

Aluminum-toxicity responses in *Phaseolus vulgaris* L. genotypes

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Abstract: Al-toxicity in acid soils is one of the major problems in crop production worldwide. The acidification of soils is also a serious problem in Hungary. The liming materials are expensive for most small farmers so to reduce farmer's dependence on lime we need to find significant differences in Al-toxicity resistance in plants. Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for people, and it is the second source of protein. Therefore, the examination of common bean in the case of Al-toxicity has great importance. Five genotypes were investigated in this experiment (Apache, Aztec, Bill Z, Fargo, Grand Mesa). Five-day-old uniform seedlings were transferred to 4.5 L pots with constantly aerated simplified nutrient solution containing 0.5 mM CaCl_2 , 0.5 mM KCl and 8 μM H_3BO_3 . This solution allows optimum root elongation for short treatment. After 24 h of root growth, the pH of the solution was decreased from 5.5 to 5.0, after 48 h from 5.0 to 4.5 to avoid pH stress. After 3 days, the first 20 mm of the root apex was marked with permanent marker and plants were treated with 0 and 20 μM AlCl_3 . The distances from the root apex were measured after 4 h, 8 h, 24 h, 48 h and 72 h after treatment. Moreover, relative chlorophyll content, dry weight of root and shoot and enzymatic assays (lipid peroxidation, SOD=superoxide dismutase, POX=peroxidase, ROS=reactive oxygen species) were measured. According to the measured parameters of root growth and various enzymatic activities, we found that there is a strong genotype effect regarding the bean plant's response to Al stress. Some bean genotypes were less affected by the Al-toxicity stress than other genotypes.

Keywords: aluminium-toxicity, bean, oxidative stress, root growth

Introduction (Times New Roman, 10 pt, bold, left)

Aluminum (Al) toxicity is a major factor limiting plant growth especially on acid soils. Common bean (*Phaseolus vulgaris* L.) growing area of about 40 % of Latin America and 30-50 % of central, eastern and southern Africa are affected by Al toxicity resulting in yield reduction from 30 to 60 % (CIAT, 1992). The bean is not the most important legumes in Hungary, it is grown only around 750-800 ha in 2015 and the yield was approx 1500 tonnes (KSH, 2015) but it has important role in human nutrition because of its high protein and nutrient content.

Common bean needs significant improvement in Al resistance to reduce farmer's dependence on lime and fertilizers (Rao, 2001). Genotypic differences in seed yield of 5000 common bean germplasm accessions and breeding lined have been observed in field screening on Al-toxic soils that were amended with or without lime (Rao et al., 2004). Significant genotypic differences in Al resistance in common bean were also reported based on Al-inhibited root elongation in nutrient solution (Rangel et al., 2005; Manriquen et al., 2006).

The primary effect of Al is an inhibition of root growth (Foy, 1988), an effect that can be seen within hours of treatment (Blamey et al., 2004). The major site of Al perception and response in the root apex (Ryan et al., 1993), and particularly, the distal part of the transition zone (1-2 mm) is the most Al-sensitive apical root zone (Kollmeier et al., 2000).

Al-toxicity such as one of the environmental stresses induces the formation of reactive oxygen species (ROS) in plant cells (Breusegem et al., 2001). Under normal

physiological conditions, cell produce ROS by means of the reduction of molecular oxygen (Hippeli et al., 1999), but under conditions of environmental stress this production is increased.

All cells possess a defensive system, consisting of various enzymes such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and reductase. These enzymes efficiently reduce SOD under normal circumstances, but if complete reduction does not occur, as under conditions of increased production, the result may be a state of oxidative stress leading to the oxidation of biomolecules (e.g. lipids, proteins etc) (Schieber and Chandel, 2014).

The main objective of the study was to prove that there is a strong genotype effect of Al toxicity and sensitivity in five common bean genotypes.

Materials and methods

Seeds of common bean genotypes Apache, Aztec, Bill Z, Fargo and Grand Mesa from Pinto line were germinated between filter paper, in an upright position. Five-day-old uniform seedlings were transferred to 4.5 litre pots with constantly aerated simplified nutrient solution containing 0.5 mM CaCl₂, 0.5 mM KCl and 8 µM H₃BO₃. This solution allows optimum root elongation for three days at least. After 24 h of root growth, the pH of the solution was lowered to 5.0, after 24 h 4.5 and keeps this pH until at the end of the experiment. The experimental design was a completely randomized design with three pots per treatments, each pot contained 4 plants.

Plants were cultured in a growth chamber with controlled environmental conditions of a 16/8-h light/dark regime, 20/15°C day/night temperature and photon flux density of 574 µmol m⁻²s⁻¹ photosynthetic active radiation at the plant level.

Two hours before Al treatment, tap roots were marked 2 cm behind the root tip using permanent marker, which did not affect root growth during the experimental period. Afterwards, the plants were transferred to simplified nutrient solution containing 0 or 20 µM AlCl₃. Root elongation was measured at 4, 8, 24, 48 and 72 h of Al treatment using a 1-mm scale.

The shoots and roots of common bean were dried at 60 °C for 3 days and measured with analytical scale.

The enzymes antioxidants analysed in the roots were superoxide dismutase, peroxidase, lipid peroxidation and the amount of ROS. Activity of superoxide dismutase was determined by the method of Misra and Fridovich (1972). Assay of peroxidase as proposed by Reddy et al. (1995) was adopted for evaluating the activity of peroxidase. For the measurement of lipid peroxidation in root, the TBA test which determines MDA as an end product of lipid peroxidation was used according to Heath and Packer (1968).

To determine the amount of total ROS (reactive oxygen species) 2,7-dichlorofluorescein diacetate was used.

Protein was measured by the method of Bradford (1976). Microsoft Office Excel 2003 and Sigma Plot 12.0 version were used to the statistical analysis.

Results and discussion

The toxic effect of Al is firstly root related. The dry weight of shoot did not changed significantly when plants were treated with 20 µM Al. The dry weight of root decreased

significantly at all examined genotypes (Table 1). The highest decreasing was observed at Apache.

*Table 1. Effect of Al treatment on the shoot and root dry weight of the common bean genotypes (Apache, Aztec, Bill Z, Fargo, Grand Mesa) grown in simplified nutrient solution containing 0.5 mM CaCl₂, 0.5 mM KCl and 8 μM H₃BO₃ without (0 μM) and with 20 μM Al for up to 72 h at 4.5 pH. Significant difference compared to the 0 μM Al treatment: ***p*<0.01, ****p*<0.001*

Genotypes	shoot dry weight (g plant ⁻¹)		root dry weight (g plant ⁻¹)	
	0 μM Al	20 μM Al	0 μM Al	20 μM Al
Apache	0.27± 0.03	0.27± 0.05	0.09± 0.02	0.04± 0.01***
Aztec	0.26± 0.02	0.23± 0.04	0.07± 0.01	0.04± 0.00***
Bill Z	0.23± 0.03	0.24± 0.04	0.08± 0.01	0.06± 0.01**
Fargo	0.27± 0.06	0.30± 0.03	0.09± 0.02	0.06± 0.01***
Grand Mesa	0.24± 0.02	0.25± 0.01	0.08± 0.01	0.05± 0.01**

The effect of Al on root-elongation rate is best shown as Al-induced inhibition of root elongation (Table 2). The genotypes showed highly significant difference in response to Al supply. The genotypes were arbitrarily ranked for Al resistance in four categories, based on the percentage of Al-induced inhibition of root elongation. Accordingly, Aztec genotype was classified as Al-hipersensitive (inhibition > 90 %). Apache and Fargo genotypes were classified as Al-sensitive (inhibition between 50 % and 90 %), Grand Mesa is intermediate (inhibition 30 – 50 %) and Bill Z genotype is Al-resistant (inhibition < 30 %). This classification based on 20 μ M Al treatment for up to 3 days, Rangel et al (2005) with modification.

Reduction of root growth is the most widely recognized symptom of Al toxicity (Foy, 1976). The most frequently genotypic differences in Al resistance entailed measurements of root growth between 24 h and 72 h.

In presence of Al root elongation of both genotypes was several inhibited (30-55 %) 4 h after the beginning of the Al treatment. After 8-h treatment, both genotypes recovered, Aztec and Fargo more than Bill Z and Grand Mesa.

Table 2. Al-induced inhibition of root elongation of five common bean genotypes (Apache, Aztec, Bill Z, Fargo, Grand Mesa) grown in a solution containing 0.5 mM CaCl₂, 0.5 mM KCl and 8 μM H₃BO₃ for 72 h at 20 μM Al, pH 4.5 (n=12)

Genotypes	Al-inhibited root elongation %				
	4h	8h	24h	48h	72h
Apache	54.68	66.06	75.93	83.33	64.38
Aztec	61.54	72.73	78.92	86.68	90.32
Bill Z	30.11	32.74	26.63	18.38	26.51
Fargo	39.76	70.07	78.13	76.65	80.59
Grand Mesa	54.24	49.64	32.18	31.15	33.71

Cumming et al. (1992) proposed that Al resistance is an inducible trait in common bean requiring a period of stress before a resistance mechanism is switched on. In fact, these authors observed an initial decline in the root elongation of an Al-resistant cultivar followed by a substantial increase after 24 h of Al exposure, while the Al-sensitive cultivar showed a steady decline in the elongation rate over the experimental time.

Resistance to Al might be achieved by chelation or detoxification of Al by organic acids, either within the plant (Al tolerant) or in the rhizosphere by root exudation (Al exclusion) (Foy, 1988).

It is proved that exposure to Al could affect production of reactive oxygen species (ROS) in plants because Al stress causes peroxidation of lipids in the plasma membrane, the effect that could be due to ROS and Al induces the expression of several genes encoding antioxidative enzymes such as glutathione S-transferase, peroxidase and superoxide dismutase (SOD). Metals, including Al, are known to act as catalysts in ROS production and to induce oxidative damage in plants (Yamamoto et al., 2002).

The activity of SOD is increased in all genotype, the increasing was significant at Fargo and Grand Mesa. There is no connection between SOD and POX activity effected by Al stress. The POX activity significantly increased in Apache, Aztec and Fargo common bean genotypes (Table 2).

Table 3. Effect of Al on enzymatic activity (SOD, POX, LP) in the roots of five common bean genotypes (Apache, Aztec, Bill Z, Fargo, Grand Mesa) grown in a solution containing 0.5 mM CaCl₂, 0.5 mM KCl and 8 μ M H₃BO₃ for 72 h at 20 μ M Al, pH 4.5 (n=4) Significant difference compared to the 0 μ M Al treatment: **p*<0.05, ***p*<0.01

Genotypes	SOD (Ug ⁻¹ FW)		POX (Δ A436 g ⁻¹ FW min ⁻¹)		LP (nmol MDA g ⁻¹ FW)	
	0 μ M Al	20 μ M Al	0 μ M Al	20 μ M Al	0 μ M Al	20 μ M Al
Apache	0.15 \pm 0.02	0.18 \pm 0.02	2.58 \pm 0.58	3.52 \pm 0.37*	5.56 \pm 1.31	5.05 \pm 0.83
Aztec	0.14 \pm 0.03	0.22 \pm 0.04	2.67 \pm 0.21	4.13 \pm 0.75*	19.90 \pm 4.53	10.48 \pm 5.30
Bill Z	0.12 \pm 0.02	0.16 \pm 0.02	2.60 \pm 0.25	2.65 \pm 0.29	7.74 \pm 3.42	10.76 \pm 3.81
Fargo	0.16 \pm 0.01	0.29 \pm 0.07*	2.99 \pm 0.18	4.65 \pm 0.92*	28.49 \pm 9.21	28.41 \pm 5.65
Grand Mesa	0.10 \pm 0.02	0.16 \pm 0.01**	0.79 \pm 0.12*	0.89 \pm 0.01	11.42 \pm 1.44	37.41 \pm 13.74*

(SOD = superoxide dismutase, ROX = peroxidase, LP = lipid peroxidation, FW = fresh weight, MDA = malondialdehyde)

Although the thiobarbituric acid (TBA) assay is the most extensively used test for the measurement of lipid peroxidation in cell membranes and isolated lipids (Girotti et al. 1985) limitations of the test have been documented (Halliwell and Gutteridge 1990).

Lipid peroxidation is less sensitive to Al than the inhibition of root elongation. Therefore, the close relationship between root elongation rate and lipid peroxidation, independent of the factor responsible for growth inhibition suggests that lipid peroxidation is the consequence rather than the primary cause of Al injury to plant roots (Cakmak and Horst, 1991).

The amount of malondialdehyde decreased in Aztec when plants were treated with 20 μ M Al. This value was around the control in Apache and Fargo. The amount of malondialdehyde significantly increased at 20 μ M Al compared to 0 μ M Al treatment (Table 3).

According to Souza et al. (2002) a short-term Al exposure has an effect on the protein content and expression in maize root. Their results are demonstrated that the total protein content along the root apex was not affected by Al in the Al-tolerant line, but decreased in the sensitive line. To the contrary, we observed different effect of Al treatment on protein content in common bean root. The amount of protein increased when plant were treated with Al, significant increment was observed in all genotypes. The amount of

reactive oxygen species was calculated based on protein content (Table 4). The amount of ROS increased in Apache, Aztec, Bill Z and Grand Mesa genotypes.

*Table 4. Effect of Al toxicity on protein content and ROS in the roots of five common bean genotypes (Apache, Aztec, Bill Z, Fargo, Grand Mesa) grown in a solution containing 0.5 mM CaCl₂, 0.5 mM KCl and 8 μ M H₃BO₃ for 72 h at 20 μ M Al, pH 4.5 (n=5) Significant difference compared to the 0 μ M Al treatment: * p <0.05, ** p <0.01, *** p <0.001*

Genotypes	protein (mg protein/FW)		ROS (RFU μ g protein)	
	0 μ M Al	20 μ M Al	0 μ M Al	20 μ M Al
Apache	6.36 \pm 0.23	7.67 \pm 0.35***	77.43	102.66
Aztec	10.02 \pm 0.88	14.58 \pm 2.07**	4.03	43.59
Bill Z	7.19 \pm 0.30	11.02 \pm 0.46**	36.40	56.39
Fargo	8.57 \pm 0.42	12.00 \pm 2.45*	59.81	38.51
Grand Mesa	8.71 \pm 0.43	9.87 \pm 0.38**	67.77	71.45

(ROS = reactive oxygen species, RFU = relative fluorescence unit)

Conclusions

Al³⁺ solubilized in acidic soil is extremely toxic in terms of root elongation, and is believed to be the primary factor inhibiting plant growth. Therefore, intensive research has been conducted in order to ascertain the mechanisms inherent to the Al toxicity and tolerance, on scales from the global to the molecular. Many of the biological activities of the plant are altered via the Al toxicity. So through selection and breeding process strategies, it is possible to develop Al tolerant plant.

According to the measured parameters of root growth and various enzymatic activities, we found that there is a strong genotype effect regarding the bean plant's response to Al stress. Some bean genotypes were less affected by the Al-toxicity stress than other genotypes. At this point, we have not determined the critical molecular mechanisms that explain these genotypic differences. This will require some targeted molecular biology examinations.

Over the past decades many researches has been done for significant progress towards the goal of developing crops better suited for cultivation with Al toxicity in acid soil.

With further identification of molecular markers linked with Al-tolerance gene it is possible to develop better Al tolerant crop.

These measures in the field of research can be able to solve the problem of food scarcity due to abiotic stress and thus give food security to the malnourished population in the developing third world countries.

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