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Effect of Aluminium Stress on Germination and Mineral Nutrition of Kidney Bean Cultivars with Different Sensitivity to Aluminium

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Aluminium is the most important element in the soil as a stable complex with oxygen and silicates. When pH is below 5, aluminium dissolves in soil water and is absorbed by plant roots. Aluminium toxicity is a major constraint to agricultural production in the world, because 50% of the world's potential lands are acidic. Hence this study was conducted to investigate the toxic effects of aluminium on the germination and nutrient uptake in five cultivars of kidney beans (Derakhshan, Goli, Akhtar, Sayad and Naz) at three concentrations (30, 40, and 50mM) aluminium nitrate, $Al(NO_3)_3$. Due to aluminium toxicity, reduced germination and growth of seedlings was recorded in all cultivars. Absorption of various nutrients, such as Fe, Ca, Mg, K, p, N decreased in roots and shoots of all cultivars. P and Mg Contents of shoots were less affected in all cultivars. Two cultivars including Derakhshan and Goli were better in terms of growth and accumulation of minerals than the other three. In General, germination and nutrient accumulation was inhibited in kidney bean due to the presence of aluminium.

Key words: Germination, Aluminum, Nutrients, Kidney beans.

Although aluminium is not considered as an essential nutrient, it is one of the most abundant minerals in the soil which composes approximately 7% of the minerals (Vardar and Unal, 2007). Bioavailability of aluminium, hence its toxicity is limited to acidic environments. Acidic soils with pH≤5 are the main limitations in agriculture. Production of food crops, particularly corn, is negatively influenced by acid soils (Kochian *et al*. 2005). Some agricultural practices, such as removal of crops, nitrogen leakage below the root zone, improper use of nitrogen fertilizers, and accumulation of organic materials cause the agricultural soil become more acidic (Silva, 2012). Regular beans (*Phaseolus vulgaris* L.) are the most important legume for human nutrition around the world as a major source of calories and protein, especially for low-income countries of the tropics facing food shortages (Graham, 1978; Rao, 2001; Beebe, 2012). Under field conditions, regular bean often experience different abiotic stresses such as drought, aluminium and manganese toxicities, low soil fertility and high temperatures (Thung and Rao, 1999; Kshitani *et al*. 2004; Beebe, 2012; Yang *et al*. 2013). Although low fertility of acidic soils is due to a combination of mineral aluminium and manganese toxicities and shortage of mineral phosphors, calcium, magnesium and molybdenum, aluminium toxicity is the most important factor limiting crops on 67% of the areas with acidic soils (Vardar and Unal, 2007; Zheng *et al*. 2014). When pH is below 5.5, Amino silicate crystals and mineral aluminium hydroxide start dissolving and hydroxy- aluminium cations and release $Al(H_2O)^{3+}_6$

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which compete with other cations. In these conditions, they produce the ion Al^{3+} as well as a variety of molecules $AloH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$ and $Al(OH)_4$ (Panda and Matsumoto, 2007). Mononuclear species Al^{3+} and Al_{13} are considered as the most toxic types (Silva, 2012). Although some plants (such as pineapple and tea) are known to be resistant against high levels of exchangeable aluminium, aluminium is a limiting factor for most plants (Silva, 2012). In micromolar concentrations, primary root length and lateral root development which are fast (within a few minutes) are inhibited by aluminium; water and food intake are also low (Blankaflor *et al*. 1998; Barcelo *et al*. 2002). Exposure to aluminium results in altered root morphology and whereby root death (Ciamporova, 2002). Generally, the zone distant from cell division zone in root tips is sensitive aluminium stress (Kumar panda *et al*. 2009). During the past decade, extensive physiological studies suggest that plants have two main internal and external strategies for aluminium detoxification and tolerance (Gupta *et al*. 2014). External mechanism (mechanism of elimination) prevents aluminium absorption by physical or biochemical methods; for example, cell wall thickens under aluminium stress, which can effectively prevent aluminium intake (Gupta *et al*. 2014). Secretion of organic acids from roots and external aluminium chelate limits aluminium absorption (Brunner and Sperisen, 2013). However, secretory patterns are temperature-sensitive in secreted organic acid species (Ma and Furukawa, 2003). internal mechanism means that plants are able to detoxify cell aluminium by forming harmless complexes with organic ligands such as organic acids; then, plants secrete them to special organelles such Vacuoles so that they can quickly fix any damage (Delhaize *et al*. 2012; Sharma and Chakraverty, 2013). To secrete organic acids which are able to chelate aluminium plays an important role in external and internal detoxification of aluminium (Brunner and Sperisen, 2013). For the efficient use of nutrients, particularly phosphorus and calcium, aluminium toxicity tolerance is the key feature which allows plants adapt to acidic soils (Ribeiro and de Almedia, 2013). Existing species and genotypes within species are different in aluminium resistance. For most plants, fertilizing and soil amendment effort (such as limestone) may not be sufficient to reduce aluminium toxicity (for example, soil still remains highly acidic). In most countries, these strategies may be limited to economy (Silna, 2012). Purpose of this study is to determine the difference in response of bean to aluminium increase during germination and growth and examine some physiologic properties involved in stress resistance in different levels of aluminium concentration. This is an answer to the question that which cultivar of the plant grows better in acidic soils.

MATERIALS AND METHODS

To investigate the effect of aluminium on germination, seeds were placed in 8cm Petri dishes inside the incubator at 25°C for a week. Initial tests have revealed that these conditions were suitable for plant germination (Yang *et al*. 1996). Three 10-seed replications (three Petri dishes) containing four treatment groups (control, 30, 40 and 50mM) was formed for cultivars (Derakhshan, Goli, Akhtar, Sayad, and Naz). To examine the effects of aluminium on bean seedlings, the seed was provided from, Agricultural Jihad, Markazi Province, Iran, and it was planted. In this step, some baskets with 2×4mm pores were selected. Containers containing water were used as a nutrient solution until the two-leaf stage; then, seeds of both cultivars were planted in baskets. The number of seeds planted in a basket was approximately 100. Baskets containing seeds cultivated in vitro were in the dark for 24h and then moved to light. They were irrigated every morning. After one week, the plants uniform in size were selected and transferred from baskets to dark containers after rinsing them with distilled water (350ml) containing half-strength Hoagland solution (hydroponic medium). After 24 hours, they were exposed to four different aluminium treatments with concentrations of 30, 40 and 50mM. To avoid choking roots and deliver enough oxygen to the roots, the hydroponic medium containing plants was aerated for 2 hours per day through the air pump. Plants were grown under regulated conditions, that is, 16h day length, 190¼ml photons light intensity per m^2s, 26.22! temperature frequency (night/day), 65± 5 % relative humidity and nutrient solution pH in the range of 6.5 for 20 days. After treatment, plants were harvested. Roots and shoots were separated and washed by distilled water without

ions. Samples used to determine plant growth and measure elements were dried at 70°C. The amount of nickel (Ni), calcium (Ca), magnesium (Mg), and iron (Fe) was measured by the relevant standards using atomic absorption spectroscopy (Atomic Absorption Varian, Spectr AA-200). Amount of potassium of the prepared samples was measured by standard curve using flame photometer (Flame Photometer, model 410, Sherwood Company). The amount of nitrogen (N) and phosphorus (P) was measured by the relevant standards by a spectrophotometer (UV-120-01, Shimadzu). Results were analysed using SPSS, Duncan test and ANOVA.

RESULTS

Results of seed germination of five cultivars of beans in aluminium nitrate treatments are presented in Figure 1.

According to the results, increasing the aluminium nitrate concentration in Hoagland solution reduced germination and growth of seedlings in all cultivars.

For Derakhshan, average germination was 11.2%, 40.9% and 53.2% of the control under 30, 40 and 50mM aluminium nitrate, respectively, which shows a decrease compared to control.

For Goli, average germination was 14.8%, 45.1% and 63.2% of the control under 30,

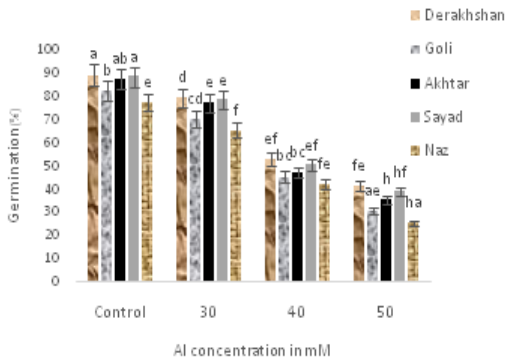

Fig. 1. Comparison of seed germinations of five bean cultivars under different treatments of aluminium (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

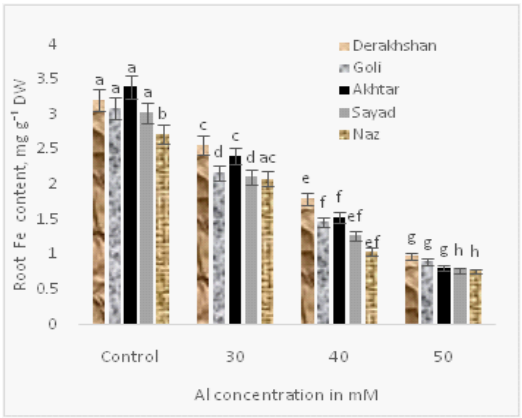

Fig. 2. Variations in iron content of root (mg.g^{-1}. DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

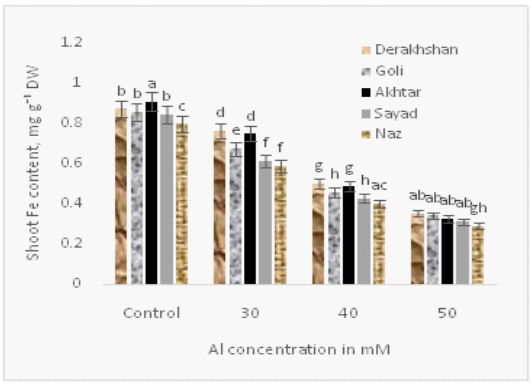

Fig. 3. Variations in iron content of shoots (mg.g^{-1}.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

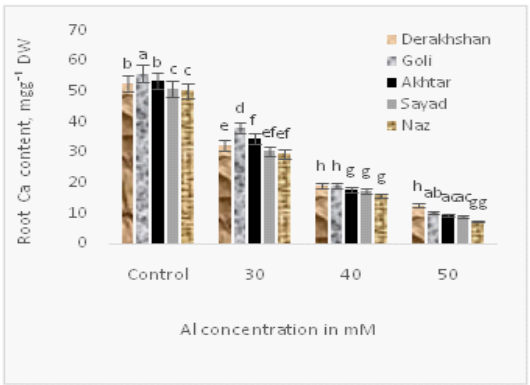

Fig. 4. Variations in calcium content of root (mg.g-1.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

40 and 50mM aluminium nitrate, respectively, which shows a decrease compared to control. This decrease was also observed for Akhtar, Sayad and Naz, significantly.

The reduction was 17.8%, 45.5% and 67.5% for Naz under 30, 40 and 50mM aluminium nitrate, respectively, which is the highest decrease among cultivars (Figure 1).

As the results show, increase in aluminium concentration significantly reduces iron ion in root and shoot of all cultivars compared to control ($P<0.05$); however, the decrease is different in five studied cultivars (Figure 2 and 3). Obviously, the decrease percentage in iron content of the root under 50mM aluminium is the highest rate of decrease.

As Figure 4 shows, calcium uptake by roots strongly decreased by presence of aluminium in nutrient solution. Significant reduction is maximized in 50mM treatments.

By increasing aluminium concentration in the culture medium, calcium content of shoots significantly decreased (Figure 5). Under Aluminium treatments, calcium content of shoots reduced by 83% in Naz under high aluminium concentration (50mM) compared to control.

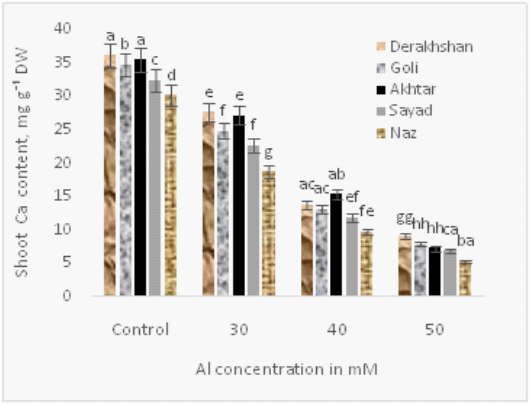

Fig. 5. Variations in calcium content of shoots (mg.g-1.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

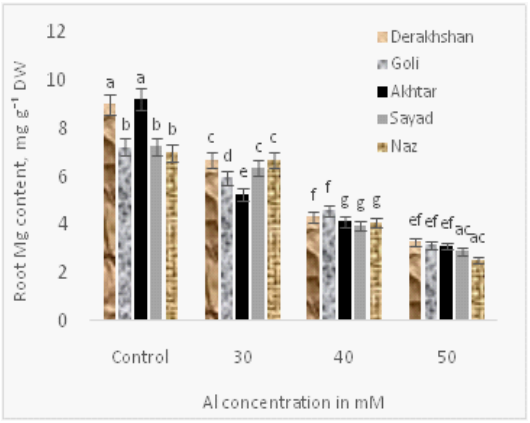

Fig. 6. Variations in magnesium content of root (mg.g-1.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test.

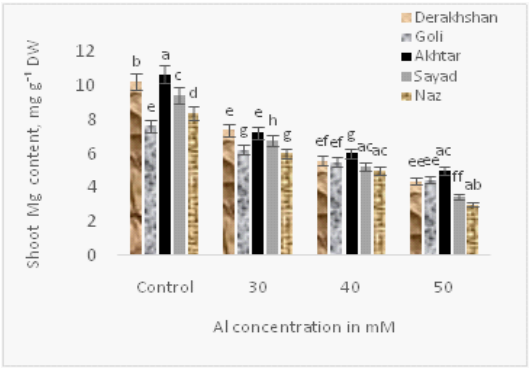

Fig. 7. Variations in magnesium content of shoots (mg.g-1.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

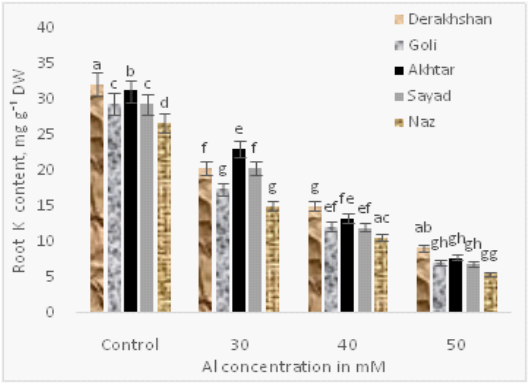

Fig. 8. Variations in potassium content of root (mg.g-1.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

Obviously, calcium content of roots and shoots treated with 50mM aluminium is higher in Derakhshan than other cultivars

According to Figure 6, the increase in aluminium concentration decreased the magnesium content of roots under three different treatments of aluminium. Increase in aluminium concentration of the nutrient solution decreased magnesium of shoots (Figure 7). As the tables show, decrease in magnesium ion of shoots was significant for five bean cultivars under different nitrate aluminium concentrations in 5%. However, the interaction of aluminium nitrate and cultivar concerning magnesium ions is insignificant in leaves.

As shown in Figure 8, potassium content of roots significantly reduced along with the aluminium concentration in the growth medium. The highest decrease occurred for Naz under high aluminium concentration (50mM) in which potassium content of roots decreased by 81%, compared to control. As roots, increase in aluminium concentration significantly decreased aluminium content of shoots (Figure 9). Under 50mM aluminium, potassium content of shoots decreased by 66% compared to control plants.

As aluminium concentration increased in

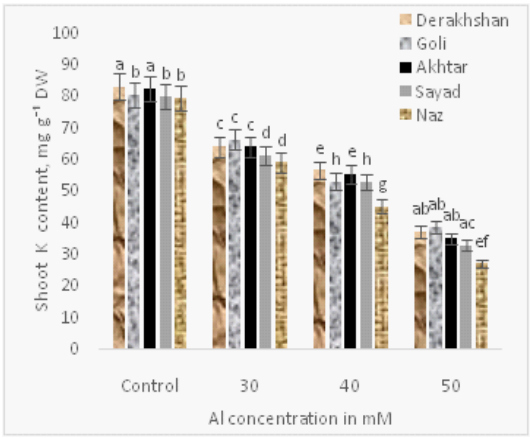

Fig. 9. Variations in potassium content of shoots (mg.g-1.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

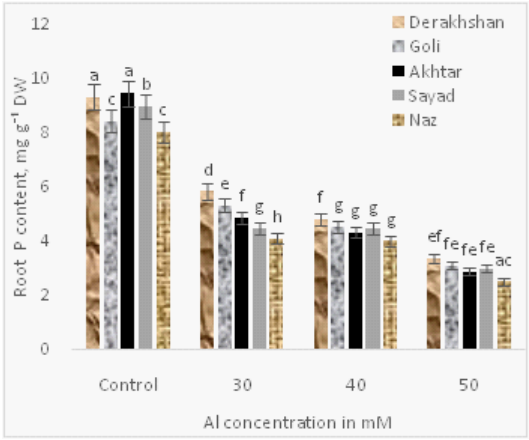

Fig. 10. Variations in phosphorus content of root (mg.g-1.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test.

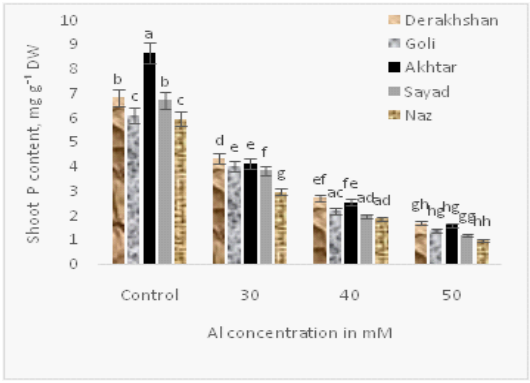

Fig. 11: Variations in phosphorus content of shoots (mg.g-1.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

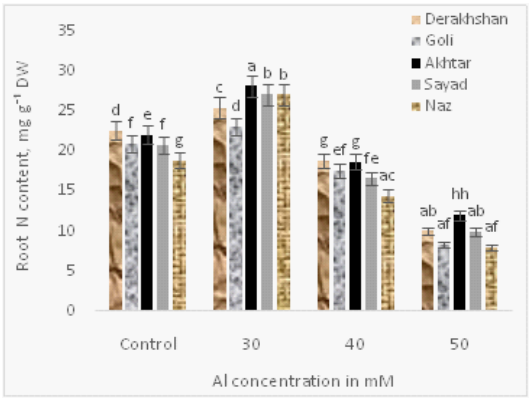

Fig. 12. Variations in nitrogen content of root (mg.g-1. DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test.

culture medium, Phosphorus content of roots and shoots significantly reduced (Figure 10 and 11). This reduction can be found in root and shoots of all bean cultivars compared to control. However,

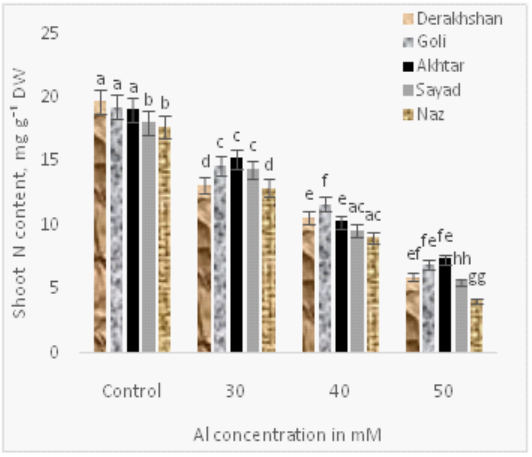

Fig. 13. Variations in nitrogen content of shoots (mg.g-1.DW) in bean cultivars under different aluminium nitrate treatments (X±S.E., n=3); there is a significant difference in mean with dissimilar letters (p=0.05) using Duncan test

showed that cultivars show different germination percentages in aluminium-containing environments (the first site to bind aluminium is probably matrix, which is mainly composed of homo polymers of galacturonic acid (Mihnen, 2008; Horst *et al*. 2010).

Aluminium tightly binds to pectin by Ca^{+}, which attaches it to the cell wall (Franco *et al*. 2004). This makes aluminium attaches to the wall through displacement of calcium; this makes the wall more rigid and does not allow the normal development (Tabuchi and Matsumoto, 2001; Brunner and Sperisen, 2013). Germination can be reduced by heavy metals interfering with metabolic processes related to normal development of seedlings during germination. Recent studies indicate severe hormonal disorders in germinating seeds exposed to different heavy metals. In seeds treated by heavy metal, endogenous Abscisic acid (ABA) content significantly increases (Atici *et al*. 2005). Probably, heavy metal toxicity and damages are in part due to increase in endogenous ABA levels during seed germination and growth (Shama and Kumar, 2002). under natural conditions, gibberellin content including GA_3 increases in the reduction is different in those five cultivars. Obviously, there is no significant difference in phosphorus content of shoots under 40 and 50mM treatments.

As Figure 12 shows, 30mM aluminium treatment significantly increased nitrogen content of root. In addition, 50mM aluminium treatment slightly (while significantly) decreased nitrogen content of the root compared to control. Changes in nitrogen content of shoots are shown in Figure 13. Aluminium in the medium led to a significant reduction in nitrogen content of shoots in all cultivars.

DISCUSSION

Seed germination is a complex process which begins with the absorption of water to activate enzyme proteins after a short pause. Germination is regulated by interaction of hormonal and environmental factors only in appropriate circumstances (Atici *et al*. 2005). Heavy metal stress is a major abiotic stress which can affect seed germination (Cassierra-Posada *et al*., 2009). The present results also indicate that increase in the aluminium concentration in the medium led to a decrease in the rate of seed germination in *Phaseolus vulgaris* L. Therefore, reduction or inhibition of germination appears to be one of the most important effects of aluminium.

Several researchers reported reduced seed germination under aluminium stress and other heavy metals in different plant species, which is consistent with current results (Gupta *et al*. 2014; Inostroza-Blancheteau *et al*. 2012; Panda *et al*. 2009; Atici *et al*. 2005; Seregin and Kozhenikova, 2005; Kopyra and Gwozdz, 2003; Ali *et al*. 2000; Madhava Rao and Sresty, 2000; Lima and Compelend, 1990; Naraganan and Symala, 1989; Nosko *et al*. 1988)

The results of this study showed that the highest and lowest germination percentages belong to Derakhshan and Naz, respectively, under the highest aluminium concentration. These results are consistent with studies conducted on germination of seven Amaranthus subspecies under different concentrations of heavy metals such as Hg, Ni, Cd, Cu, Zn, Pb (Bigaloev, 2003). Reports of the inhibitory effect of aluminium on germination of 6 wheat cultivars by Alamgir *et al* (2009)

germinating seeds (Atici *et al*. 2005), while heavy metal stress severely decreases GA_3 content in germinating seeds. In addition, endogenous cytokinins decrease in some germinating seeds under heavy metal stress (Atici *et al*. 2005). Therefore, one of the most important factors in reduction of seed germination under stress of heavy metals is their hormonal imbalance.

Another factor in reducing seed germination under heavy metals, in addition to reducing water absorption, is to inhibit or destroy the activity of the protein structures by binding heavy metals and sulfhydryl groups (Hall, 2002; Capuana, 2011). Evidence show that some heavy metals reduce the decomposition of food supplies by reducing the activity of ±-amylase and acid phosphatase, causing defects in seed germination (Mihoub *et al*., 2005). Li *et al* (2011) reporting the reduction in seed germination of plants under heavy metal stress attributed the main factor of this reaction to accumulation of reactive oxygen species (ROS) in increasing concentrations of heavy metals and oxidative stress. Seeds work differently; some heavy metals prevent germination and growth by inhibiting hydrolysis of starch endosperm; some others damage to the embryo in the seed (Mishra and Choudhuri, 1997).

Purcell *et al* (2002) observed aluminium inhibition of water uptake, growth and grain yield in soybeans. In a study of two wheat and two corn cultivars, Cassierra and colleagues (2009) in Colombia reported that highest penetration and stress of Al^{3+} occurs when germination takes place; that is, germination decreased by 35% and 31% for corn and 15% and 18% for wheat. It was found that increased concentration of Al^{3+} in seed and gemma increases Callose deposition (Zheng *et al*. 2014). Callose may block cell-by-cell transfer by blocking plasmodesmata (Panda *et al*., 2009). Considering the possible mechanisms in the inhibitory effects of heavy metals such as aluminium on the germination process, reduced germination of kidney bean can be explained under aluminium concentrations.

Many signs of Al toxicity such as chlorosis and necrosis of leaves (Vitorello *et al*. 2005), reduced or stopped growth of roots and shoots are caused by disorder or imbalance in the mineral nutrition of plants (Ribeiro *et al*. 2013). The present results indicate a decrease in K, P, Ca, Mg, Fe contents under Al treatment in roots and shoots. Nevertheless, the changes are different for N content. Under 30mM aluminium nitrate, N content of the roots increased; under 40 and 50mM treatments, N content slightly decreased. N content of shoots significantly decreased under all aluminium treatments. This reduction was more evident in susceptible cultivars. Mihailovic *et al* (2008) showed that Al inhibits the uptake of Ca^{2+}, Mg^{2+}, N by the root, which is consistent with our results.

Long exposure to Al stops root growth which is totally due to the lack of nutrients, particularly P, K, Ca and Mg (Haung & Vitarello, 1990). Al intake inhibits Mg, Ca, K, N, Fe, P and Zn in sorghum and K, Mg, Ca, P, Fe, Cu and Zn in the corn (Baligar and Fageria, 1997) and inhibits Cu, Fe, Mn and Zn intake in the cocoa plant varieties (Baligar and Fageria, 2005). Al function reduces N, P, K, Ca and Mg content in different parts of the plant (Ribeiro *et al*. 2013). K concentration decreases in roots and shoots of different lines, under aluminium treatment; it is more evident in aluminium sensitive line (Giannakoula *et al*. 2008). Reduced nutrient intake is a result of metabolic disorders caused by Al which influences the structure and activities of the enzyme in cell membranes (Seregin an Ivanov, 2001). The membrane permeability is influenced and thus the balance of ions may change in the cytoplasm.

According to Drazic *et al* (2004), one of the reasons for the change in potassium content is imbalanced aquatic plants exposed to heavy metals. Heavy metals directly or indirectly cause membrane lipid peroxidation. This process leads to the collapse of plasma membrane and thus K^+ leakage (Milon *et al*., 2003; Baccouch *et al*., 2001).

The first signs of aluminium toxicity in plants are inhibited calcium and magnesium intake, reduced flow of K, formation of Callose and excretion of organic acids (Brunner and Sperisen, 2003 Rengel, 1996). Inhibition of magnesium uptake is the result of competition between adsorption sites on the roots (Kochian, 1995). Effect of Al on magnesium content is partly due to the ability of Al to communicate with magnesium-regulated sites. These two elements have similar ionic radii; in biological systems, displacement of magnesium by aluminium is possible (Martin, 1994). According to Ghnava *et al* (2005), another limitation of Ca^{2+} transfer to leaves can be due

to the closure of calcium in the form of oxalate crystals in woody plants exposed to heavy metals.

Providing similar results, Jemo *et al* (2007) believe that Al limits P intake by root system.

Reduced amount of P in rice (Macedo *et al*., 2009 EC) and cocoa (Ribeiro *et al*., 2013) have been reported under aluminum stress. According to above, Al interrupts or imbalances mineral nutrition of different kidney bean cultivars; its effects are more evident in the Al-sensitive cultivar (Naz).

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