the (a) root and (b) shoot of rice seedlings grown in solution culture at 96 h of treatment. Otherwise as Fig. 53.

On the contrary, Al stress decreased superoxide dismutase (SOD) activity in the root of rice. 10 to 150 µM Al inhibited SOD activity by 14.8% to 50.0% in the root of rice at 96 h of exposure (Fig. 55a).

In the shoot of rice, Al (10-150 µM) decreased SOD activity by 15.7% to 47.0% at 96 h of treatment (Fig. 55b).

Effects of aluminium toxicity on peroxidase, catalase and superoxide dismutase activities in chickpea seedlings: Al (10 µM) increased peroxidase activity in the root of chickpea seedlings by 29.5% at 96 h of treatment. 50 to 150 µM Al caused a 3.7- to 4.8-fold stimulation of peroxidase activity in the root (Fig. 56a).

In the leaves of chickpea, 10 to 150 µM Al increased peroxidase activity by 2- to 13-fold at 96 h of application (Fig. 56b).

Al (10 to 150 µM) progressively increased the activity of catalase from 98.9% to 6.6-fold in the root of chickpea at 96 h of exposure (Fig. 57 a).

In the leaves of chickpea, all the concentrations of Al (10 to 150 µM) increased catalase activity. The maximum stimulation of catalase activity in the leaves was 2.3- to 6.5-fold following exposure to 50 to 150 µM Al (Fig. 57 b).

Al (10 µM) increased SOD activity in the root of chickpea by 48.7% at 96 h of exposure. SOD activity increased with the increase in Al concentration from 50 to 150 µM. A dramatic 14.8-fold increase in SOD activity was recorded in the root following 150 µM Al treatment (Fig. 58a). Similarly, SOD activity was increased in the leaves following 10 to 150 µM Al application. 100 and 150 µM Al caused a dramatic 9.5- and 14.6-fold stimulation of SOD activity in the leaves, respectively, at 96 h of exposure (Fig. 58b).

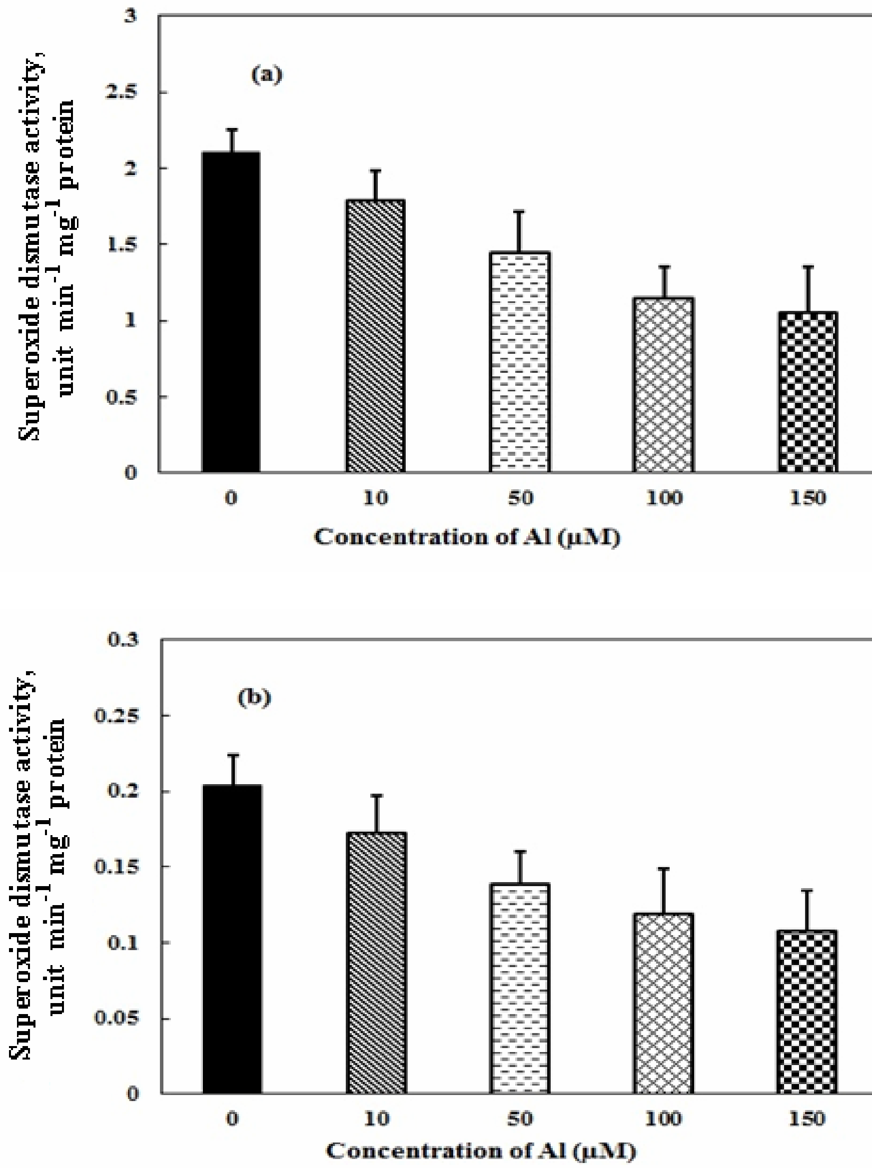
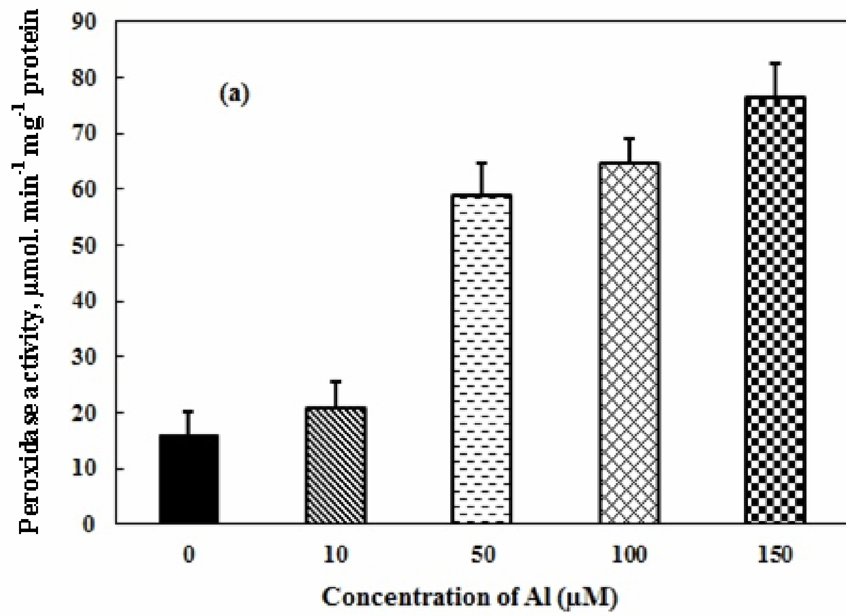


Fig. 55. The effect of different concentrations of aluminium on the activity of superoxide dismutase in the (a) root and (b) shoot of rice seedlings grown in solution culture at 96 h of treatment. Otherwise as Fig. 53.



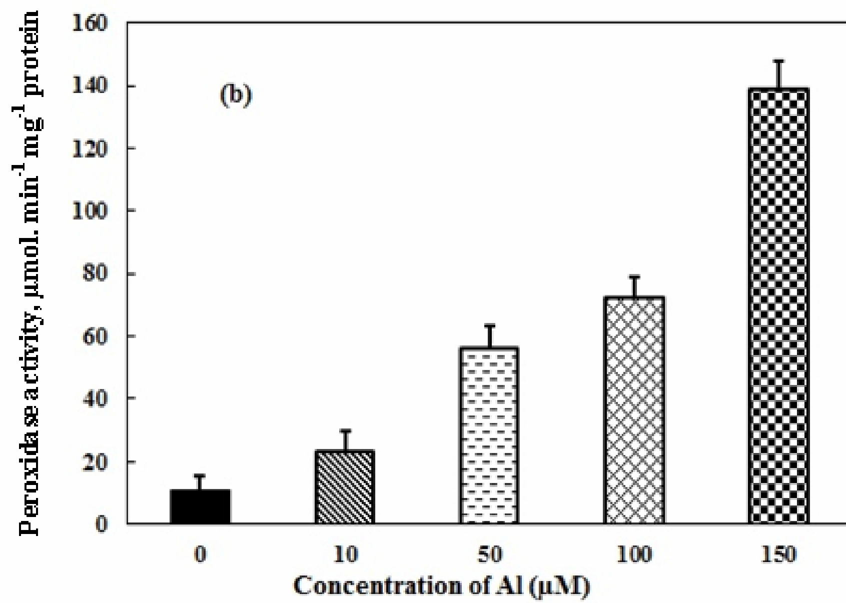


Fig. 56. The effect of different concentrations of aluminium on the activity of peroxidase in the (a) root and (b) leaves of chickpea seedlings grown in solution culture at 96 h of treatment. Otherwise as Fig. 53.

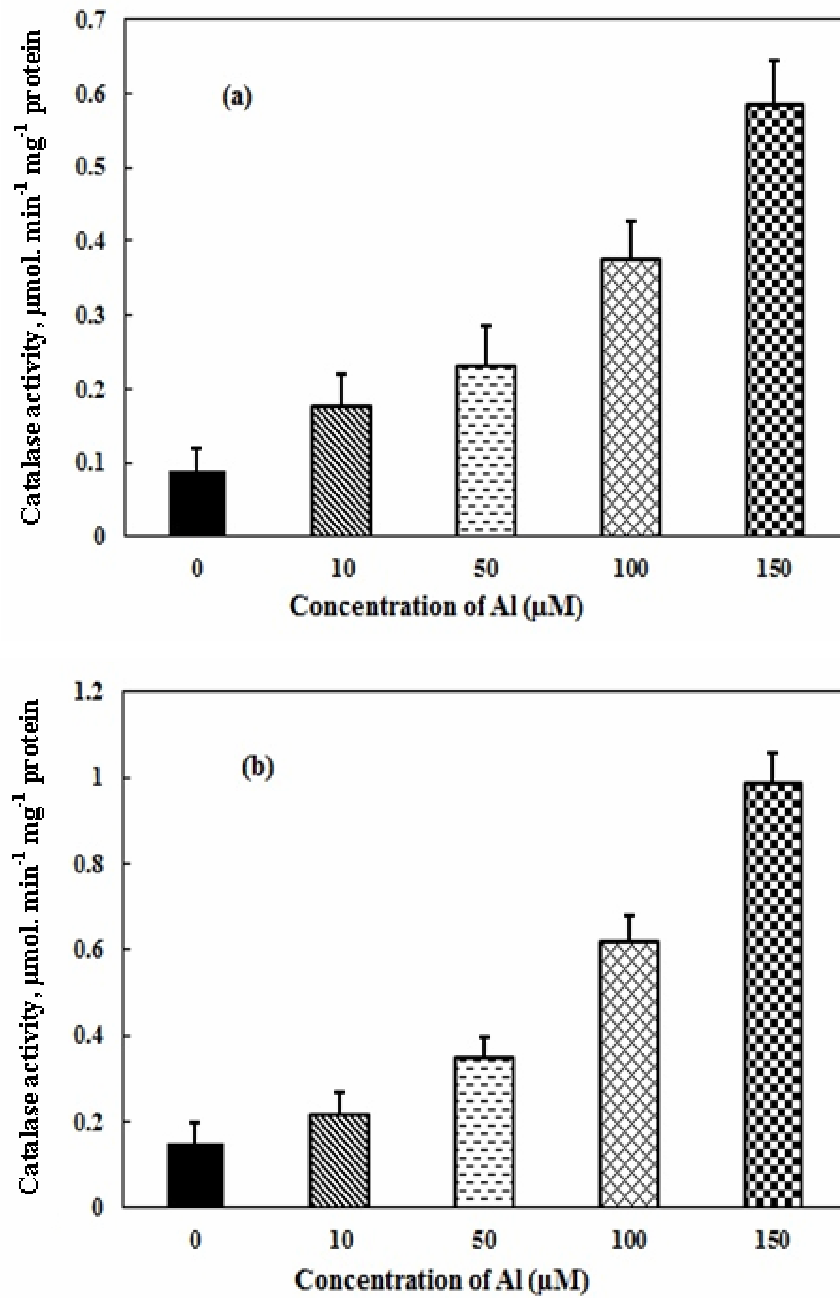


Fig. 57. The effect of different concentrations of aluminium on the activity of catalase in the (a) root and (b) leaves of chickpea seedlings grown in solution culture at 96 h of treatment. Otherwise as Fig. 53.

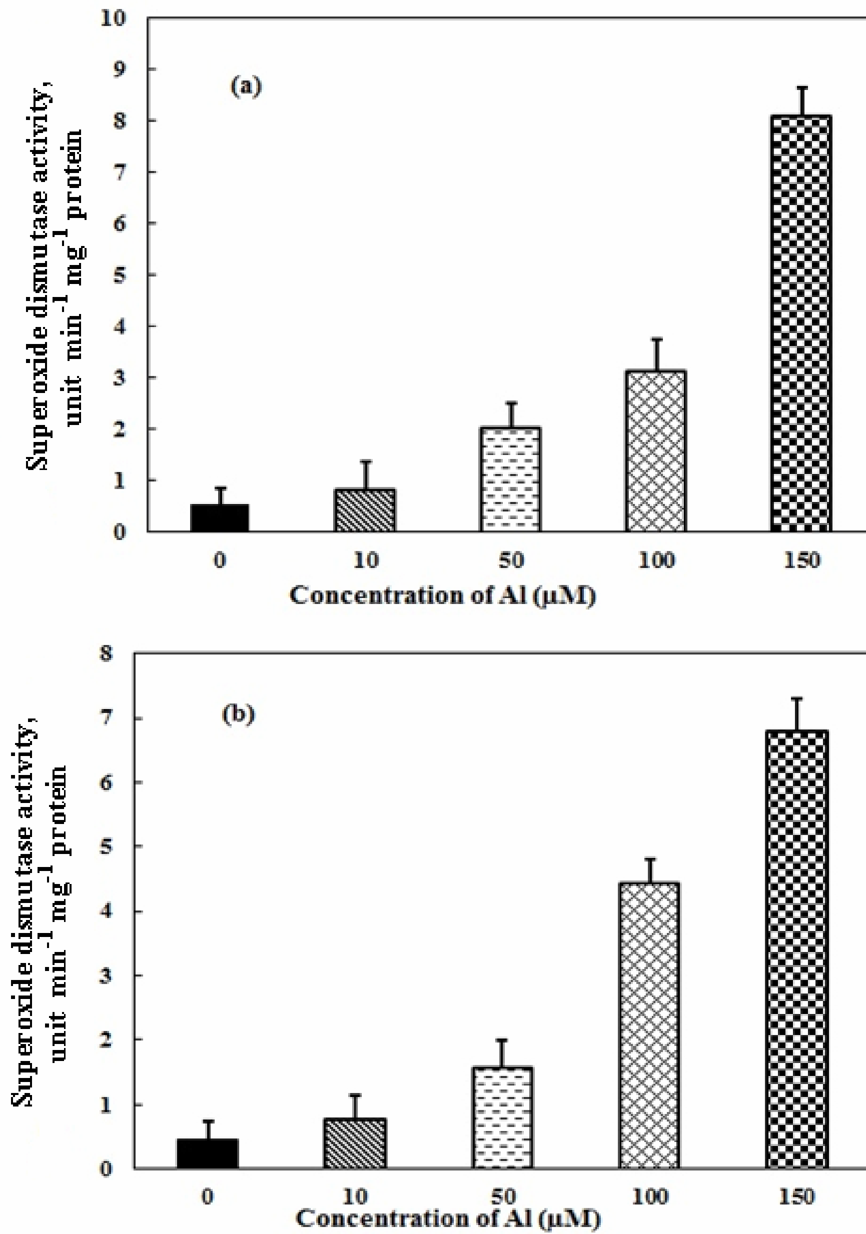


Fig. 58. The effect of different concentrations of aluminium on the activity of superoxide dismutase in the (a) root and (b) leaves of chickpea seedlings grown in solution culture at 96 h of treatment. Otherwise as Fig. 53.

5.3c Effects of aluminium toxicity on phenolic compounds and chlorophyll a, chlorophyll b and carotenoid contents in rice and chickpea plants grown in sand culture

Effects of aluminium toxicity on accumulation of phenolic compounds in rice and chickpea plants grown in sand culture: Al (50 µM) increased accumulation of phenolic compounds in the root of rice plants by 36.0% to 2.3-fold from 7 to 28 day of treatment. 100 and 150 µM Al caused a 48.9% to 5-fold and 2.6-fold to 6.9- fold increase in phenolic compounds, respectively, in the root of rice plants from 7 to 28 day of application (Fig. 59a).

In the shoot of rice, 50, 100 and 150 µM Al caused a 67.9% to 2.2-fold, 2.7- to 3.7-fold and 3.5- to 3.7-fold increase in accumulation of phenolic compounds, respectively, from 7 to 28 day of treatment (Fig. 59b).

In chickpea plants, Al caused a dramatic increase in the accumulation of phenolic compounds. For example, 50 µM Al increased the accumulation of phenolic compounds by 2.8- to 4.6-fold in the root from 7 to 28 day of application. Similarly, 100 and 150 µM Al caused a 3.8- to 6-fold and 7.5- to 10.5-fold increase in the root, respectively, from 7 to 28 day of treatment (Fig. 60a).

In the stem of chickpea, 50, 100 and 150 µM Al increased the accumulation of phenolic compounds by 2.9- to 3.8-fold, 3.9- to 4.9-fold and 7.5- to 6.9-fold, respectively, from 7 to 28 day of treatment (Fig. 60b).

Al (50 µM) caused an increase in the accumulation of phenolic compounds by 2.8- to 3.7-fold in the leaves of chickpea from 7 to 28 day of application. At concentrations of 100 and 150 µM Al, a 3.7- to 4.9-fold and 5.8- to 7.5-fold increase in the accumulation of phenolic compounds, respectively, was observed in the leaves from 7 to 28 day of exposure (Fig. 60c).

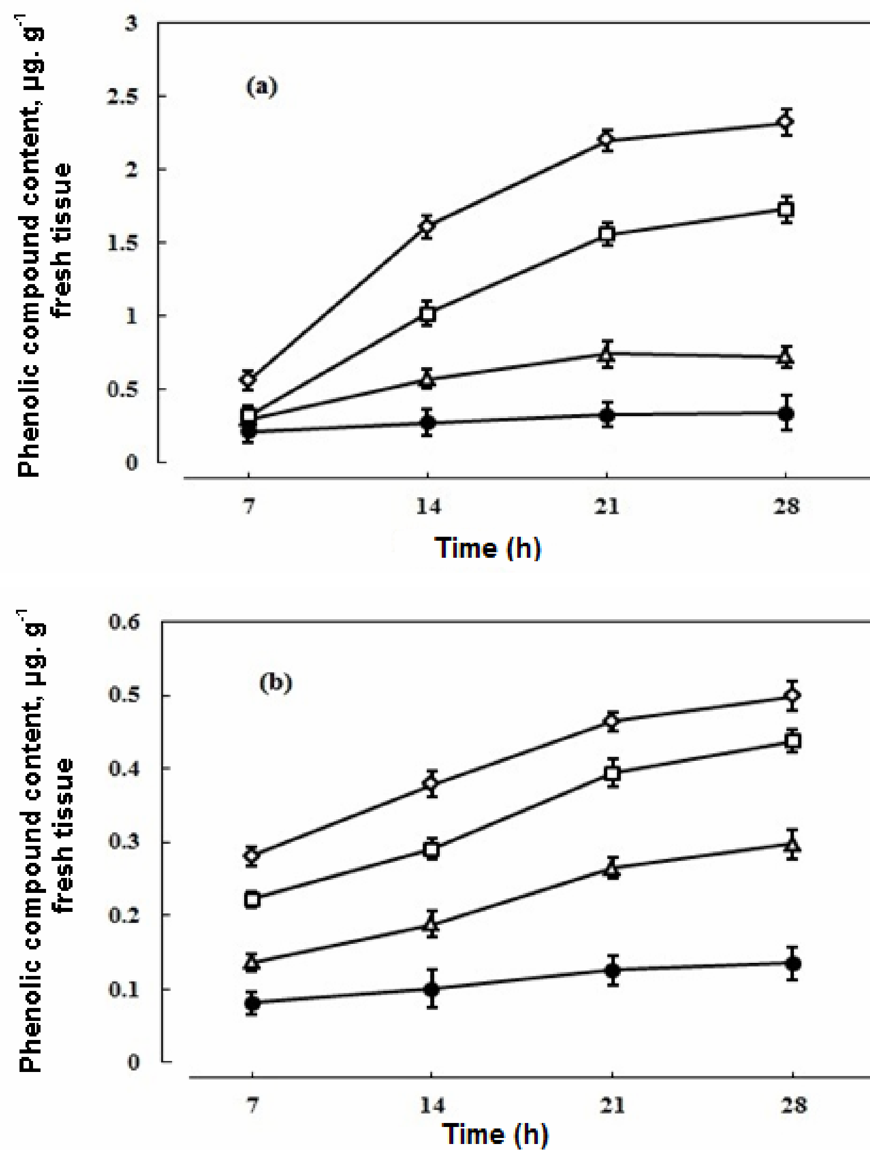


Fig. 59. The effect of different concentrations of aluminium on the accumulation of phenolic compounds in the (a) root and (b) shoot of rice seedlings grown in sand culture. ● represents control; Δ 50µM Al; □ 100µM Al; ◊ 150µM Al. Each value is the mean of three replicates ± standard error.

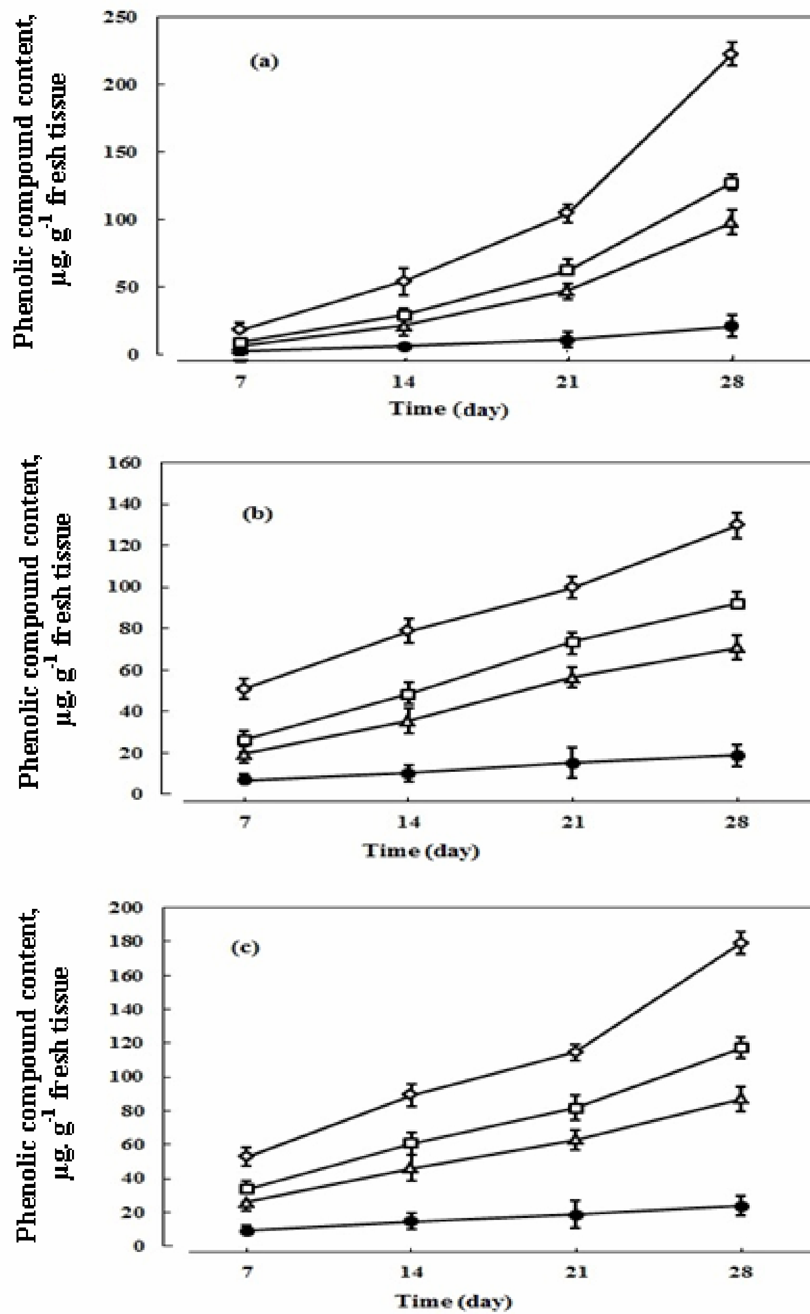


Fig. 60. The effect of different concentrations of aluminium on the accumulation of phenolic compounds in the (a) root, (b) stem and (c) leaves of chickpea plants grown in sand culture. Otherwise as Fig. 59.

Effects of aluminium toxicity on chlorophyll a, chlorophyll b and carotenoid contents in the leaves of rice and chickpea plants grown in sand culture: Al (50 µM) decreased chlorophyll a content by 4.5 to 16.0% in the leaves of rice seedlings from 14 to 28 day of treatment. A 9.0 to 22.0% inhibition of chlorophyll a content in the leaves of rice was obtained following 100 µM Al treatment from 14 to 28 day of exposure. 150 µM Al caused a maximum of 11.6 to 30.0% inhibition of chlorophyll a content in the leaves from 14 to 28 day of application (Fig. 61a).

Chlorophyll b content in the leaves of rice plants was decreased by 8.8 to 19.0% from 14 to 28 day following application of 50 µM Al. Al (100 µM) also caused 19.0 to 31.0% inhibition of Chlorophyll b content in the leaves from 14 to 28 day of treatment. A maximum of 25.9 to 37.0% inhibition of Chlorophyll b content in the leaves of rice was observed when exposed to 150 µM Al from 14 to 28 day of application (Fig. 61b).

Al (50-150 µM) decreased carotenoid content in the leaves of rice plants from 14 to 28 day of treatment. 150 µM Al caused 8.0 to 12.8% inhibition of carotenoids in the leaves from 14 to 28 day of application (Fig. 61c).

In the leaves of chickpea plants, 50 µM Al decreased chlorophyll a content from 6.0 to 21.0% from 7 to 28 day of treatment. Degree of inhibition of chlorophyll a content in the leaves increased with the increase in Al concentrations. 100 and 150 µM Al caused a 12.8 to 27.8% and 19.5 to 37.0% inhibition of chlorophyll a content in the leaves, respectively, from 7 to 28 day of application (Fig. 62a).

Al (50 µM) decreased chlorophyll b content in the leaves of chickpea by 6.0 to 18.9% from 7 to 28 day of exposure. Chlorophyll b content in the leaves was inhibited by 16.0 to 30.9% following 100 µM Al treatment from 7 to 28 day of application. A maximum of 22.5 to 42.9% inhibition of chlorophyll b content in the leaves of chickpea was obtained under the influence of 150 µM Al from 7 to 28 day (Fig. 62b).

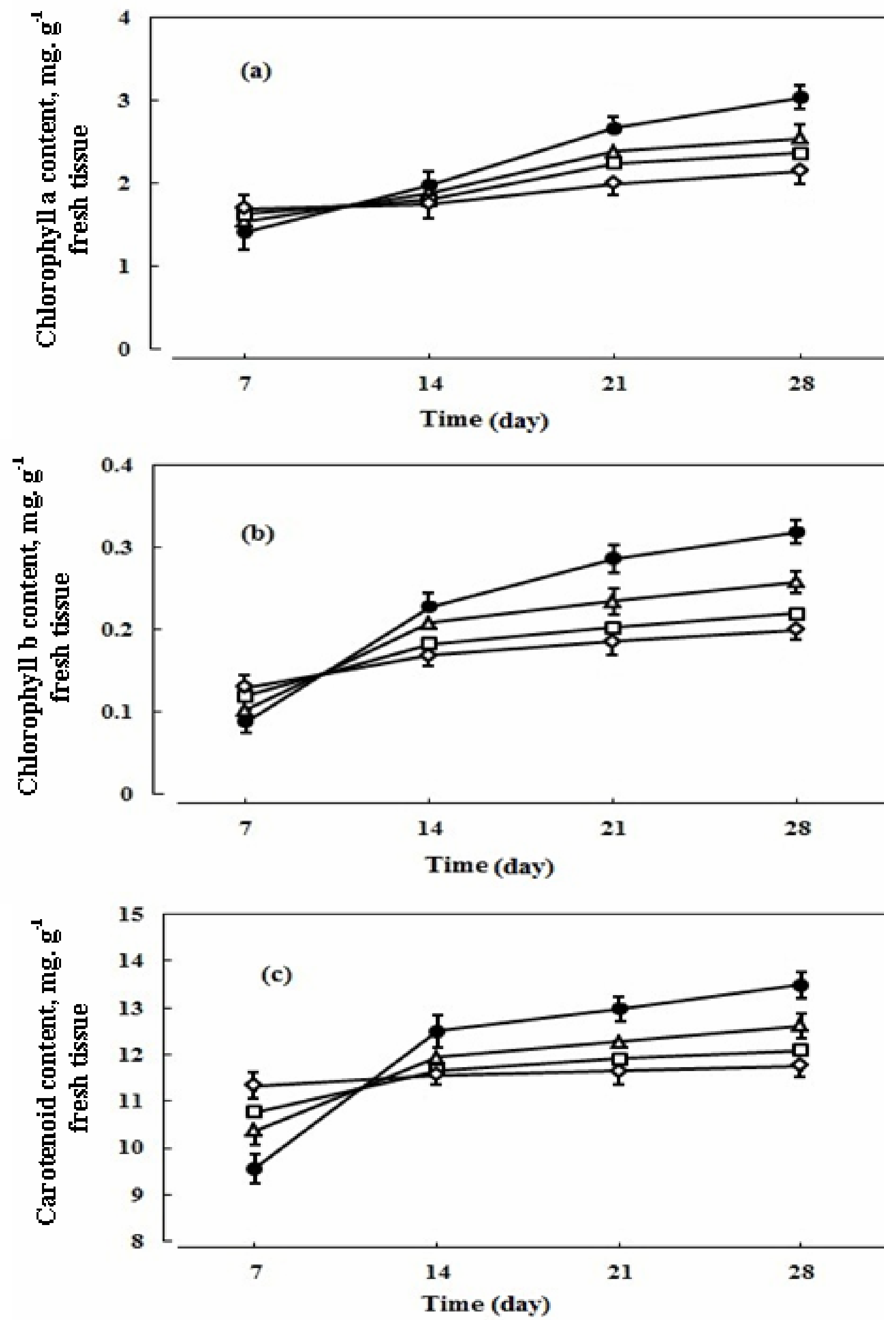


Fig. 61. The effect of different concentrations of aluminium on (a) chlorophyll a, (b) chlorophyll b and (c) carotenoid content in the shoot of rice plants grown in sand culture. Otherwise as Fig. 59.

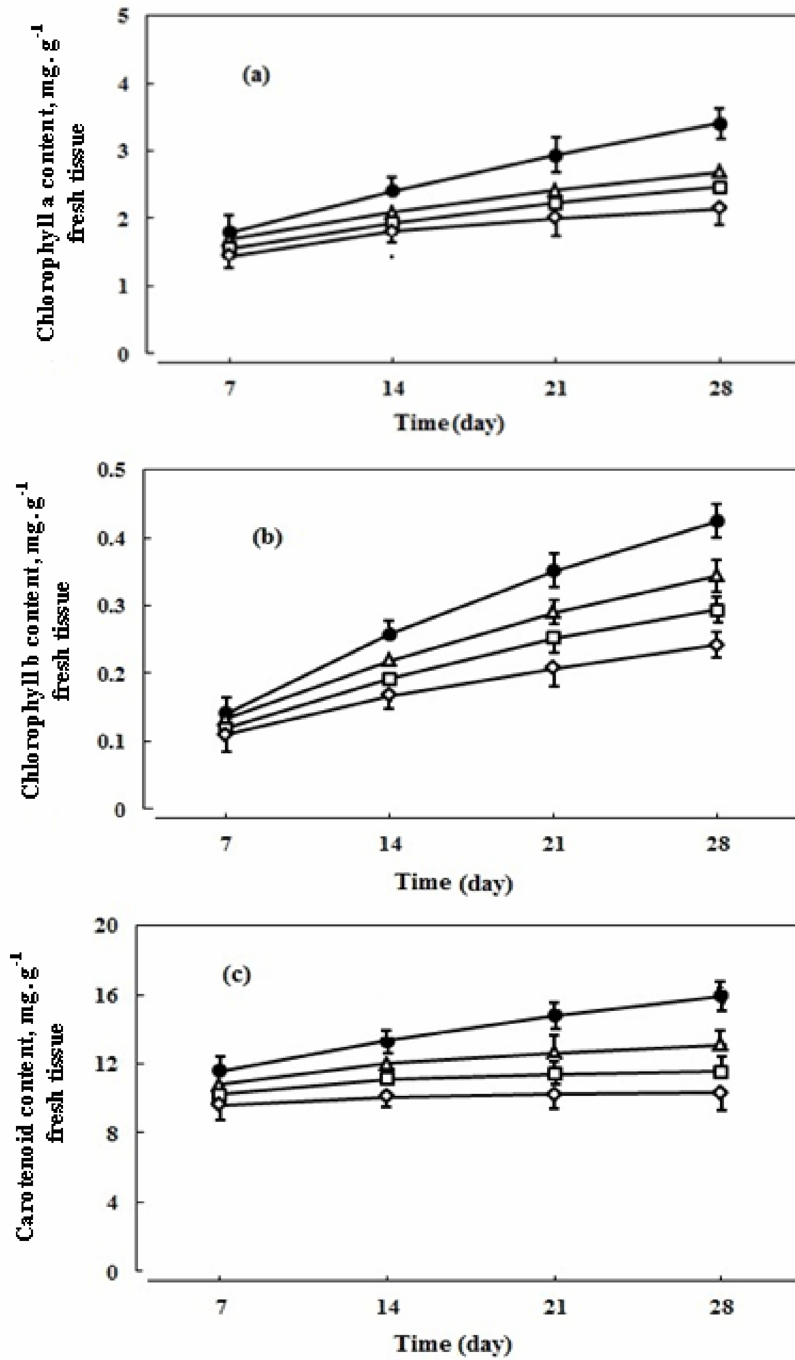


Fig. 62. The effect of different concentrations of aluminium on (a) chlorophyll a, (b) chlorophyll b and (c) carotenoid content in the leaves of chickpea plants grown in sand culture. Otherwise as Fig. 59.

Carotenoid content was consistently decreased in the leaves of chickpea plants bathed in 50, 100 and 150 µM Al solution. 50 µM Al inhibited carotenoid content in the leaves from 6.7 to 17.8% from 7 to 28 day of treatment. Carotenoid content in the leaves was decreased by 11.7 to 27.5% when subjected to 100 µM Al from 7 to 28 day of application. 150 µM Al decreased carotenoid content in the leaves by 17.0 to 35.0% from 7 to 28 day of treatment (Fig. 62c).

5.4 Discussion

Effects of aluminium toxicity on the accumulation of reducing sugar in rice and chickpea seedlings grown in solution culture: Different concentrations of aluminium (10-150 µM) increased reducing sugar content in the root and shoot of rice (Fig. 43), and the root, stem and leaves of chickpea (Fig. 44). Similarly, lower concentration of Al was found to increase reducing sugar content in barley (Abdalla 2008).

Effects of aluminium toxicity on total sugar content in rice and chickpea seedlings grown in solution culture: Al treatment caused a stimulation of total sugar content in the root and the shoot of rice (Fig. 45), and root, stem and leaves of chickpea (Fig. 46). This result is in agreement with the work of Cambraia and coworkers (1983) who found that Al increased the total sugar content in sorghum.

Effects of aluminium toxicity on the accumulation of proline in rice and chickpea seedlings grown in solution culture: Al stress increased proline content in the root and shoot of rice (Fig. 47), and the root, stem and leaves of chickpea (Fig. 48). Proline level was found to increase under different stress conditions. Al-induced increase in proline content indicates that plant is stressed. Similarly, Al was found to increase proline content in *Stylosanthes guianensis and S. macrocephala* (Amaral *et al.* 2013).

Effects of aluminium toxicity on total amino acid content in rice and chickpea seedlings grown in solution culture: Al treatment increased total amino acid content in the root and shoot of rice and chickpea seedlings (Figs. 49 and 50). The increase in total amino acids might be due hydrolysis of protein which might result in observed decrease in protein content (Figs. 51 and 52).

Effects of aluminium toxicity on the accumulation of protein in rice and chickpea seedlings grown in solution culture: Al stress decreased protein content in the root and shoot of rice (Fig. 51) and root and stem of chickpea (Fig. 52). Similarly, da Cruz *et al.* (2011) found that Al toxicity inhibited total soluble protein in *Sorghum bicolor*.

Effects of aluminium toxicity on the activity of antioxidant enzymes - peroxidase, catalase and superoxide dismutase (SOD) in rice and chickpea seedlings grown in solution culture: Aluminium stress caused a dramatic increase in the activity of peroxidase and catalase but decreased that of SOD in the root and the shoot of rice seedlings (Figs. 53-55). However, Al treatment caused a few folds stimulation of the activity of peroxidase, catalase and SOD in the root and leaves of chickpea seedlings (Figs. 56-58). Similar aluminium toxicity-induced stimulation of the activities of peroxidase, superoxide dismutase was found in tomato. On the contrary, Al caused an inhibition of catalase activity in tomato (Surapu *et al*. 2014).

Giannakoula *et al.* (2010) and Ma *et al.* (2012) working with two maize and rice cultivars with different tolerance to Al, respectively, showed that the improvement in protection against Al toxicity was obtained by an increase in the activity of the antioxidant enzymes. Lee *et al.* (2001) suggested that SOD might function in signaling of oxidative stress which might lead to the induction of antioxidant enzymes associated with an ½ O_2 scavenging system.

Effects of aluminium toxicity on accumulation of phenolic compounds in rice and chickpea plants grown in sand culture: Aluminium (50-150 μM) caused a

dramatic stimulation of phenolic compounds which was as high as 5- to 10.5-fold in rice and chickpea plants (Figs. 59 and 60). Phenolic compounds might act as a detoxifier of aluminium toxicity.

Effects of aluminium toxicity on chlorophyll a, chlorophyll b and carotenoid contents in the leaves of rice and chickpea plants grown in sand culture: Al (50 to 150 µM) decreased chlorophyll a, chlorophyll b contents in the leaves of rice and chickpea plants at 14 to 28 day of treatment (Figs. 61a, b and 62a, b). Similarly, Al inhibited chlorophyll a, chlorophyll b contents in sunflower varieties Sinera and Sanbera (Ziaei *et al.* 2014). An inhibition of chlorophyll a and chlorophyll b contents might decrease the rate of photosynthesis. Besides chloroplast was considered as the most powerful source of reactive oxygen species (ROS) in plants (Foyer *et al.* 1994). A little difference in photosynthetic machinery by aluminium stress might lead to production of a huge amount of ROS which might generate more oxidative stress in the shoot.

Al (50-150 µM) caused a decrease in carotenoid content in the leaves of rice and chickpea (Figs. 61c and 62c). This result was in agreement with the work of Sabat *et al.* (2016) who found that Al stress inhibited carotenoid content in *Vigna radiata*.

Chapter 6

Effects of aluminium toxicity on root elongation, root and shoot growth of rice and chickpea seedlings

6.1 Introduction

6.1.1 Effects of aluminium toxicity on root elongation

Aluminium inhibited root elongation in many plants (Gupta *et al.* 2013). Aluminium caused an inhibition of root elongation in green gram (*Vigna radiata*) (Panda *et al.* 2003). On the other hand, the length and number of lateral roots in *Quercus serrata* was increased by aluminium treatment (Tomioka *et al.* 2012). Al inhibited root cell elongation (Clarkson 1965, Klimashevski and Dedov 1975).

6.1.2 Effects of aluminium toxicity on root growth and plant growth and root and shoot length

Root growth of pumpkin was inhibited after a brief exposure to Al (Dipierro *et al.* 2005).

Al caused alteration of root morphology including root thickening, disturbances of root peripheral tissue and initiation of lateral roots closer to the tips (Ciamporova 2002).

Increasing concentrations of Al in solution progressively decreased the growth of the shoot of physic nut (Steiner *et al.* 2012). Absorbed aluminium adversely affected plant growth in many plants (Gupta *et al.* 2013). With the increase in Al concentration, the biomass of hypocotyls and radicles was decreased gradually in *Jatropha curcas* seedlings (Ou-yang *et al.* 2014).

Al stress reduced root and shoot length in two rice cultivars (Bhoomika *et al.* 2013).

6.2 Materials and Methods

6.2.1 Methods of studying effects of aluminium toxicity on root elongation and number of lateral roots: In order to study the root elongation and number of lateral roots starting from germination of seeds, rice and chickpea seedlings were raised in rhizobox according to the method described in sections 2.20.1 and 2.20.2.

The sand of three rhizoboxes with seedlings were moistened with half strength Hoagland solution (pH 4.2) which was used as control and other nine rhizoboxes were moistened with 50, 100 and 150 μM $AlCl_3$ solution (pH 4.2).

The primary root length and the number of lateral roots in control and aluminium-stressed seedlings were recorded every day. The root of the seedling in the rhizobox was traced on the transparent sheet. This was done from the 1st to 5th day of germination. The length and number of lateral roots traced in the tracing paper was measured and recorded according to section 2.20.3 and 2.20.4.

6.2.2 Methods of studying effects of aluminium toxicity on the root length, shoot length and shoot/root length ratio of rice and chickpea seedlings grown in solution culture: Seedlings were grown in solution culture according to the method described in section 2.6. Seven-day-old seedlings were transferred to half strength Hoagland solution (control) (pH 4.2) and 10, 50, 100 and 150 μM $AlCl_3$ solution made in half strength Hoagland solution (pH 4.2). Length of root and shoot of the seedlings were measured in cm with a scale at 3, 6, 24, 48, 72 and 96 h of aluminium treatment.

6.2.3 Methods of studying effects of aluminium toxicity on the dry weight of root and shoot and shoot/root dry weight ratio of rice and chickpea seedlings grown in solution culture: Rice and chickpea seedlings were grown in solution culture for 7 days according to section 2.6. Aluminium treatment was applied to 7-day-

old seedlings and samples were collected after 3, 6, 24, 48, 72 and 96 h of aluminium exposure and the dry weight of tissue was recorded according to the method outlined in section 2.8.

6.3 Results and Discussion

6.3.1 Effects of aluminium toxicity on primary root length, number of lateral roots in rice and chickpea seedlings grown in rhizobox

Al (50 µM) decreased primary root length of rice by 24.4 to 59.0% from 1 to 5 day of treatment. Similarly, 100 and 150 µM Al caused a 31.7 to 67.3% and 31.7 to 70% inhibition of primary root length of rice, respectively, from 1 to 5 day of application (Fig. 63).

In chickpea seedlings, 50 µM Al inhibited primary root length by 33.9 to 51.6% from 1 to 5 day of treatment. Exposure to 100 and 150 µM Al resulted in 64.5 to 82.6% and 66.0% to 83.0% inhibition of primary root length of chickpea, respectively, from 1 to 5 day (Fig. 64).

Al (50 µM) decreased the number of lateral roots of rice from 50.0 to 48.0% from 1 to 5 day of treatment. 100 µM Al caused a decrease in the number of lateral roots from 75.0 to 72.0% over a time period of 1 to 5 day. Similar magnitude of inhibition of the number of lateral roots of rice was recorded at 150 µM Al application (Fig. 65).

Al (50 µM) decreased the number of lateral roots of chickpea seedlings by 60.0 to 41.0% from 2 to 5 day of application. 100 µM Al caused an inhibition of the number of lateral roots by 80.0 to 77.0% from 2 to 5 day of treatment. Exposure to 150 µM Al also showed similar inhibition of the number of lateral roots of chickpea from 3 to 5 day of application (Fig. 66).

Meda and Furlani (2005) found that Al reduced the root elongation by 50% in tropical leguminous plant. Similar aluminium-induced inhibition of the root length was recorded in wheat (Jamal *et al.* 2006). Ryan *et al.* (1993) showed that

20 μM $AlCl_3$ inhibited root elongation of corn root by 50.0%. Decrease in the number of lateral roots would decrease the ion absorption area of the root system.

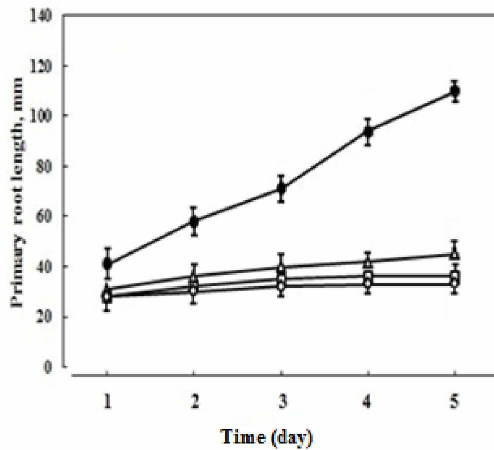


Fig. 63. The effect of different concentrations of aluminium on the primary root length of rice seedlings grown in rhizobox. ● represents control; Δ 50 μM Al; □ 100 μM Al; ◊ 150 μM Al. Each value is the mean of three replicates ± standard error.

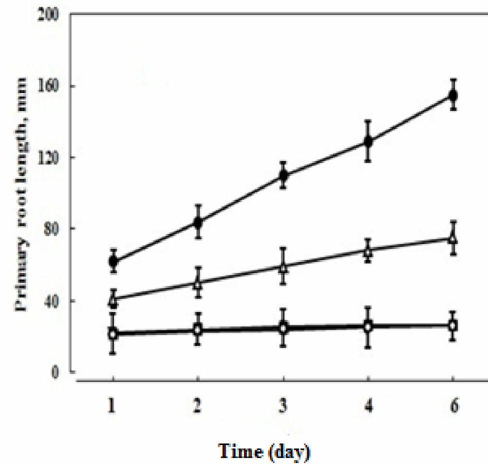


Fig. 64. The effect of different concentrations of aluminium on primary root length of chickpea seedlings grown in rhizobox. Otherwise as Fig. 63.

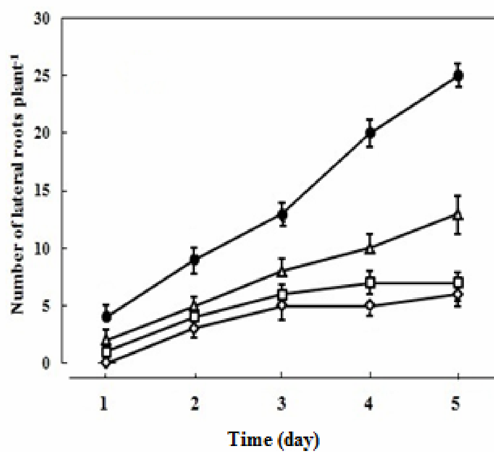


Fig. 65. The effect of different concentrations of aluminium on the number of lateral roots of rice seedlings grown in rhizobox. Otherwise as Fig. 63.

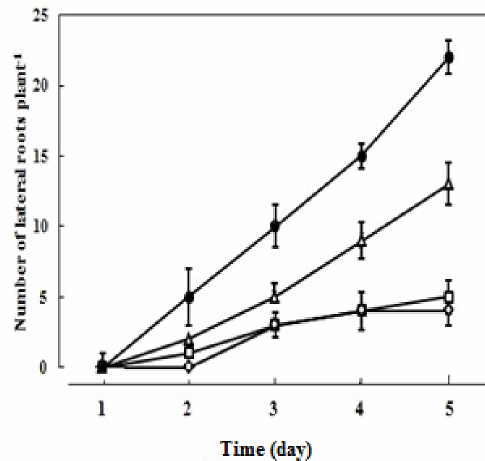


Fig. 66. The effect of different concentrations of aluminium on the number of lateral roots of chickpea seedlings grown in rhizobox. Otherwise as Fig. 63.

6.3.2 Effects of aluminium toxicity on the root length, shoot length and shoot/root length ratio in rice and chickpea seedlings grown in solution culture

Exposure of rice seedlings to 10 μM Al inhibited the root length by 16.4 to 40.6% from 3 to 96 h of exposure. The root length was decreased with the increase in Al concentration from 50 to 150 μM Al. The highest inhibition of the root length was exerted by 150 μM Al which ranged from 43.4 to 61.6% over a period of 3 to 96 h of treatment (Fig. 67a).

Al (50 μM) decreased the shoot length of rice by 8.8 to 22.3% from 3 to 96 h of treatment. 100 and 150 μM Al inhibited the shoot length by 13.5 to 27.9% and 17.7 to 32.8%, respectively, from 3 to 96 h of application (Fig. 67b).

Al, at a concentration of 10 μM, increased shoot/root length ratio of rice by 14.2 to 47.2% from 3 to 96 h of treatment. The shoot/root length ratio increased with the increase in Al concentrations. The maximum stimulation of shoot/root length ratio was recorded at 150 μM Al which ranged from 45.8 to 76.4% from 3 to 96 h of application (Fig.67c).

In chickpea seedlings, 50 μM Al decreased the root length by 7.3 to 41.6% from 3 to 96 h of treatment. The magnitude of inhibition of the root length increased with the increase in Al concentrations. 150 μM Al caused the highest inhibition of the root length of chickpea ranging from 32.9 to 60.0% from 3 to 96 h of exposure (Fig. 68a).

Al, at concentrations of 50 and 100 μM, decreased the shoot length of chickpea by 5.6 to 26.0% and 9.0 to 28.8%, respectively, from 6 to 96 h of treatment. Al (150 μM) inhibited the shoot length by 11.6 to 34.0% from 3 to 96 h of application (Fig. 68b).

The shoot/root length of chickpea seedlings was increased by all the concentrations of Al (10-150 μM) used. 100 and 150 μM Al increased the shoot/root length by 19.9 to 48.0% and 31.9 to 65.0%, respectively, from 3 to 96 h of treatment (Fig. 68c).

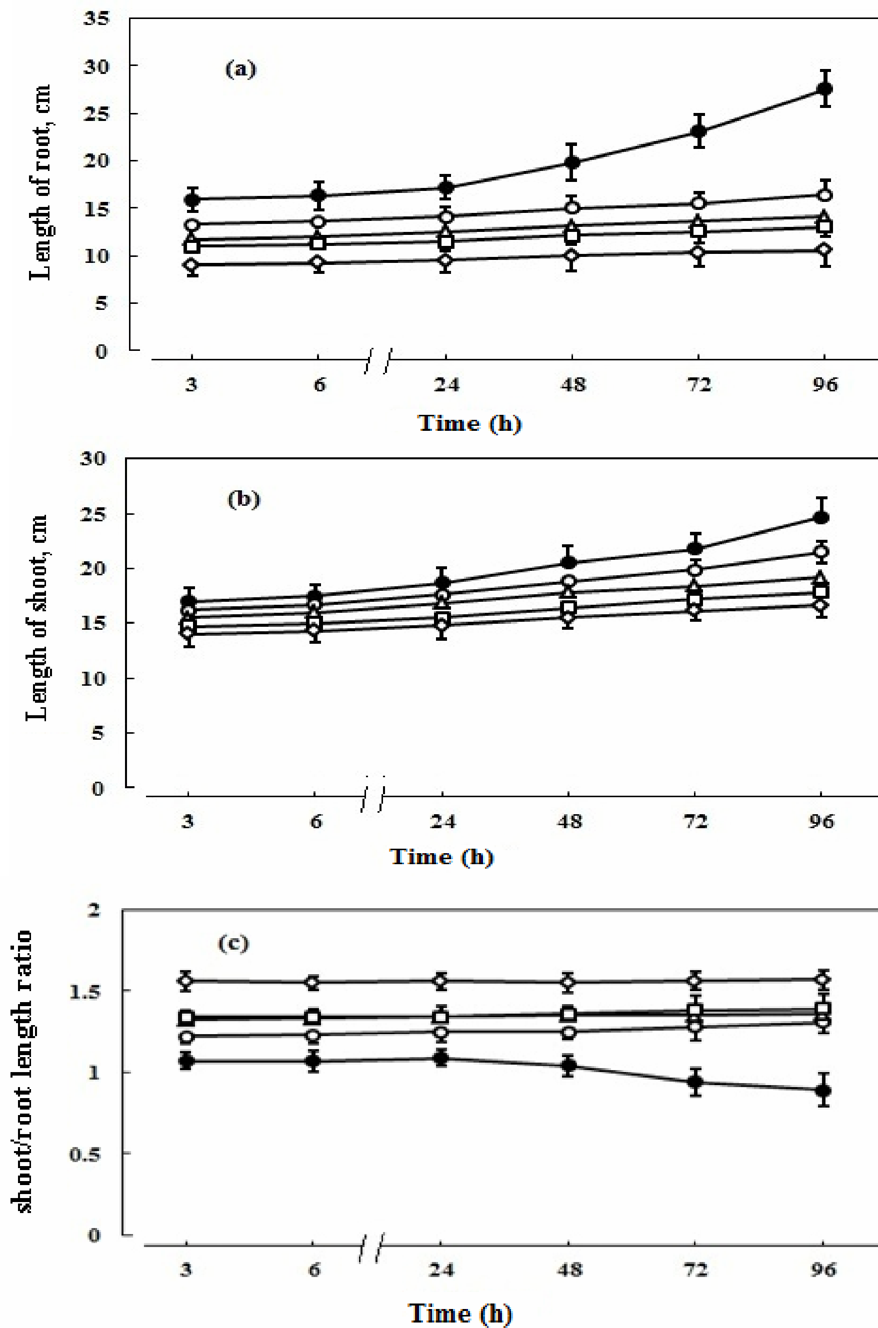


Fig. 67. The effect of different concentrations of aluminium on the (a) root length, (b) shoot length and (c) shoot/root length ratio of rice seedlings grown in solution culture. ● represents control; ○ 10 µM Al; Δ 50 µM Al; □ 100 µM Al; ◊ 150 µM Al. Each value is the mean of three replicates ± standard error.

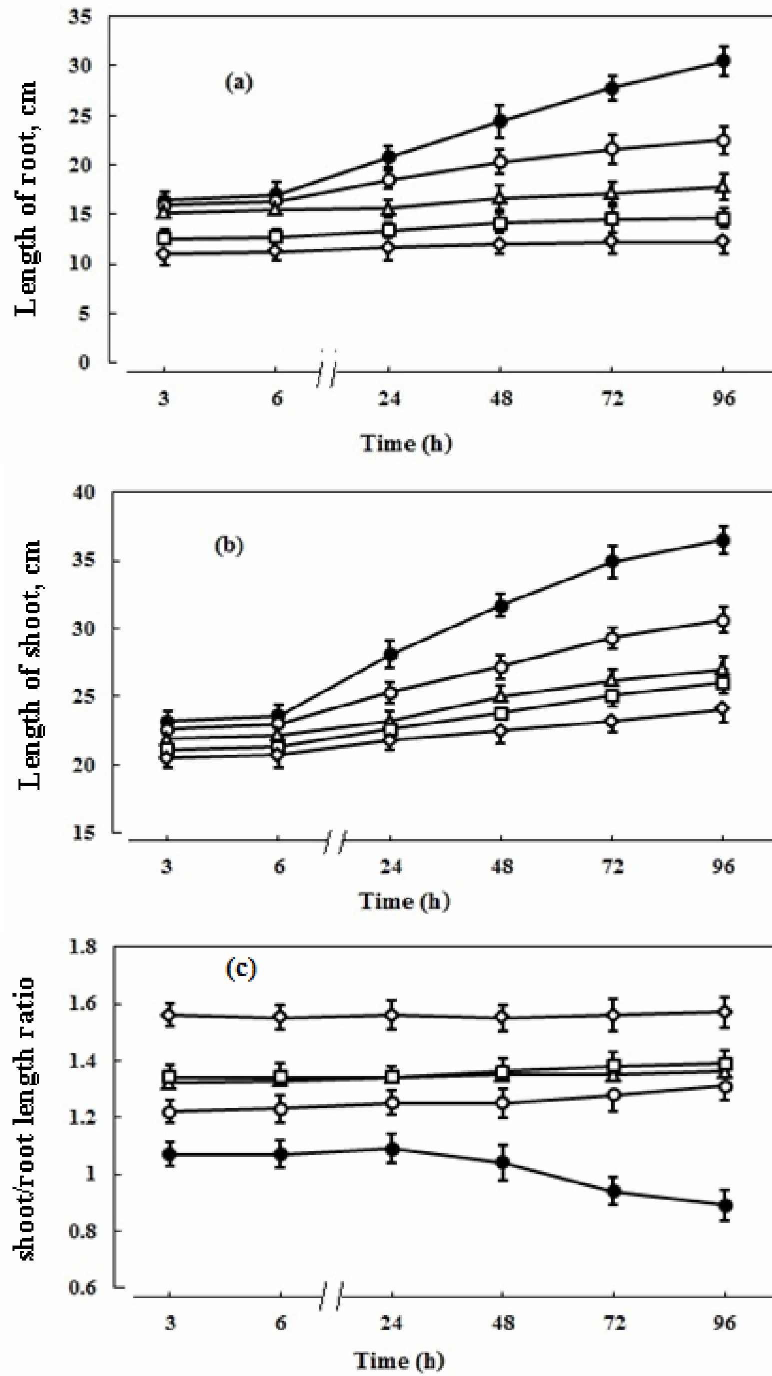


Fig. 68. The effect of different concentrations of aluminium on the (a) root length, (b) shoot length and (c) shoot/root length ratio of chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

Al (10-150 μM) decreased the length of rice and chickpea seedlings (plates 3 and 4).

Al (50 μM) decreased the length of rice seedlings by 10.0 to 27.5% from 3 to 96 h of treatment. The degree of inhibition of the length of rice seedlings increased with the increase in Al concentrations, from 50 to 150 μM. The maximum inhibition of length of seedlings was observed at 150 μM Al which ranged from 30.1 to 48.0% from 3 to 96 h of application (Fig. 69).

Al (10 and 150 μM) decreased the length of chickpea seedlings. 100 μM Al inhibited the length of chickpea seedlings by 15.2 to 39.4% from 3 to 96 h of exposure. Similarly, 150 μM Al decreased the length of chickpea seedlings by 20.5 to 45.8% from 3 to 96 h of application (Fig. 70).

Earlier, it was reported that Al stress inhibited the root growth of wheat (Foy 1988, Jones and Kochian 1995) and barley (Alam 1981).

Similarly, Kinraide and coworkers (1985) reported that a 60% reduction in the root growth was observed in 2-day-old Dayton barley exposed to less than 1 μM Al. However, Hecht-Buchholz and Schuster (1987) did not observe reduction of the root growth in 18-day-old seedlings of same barley variety. Mahapatra and coworkers (2015) found that Al decreased the root length and shoot length of *Vigna radiata*.

6.3.3 Effects of aluminium toxicity on the dry weight of root and shoot and shoot/root dry weight ratio in rice and chickpea seedlings grown in solution culture

Al, at concentrations of 10 and 50 μM, decreased the dry weight of root of rice seedlings by 33.3 to 44.4% and 33.3 to 55.6%, respectively, from 3 to 96 h of treatment. Similarly 150 μM Al inhibited the dry weight of root by 50 to 66.7% from 3 to 96 h of application (Fig. 71a).

Plate 3. Effects of aluminium toxicity on the root and shoot length of rice seedlings grown in solution culture.

Plate 4. Effects of aluminium toxicity on the root and shoot length of chickpea seedlings grown in solution culture.

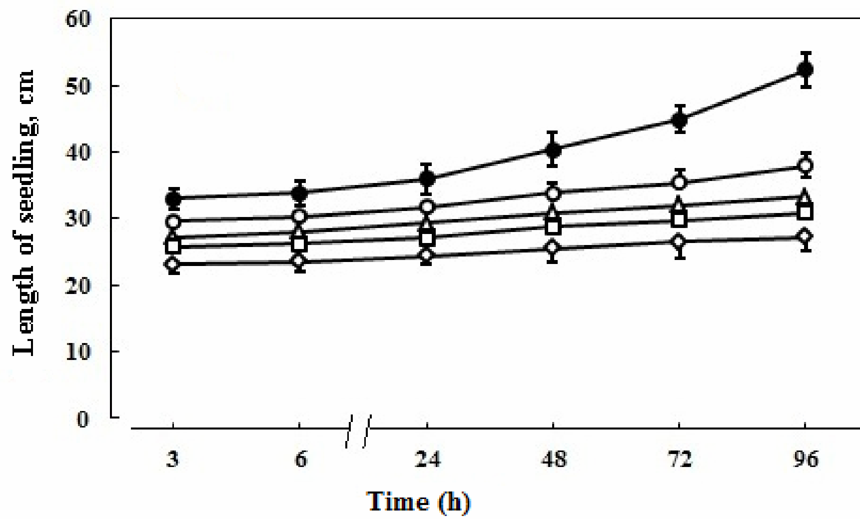


Fig. 69. The effect of different concentrations of aluminium on the length of rice seedlings grown in solution culture. Otherwise as Fig. 67.

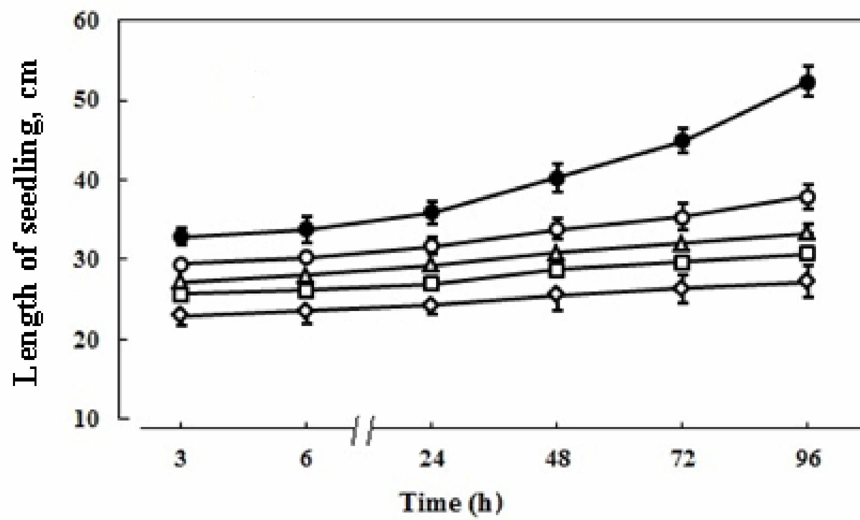


Fig. 70. The effect of different concentrations of aluminium on the length of chickpea seedlings grown in solution culture. Otherwise as Fig. 67.

In rice seedlings, Al (10 μM) decreased the dry weight of shoot by 16.7 to 22.0% from 24 to 96 h of exposure. The dry weight of shoot of rice decreased with the increase in Al concentration. A maximum of 40.0 to 44.5% inhibition in the shoot dry weight was observed following 150 μM Al treatment (Fig. 71b).

In rice, 10 μM Al increased shoot/root dry weight ratio of rice by 20.0 to 40.0% from 3 to 96 h of application. The stimulation of shoot/root dry weight ratio increased with the increase in Al concentrations. 150 μM Al caused the maximum 20.0 to 70.0% enhancement of shoot/root dry weight ratio from 3 to 96 h of exposure (Fig. 71c).

Similarly, in chickpea seedlings, 50 μM Al decreased the dry weight of root by 5.9 to 22.0% from 3 to 96 h of treatment. 100 and 150 μM Al caused 11.8 to 37.0% and 17.7 to 44.4% inhibition of the root dry weight, respectively, from 3 to 96 h of application (Fig. 72a).

The dry weight of stem of chickpea seedlings decreased gradually with the increase in Al concentrations from 10 to 150 μM. A maximum of 27.3 to 45.5% inhibition of the dry weight of stem was recorded at 150 μM Al at 3 to 96 h of treatment (Fig. 72b).

In chickpea seedlings, 50 μM Al decreased the dry weight of leaves by 7.7 to 16.7% following 3 to 96 h of application. Similarly, 100 and 150 μM Al decreased the dry weight of leaves by 15.4 to 29.2% and 15.4 to 33.3%, respectively, from 3 to 96 h of application (Fig. 72c).

However, shoot/root dry weight ratio of chickpea seedlings was increased with the increase in concentration of Al from 10 to 150 μM. In chickpea, the maximum stimulation of shoot/root dry weight ratio was observed following 150 μM Al treatment which ranged from 6.3 to 12.4% over a exposure period of 96 h (Fig. 72d).

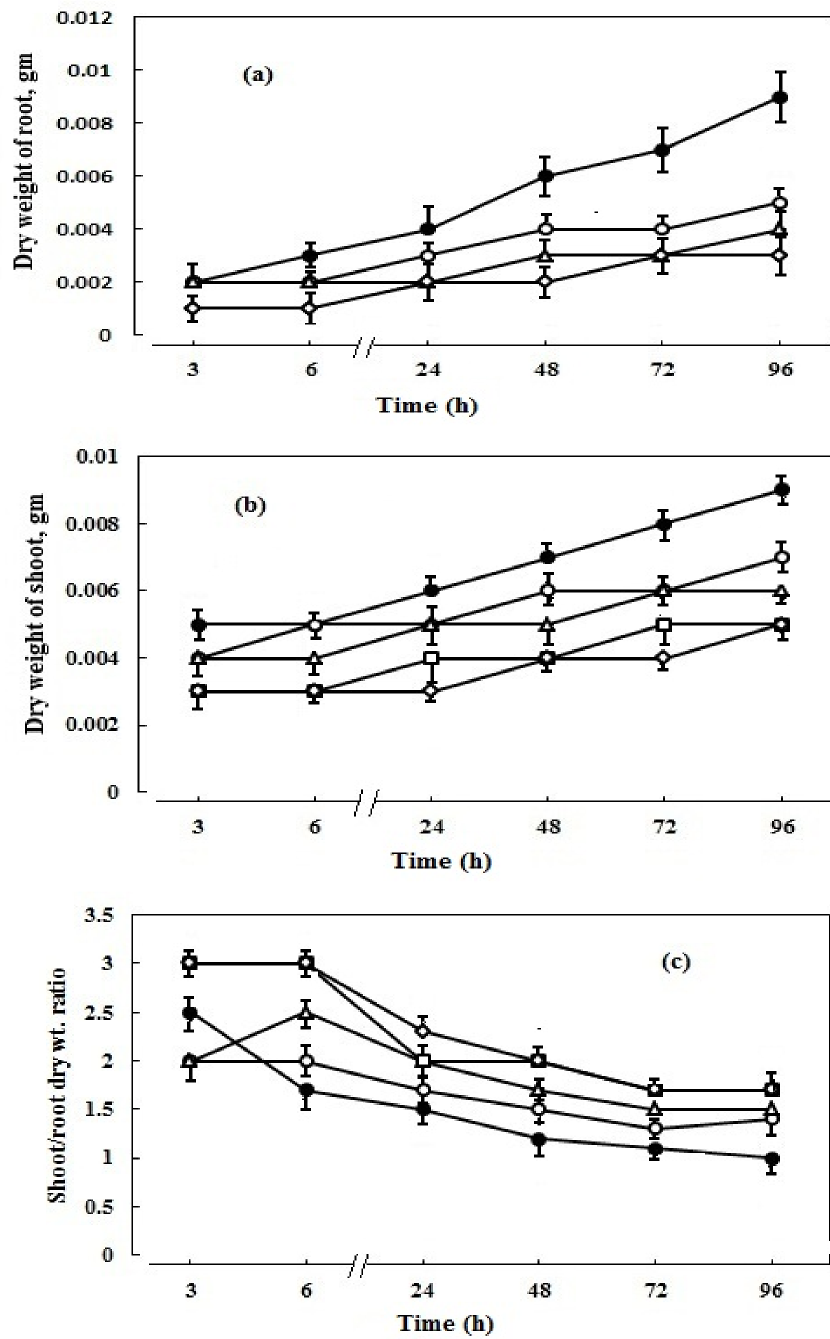


Fig. 71. The effect of different concentrations of aluminium on dry weight of the (a) root, (b) shoot and (c) shoot/root dry weight ratio of rice seedlings grown in solution culture. Otherwise as Fig. 67.

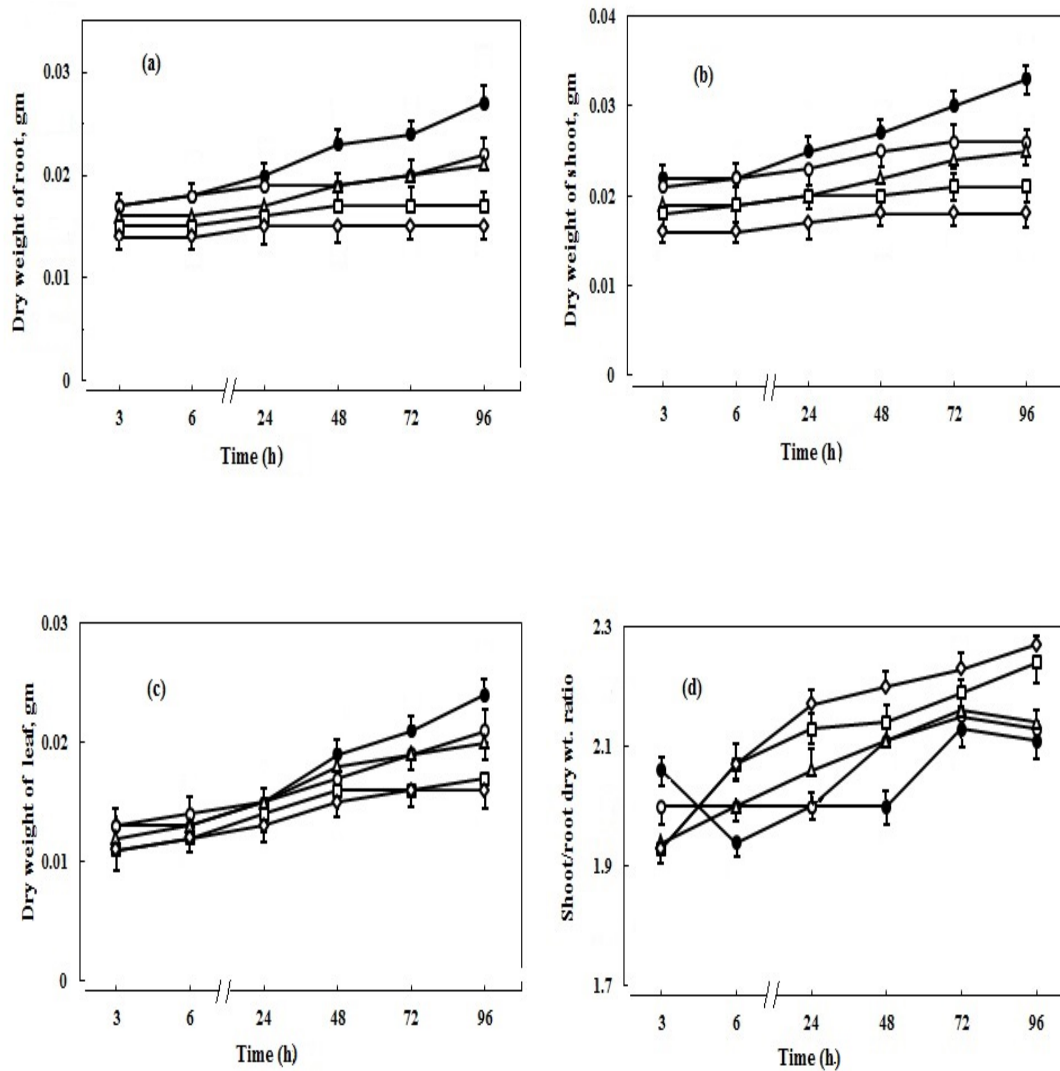


Fig. 72. The effect of different concentrations of aluminium on dry weight of the (a) root, (b) stem, (c) leaf and (d) shoot/root ratio of chickpea seedling grown in solution culture. Otherwise as Fig. 67.

Similarly, Al decreased the dry weight of cultured cells of tobacco (Abdel-Basset *et al.* 2013). On the contrary, Symeonidis *et al.* (2004) found that Al increased the dry weight of melon (*Cucumis melo*).

Increase in shoot/root dry weight ratio in rice and chickpea seedlings (Figs. 71c and 72d) indicates that growth of the shoot is higher than that of the root under the influence of aluminium.

Chapter 7

Effects of aluminium toxicity on the anatomical structures in rice and chickpea plants and its relation with ion transport

7.1 Introduction

Plants respond to various stresses through morphological, anatomical and physiological adjustments that help them to cope with this stress. Wagatsuma *et al.* (1987) reported that the cells of the epidermis and outer cortex of maize were damaged and partially detached in barley plant after 6 days of exposure to Al treatment.

Cha and Lee (1996) reported that Al-treated root accumulated phenolic material in root tissue *of Alnus hirsute* plant. Longitudinally elongated cell and larger number of intercellular spaces were found in maize root at 30 and 75 µM Al treatment (Batista *et al.* 2012). The size of protoxylem and metaxylem vessels were smaller and showed deformed appearance in maize plant after aluminium treatment (Batista *et al.* 2012).

Due to Al exposure, reduced length was found in the meristematic and elongation zones of barley root (Kochian 1995) and the root cortex of wheat (Sasaki *et al.* 1996) and maize (Budiková 1999).

Gomes *et al.* (2011) reported that, leaf epidermis thickness of the adaxial and abaxial surface of *Brachiaria decumbens* (signal grass) increased, but McQuattie and Schier (1993), found that the size of leaf mesophyll cells of pitch pine seedlings was reduced.

Aluminium treatment resulted in closure of stomata (Rengel 1992, Kochian 1995). In Roman nettle, stomatal sizes were significantly decreased due to aluminium stress compared to that of control (Özyiğit and Akinci 2009).

The effect of aluminium stress on the changes in anatomical structures of root, stem and leaf in rice and chickpea were studied in order to establish its correlation with the effect of aluminium toxicity on transport of ions.

7.2 Materials and Methods

For studying anatomical structures, rice and chickpea plants were grown in sand culture in net house under natural environmental condition according to the method described in section 2.5. Plants were subjected to 150 and 300 μM Al and half strength Hoagland solution was used as control. Root, stem and leaf of 28-day-old plants were collected according to section 2.23.

Free hand sectioning was done and the sections were stained with saffranin. Transverse sections of the root, stem and leaf were studied with a compound microscope. Photographs of sections were taken using a camera (Axiocam ERc 5s) at different magnification (5X, 10X and 40X).

Leaf stoma and trichome of 28-day-old control and Al-treated plants grown in sand culture were also studied.

7.3 Results

7.3.1 Effects of aluminium toxicity on anatomy of the root of rice

Transeverse section of the middle part of the root of aluminium-stressed rice plant showed following anatomical structures:

In the root of rice, significant structural changes occurred due to Al stress. Under aluminium stressed condition, root length was decreased as compared to that of control root.

Epidermis: Epidermis was uniseriate and composed of parenchyma cells in both control and aluminium treated roots (Plate 5a, b and c). Epidermal cells were larger in size in Al-treated plant. Some of the epidermal cells grew out in the form of unicellular hair.

Exodermis: A specialized layer of parenchyma cells constituted the exodermis which lied beneath the epidermis in both control and aluminium treated plants.

Sclerenchyma: Adjoining to exodermis, a tissue of thick-walled cells formed a distinct sclerenchymatous cylinder in both control and Al-treated plants. Sclerenchymatous layer was uniserate both in control and 150 μM Al treated plant but biseriate in 300 μM Al-treated plant (Plate 6a, b and c).

Cortex: Cortex usually consisted of large thin-walled parenchyma cells surrounded on the outside by the epidermis and on the inside by the endodermis in the root of rice plants. In control root, there were no airspaces but in Al-treated plant root, cortical cells were longitudinally elongated with large airspaces (Plate 6a, b and c).

Endodermis: Endodermis is a specialized cylinder of cells composed of a single layer of barrel shaped parenchyma cells forming the inner boundary of the cortex which separates the outer cortex from the central core. In both control and Al-treated roots, the endodermis was composed of single layer of cells (Plate 7a, b and c).

Pericycle: Pericycle is a layer of plant tissue beneath the endodermis which surrounds the conducting tissue both in roots of control and Al-treated roots (Plate 7a, b and c).

Vascular tissue: Vascular system consisted of groups of phloem tissue, metaxylem vessel, protoxylem vessel and non-lignified pith. In control plant root, the number of metaxylem vessels were four whereas in 150 and 300 μM Al-stressed root, the number was reduced to three and two, respectively. On the other hand, in 300 μM Al-treated root, the diameter of metaxylem vessels were increased (Plate 5a, b and c, and 7a, b and c).

Pith: Pith is the soft, central cylinder of thin walled parenchymatous tissue in the root, which is prominent both in control and Al-stressed condition (Plate 7a, b and c).

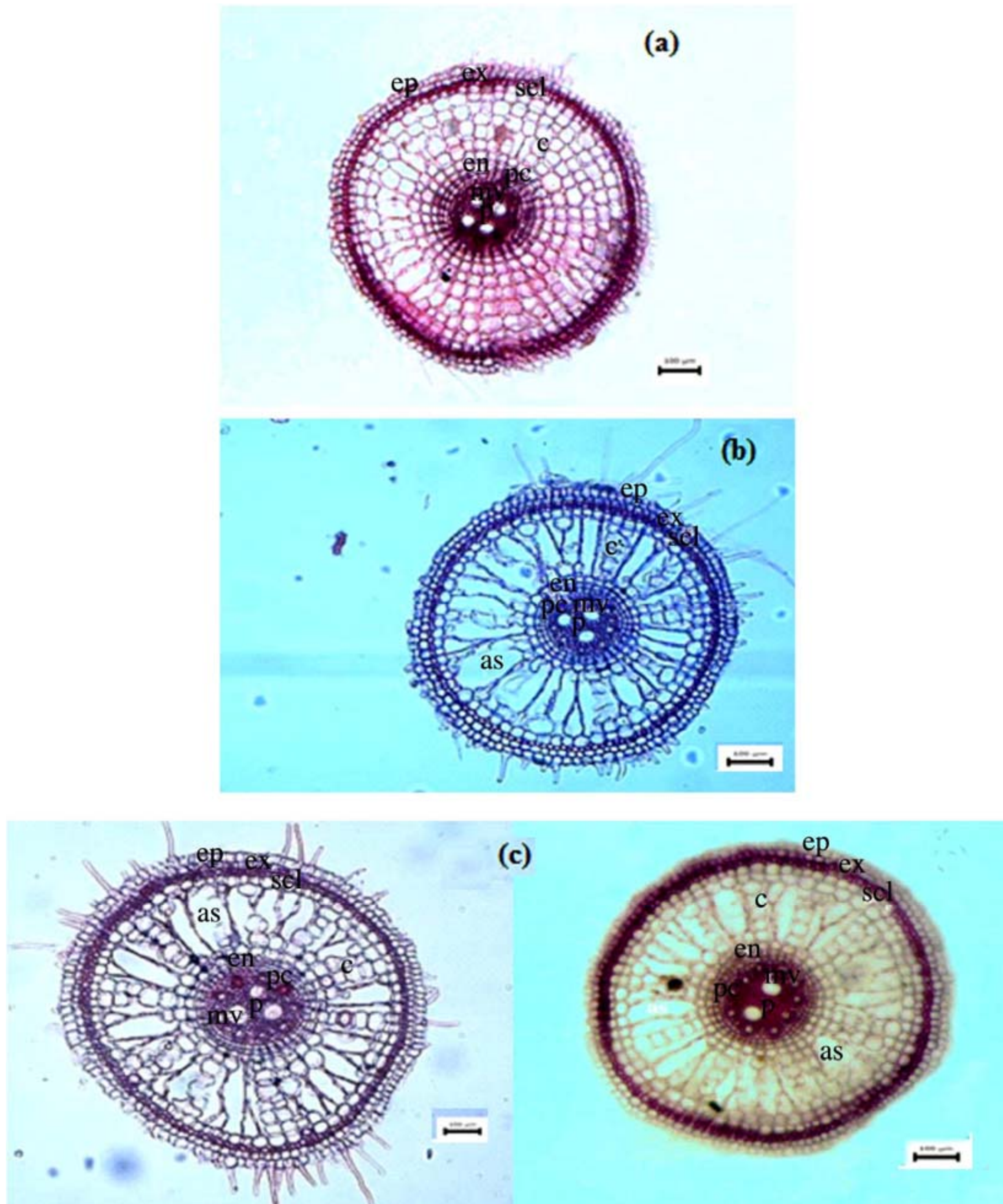


Plate 5. Transverse section of the root of rice (a) control, (b) 150 μM Al and (c) 300 μM Al-treated plant showing epidermis (ep), exodermis (ex), sclerenchyma (scl), cortex (c), endodermis (en), pericycle (pc), metaxylem vessel (mv), pith (p) and air space (as). Bar = 100 μm.

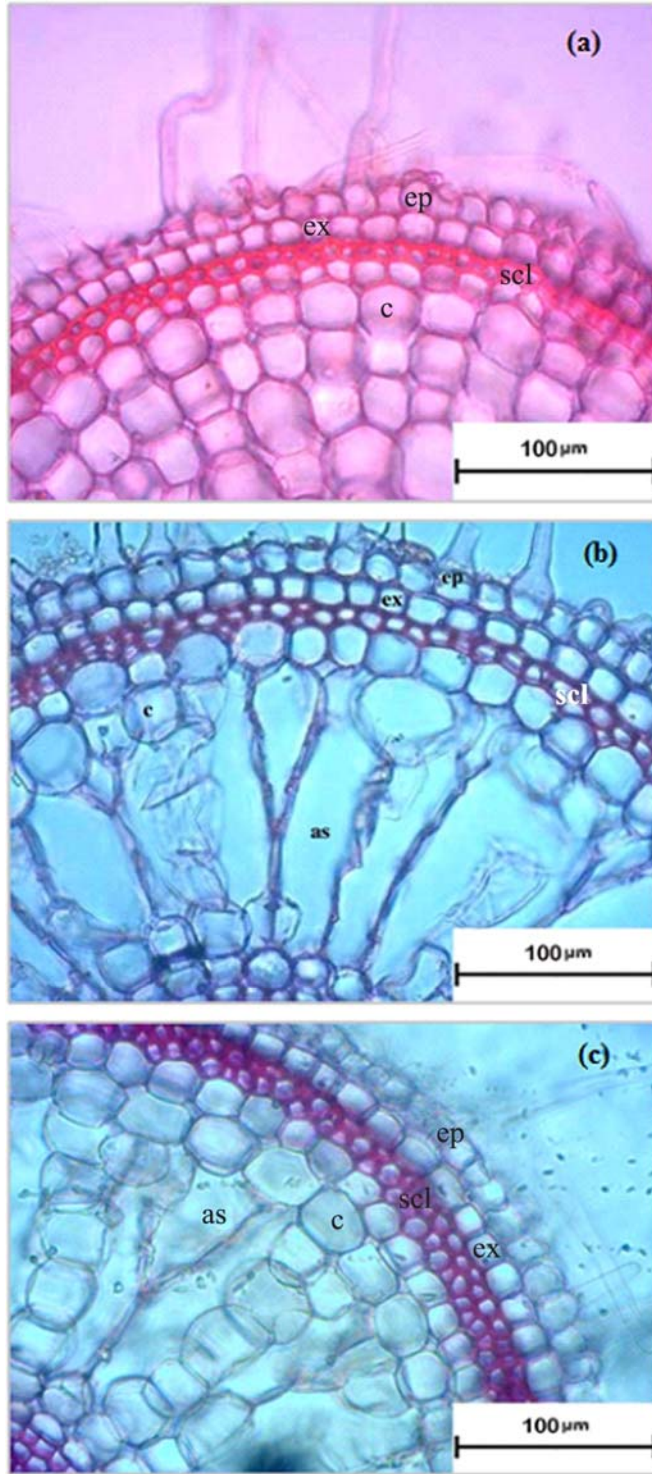


Plate 6. Same as plate 5 but of higher magnification showing epidermis (ep), exodermis (ex), sclerenchyma (scl), cortex (c) and air space (as). Bar = 100 µm.

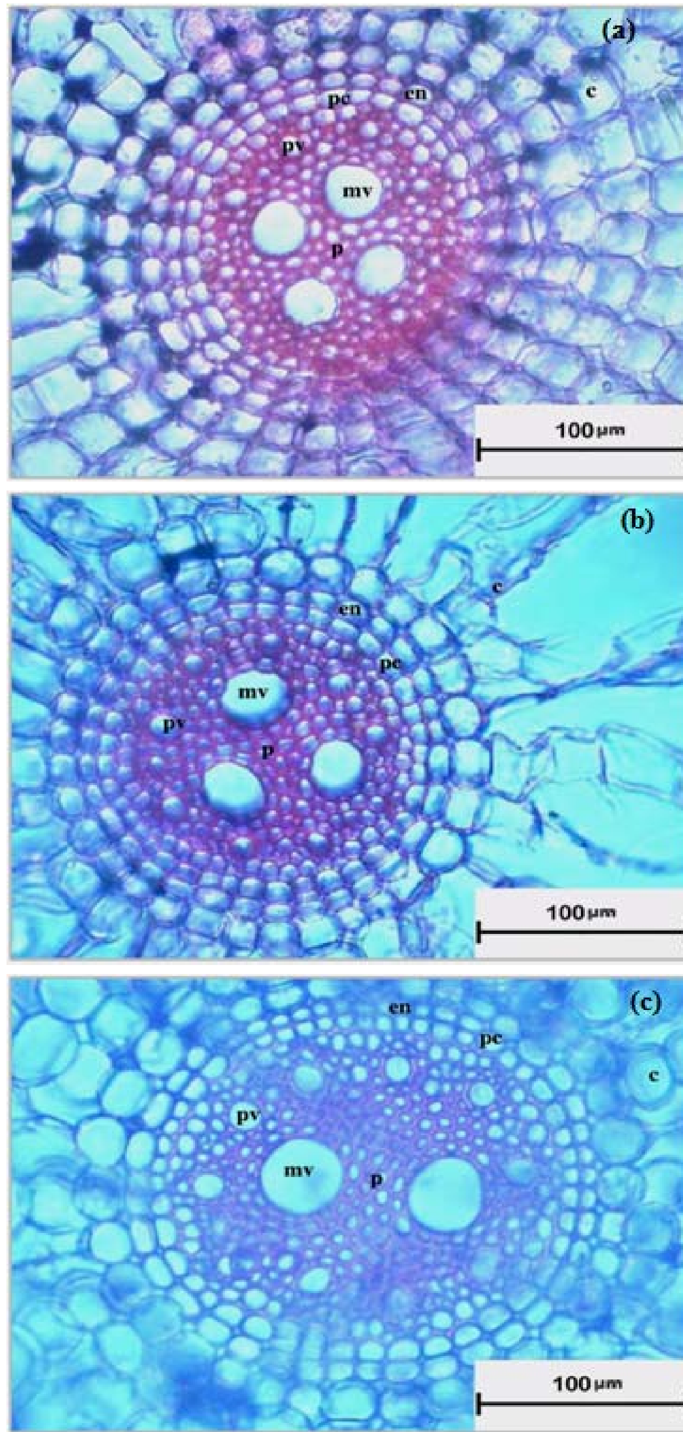


Plate 7. Same as plate 5 but of higher magnification showing cortex (c), endodermis (en), pericycle (pc), metaxylem vessel (mv), protoxylem (pv) and pith (p). Bar = 100 µm.

7.3.2 Effects of aluminium toxicity on anatomy of the stem (internode) of rice

Transverse section of the internode of the stem of 28-day-old rice plant showed the following anatomical structures:

Epidermis: Epidermis was monoseriate and composed of long elongated cells in both control and Al-treated stem (Plate 8a, b and c).

Sclerenchyma: Adaxial to epidermis thick layer of sclerenchymatous cells were found in Al-treated plant (Plate 8a, b and c). Sclerenchymatous layer was many in number in 300 µM Al-treated plant.

Vascular bundles: In the rice internode, two types of vascular bundles were found and they were arranged in two rings. The abaxial bundles were attached to the adaxial sclerenchyma cells of hypodermis, size of the vascular bundles were decreased in Al-treated plant.

In both small and large bundles two metaxylem vessels, one protoxylem vessel were found. The protoxylem lacuna was found in large bundle, vascular bundles were covered by bundle sheath. The metaxylem vessels of Al-treated plant were smaller in size than that of control plant. Phloem was present in all large and small bundles (Plate 8a, b and c). Phloem area decreased in Al-treated plant.

Ground tissue: The ground tissue includes all tissues that are neither dermal nor vascular and usually consists of parenchyma, collenchyma and sclerenchyma cells. Larger parenchyma cells were observed both in control and Al-treated stem (Plate 8a, b and c).

7.3.3 Effects of aluminium toxicity on anatomy of the leaf blade of rice

Transverse section of the middle portion of the 4th leaf of 28-day-old rice plant showed the following anatomical structures:

Tissues of the leaf blade consisted of epidermis, vascular bundle, mesophyll tissue and bulliform cells.

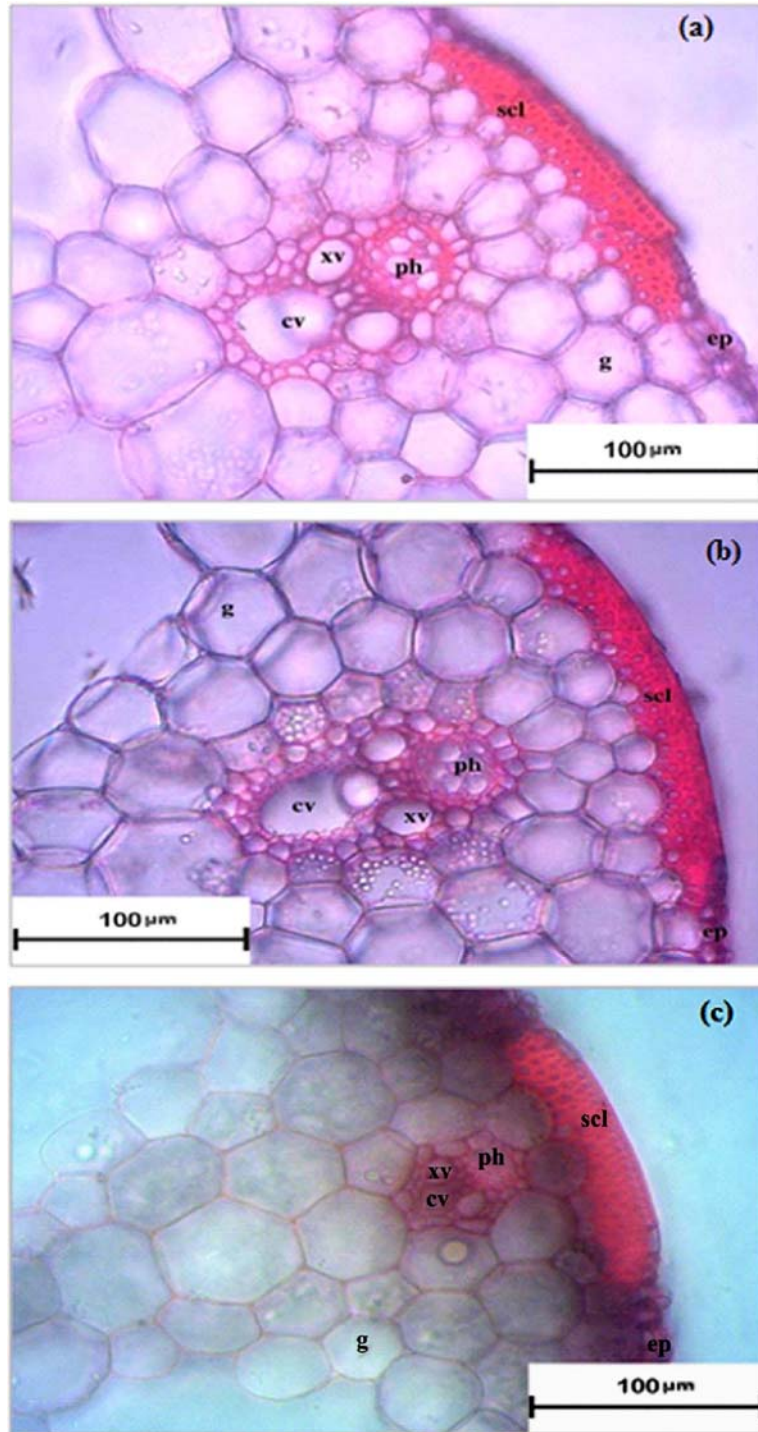


Plate 8. Transverse section of the stem (internode) of rice (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing epidermis (ep), sclerenchyma (scl), ground tissue (g), phloem (ph), xylem vessel (xv) and protoxylem cavity (cv). Bar = 100 µm.

Epidermis: In rice leaf, both upper and lower epidermis was present. Epidermis was composted of parenchyma cells and was uniseriate.

Parenchyma cells: In the control leaf (midrib region), parenchyma cells were well developed but in the leaf of Al-treated rice leaf, poorly developed parenchyma cells were observed (Plate 9a, b and c). Midrib region was composed of five layers of parenchyma cells with large cavities.

Mesophyll tissue: Mesophyll cells were arranged in 3-4 layers in control plant. Mesophyll tissue of the leaf was fewer in number in Al-treated plant. Amount of chlorophyll was also reduced in the leaf of Al-stressed plant as compared to that of control (Plate 11a, b and c).

Vascular bundles: Large and small vascular bundles were found in the leaf blade of rice plant. The midrib region consisted of vascular bundle and colorless thin walled parenchyma cells. Number of vascular bundles decreased in midrib region of Al-stressed plant. Vascular bundles of the leaf blade were completely surrounded by parenchymatous bundle sheath. Diameter of metaxylem vessel was also decreased in the leaf of Al-treated plant (Plate 10a, b and c).

Bulliform cells: Bulliform cells are large, thin-walled, colorless, bubble-shaped epidermal cells that occur in groups on the adaxial surface of the leaf of rice. In the leaf blade, bulliform cells were observed in the leaf of control and Al-treated rice plant. Size and frequency of bulliform cells were increased in the leaf of Al-treated plant. Midrib region lacked bulliform cells (Plate 11a, b and c).

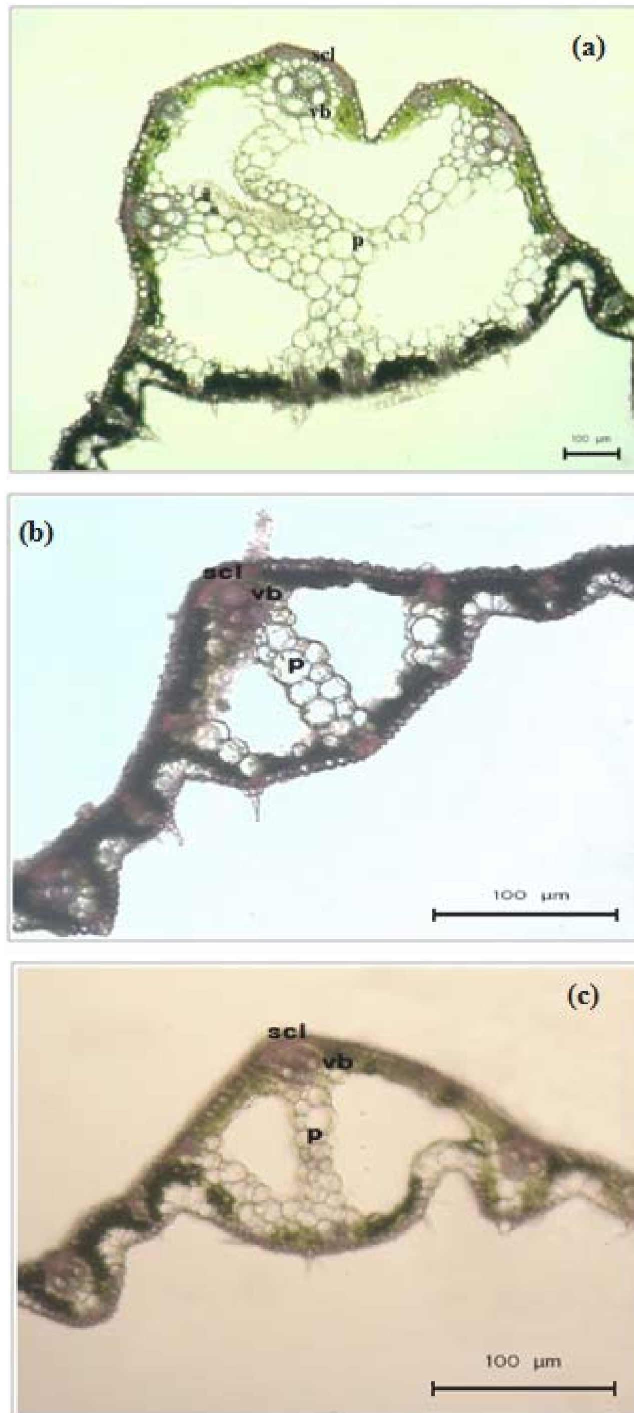


Plate 9. Transverse section of the leaf (midrib area) of rice (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing sclerenchyma (scl), vascular bundle (vb) and parenchyma cell (p). Bar = 100 µm.

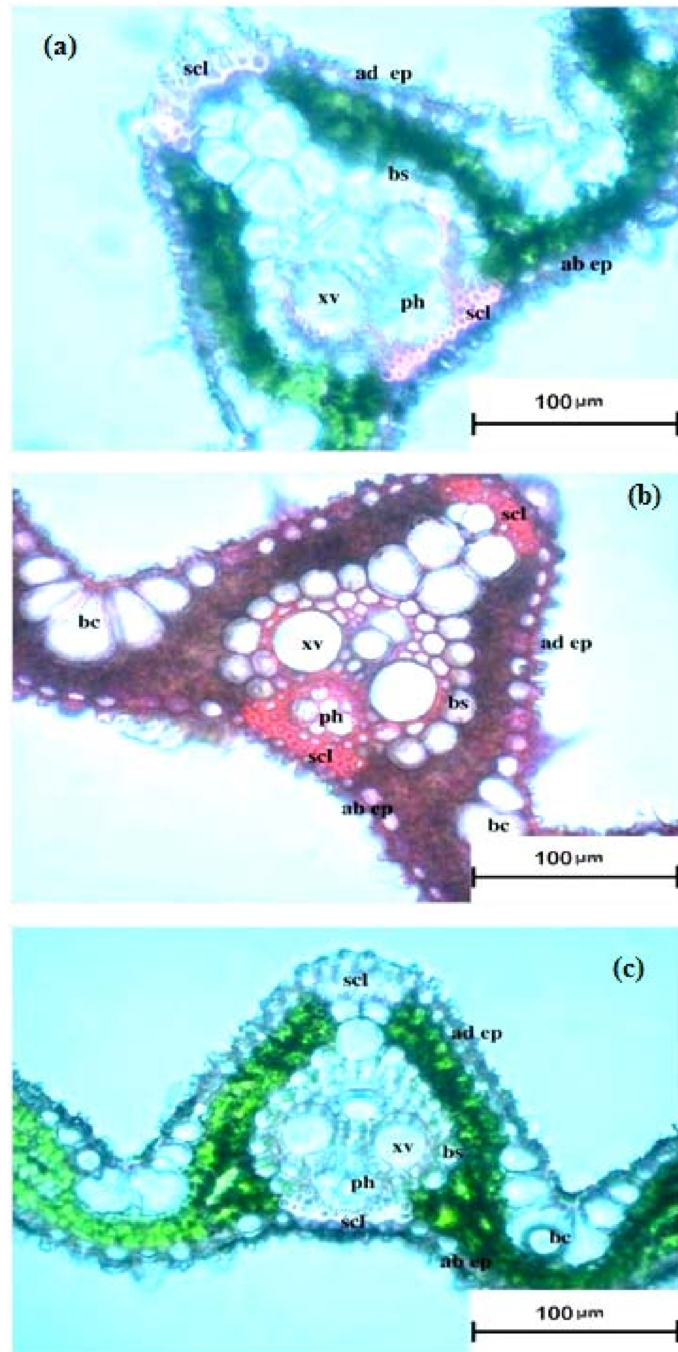


Plate 10. Transverse section of the leaf blade of rice (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing adaxial surface epidermis (ad ep), abaxial surface epidermis (ab ep), sclerenchyma (scl), xylem vessel (xv), phloem (ph), bundle sheath (bs) and bulliform cell (bc). Bar = 100 µm.

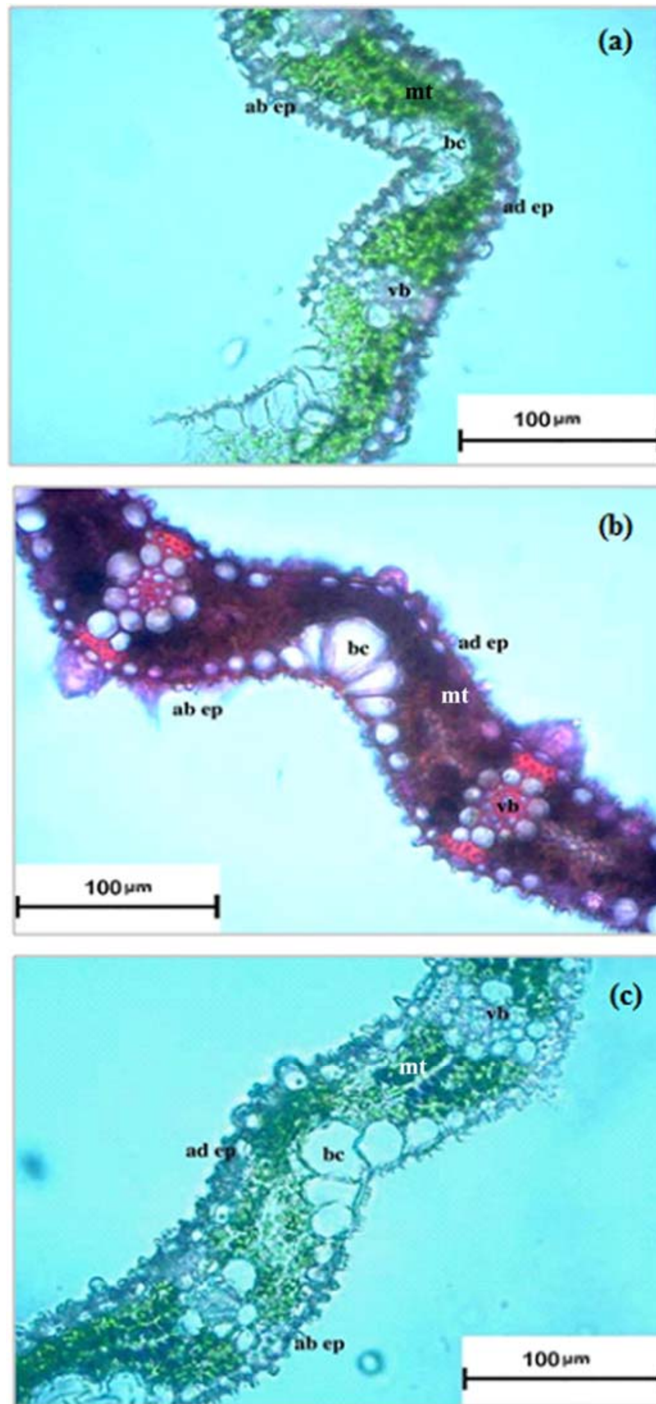


Plate 11. Transverse section of the leaf blade of rice (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing adaxial face epidermis (ad ep), abaxial face epidermis (ab ep), vascular bundle (vb), mesophyll tissue (mt) and bulliform cell (bc). Bar = 100 µm.

7.3.4 Effects of aluminium toxicity on stomata and trichomes of the leaves of rice

Structures of stomata and trichomes of the leaves of the 4th node from the top of 28-day-old rice plants are as follows:

Stoma is a tiny pore in a leaf surrounded by a pair of guard cells. Graminaceous type of stomata were observed in the leaf of rice plant. In the leaf of Al-treated plant, the number of the stomata was increased whereas stomatal opening was reduced. Due to aluminium treatment, the guard and subsidiary cells also became reduced in size than those of control plant (Plate 12a, b and c).

Trichome is a hairlike or bristlelike, non-glandular outgrowth from the epidermis of a leaf. A large number of trichomes were found in the leaf of control plant. As compared to control, Al treatment increased the number trichomes but decreased the size of trichomes (Plate 13a, b and c).

7.3.5 Effects of aluminium toxicity on anatomy of the root of chickpea

Due to aluminium treatment, the length of primary root and the number of lateral roots of chickpea were reduced than that of the control root.
The following anatomical structures were shown in transverse section of the middle part of the root of 28-day-old chickpea:

Epidermis: The epidermis is a protective outer covering of the root. In the root of control chickpea plant, isodiametric single layered epidermis was observed but in the root of Al-treated plant, the broken epidermal layer was found (Plate 14a, b and c).

Cortex: Cortex is the layers of tissue located between the epidermis and the vascular bundles. In the root of Al-treated chickpea plant, cortex cells occupied smaller area than that of control root. The cortex composed of 16-17 layers of cells in the root of control plant (Plate 15a) whereas its thickness was 12-13 layers of cells in the root of 300 μM Al-treated plant (Plate 15c). Phenolic material was accumulated in the cortex cell of Al-treated plant root (Plate 14b and c).

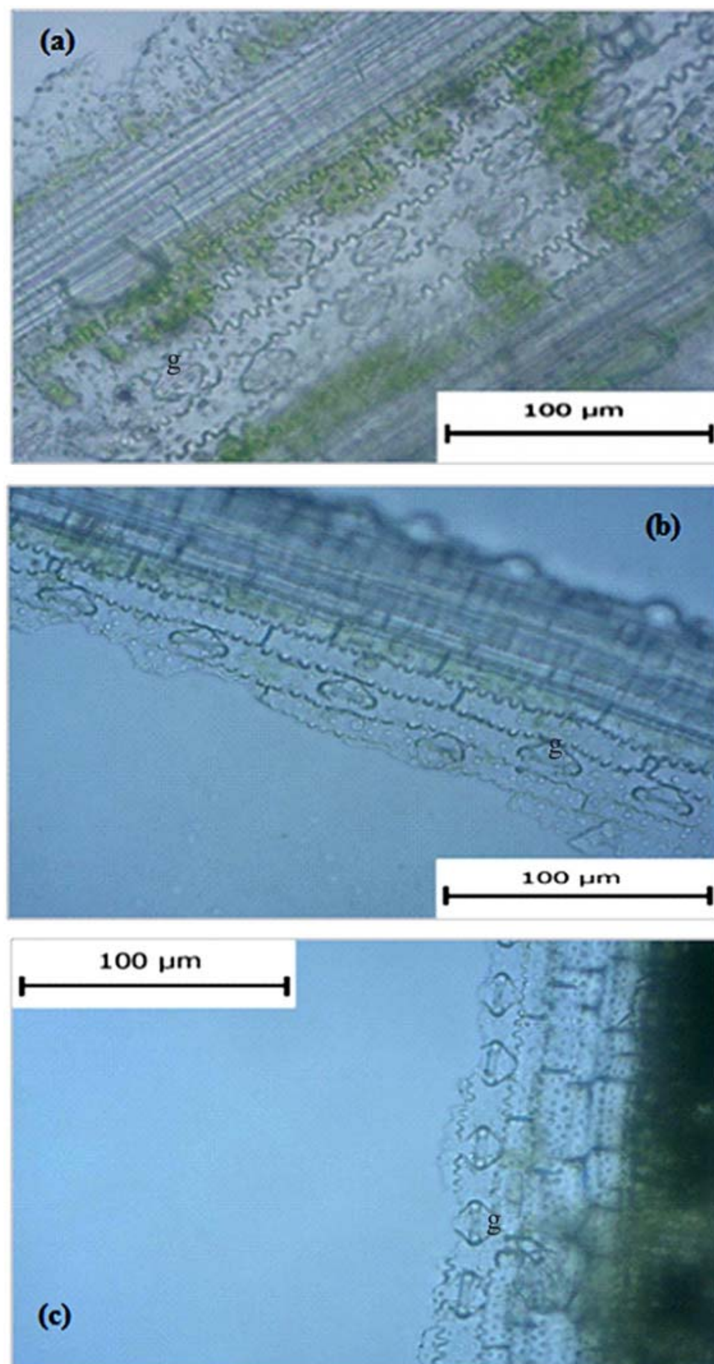
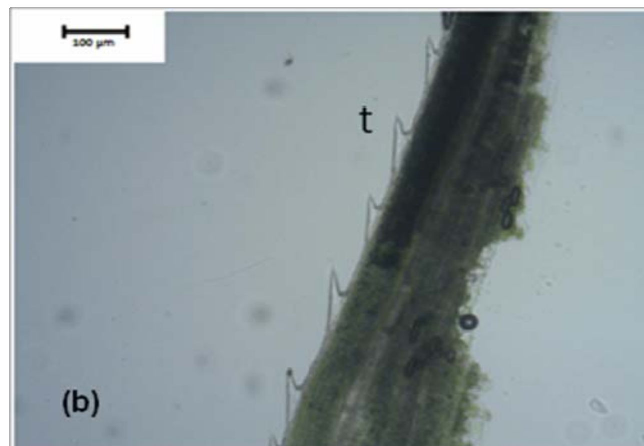


Plate 12. Peel of the leaf of rice (a) control, (b) 150 µM and (c) 300 µM Al-treated plant showing stomata and guard cell (g). Bar = 100 µm.



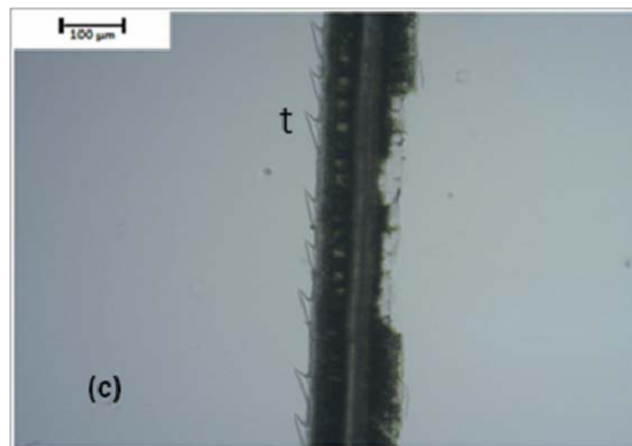


Plate 13. Peel of the leaf of rice (a) control, (b) 150 µM and (c) 300 µM Al-treated plant showing trichomes (t). Bar = 100 µm.

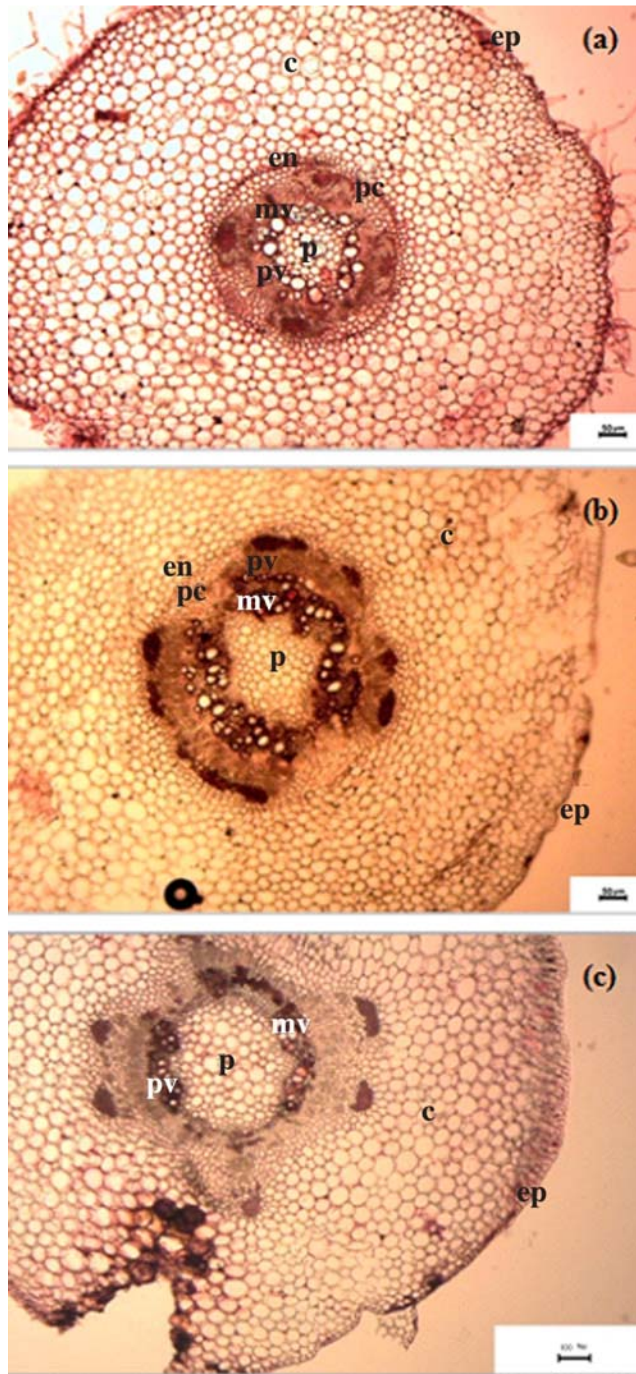


Plate 14. Transverse section of the root of chickpea (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing epidermis (ep), cortex (c), endodermis (en), pericycle (pc), metaxylem vessel (mv), protoxylem vessel (pv) and pith (p). Bar = 100 µm.

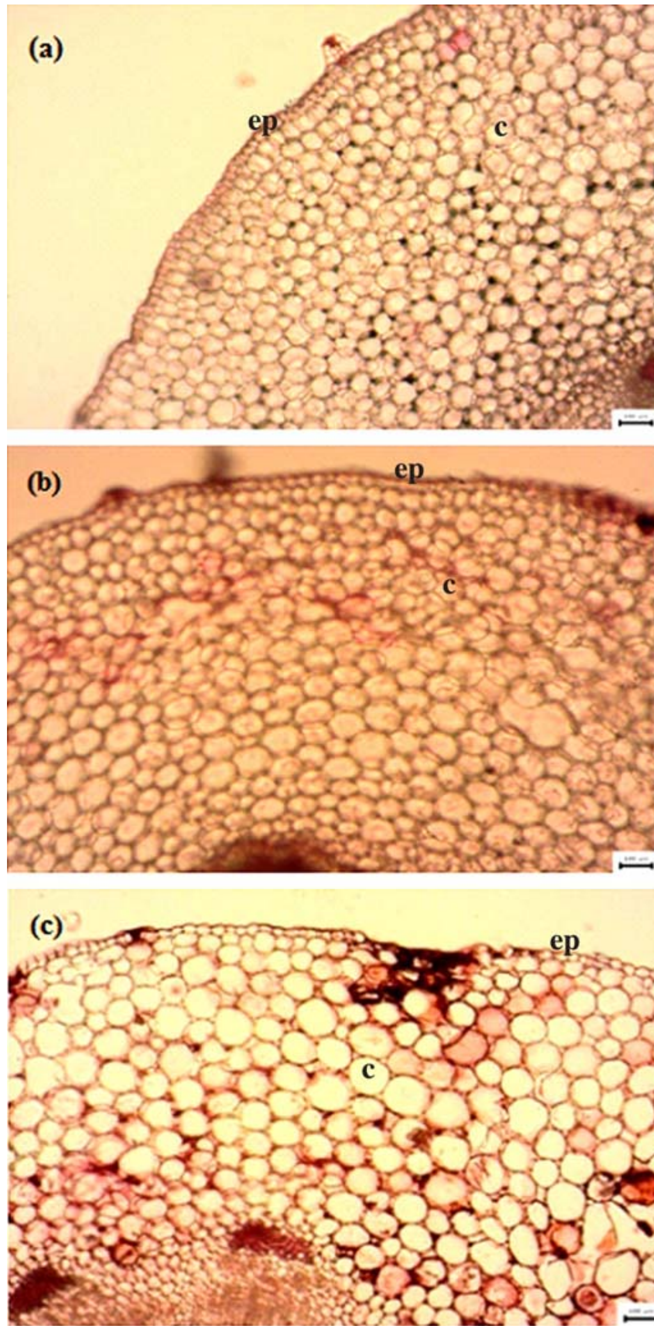


Plate 15. Same as plate 14 but of higher magnification showing epidermis (ep) and cortex (c). Bar = 100 µm.

Endodermis: Endodermis is the layer of parenchyma-cells which are united to form the sheath surrounding a vascular bundle. Endodermis consisted of single layered cells both in the root of control and Al-stressed chickpea plant (Plate 15a, b and c).

Pericycle: Pericycle layer is composed of thin parenchyma cells lying just within the endodermis and enclosing the vascular tissue. Single layered pericycle was found in the root of both control and Al stressed chickpea plant (Plate 16a, b and c).

Vascular tissue: The most significant structural changes occurred in vascular system. Smaller sized and irregular structured metaxylem vessels were found in the root of 150 and 300 μM Al-treated chickpea plant. The number of metaxylem vessels were also reduced in the root of Al-treated plant. A huge amount of phenolic material were found in the vessel in the root of Al-treated plant (Plate 17a, b and c). As compared to that of control root, larger group of sclerenchyma cells were superimposed upon the phloem tissue (Plate 18a, b and c). Phloem was also smaller in size in the root of Al-treated plant.

Pith: No remarkable change was observed in pith in the root of Al-treated rice and chickpea (Plate 18a, b and c).

7.3.6 Effects of aluminium toxicity on anatomy of the stem of chickpea

Transverse section of the stem of 28-day-old chickpea plant showed the following anatomical structures:

Epidermis: Monoseriate epidermis was observed in the stem of control and Al-treated chickpea plant. In control stem, regular shaped epidermal cells were found but Al resulted in irregular shaped epidermal cells (Plate 20a, b and c).

Cortex: Cortical cells are lying between the epidermis and the endodermis of stem. Cortex consisted of several layers of parenchyma cells. The cortical cells of Al stressed stem occupied smaller area than that of the control stem in chickpea (Plate 19a, b and c).

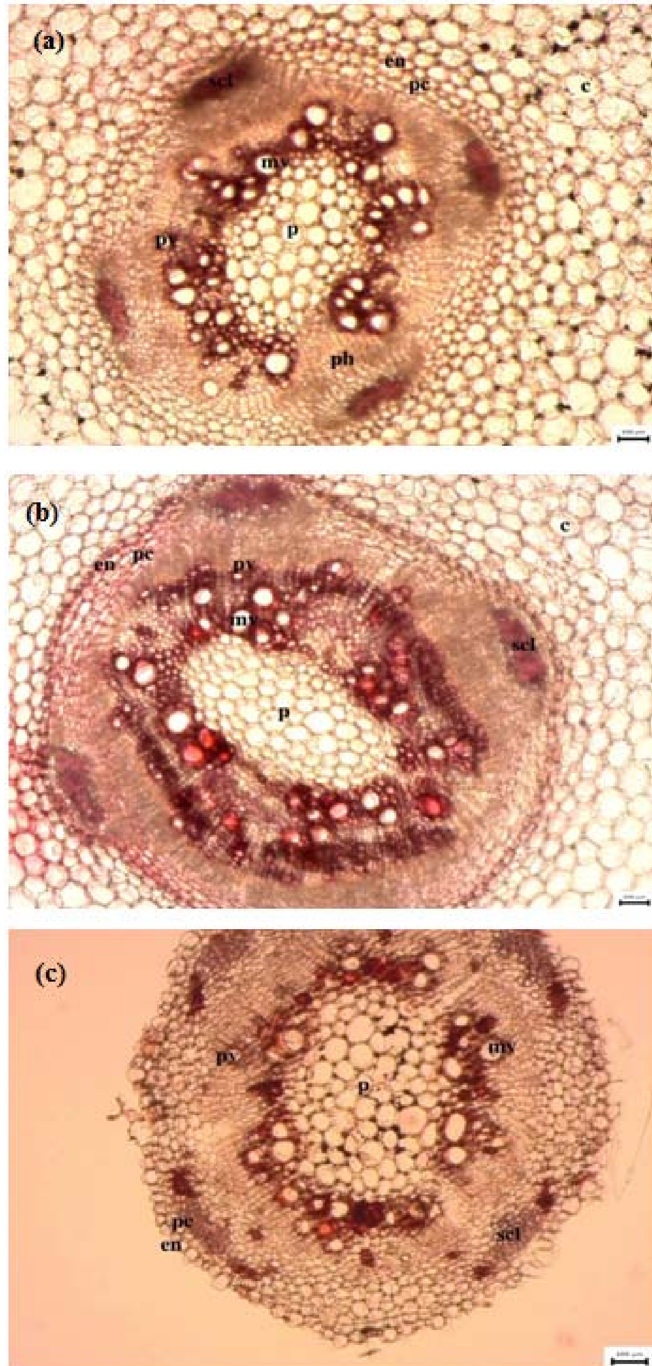


Plate 16. Same as plate 14 but of higher magnification showing cortex (c), endodermis (en), pericycle (pc), sclerenchyma (scl), metaxylem vessel (mv), protoxylem vessel (pv), phloem (ph) and pith (p). Bar = 100 µm.

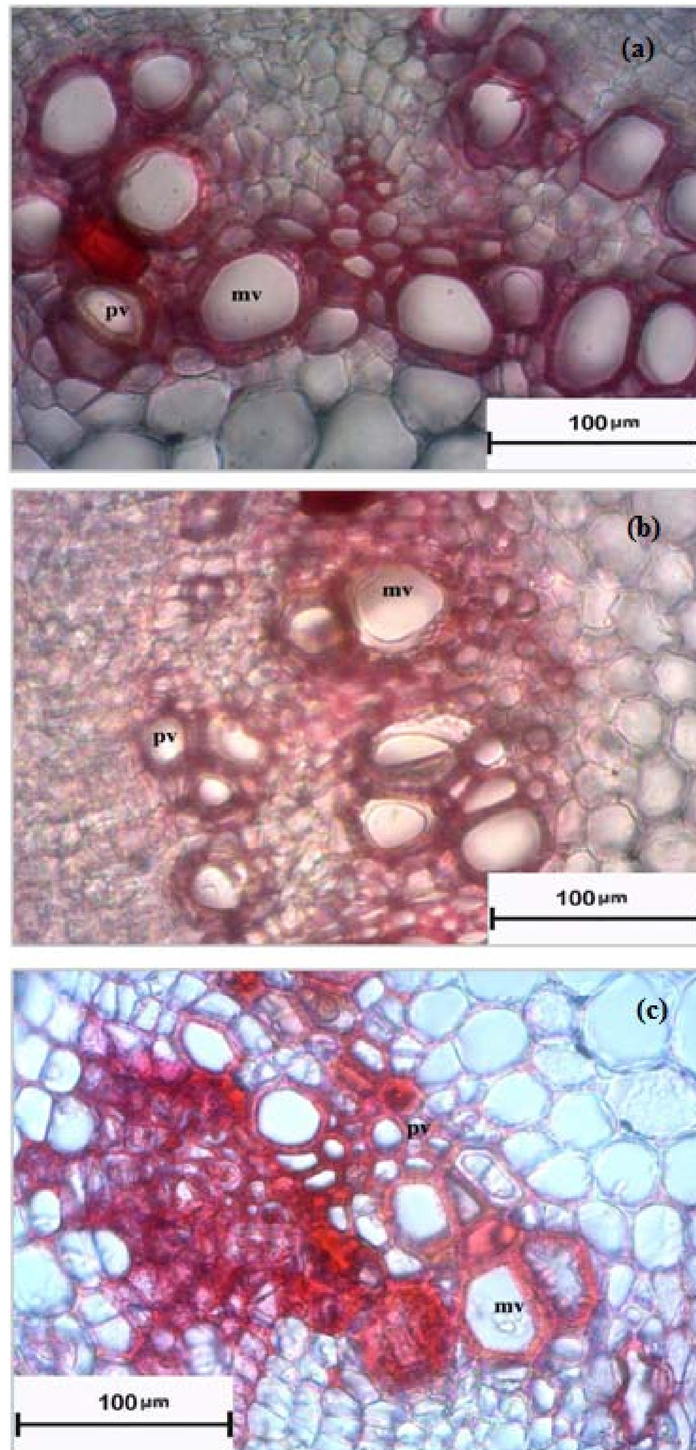


Plate 17. Same as plate 14 but of higher magnification showing metaxylem vessel (mv) and protoxylem vessel (pv). Bar = 100 µm.

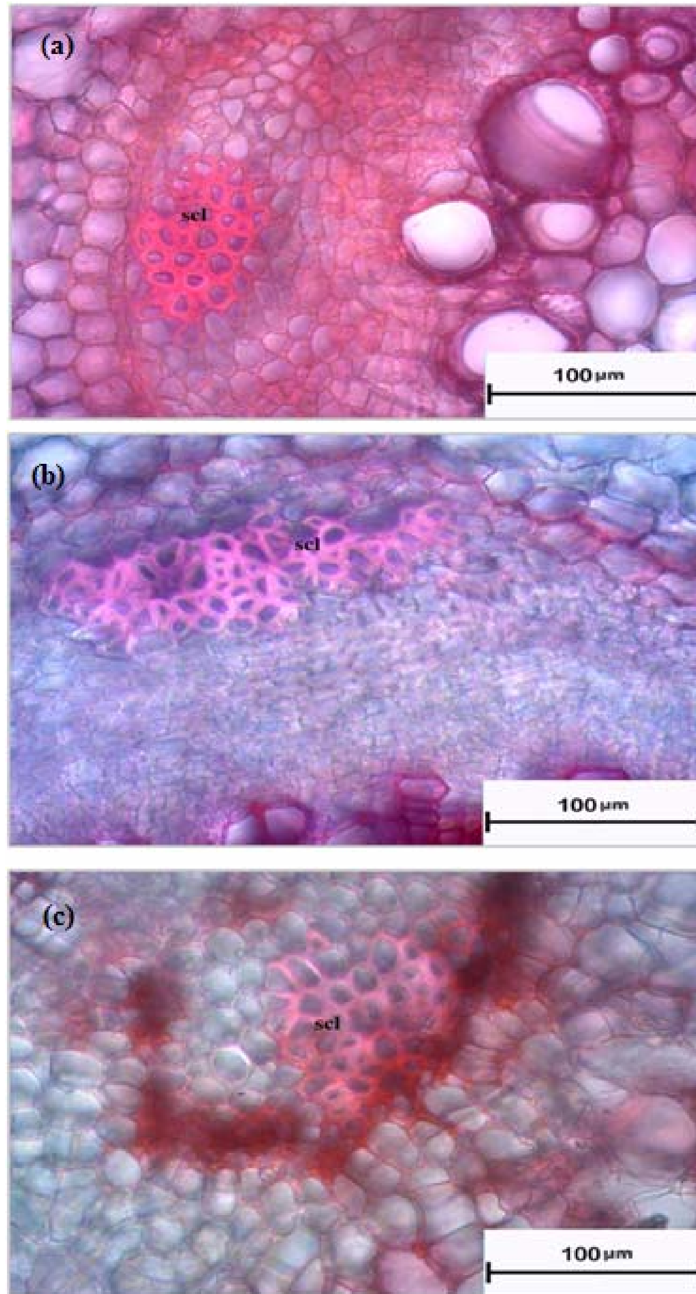


Plate 18. Same as plate 14 but of higher magnification showing sclerenchyma (scl). Bar = 100 µm.

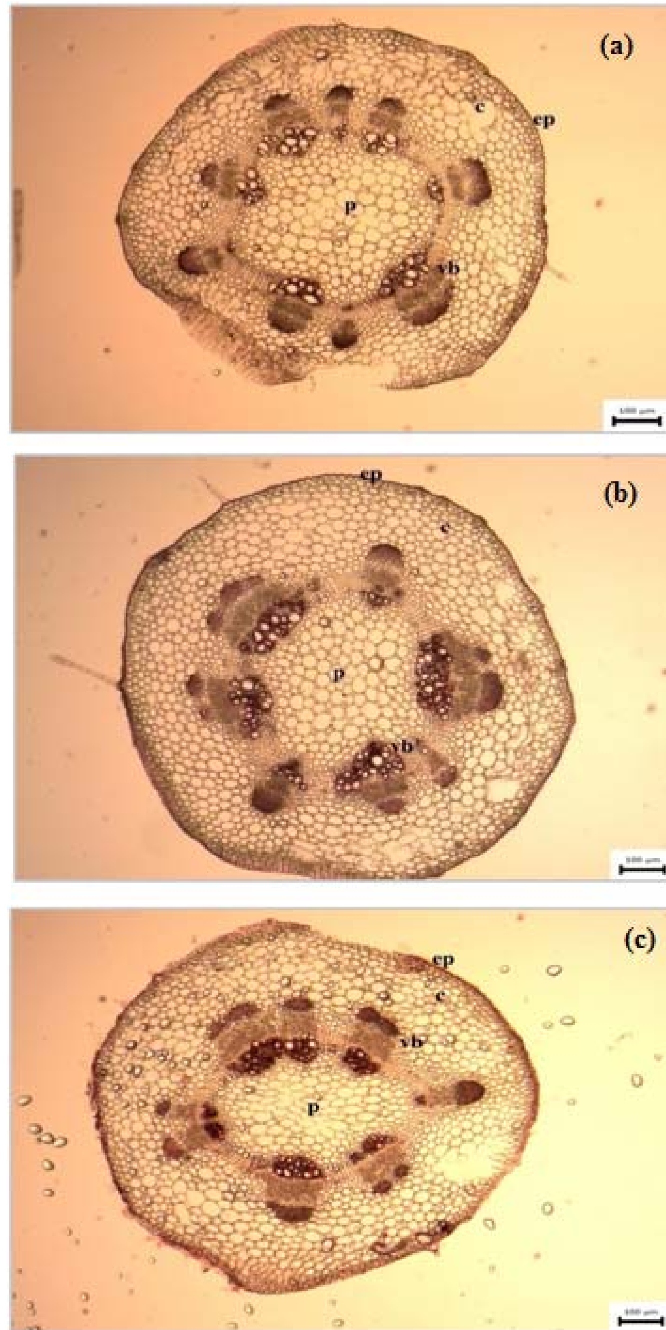


Plate 19. Transverse section of the stem of chickpea (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing epidermis (ep), cortex (c), vascular bundle (vb) and pith (p). Bar = 100 µm.

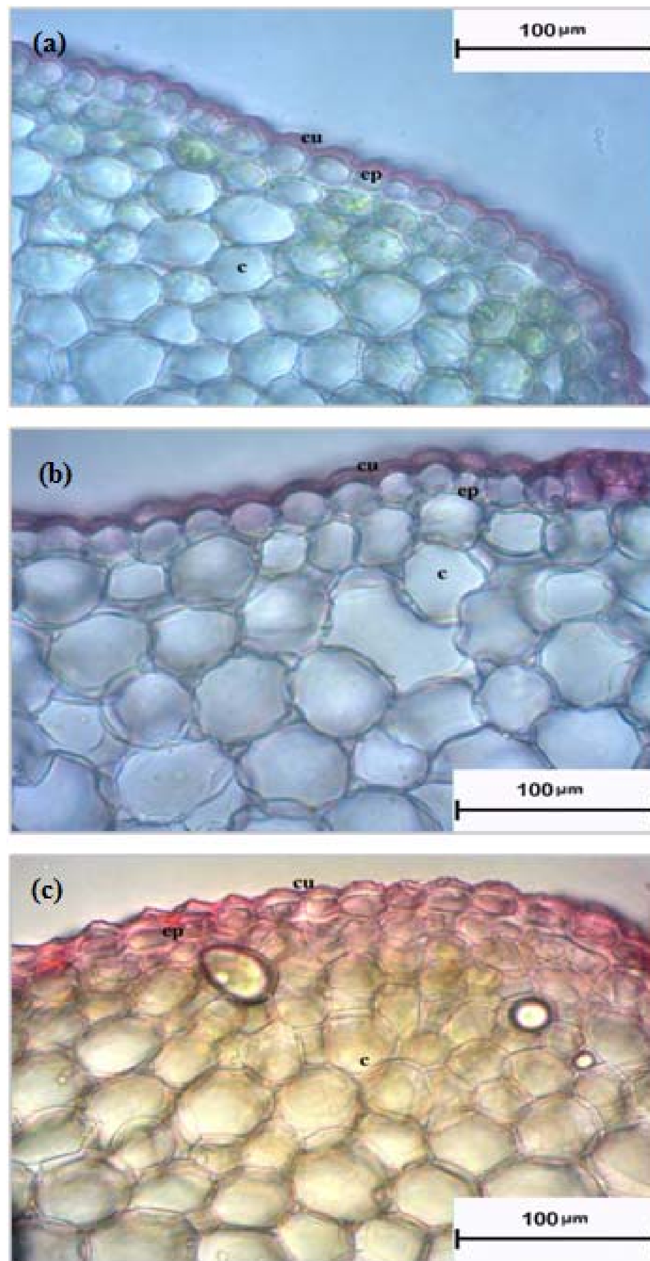


Plate 20. Same as plate 19 but of higher magnification showing cuticle (cu), epidermis (ep) and cortex (c). Bar = 100 µm.

Vascular bundle: In the stem of chickpea, application of Al reduced number of vascular bundles which were radially arranged. In some vascular bundles of Al-treated plant, there was poor development of xylem vessels. Metaxylem vessels were reduced in the plant treated with aluminium than that of control plant. Reduced number and smaller sized metaxylem vessels were observed in the stem of Al-treated chickpea plant. Storage of phenolic compounds were found around the vessels in the stem of Al- treated chickpea plant (Plate 21a, b and c).

Secondary growth was observed in the stem of both control and Al-stressed plant. In Al-treated plant, cambium ring was thin compared to control. Sclerenchyma cells were found above the phloem tissue. As compared to control, larger area of sclerenchyma cells were noticed in the stem of Al-stressed plant. A large amount of phenolic compounds was found in the stem of Al-treated chickpea plant (Plate 22a, b and c, and 23 a, b and c).

Pith: The pith is made up of parenchyma cells. Smaller pith area was observed in Al-treated chickpea plant as compared to that of control plants (Plates 19a, b and c, and 21a, b and c).

7.3.7 Effects of aluminium toxicity on anatomy of the leaves of chickpea

Transverse section of the middle portion of the 4th leaf of 28-day-old chickpea plant showed the following structures:

Epidermis: As compared to control plant the epidermal cells were irregular and disorganized in Al-stressed plant. In the leaf of Al-treated plant, the epidermal cells were comparatively smaller than those of the leaf of control plant (Plate 25a, b and c).

Mesophyll tissue: Mesophyll tissue is soft chlorophyll-containing tissue of a leaf between the upper and lower layers of epidermis. These are a type of ground tissue and found as two distinct types in the dorsiventral leaves - palisade parenchyma and spongy parenchyma mesophyll cells. Palisade parenchyma cells

consists of relatively elongated cells have a lot of chloroplasts in those and these are usually only one layer of cells, and located near the adaxial surface epidermis. Whereas the spongy mesophyll cells contain large intercellular spaces.

Less amount of chlorophyll was present in Al-treated plants than that of control plant (Plate 24a, b and c). In the leaf of chickpea, amount of palisade and spongy parenchyma decreased following aluminium treatment. In the leaf of Al-treated chickpea plants below the adaxial surface epidermis, palisade parenchyma was reduced, and above the abaxial surface epidermis, spongy parenchyma was also reduced (Plate 24a, b and c, and 25a, b and c).

Vascular bundle: Vascular area of the leaf of chickpea consisted of xylem and phloem. The phloem area in Al-treated leaf of chickpea became smaller in relation to that of the leaf of control plant. In 300 μM Al-treatment, the number of xylem vessel in the leaf was reduced as compared to that of control plant (Plate 25a, b and c).

7.3.8 Effects of aluminium toxicity on stomata and trichomes of the leaves of chickpea

In the leaf of 28-day-old control plant, almost all the stomata were open whereas Al treatment caused closure of stomata (Plate 26a, b and c).

In Al-treated leaf, number of trichome was higher as compared to that of the leaf of control plant. Both glandular and non-glandular trichomes were more common on the leaves of plant (Plate 27a, b and c).

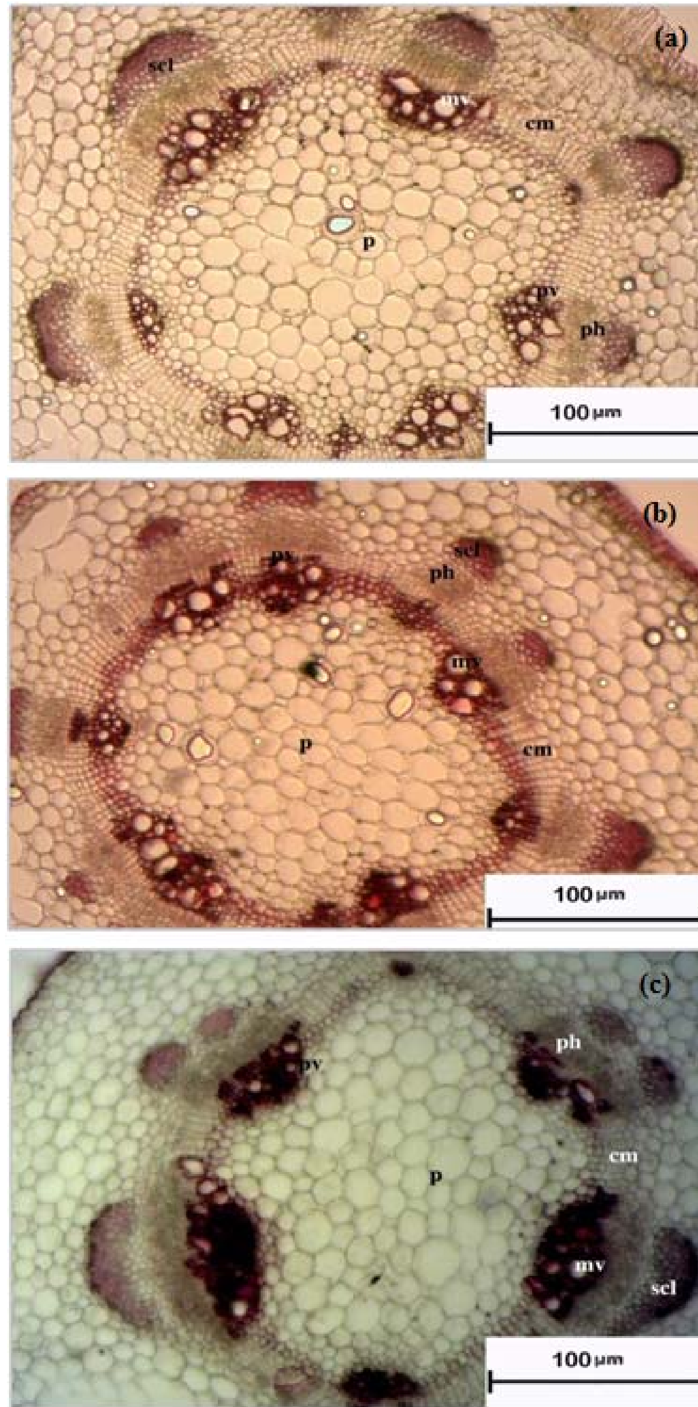


Plate 21. Same as plate 19 but of higher magnification showing sclerenchyma (scl), metaxylem vessel (mv), protoxylem vessel (pv), cambium (cm), phloem (ph) and pith (p). Bar = 100 µm.

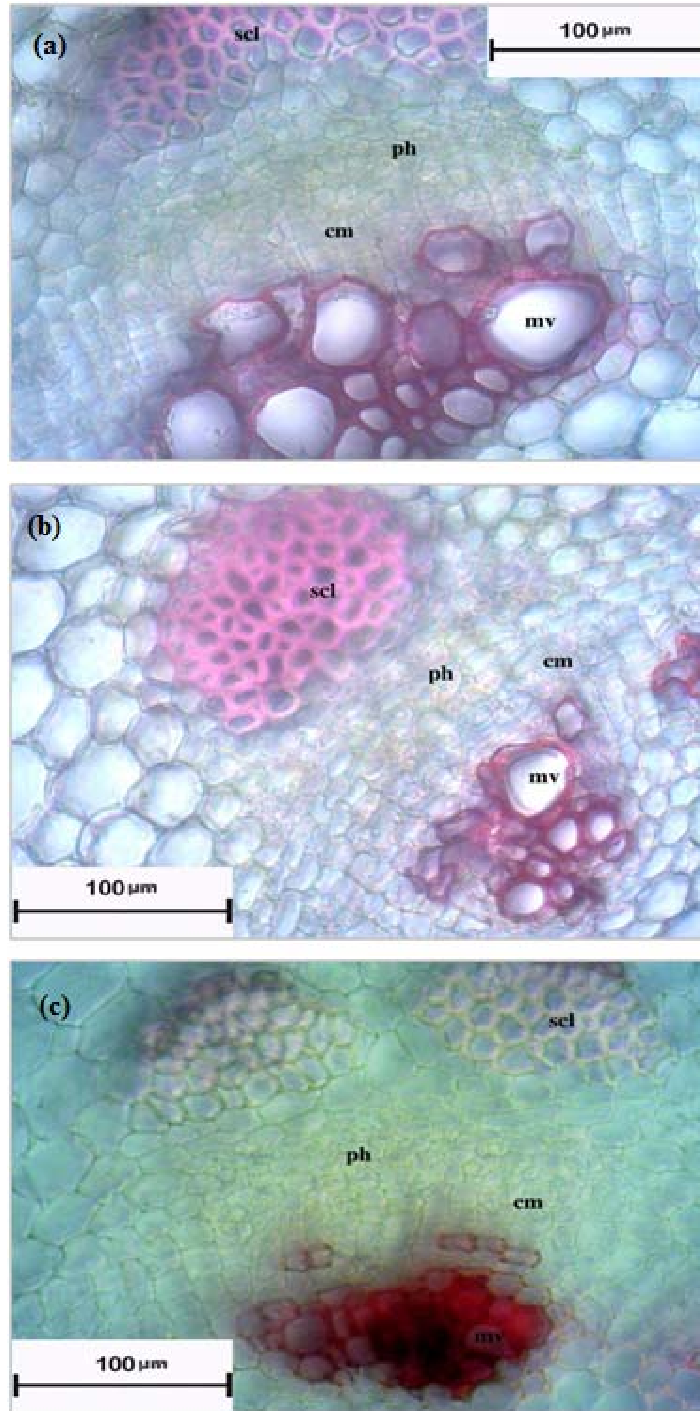


Plate 22. Same as plate 19 but of higher magnification showing sclerenchyma (scl), metaxylem vessel (mv), cambium (cm) and phloem (ph). Bar = 100 µm.

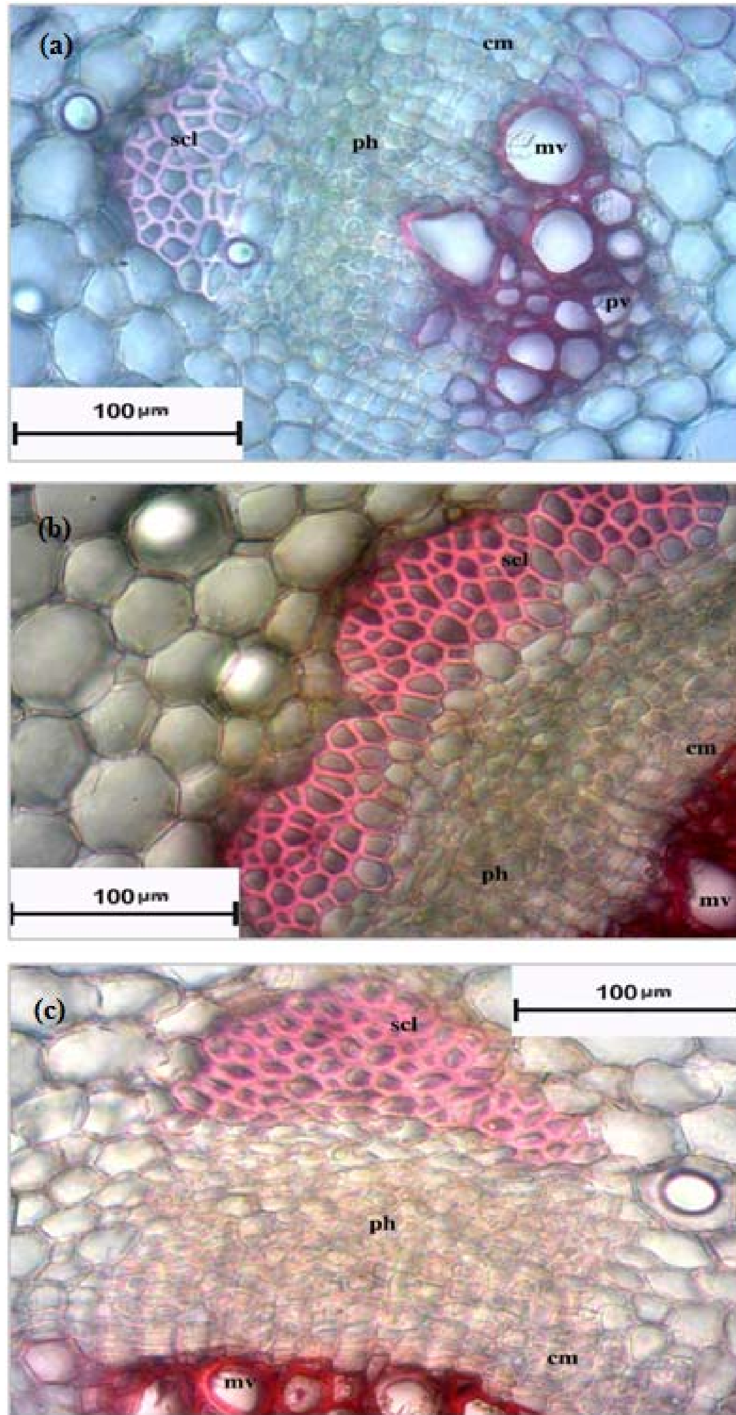


Plate 23. Same as plate 19 but of higher magnification showing sclerenchyma (scl), phloem (ph), cambium (cm), metaxylem vessel (mv) and protoxylem vessel (pv). Bar = 100 µm.

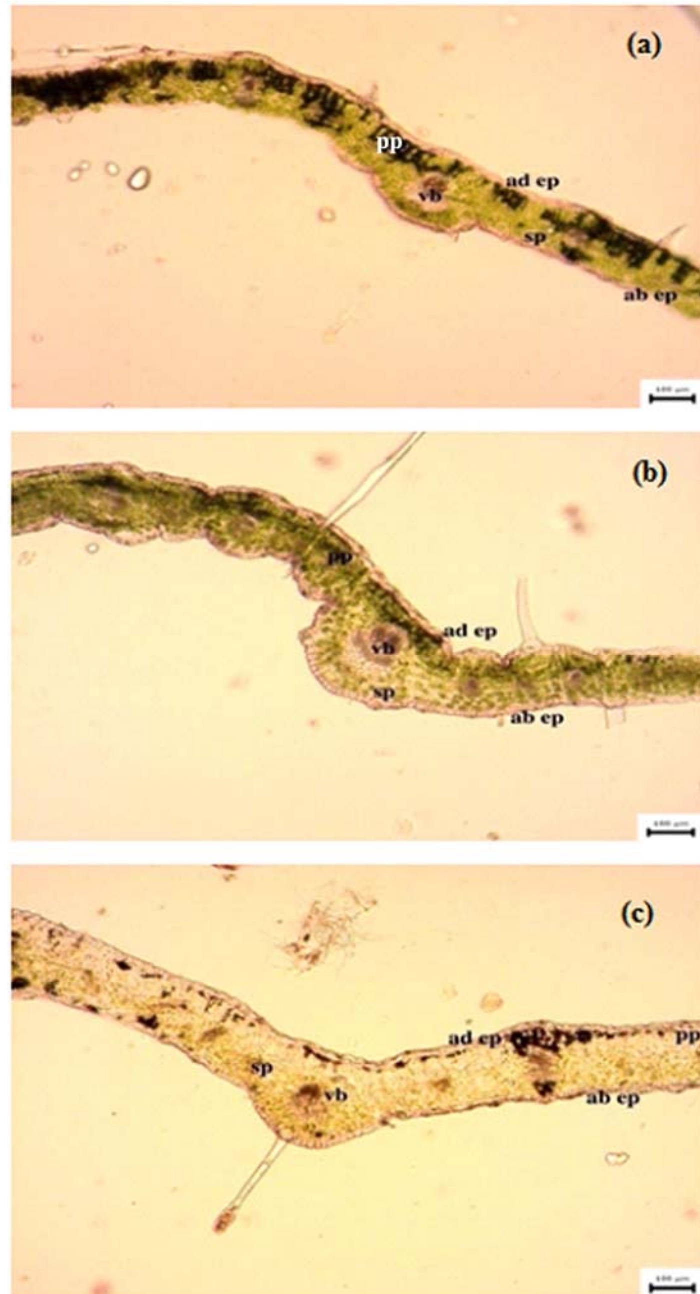


Plate 24. Transverse section of the leaf of chickpea (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing adaxial surface epidermis (ad ep), abaxial surface epidermis (ab ep), palisade parenchyma (pp), spongy parenchyma (sp) and vascular bundle (vb). Bar = 100 µm.

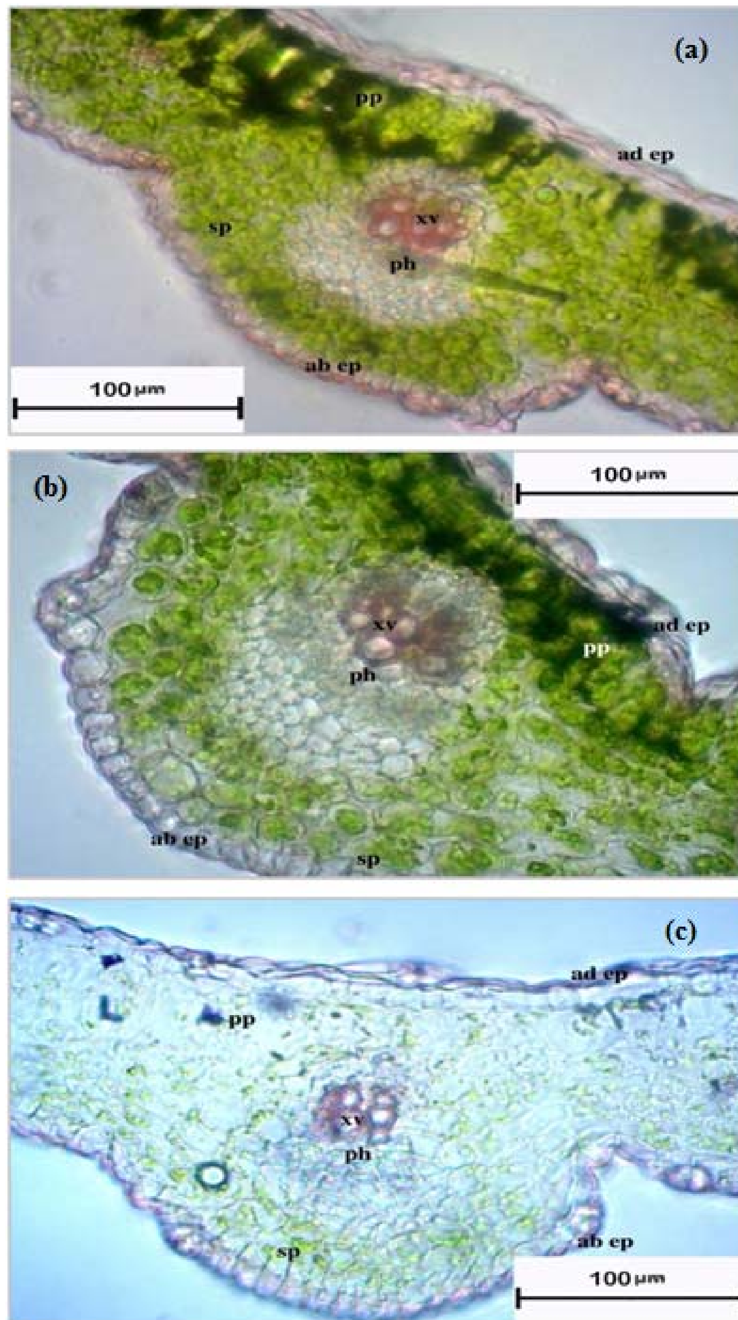


Plate 25. Same as plate 24 but of higher magnification showing adaxial face epidermis (ad ep), abaxial surface epidermis (ab ep), xylem vessel (xv), phloem (ph), palisade parenchyma (pp) and spongy parenchyma (sp). Bar = 100 µm.

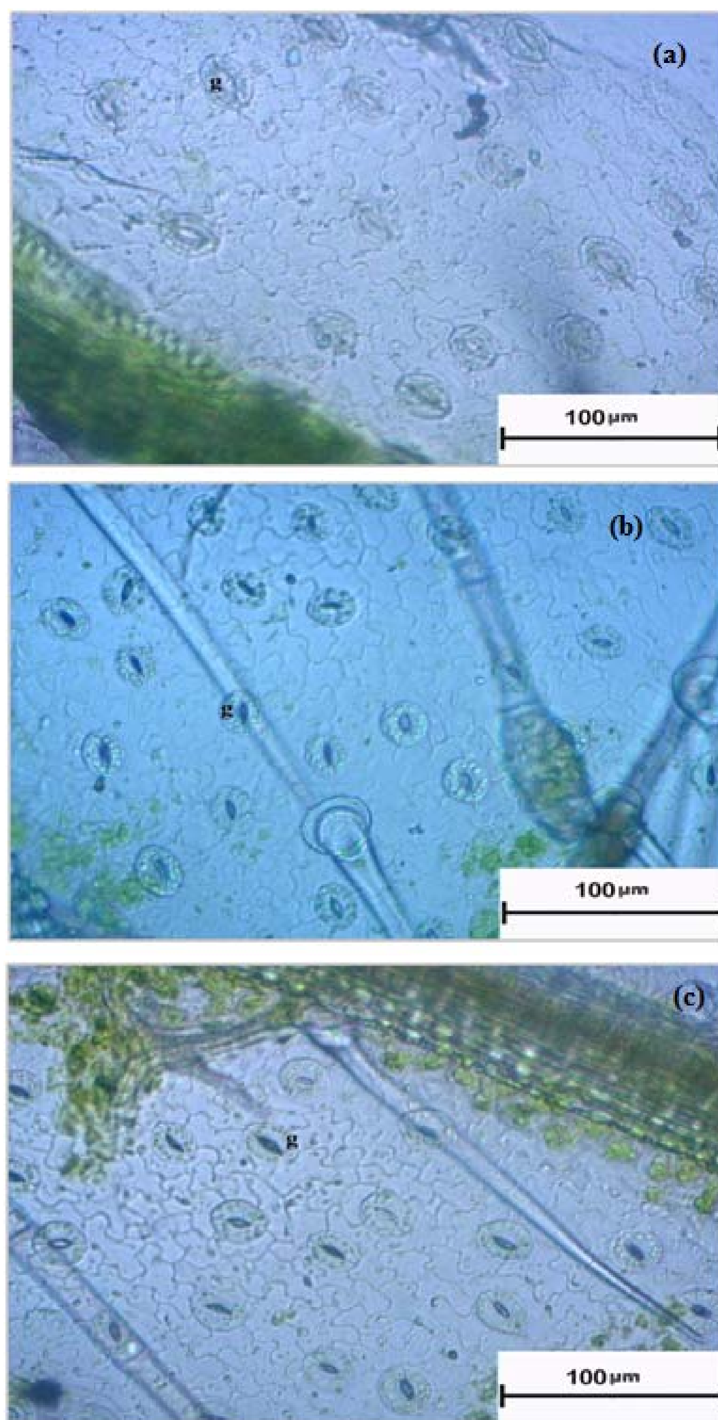


Plate 26. Peel of the leaf of chickpea (a) control, (b) 150 μM and (c) 300 μM Al-treated plant showing open and closed stomata and guard cell (g). Bar = 100 μm.

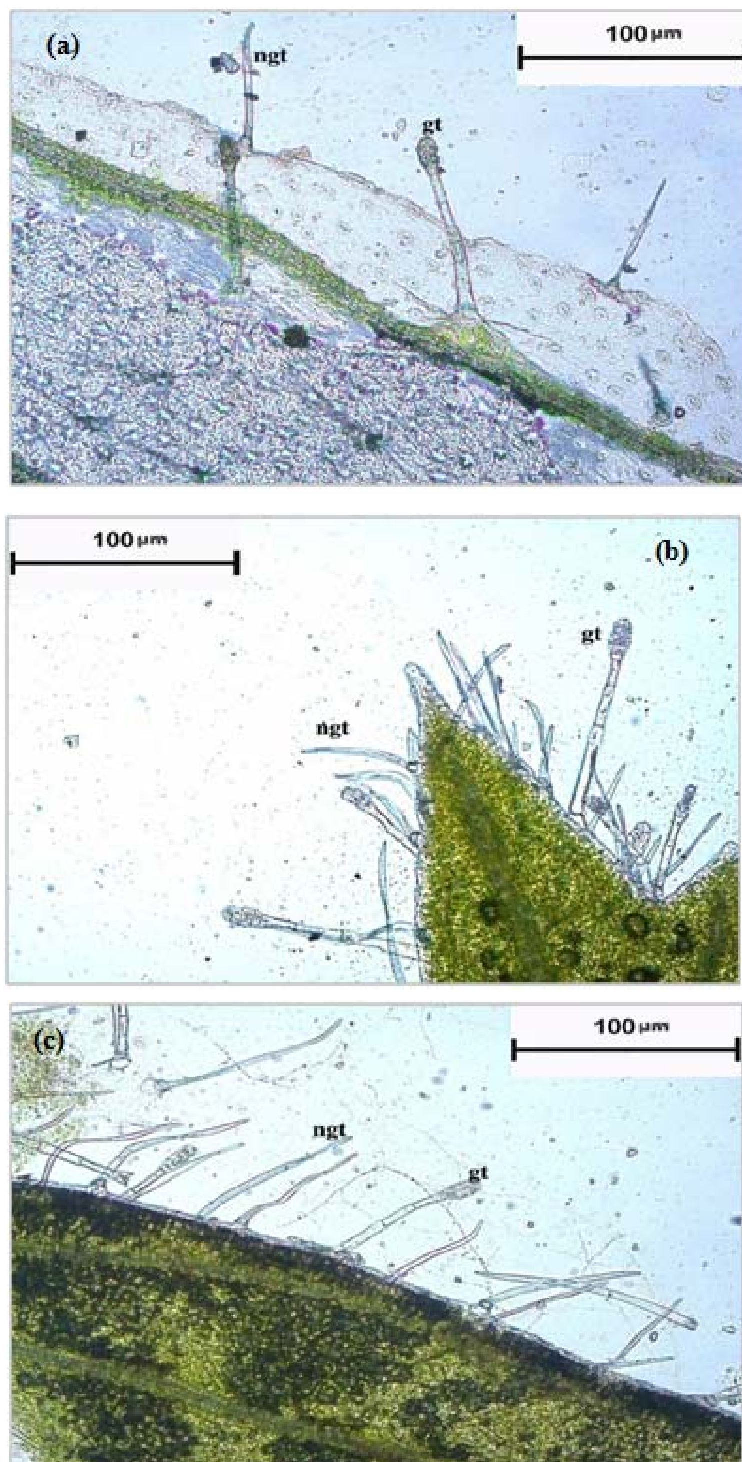


Plate 27. Peel of the leaf of *Cicer aeriatinum* L. (a) control, (b) 150 µM and (c) 300 µM Al-treated showing glandular (gt) and nonglandular trichomes (ngt). Bar = 100 µm.

7.4 Discussion

7.4.1 Effects of aluminium toxicity on the anatomy of root, stem and leaves of rice and chickpea

Epidermis was monoseriate and composed of long elongated cells in control root and stem of rice and chickpea plant but due to aluminium (Al) treatment the root and the stem of rice (Plates 6 and 8) and chickpea (Plates 15 and 20) plants were affected. Al exposure triggered different changes in the shape of epidermal cells of root. The epidermal cells were of typical size in longitudinal sections of the control roots but epidermal cells of Al-treated roots lost their tissue features and appeared shorter and wider than the cells in control rice roots. These changes were also observed in cortical cells of rice root (Alvarez *et al.* 2012). Similar alterations in other species had been documented by Horst (1995), Barceló and Poschenrieder (2002) and Gunsé *et al.* (2003). Budíková *et al.* (1997) reported that the epidermis and peripheral cortex layers were more affected than the central cylinder cells in Al treated root.

In rice root, the cortical cells presented a higher amount of intercellular space and its cells were elongated longitudinally (Plates 5 and 6). Similar result was also found in maize by Batista *et al.* (2012).

In the root of rice and chickpea, the number of metaxylem vessels was reduced than that of the control plants (Plates 5, 7, 14 and 16). Similarly Batista *et al.* (2012) found that in the vascular bundle, the metaxylem and protoxylem had no secondary walls and their diameter was much smaller compared to that of control plants.

No significant changes were observed in the pith area of rice and chickpea root and stem (Plates 7, 14 and 16). Batista *et al.* (2012) reported that the region of the pith was reduced and poorly developed in corn root due to Al toxicity.

Irregular and disorganized epidermal layer was found in rice and chickpea leaves (Plates 10 and 25). On the other hand, Özyiğit *et al.* (2013) observed no significant differences between epidermis (adaxial and abaxial) of control plant and epidermis (adaxial and abaxial) of the leaves of Al-treated cotton plant. Similarly, Özyiğit *et al.* (2013) found that after Al exposure a structural degradation of palisade and spongy parenchyma caused.

The leaf sheaths of the rice plants exposed to Al had a uniseriate epidermis coated with a thin cuticle layer and the cells of both the epidermis and the mesophyll tissues were less developed (Plates 10, 11, 24 and 25). Similar result was also observed in corn leaves by Batista *et al.* (2012). Ruan *et al.* (2011) reported that Al toxicity resulted in reduced leaf thickness in *Acacia melanoxylon*. Under high Al concentration, damage of mesophyll cells of oilseed rape was observed by Qian *et al.* (2014). de Almeida *et al.* (2015) found that after Al exposure, leaf issue thickness of cacao seedlings was increased. Collapse of palisade and spongy parenchyma cells with intense vacuolization induced by toxic metals (Zn and Cd) was observed by Sridhar *et al.* (2005) and Zhao *et al.* (2000).

The number of stomata were significantly reduced in 100 and 200 μM Al-treated plants. Al stress reduced stomatal aperture (Plate 26b and c) and also reduced the chlorophyll content (Plates 10, 11, 24 and 25). In Al-sensitive plants, presence of Al was found to reduce stomatal conductance in tea (Mukhopadyay *et al.* 2012) and chlorophyll content in tea, maize and barley (Mukhopadyay *et al.* 2012, Mihailovic *et al.* 2008, Abdalla *et al.* 2008). Al treatment was found to induce stomatal closure (Sivaguru *et al.*1999, Delhaize and Ryan 1995, Vardar and Ünal 2007).

7.4.2 Relationship between the effect of aluminium toxicity on ion transport with Al-induced changes in anatomical structures in rice and chickpea

Aluminium toxicity-induced changes in anatomical structures in the root, stem and leaves of both rice and chickpea (Plates 5-11, 14-25) might be related to observed Al-induced changes in ion transport in these plants (Figs. 13-47, Chapter 4).

Al-induced decrease in number and diameter of xylem vessels (Plates 5, 7, 10, 14, 16-17, 19, 21-22, 25) would decrease the translocation of ions from the root to the shoot (Figs. 13-42, Chapter 4) and thus adversely affect the distribution of ions in different parts of the plant.

Closure of stomata by aluminium (Plates 8 and 22) might be due to increase in abscisic acid level by aluminium stress. Closure of stomata might decrease the rate of photosynthesis due to decrease in CO_2 diffusion through the stomata.

Chapter 8

CONCLUSION

In this concluding chapter, all the results are co-ordinated to obtain a composite picture on the effect of aluminium toxicity on ion transport and its correlation with its effect on biochemical changes and anatomical structure, and growth.

Aluminium toxicity decreased germination of rice and chickpea seeds (Table 1 and 2). Al treatment was found to decrease K^+ accumulation but caused a dramatic increase in Cl^- and Al^{3+} accumulation in the radicle and plumule (Figs. 1-12). The stimulation of Cl^- and Al^{3+} accumulation with concomitant inhibition of that of K^+ in the radicle and plumule was responsible for Al stress-induced inhibition of germination of rice and chickpea seeds.

Aluminium stress decreased accumulation of K^+ but increased that of Na^+ in different organs of intact rice and chickpea seedlings (Figs. 13-16). In normal condition, uptake of K^+ is higher than that of Na^+ which is called K^+/Na^+ selectivity. But under aluminium toxicity, uptake of Na^+ is higher than that of K^+. Therefore, aluminium toxicity alters K^+/Na^+ selectivity.

Aluminium toxicity-induced increase in accumulation of Cl^- in intact rice and chickpea seedlings (Figs. 17-18) might be toxic for metabolic activities resulting in inhibition of growth.

Aluminium-stress decreased accumulation of NO_3^- in intact rice and chickpea seedlings (Figs. 19-20) which might decrease the synthesis of protein. Simon *et al.* (1994a) suggested that accumulation of excess Al^{3+} might limit the nitrate absorption rate due to the inhibition of its carrier.

Aluminium stress inhibited Ca^{2+} accumulation in different parts of rice and chickpea seedlings (Figs. 21 and 22). The inhibition of Ca^{2+} accumulation in the root (Fig. 21a) might impair the permeability characteristic of plasmamembrane

because Ca^{2+} is responsible for maintaining this unique characteristic of plasmamembrane. Furthermore, Al stress-induced Ca^{2+} uptake involves a limitation of its transport to the leaves, this reduced transport of Ca^{2+} to the shoot helps to maintain a normal calcium concentration in the root cells (Huang *et al.* 1995). Interaction between Al^{3+} and Ca^{2+} have long been implicated in Al phytotoxicity because symptoms of severe Al toxicity in the field resemble those of Ca^{2+} deficiency and supplementation of Ca^{2+} can substantially alleviate Al-stress symptoms (Foy 1988 and Rengel 1992).

Aluminium toxicity decreased the accumulation of Mg^{2+} in rice and chickpea seedlings (Figs. 23 and 24). Al-induced inhibition of Mg^{2+} uptake was found to be associated with Al-induced Mg^{2+} deficiency (Huang *et al.* 1992a) by blocking binding sites of transport proteins (Rengel and Robinson 1989a).

Mg^{2+} is a constituent of chlorophyll. So, Al-induced decrease in Mg^{2+} accumulation would decrease chlorophyll synthesis.

Ca^{2+} and Mg^{2+} was reported to alleviate aluminium toxicity (Keltjens and Tan 1993). Therefore, Al-induced inhibition of Ca^{2+} and Mg^{2+} in rice and chickpea seedlings would aggrevate the aluminium stress.

Aluminium toxicity decreased Fe^{2+} accumulation in different parts of rice and chickpea seedlings (Figs. 25 and 26). Iron is the constituent of respiratory enzyme cytochrome oxidase. Therefore, Al-induced decrease in Fe^{2+} accumulation would reduce respiration resulting in a decrease in ion transport which is dependent on respiratory energy.

Aluminium stress increased accumulation of Al^{3+} in different parts of rice and chickpea seedlings (Figs. 27 and 28). The dramatic increase in the concentration of Al^{3+} in the root tissue would hinder the absorption of ions. The massive accumulation of Al^{3+} in the root and shoot tissue indicates that these particular varieties of rice and chickpea are moderately aluminium tolerant. It is well

known that there are two classes of aluminium tolerance mechanisms: some are those that exclude Al^{3+} from the root apex and others are those that allow the plant to tolerate Al accumulation in the root and the shoot cytoplasm. This view is supported by Taylor (1991) who proposed that tolerance strategies identified can be separated into the mechanism that involves exclusion of Al from the root apex and the mechanism that allow the plant to tolerate Al within the cells.

The effect of aluminium toxicity on K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+} and Fe^{2+} was studied in solution culture under controlled environmental condition (chapter 4a) and in sand culture under natural environmental condition (chapter 4b). The accumulation of K^+, Ca^{2+}, Mg^{2+} and Fe^{2+} was decreased in both solution (Figs. 13-14 and 21-26; section 4a) and sand culture (Figs. 31-32 and 37-42; section 4b). On the contrary, Al stress increased the accumulation of Na^+ and Cl^- in both solution (Figs. 15-18; section 4a) and sand culture (Figs. 33-36; section 4b). Therefore, it is apparent from the above comparison that the effect of aluminium stress on ion transport in rice and chickpea plants grown in solution culture under controlled environmental condition might be reconciled with that in plants grown in sand culture under natural environmental condition. Furthermore, it is suggested that the results on the effect of aluminium toxicity on ion transport in solution and sand culture might be reproducible even when plants are grown in acid soil in field condition.

Aluminium toxicity increased reducing sugar (Figs. 43-44) and total sugar (Figs. 45-46) in rice and chickpea plants. Increase in reducing and total sugar level caused by Al might help to maintain the osmotic potential of cell sap under aluminium stress condition. Furthermore, Sato *et al.* (2004) concluded that carbohydrate accumulation affects the maintenance of cellular membrane and osmotic regulation.

Aluminium toxicity increased proline content in rice and chickpea seedlings (Figs. 47 and 48). Increase in proline content acts as an indicator of stress. Al-

induced increase in proline content in rice and chickpea seedlings indicates that both the plant species were stressed due to exposure to aluminium. Moreover, nonenzymatically synthesized compounds such as proline are able to strengthen metal-detoxification capacity of intracellular antioxidant enzymes.

Aluminium stress increased total amino acid in rice and chickpea seedlings (Figs. 49 and 50). The increase in total amino acid might be related to the decrease in protein content under aluminium stress. The increase in total amino acid by Al might be due to conversion of protein to amino acid. The increase in total amino acid might have probably been caused by the increase in the activity of protease enzyme which breaks reserve proteins.

Aluminium toxicity decreased protein content in rice and chickpea seedlings (Figs. 51 and 52). During the stress caused by aluminium, this element acts as a limiting factor for the assimilation of nitrogen once there is a reduction in nitrate reductase activity which is the first enzyme associated with the nitrogen metabolism, This, in turn, would cause a reduction in synthesis of protein (da Cruz *et al.* 2011).

Aluminium stress increased the activities of antioxidant enzymes peroxidase and catalase in rice (Figs. 53 and 54) and that of peroxidase, catalase and superoxide dismutase (SOD) in chickpea (Figs. 56-58). Peroxidase, catalase and SOD are key enzymes in antioxidative defense system. Reactive oxygen species (ROS) is closely related to the response of plants to heavy metals (Nagajyoti *et al.* 2010). Al triggers an increased production of ROS which includes singlet oxygen ($\frac{1}{2}$ O_2^-), superoxide radical (O_2), hydroxyl radical (OH) and H_2O_2 in the tissue. These ROS cause oxidative damage to cellular organelles and biomolecules, and thus lead to several metabolic alteration (Ma 2007, Jain *et al.* 2008). Free radical scavenging enzymes such as peroxidase, catalase and SOD keep the cellular level of ROS under control and help to avoid oxidative damage. Al toxicity-induced increase in peroxidase, catalase and SOD plays a vital role in aluminium

stress tolerance. Antioxidant enzyme activities serve as a key biochemical indicator to assess the sensitivity of plants under stress condition.

Aluminium toxicity increased the accumulation of phenolic compounds in rice and chickpea plants (Figs. 59 and 60). Phenolics might act as a detoxifier of aluminium toxicity.

Aluminium stress decreased chlorophyll a, chlorophyll b and carotenoid contents in rice and chickpea plants (Figs. 61 and 62). The content of photosynthetic pigments is decreased due to the destruction of chloroplast structure by Al stress. Besides, aluminium toxicity induced chlorophyll photo-oxidation. The destruction of prematerial of chlorophyll synthesis causing reduction in biosynthesis of chlorophyll might lead to the decrease in chlorophyll content. One of the causes of reduction of photosynthetic pigments during Al-stress is the production of ROS that cause breakdown and decrease of pigments.

It is suggested that decrease in chlorophyll a and b content might decrease photosynthesis resulting in a decrease in ion transport because ion transport is dependent on photosynthesis for energy.

Aluminium toxicity decreased primary root length and number of lateral roots in rice and chickpea seedlings (Figs. 63 and 64). Aluminium-induced inhibition of root growth often precedes or coincides with a decline in cell division (Frantzios *et al.* 2001). Therefore, the rapid Al-induced inhibition of root elongation is likely to be caused by inhibition of cell elongation rather than that of cell division. Inhibition of root growth requires the root apex, in particular the distal part of elongation zone within apex (Kollmeier *et al.* 2000) directly exposed to Al. This observation indicates that root apex is a critical site of perception and expression of Al toxicity.

Aluminium stress decreased dry weight of the root of rice and chickpea seedlings (Figs. 71a and 72a). Long term inhibition of root growth is considered

to be primarily the result of inhibited cell elongation, at least in early stages of toxicity, while reduced cell division can obviously affect root growth at later stages (Kochian 1995, Barceló and Poschenrieder 2002 and Ciamporova 2002). Matsumoto (1991) suggested that inhibition of root growth is associated with a reduction in mitotic activity of the meristematic zone. The accumulation of Al in the cell nucleus was observed in several plant species (Liu and Jiang 1992). Matsumoto (1991) suggested that the formation of DNA-Al complex in the nucleus would be responsible for the inhibition of the cell division leading to the inhibition of root growth.

Aluminium toxicity increased shoot/root dry weight ratio in rice and chickpea seedlings (Figs.71c and 72d). Increase in shoot/root ratio indicates that shoot growth is higher than that of root growth under aluminium stress.

Furthermore, Al stress-induced decrease in primary root length and number of lateral roots (Figs. 63 and 64) and inhibition of long term root growth (Figs. 71a and 72a) cause a decrease in root surface area. The decrease in root surface area would lead to a decrease in absorption of ions.

Aluminium stress decreased the number and diameter of xylem vessels of the root, stem and leaf of rice and chickpea plants (Plates 5, 7, 8, 10, 16, 17, 21, 22 and 25). Aluminium-induced decrease in number and diameter of xylem vessels would decrease the translocation of ions from the root to the shoot and thus adversely affect the distribution of ions in different parts of the plant. This would, in turn, slow down the metabolic processes in different organs of plant because ions act as cofactors of enzymes involved in numerous metabolic reactions.

Aluminium toxicity caused the closure of stomata in rice and chickpea plants (Plates 12 and 26). Al stress-induced closure of stomata might decrease the rate of photosynthesis due to the decrease in CO_2 diffusion through the stomata.

In a nut shell, Al-induced decrease in protein (Figs. 51 and 52) which acts as a respiratory substrate might reduce supply of energy. Furthermore, proposed decrease in photosynthesis brought about by Al-induced inhibition of chlorophyll content (Figs. 61 and 62) and closure of stomata (plates 12 and 26) would also cause reduction in supply of energy. The shortage of energy supply, in turn, might decrease energy dependent ion transport (chapter 4) which would lead to observed Al-induced inhibition of growth (chapter 6) of rice and chickpea seedlings.

Finally, the fundamental informations obtained in the course of the present investigation on the effect of aluminium toxicity on ion transport and its correlation with biochemical changes and anatomical structures in rice and chickpea plants might be useful for solving the problem of aluminum toxicity prevailing in Bangladesh.

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EFFECTS OF ALUMINIUM TOXICITY ON GERMINATION OF SEEDS AND ITS CORRELATION WITH K^+, Cl^- AND Al^{3+} ACCUMULATION IN RADICLE AND PLUMULE OF *ORYZA SATIVA* L. AND *CICER AERIATINUM* L.

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Keywords: Aluminium toxicity, Germination, K^+, Cl^-, Al^{3+} accumulation, Plumule, Radicle, Rice, Chickpea

Abstract

Aluminium at concentrations of 10, 50, 100 and 150 µM inhibited germination of rice and chickpea seeds. Al (10 to 150 µM) decreased accumulation of K^+ in the radicle and plumule of germinating rice and chickpea seeds from 48 to 96 hrs of treatment. The degree of inhibition increased with the increase in Al concentration. On the other hand, Cl^- accumulation was increased in the radicle and plumule of rice and chickpea seedlings following 10 to 150 µM Al treatment. A maximum of 2- to 2.4-folds increase in accumulation of Cl^- was observed under Al stress. A 72 hrs exposure to 10 and 100 µM Al caused a 2.3-folds and 3.8-folds increase in accumulation of Al^{3+} in the radicle and a 1.6- to 2.0-folds increase of that in the plumule of rice seedlings. In the germinating chickpea seeds, Al treatment caused a 2- to 3.4-folds and a 2- to 3-folds increase in accumulation of Al in the radicle and plumule, respectively. Correlation between Al-induced seed germination with K^+, Cl^- and Al^{3+} accumulation is discussed.

Introduction

Germination potential of seeds is an important factor for growing plants in adverse soil condition like aluminium toxicity. There are a few reports on the effect of aluminium stress on germination of seeds. Al^{3+} decreased seed germination in maize (Nasr 2013). Aluminium at a concentration of 500 ppm had inhibitory effect on wheat seed germination (Alamgir and Akhter 2009). Al toxicity inhibited seed germination in a few plants (Delhaize and Ryan 1995). 50 µM Al treatment decreased germination percentage in maize (Gumze *et al.* 2007). Al significantly reduced germination of pea (*Pisum sativum* L.) seeds (Singh *et al.* 2011). On the contrary, aluminium toxicity had no effect on germination of wheat (Jamal *et al.* 2006). Significant effect of 50 - 200 µg Al was not found on germination of tobacco seeds. However, germination time was delayed with increasing Al concentration (Varder *et al.* 2006). There are no reports on the mechanism of aluminium-induced inhibition of seed germination. Accumulation of K^+, Cl^- and Al^{3+} in plumule and radicle may have some relation with the inhibition of germination of seeds by aluminium.

So in this study, the effect of aluminium on seed germination and its correlation with K^+, Cl^- and Al^{3+} accumulation in plumule and radicle is reported.

Materials and Methods

Rice (*O. sativa* var. BRRI Dhan-53) and chickpea (*C. arietinum* var. Bari Chhola-7) were taken as experimental plant materials. Seeds of rice were obtained from Bangladesh Rice Research Institute (BRRI) and that of chickpea were procured from Bangladesh Agricultural Research Institute (BARI), respectively.

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Four different concentrations (10, 50, 100 and 150 µM) of $AlCl_3$ were prepared using half strength Hoagland solution and the pH of each solution was adjusted to 4.2 with 0.2N H_2SO_4. Half strength Hoagland solution having pH adjusted to 4.2 was used as control.

The seeds were surface sterilized to avoid fungal infection by soaking the seeds with 5.25% sodium hypochlorite for three minutes. The sterilized seeds were submerged in distilled water and aerated for 30 min with an air compressor. Thirty such sterilized seeds were placed on Whatman filter paper contained in a petri dish. Three replicates were used for each treatment. Filter papers were soaked with 10, 50, 100 and 150 µM $AlCl_3$ (pH 4.2) and half strength Hoagland solution (pH 4.2) was used as control. The chickpea and rice seeds were allowed to germinate in dark at 25°C ± 1°C and 30 ± 1°C, respectively. Seeds were considered to be germinated when radicles and plumules could be clearly distinguished. Germination of seeds was recorded at 48, 72 and 96 hrs of Al treatment.

Radicles and plumules of the germinated seeds were separated from cotyledons at 48, 72 and 96 hours from the time of sowing. K^+ and Cl^- were extracted from dry tissue (radicle and plumule) by boiling in a hot water bath following Samad and Karmoker (2013). Al^{3+} was extracted from dry tissue by boiling in a mixture of nitric acid and perchloric acid (4 : 1) using a hot sand bath. Al^{3+} was measured using atomic absorption spectrophotometer (Shimadzu, AA7000, Japan).

Results and Discussion

In rice, aluminium concentration of 10, 50, 100 and 150 µM decreased germination of seeds by 12, 25, 30 and 34%, respectively at 48 hrs of treatment. At 72 hrs of treatment, aluminium (50 - 150 µM) inhibited germination of seeds by 13 to 28%. At 96 hrs of treatment, 50 - 150 µM aluminium decreased germination of rice seeds by 6 to 21% (Table 1).

Table 1. Effects of different concentrations of $AlCl_3$ on germination of seeds of rice. Each value is the mean of three replicates ± standard error.

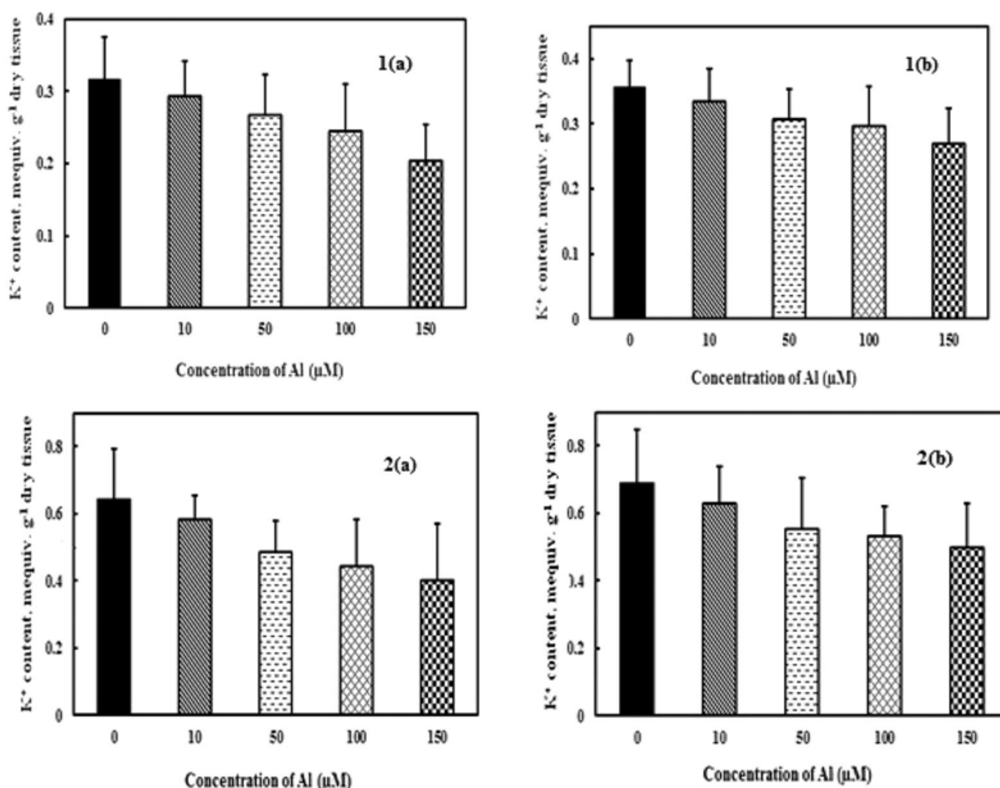
Duration of treatment (hrs)	% germination concentration of $AlCl_3$ (µM)				
	0	10	50	100	150
48	96 ± 0.359	88 ± 0.546	75 ± 0.333	70 ± 0.577	66 ± 0.530
72	100 ± 0.333	95 ± 0.571	87 ± 0.882	79 ± 0.498	72 ± 0.672
96	100 ± 0.333	98 ± 0.333	94 ± 0.571	85 ± 0.667	79 ± 0.571

In chickpea, aluminium (10 - 150 µM) inhibited seed germination by 20 to 42% at 48 hrs of treatment. At 72 hrs of treatment, 50 - 150 µM aluminium decreased germination of chickpea seeds by 10 to 21%. Aluminium (50 - 150 µM) inhibited seed germination by 7 to 16% at 96 hrs of treatment (Table 2). Aluminium-induced seed germination is supported by the work of Nasr (2013) and Alamgir and Akhter (2009) who recorded inhibition of seed germination of maize and wheat seeds following aluminium treatment.

In rice, accumulation of K^+ in the radicle was decreased by 7% at 10 µM Al treatment and the degree of inhibition increased with the increase in aluminium concentration from 10 - 150 µM and the maximum inhibition was 35% at 150 µM Al at 72 hrs of treatment (Fig. 1a). Similar pattern of inhibition of K^+ accumulation was observed in the plumule of rice following different concentrations of aluminium (10 - 150 µM) treatment at 72 hrs of treatment. The degree of inhibition of K^+ accumulation in the plumule increased with the increase in aluminium concentration from 10 - 150 µM ranging from 6 - 25% (Fig. 1b).

Table 2. Effects of different concentrations of $AlCl_3$ on germination of seeds of chickpea. Each value is the mean of three replicates ± standard error.

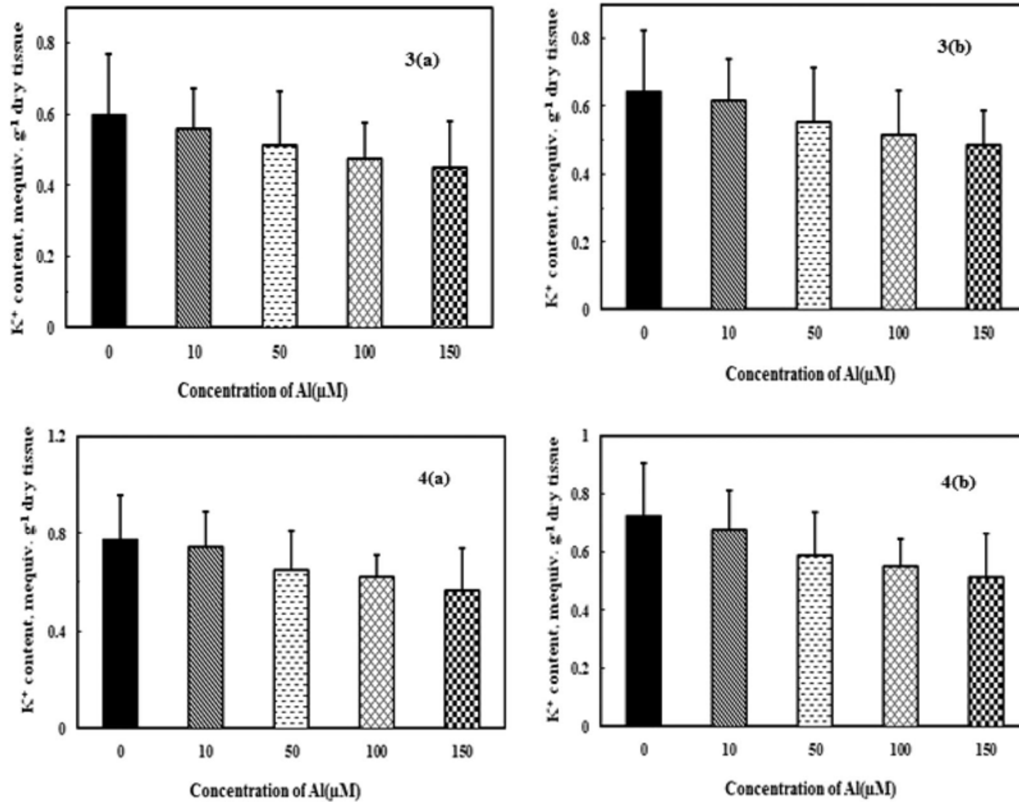
Duration of treatment (hrs)	% germination concentration of $AlCl_3$ (µM)				
	0	10	50	100	150
48	94 ± 0.577	80 ± 0.577	72 ± 0.882	66 ± 0.946	58 ± 0.882
72	100 ± 0.333	94 ± 0.495	90 ± 0.651	85 ± 0.333	79 ± 0.577
96	100 ± 0.333	96 ± 0.568	93 ± 0.333	89 ± 0.577	84 ± 0.333



Figs 1-2: 1. The effect of different concentrations of aluminium (Al) on the accumulation of K^+ in (a) radicle and (b) plumule of germinating rice seeds at 72 hrs of treatment . epresents control;) µM Al, 50 µM Al, 100 µM Al and 150 µM Al. Each value is the mean of three replicates. Bars represent ± standard error of the mean value. 2. The effect of different concentrations of aluminium (Al) on the accumulation of K^+ in (a) radicle and (b) plumule of germinating rice seeds at 96 hrs of treatment. Otherwise as in Fig. 1.

Similarly, 10 - 150 µM aluminium inhibited K^+ content in the radicle and in the plumule of rice at 96 hrs of treatment. In this case also the degree of inhibition of K^+ accumulation in the radicle and plumule increased with the increase in aluminium concentration from 10 - 150 µM at 96 h of treatment. The inhibition of K^+ content in the radicle ranged from 9 - 38% and that of K^+ in the plumule ranged from 9 - 28% at Al concentration ranging from 10 - 150 µM (Fig. 2a,b).

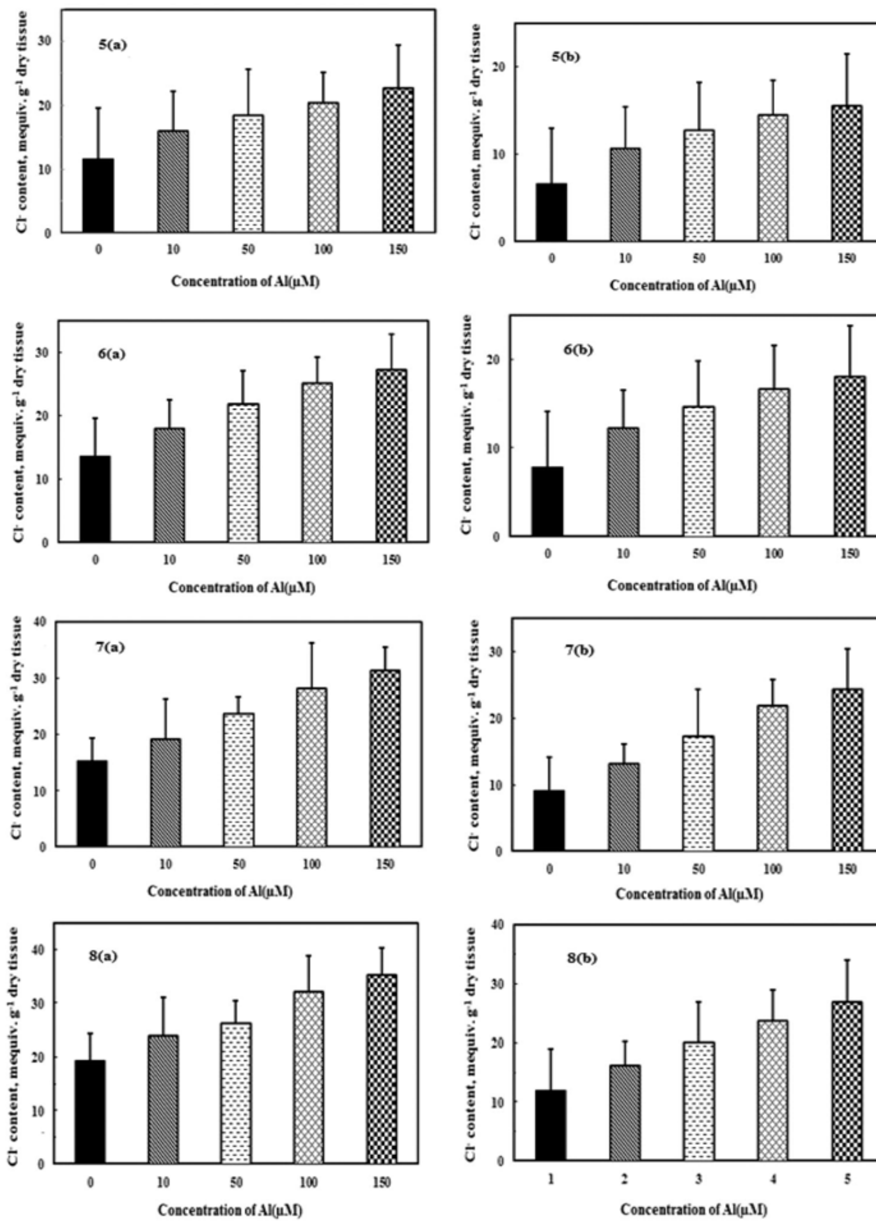
In chickpea, accumulation of K^+ decreased in the radicle by 5 - 24% following 10 - 150 µM aluminium treatment at 72 hrs of treatment (Fig. 3a). Similar magnitude of inhibition of K^+ (4 to 24%) was observed in the plumule of chickpea following Al treatment at 72 hrs of treatment (Fig. 3b).



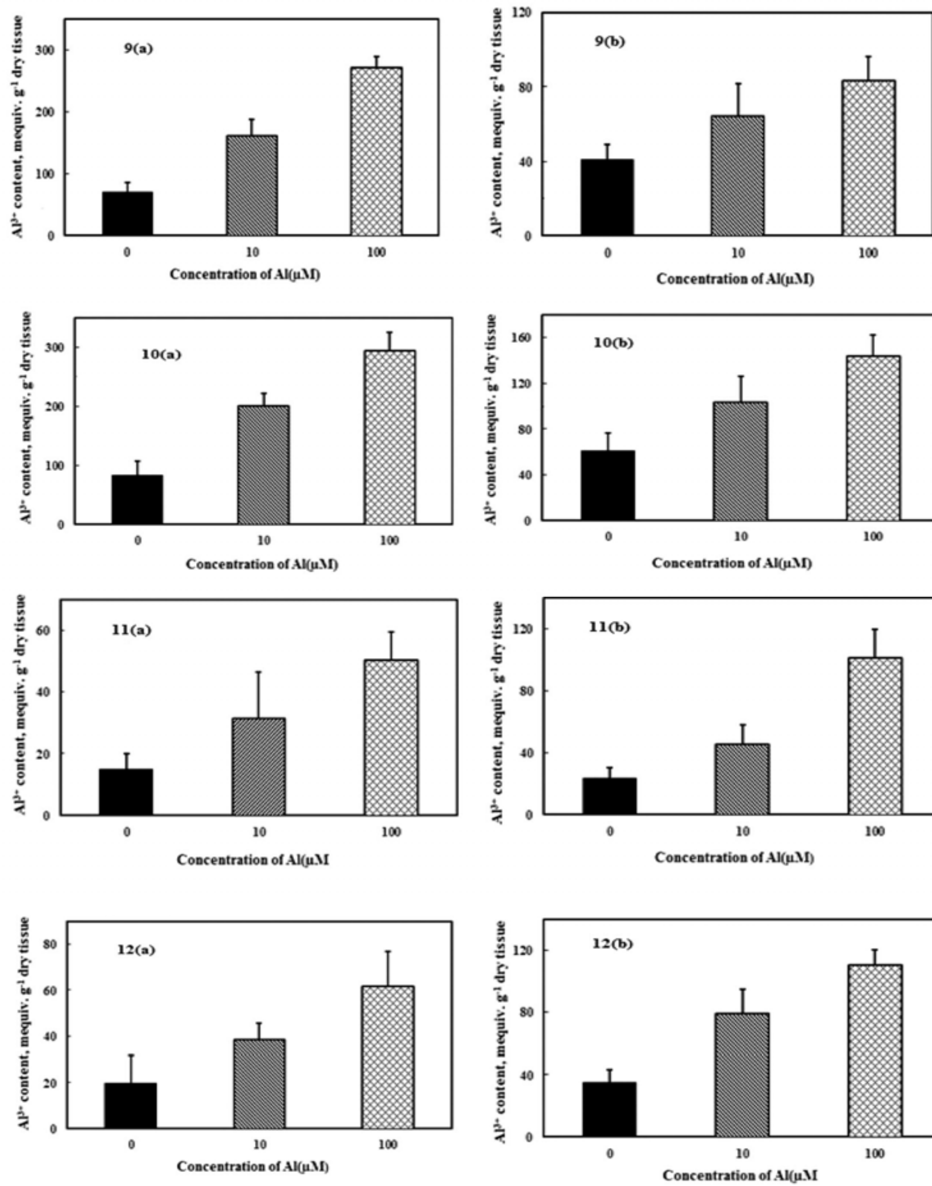
Figs 3-4: 3. The effect of different concentrations of aluminium (Al) on the accumulation of K^+ in (a) radicle and (b) plumule of germinating chickpea seeds at 72 hrs of treatment. Otherwise as Fig. 1. 4. The effect of different concentrations of aluminium (Al) on the accumulation of K^+ in (a) radicle and (b) plumule of germinating chickpea seeds at 96 hrs of treatment. Otherwise as in Fig. 1.

Similarly, 10 - 150 µM aluminium inhibited K^+ content in the radicle and plumule of chickpea at 96 hrs of treatment. The degree of inhibition increased with the increase in aluminium concentration from 10 - 150 µM at 96 hrs of treatment. The inhibition of K^+ content in the radicle of chickpea ranged from 3 - 27% and that of the plumule of chickpea ranged from 6 - 29% at Al concentration ranging from 10 - 150 µM (Fig. 4a, b). This result is supported by Horbowicz *et al*. (2011) who found that high concentration of Al in Hoagland solution decreased K^+ content in cotyledons and hypocotyls of common buckwheat (*Fagopyrum esculentum* Moench).

Aluminium at concentrations of 10, 50, 100 and 150 µM increased accumulation of Cl^- by 38, 60, 77 and 96%, respectively in the radicle of rice at 72 hrs of treatment as compared to control (Fig. 5a). In the plumule of rice, 62% to 2.4-folds increase in Cl^- accumulation was observed at 72 hrs of application of 10 - 150 µM aluminium (Fig. 5b).



Figs 5-8: 5. The effect of different concentrations of aluminium (Al) on the accumulation of Cl^- in (a) radicle and (b) plumule of germinating rice seeds at 72 hrs of treatment. Otherwise as in Fig. 1. 6. The effect of different concentrations of aluminium (Al) on the accumulation of Cl^- in (a) radicle and (b) plumule of germinating rice seeds at 96 hrs of treatment. Otherwise as in Fig. 1. 7. The effect of different concentrations of aluminium (Al) on the accumulation of Cl^- in (a) radicle and (b) plumule of germinating chickpea seeds at 72 hrs of treatment. Otherwise as in Fig. 1. 8. The effect of different concentrations of aluminium (Al) on the accumulation of Cl^- in (a) radicle and (b) plumule of germinating chickpea seeds at 96 hrs of treatment. Otherwise as in Fig. 1.



Figs 9-12: 9. The effect of different concentrations of aluminium (Al) on the accumulation of Al^{3+} in (a) radicle and (b) plumule of germinating rice seeds at 72 hrs of treatment ■ represents control, ▧ 10 µM Al ▩ 100 µM Al. Each value is the mean of three replicates. Bars represent ± standard error of the mean value. 10. The effect of different concentrations of aluminium (Al) on the accumulation of Cl^- in (a) radicle and (b) plumule of germinating rice seeds at 96 hrs of treatment. Otherwise as in Fig. 9. 11. The effect of different concentrations of aluminium (Al) on the accumulation of Cl^- in (a) radicle and (b) plumule of germinating chickpea seeds at 72 hrs of treatment. Otherwise as in Fig. 9. 12. The effect of different concentrations of aluminium (Al) on the accumulation of Cl^- in (a) radicle and (b) plumule of germinating chickpea seeds at 96 hrs of treatment. Otherwise as in Fig. 9.

Similarly, 10 - 150 µM aluminium caused a 62% to 2.0-folds increase in Cl^- content in the radicle (Fig. 6a) and a 57% to 2.3-folds increase in the accumulation of Cl^- in the plumule of rice at 96 h of treatment (Fig. 6b).

In chickpea, 10 to 150 µM aluminium caused a 26% to 2-folds increase in Cl^- accumulation in the radicle at 72 hrs of treatment (Fig 7a). In the plumule of chickpea seeds, 10 - 150 µM aluminium caused a 45% - 2.7-folds increase in Cl^- accumulation at 72 hrs of treatment (Fig. 7b).

A 24 to 83% increase in Cl^- accumulation in the radicle was observed at 96 h following 10 and 150 µM aluminium application (Fig. 8a). Similarly, 36% - 2.2-folds increase in Cl^- accumulation in the plumule was observed at 96 hrs following 10 - 150 µM aluminium treatment (Fig. 8b).

At 72 hrs exposure of 10 and 100 µM Al caused 2.3-folds and 3.8-folds increase in accumulation of Al^{3+}, respectively in the radicle of rice (Fig. 9a). Similarly, 10 and 100 µM Al caused a 1.6-folds and 2-folds increase in Al content in the plumule, respectively at 72 hrs of treatment (Fig. 9b).

A 2.4-folds and 3.6-folds increase in Al^{3+} was recorded in the radicle of rice following 10 and 100 µM aluminium, respectively at 96 hrs of treatment (Fig. 10a). In the plumule, 10 and 100 µM Al caused 1.7-folds and 2.4-folds increase in the accumulation of Al^{3+}, respectively at 96 hrs of treatment (Fig. 10b).

Application of 10 and 100 µM Al for 72 hrs caused a 2- folds and 3.4- folds increase in accumulation of Al^{3+}, respectively in the radicle of chickpea (Fig. 11a). In the plumule, exposure of 10 and 100 µM Al for 72 hrs resulted in 2-folds and 4.3-folds increase in Al, respectively (Fig. 11b).

Similarly, a 96 hrs exposure of 10 and 100 µM aluminium increased Al^{3+} accumulation by 2- and 3.2-folds in the radicle of chickpea (Fig. 12a). In the plumule, 10 and 100 µM aluminium treatment caused a 2.2-folds and 3.1-folds increase in accumulation of Al^{3+}, respectively in the plumule at 96 hrs of treatment (Fig. 12b).

Al treatment in the germinating seeds of rice and chickpea decreased K^+ accumulation and increased Cl^- and Al^{3+} accumulation in both the radicle and plumule. It is suggested that aluminium toxicity induced increase in accumulation of Cl^- and Al^{3+} with the concomitant decrease in K^+ accumulation in the radicle and plumule might be responsible for Al-induced inhibition of germination of seeds.

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EFFECT OF ALUMINIUM TOXICITY ON THE ACCUMULATION AND DISTRIBUTION OF K^+, Na^+, Cl^- AND NO_3^- IN *ORYZA SATIVA* L. AND *CICER ARIETINUM* L.

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Key words: Aluminium toxicity, Ion transport, K^+, Na^+, Cl^- and NO_3^- accumulation, Rice, Chickpea

Abstract

Aluminium (10 to 150 μM) decreased K^+ accumulation in the root and shoot of rice and the root, stem and leaves of chickpea seedlings. On the other hand, Al, at a concentration of 10, 50, 100 and 150 μM increased Na^+ content in different parts of rice and chickpea seedlings. 150 μM Al increased Na^+ accumulation in the root by 2.1- to 2.2-folds from 3 to 96 hrs of treatment. Aluminium at a concentration of 150 μM caused a dramatic 2- and 3.4-folds increase in Cl^- accumulation in the root and shoot of rice, respectively. In chickpea, 150 μM Al increased Cl^- accumulation in the root by 2-folds. On the contrary, Al application decreased NO_3^- accumulation in different parts of rice and chickpea seedlings.

Introduction

Aluminium (Al) is the third most abundant metallic element in soil but becomes available to plants only when the soil pH drops below 5.5. When pH drops below 5.5, aluminosilicate clays and aluminium hydroxide minerals begin to dissolve, releasing aluminium-hydroxy cations Al $(H_2O)_6^{3+}$ or (Al^{3+})[1]. The mononuclear Al^{3+} species is considered as the most toxic forms[2-3]. Al toxicity is a major factor limiting plant production on acid soil[4].

Aluminium toxicity decreased K^+ content in the root but increased in the stem of *Theobahia* and in the leaves of both genotypes of *Cacao*[5]. Al treatment decreased K^+ content in the root, stem and leaves of tomato[6]. On the contrary, Al increased accumulation of K^+ in the root of sorghum[7]. Aluminium reduced NO_3 uptake in soybean[8-9] and in wheat[10]. On the other hand, Al increased absorption of nitrate in *Stylosanthes guianensis* and *S. macrocephala* [11-12].

Reports on the effect of aluminium application on the accumulation of K^+, Na^+, Cl^- and NO_3^- in rice (*Oryza sativa* L.) and chickpea (*Cicer arietinum* L.) are very rare. Therefore, in this paper, the effect of aluminium toxicity on the accumulation and distribution of K^+, Na^+, Cl^- and NO_3^- in rice and chickpea seedlings is reported.

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Material and Methods

Rice (*Oryza sativa* var. BRRI Dhan-53) and chickpea (*Cicer arietinum* var. Bari Chhola-7) were taken as experimental plant material. Seeds of rice were collected from Bangladesh Rice Research Institute (BRRI) and that of chickpea were procured from Bangladesh Agricultural Research Institute (BARI).

The seeds were surface sterilized according to Samad and Karmoker[13]. Then the seeds were spread over cotton gauge placed in a plastic lid having holes and was placed upon the beaker filled with half strength Hoagland solution. After 48 hrs of sowing, the seeds were germinated and then were transferred to light bank. Rice seedlings were grown at a day/night temperature of 30ºC ± 1ºC/25ºC ± 1ºC and day/night length of 14 hrs/10 hrs. Chickpea seedlings were grown at a day/night temperature of 2 ºC ± 1ºC /18ºC ± 1ºC and day/night length of 10 hrs/14 hrs. Light intensity was 160 μ einstein $m^{-2}s^{-1}$. The solution was replenished every 48 hrs. The solution was continuously aerated through bubbler with the help of air compressor. Seven-day-old seedlings were transferred to half strength Hoagland solution (control) and 10, 50, 100 and 150 μM $AlCl_3$ solution made in half strength Hoagland solution. The pH of all solutions including control were adjusted to 4.2 with 0.2N H_2SO_4.

Shoots, stems and leaves were collected in triplicate after 3, 6, 24, 48, 72 and 96 hrs of aluminium treatment. The K^+, Na^+, Cl^- and NO_3^- were extracted from dry tissue by boiling in water bath with two changes of 10 ml distilled water contained in test tubes. K^+ and Na^+ ions were measured by flame photometer (Jenway, PEP-7, UK) at a wavelength of 767 nm and 589 nm, respectively. Amount of Cl^- was measured by titrimetric method with 0.05 N $AgNO_3$ using 5% $K_2Cr_2O_4$ as an indicator. Nitrate was determined following the method of Cataldo *et al.* [14].

Results and Discussion

Aluminum at concentrations of 10 to 150 μM decreased K^+ accumulation in the root of rice except an initial stimulation. 10 and 150 μM Al caused 8 to 20% and 18 to 39% inhibition of K^+, respectively in the root of rice seedlings (Fig. 1a). 150 μM Al resulted in a 13 to 48% decrease in accumulation of K^+ in the shoot of rice from 6 to 96 hrs of treatment (Fig 1b).

In chickpea seedlings, 10 μM Al decreased K^+ content in the root by 8 to 24% from 3 to 96 hrs of treatment. Al-induced inhibition of K^+ accumulation in the root increased with the increase in Al concentration from 10 to 150 μM (Fig. 2a). In the stem of chickpea, maximum accumulation of K^+ in the stem occurred at 150 μM Al treatment which ranged from 25 to 44% from 3 to 96 hrs of application (Fig. 2b). Similarly, all the concentrations of Al used inhibited accumulation of K^+ in the leaves of chickpea (Fig. 2c). This result is supported by Bhalerao and Probhu[15] who found that Al decreased K^+ accumulation in maize and sorghum. On the contrary, Al increased K^+ content in *Stylosanthes*[16].

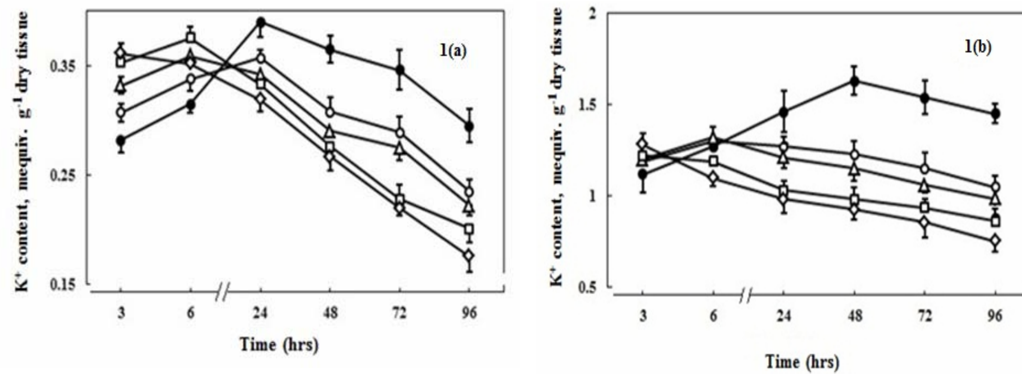

Fig. 1. The effect of different concentrations of aluminium (Al) on the accumulation of K^+ in (a) root and (b) shoot of rice seedlings. ● represents control; ○ 10 μM Al; Δ 50 μM Al; □ 100 μM Al; ◊ 150 μM Al. Each value is the mean of three replicates. Bars represent ± standard error of the mean value.

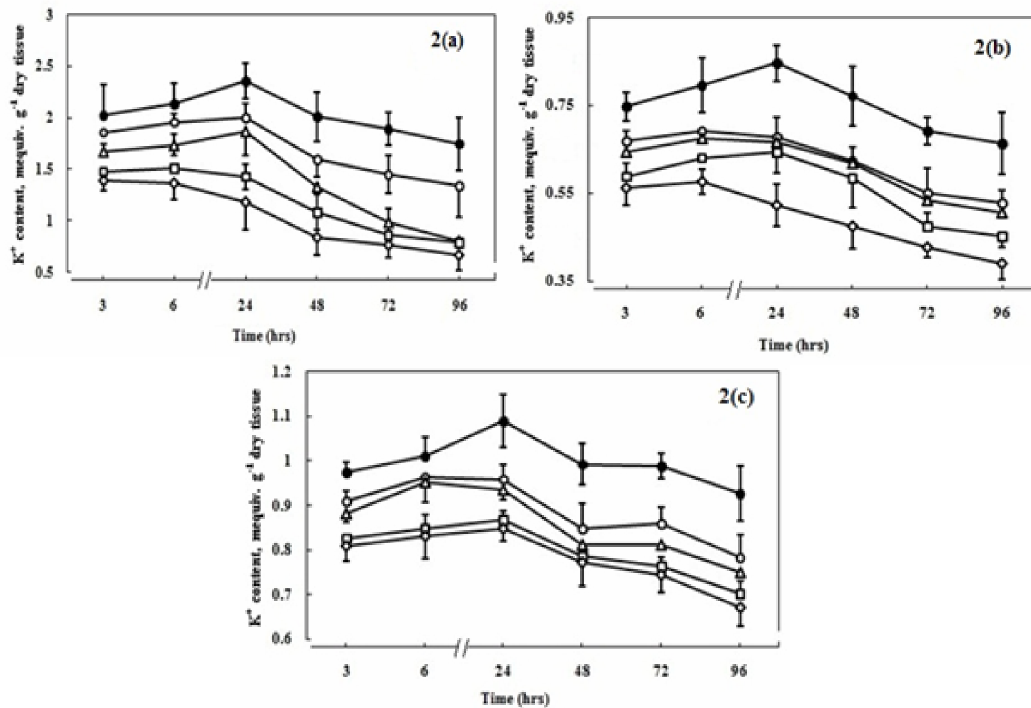

Fig. 2. The effect of different concentrations of aluminium on the accumulation of K^+ in (a) root, (b) stem and (c) leaf of chickpea seedlings. Otherwise as Fig. 1.

Aluminium at a concentration of 10 μM, increased the accumulation of Na^+ from 28 to 37% in the root of rice from 3 to 96 hrs of treatment. Highest stimulation of Na^+ accumulation in the root occurred at 150 μM Al application which ranged from 2.1- to

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2.2-folds (Fig. 3a). A 53 to 55% increase in Na^+ accumulation in the shoot of rice was observed following 150 μM Al treatment from 3 to 96 hrs of treatment (Fig. 3b).

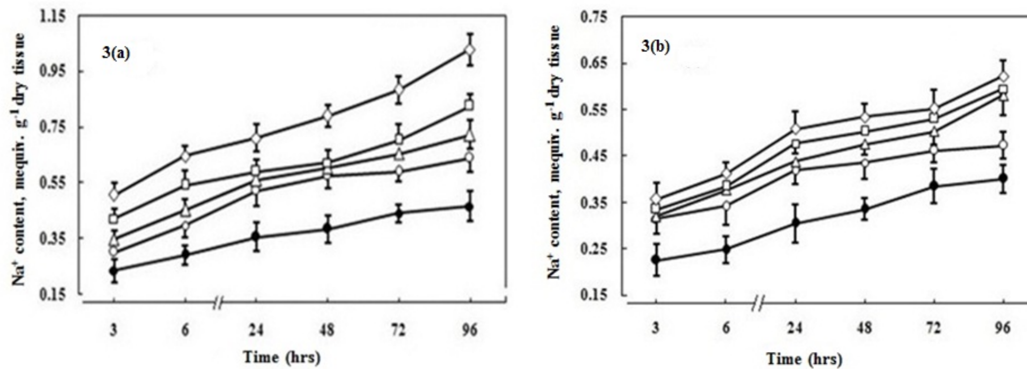

Fig. 3. The effect of different concentrations of aluminium on the accumulation of Na^+ in (a) root and (b) shoot of rice seedlings. Otherwise as Fig. 1.

In chickpea seedlings, 10, 100 and 150 μM Al increased Na^+ accumulation in the root by 9 to 16, 30 to 41 and 40 to 50%, respectively from 3 to 46 hrs of treatment (Fig. 4a). In the stem, 100 and 150 μM Al caused a 23 to 29 and 37 to 43% increase in Na^+ accumulation, respectively from 3 to 96 hrs of application (Fig. 4b). In the leaves, 100 and 150 μM Al increased accumulation of Na^+ by 36 to 52 and 53 to 72%, respectively from 3 to 96 hrs of treatment (Fig. 3c). This result is in agreement with the work of Lidon *et al.*(17) who found that 0.33 mM Al increased Na^+ content in the root of maize.

Uptake of K^+ was decreased by aluminum toxicity (Figs 1 and 2) but that of Na^+ was increased (Figs 3 and 4) in rice and chickpea seedlings. Therefore, it appears that Al alters the K^+/Na^+ selectivity.

In rice, 10 μM Al increased Cl^- accumulation in the root by 19 to 58% from 3 to 96 hrs of treatment. Chloride accumulation increased with the increase in concentration of aluminium. The highest accumulation occurred at 150 μM Al where a 61% to 2.2-folds increase in Cl^- accumulation in the root was recorded (Fig. 5a). In the shoot, 100 and 150 μM Al caused a 2- to 2.5-folds and 2.7- to 3.4-folds increase in Cl^- accumulation, respectively from 3 to 96 hrs of application (Fig. 5b).

In chickpea seedlings, 150 μM Al caused the maximum stimulation of Cl^- in the root ranging from 85% to 2-fold from 3 to 96 hrs of application (Fig. 6a). In the stem, 50 and 150 μM Al increased Cl^- accumulation by 23 to 28 and 36 to 49%, respectively from 3 to 96 hrs of treatment (Fig. 6b). In leaves 150 μM Al caused the maximum (74 to 81%) increase in Cl^- accumulation (Fig. 6c). On the contrary, Al decreased accumulation of Cl^- in maize (18).

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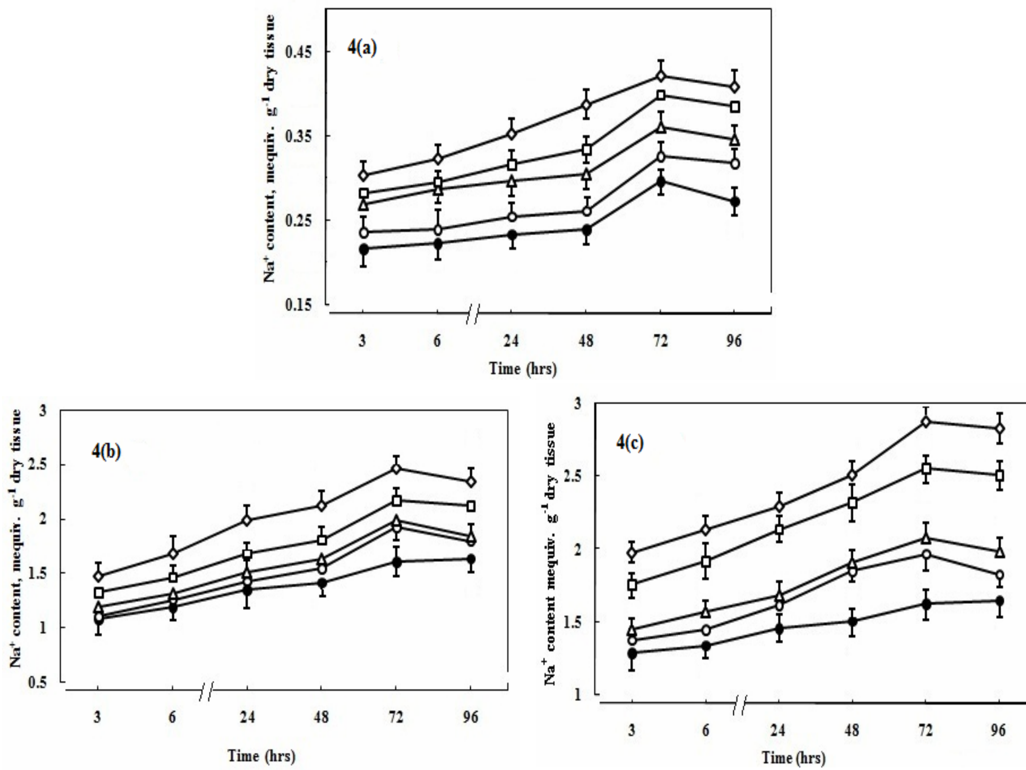

Fig. 4. The effect of different concentrations of aluminium on the accumulation of Na^+ in (a) root, (b) stem and (c) leaf of chickpea seedlings. Otherwise as Fig. 1.

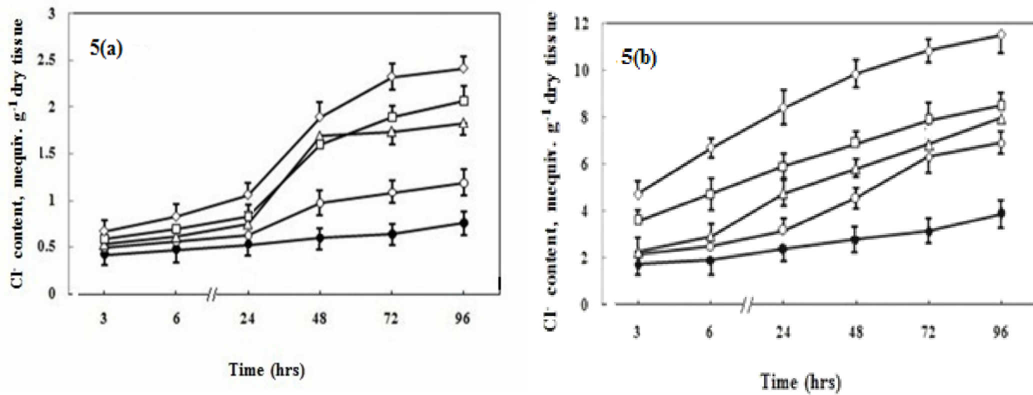

Fig. 5. The effect of different concentrations of aluminium on the accumulation of Cl^- in (a) root and (b) shoot of rice seedlings. Otherwise as Fig. 1.

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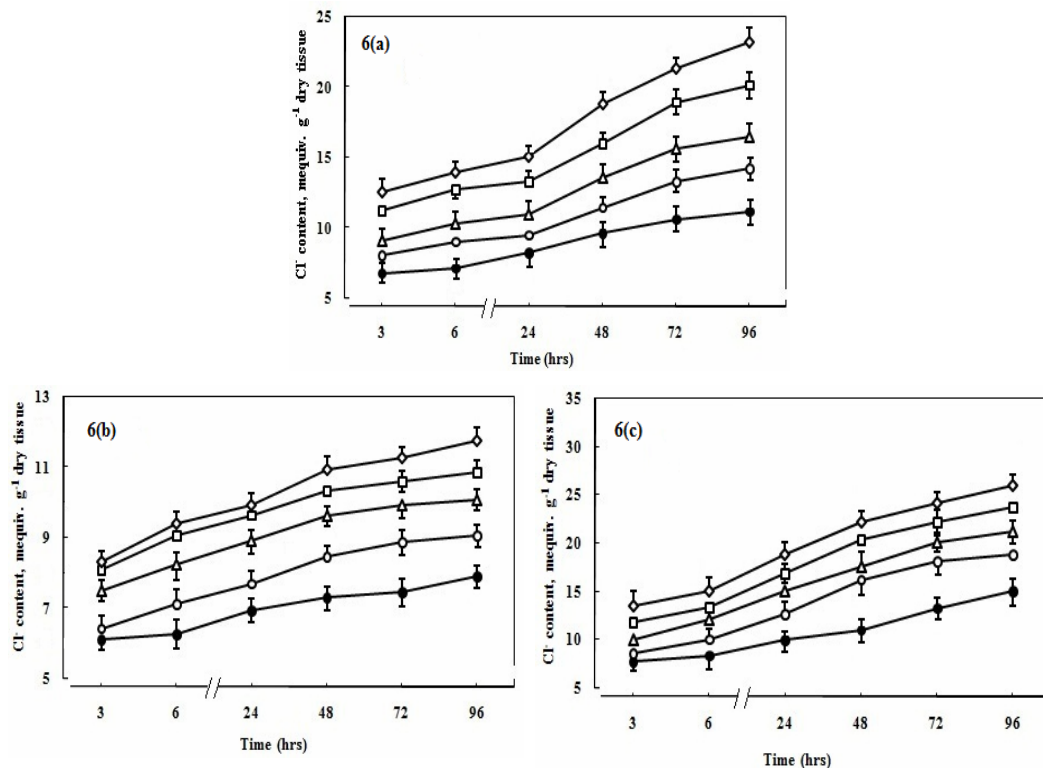

Fig. 6. The effect of different concentrations of aluminium on the accumulation of Cl^- in (a) root, (b) stem and (c) leaf of chickpea seedlings. Otherwise as Fig. 1.

In rice, the maximum inhibition of 34 to 82.8% in NO_3^- accumulation in the root was caused by 150 μM Al treatment (Fig. 7a). In shoot 50 and 100 μM Al decreased NO_3^- accumulation by 17 to 53.7 and 11.7 to 79.8%, respectively from 6 to 96 hrs of treatment (Fig. 7b).

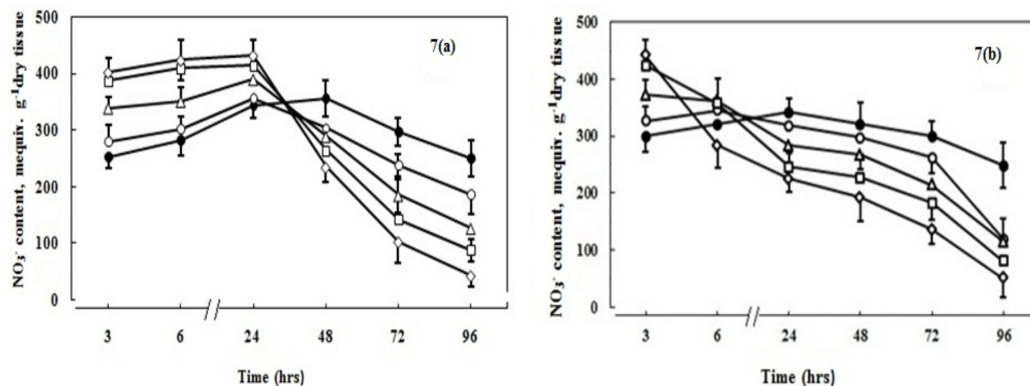

Fig. 7. The effect of different concentrations of aluminium on the accumulation of NO_3^- in (a) root and (b) shoot of rice seedlings. Otherwise as Fig. 1.

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In chickpea seedlings, 10 and 100 μM Al inhibited NO_3^- accumulation in the root by 21.7 to 50 and 27 to 61%, respectively from 48 to 96 hrs of treatment (Fig. 8a). In the stem, 10, 100 and 150 μM Al decreased NO_3^- accumulation by 7.5 to 57, 26.6 to 69 and 28.8 to 76.5%, respectively from 3 to 96 hrs of application (Fig. 8b). In the leaves, all the concentration of Al (10-150 μM) decreased NO_3^- accumulation. The highest inhibition of NO_3^- in the leaves was exerted by 150 μM Al where a 37 to 77.9% reduction was recorded from 3 to 96 hrs of treatment (Fig. 8c). Similar Al-induced inhibition of NO_3^- was found in sorghum[(19-20)].

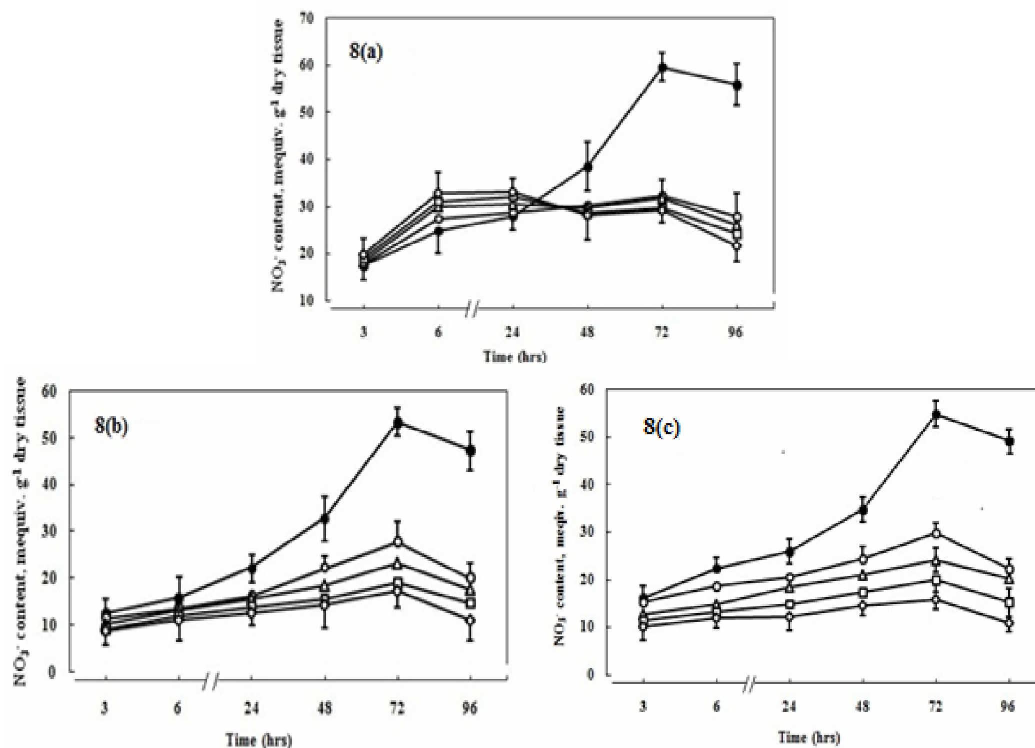

Fig. 8. The effect of different concentrations of aluminium on the accumulation of NO_3^- in (a) root, (b) stem and (c) leaf of chickpea seedlings. Otherwise as Fig. 1.

Al-induced changes in K^+/Na^+ selectivity and low level of K^+ might disrupt metabolic functions of plants. On the otherhand, Al-induced dramatic stimulation of Cl^- in rice and chickpea might be toxic for the plants.

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