Effect of aluminum and bacteria fertilizer treatment on the *Vigna radiata* root growth and photosynthetic activity on early growth stage

Dávid KACZUR[1] – Csaba BOJTOR[1] – Gerda HANKOVSZKY[1] – Szilvia VERES[2] – Brigitta TÓTH[1]

1: Institute of Nutrition, University of Debrecen, Egyetem Square 1, 4032 Debrecen, Hungary; E-mail: btoth@agr.unideb.hu

2: Department of Agricultural Botany, Crop Physiology and Biotechnology, Institute of Crop Sciences, Faculty of the Agricultural and Food Sciences and Environmental Management, University of Debrecen, 4002 Debrecen, P.O. Box 400, Hungary

Abstract: Knowing how aluminum in acidic condition may limit the growth of mung bean (*Vigna radiate)* is important because bean is one of the most important food legumes for people. Aluminum (Al) toxicity is one of the major constraints of crop production in acid soils. Five-day-old uniform seedlings were transferred to 1.7 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. The first 20 mm of the root apex was marked with permanent marker. Subsequently, plants were transferred to simplified nutrient solution containing 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. To compensate the Al-stress, bacterium fertilizer was added to the nutrient solution 1) before the Al-treatment and 2) at the same time with Al. The elongation rate was calculated. The value of potential photochemical activity was measured by the chlorophyll fluorescence method with PAM-2001 (WALZ GmbH, Germany). The dry weight of shoot and root were measured with thermo gravimetric method. According the root length elongation, the early time bacterium treatment has positive effect on root length 4 and 8 hrs after Al-treatment, but could not compensate the Al-stress for a longer time. We did not find significant difference in relative chlorophyll content (SPAD) or in the photosynthetic activity (Fv/Fm) among treatment. There results confirm the literature data, the toxic effect of Al is firstly root related.

Keywords: aluminium-toxicity, mung bean, photosynthetic activity, root growth

Introduction

Aluminum is the most abundant metal element in the earth's crust and bound aluminum will dissolve in acidic soils. In case of neutral pH, aluminum is not soluble and can be found as aluminum oxide and silicate, while phytotoxic form of aluminum will be spread in soil solution and affect root and plant growth when pH decreases. The first effect of aluminum toxicity is its negative effect on root growth (Arsintescu et al., 2001). When soluble $A1^{3+}$ content reaches 10-20 mg/kg or more, it produces severe toxic effects on plants (Kochian et al., 2004). Micromolar concentrations of Al can inhibit root elongation and consequently influence water and nutrient uptake, resulting in poor plant growth (Delhaize and Ryan, 1995). Al not only affects plant roots, but photosynthetic behavior also is subjected to Al toxicity. For Al-sensitive plants, present of Al may reduce stomatal conductance (Mukhopadyay et al, 2012) and chlorophyll content (Mihailovic et al., 2008), change chlorophyll a/b ratio (Ying and Liu, 2005), photosynthetic rate usually declines (Lazarevic et al., 2014).All heavy metals significantly lowered the leaf contents of the photosynthetic pigments (Aldoobie and Beltagi, 2013). The decrease of pigment levels, as a result of heavy metals, has been found in many plants (Van et al., 1990). According to results from broad bean, aluminum toxicity causes the reduction of root respiration and photosynthesis (Arsintescu et al., 2001). The content of photosynthetic pigment is decreased, because of the destruction of chloroplast structure, photosynthetic system and chlorophylls photo oxidation, the destruction of the pre-material of chlorophyll synthesis and the inhibition of chlorophyll biosynthesis.

During different stresses, existing chlorophyll in chloroplast is broken down and thylakoid structure disappeared (Rout et al., 2001). Many plants have different mechanisms in plants have been categorized as: 1. external via the exudation of organic acids from the radical apexes and subsequent chelation of the Al in the rhizosphere (Ma et al., 2001), 2. internal or Al-tolerant as Al chelation is produced inside the cell and then later stored and compartmentalized in cell organelles like the vacuole (Delhaize and Ryan, 1995).

Some plant species have the ability to detoxify Al in the rhizosphere by exuding organic acids. Organic acids play a role in external and internal neutralization of Al. Generally, organic acids secreted by roots are malate, citrate and oxalate. The amount of organic acids released varies between plant species, and the detoxification mechanisms in an internal tolerance (Ma et al., 2001).

It is proven that plant growth promoting bacteria (PGPB) is associated with rhizosphere (Bashan, 1998) and they have the potential to produce a large amount of organic acids (Carson et al., 1991;), which resulted in P binding by chelation, and may also be a possible mechanism for reducing Al toxicity of roots. These PGPB would enhance the growth of plant grown on soils with high Al content (Tóth et al., 2013; Panhwar et al., 2015).

Materials and methods

Seeds of mung bean were germinated between filter paper, in an upright position. Five-day-old uniform seedlings were transferred to 1.7 litre 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 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, 25/20°C day/night temperature and photon flux density of 300 μmol $m^{-2}s^{-1}$ 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.

The experimental design was a completely randomized design with three pots per treatments, each pot contained 4 plants.Afterwards, the plants were transferred to simplified nutrient solution containing 0 or 20 μM $AlCl_3$. Root elongation was measured at 4, 8, 24, 48 and 72 h of Al treatment using a 1-mm scale.

The applied bacteria fertilizer contains *Azotobacter chroococcum* and *Bacillus megaterium*. The dose of bacteria fertilizer was 2 ml dm^{-3}. The bacteria fertilizer was added to the nutrient solution from the first day of the experiment (Phy+20) and in the same time of Al-treatment on 3rd of the experiment (20+Phy). The nutrient solution was completed with $AlCl_3$ when Al-stress was examined (20μM Al). The experiment was finished on the 6th day of experiment.The shoots and roots of mung bean were dried at 65 ° C for 3 days and measured with analytical scale.

The relative chlorophyll content (SPAD-Units) was measured with SPAD-502 relative

 DOI: 10.18380/SZIE.COLUM.2017.4.1.suppl

chlorophyll meter (MINOLTA, Japan). The parameters of in vivo chlorophyll fluorescence were detected with a PAM-2001 (Walz, Germany) modulated light fluorometer as described by Schreiber et al. (1986). Samples were dark-adapted for 20 minutes. After dark adaptation, the initial fluorescence (F_o) was excited by weak light. The maximal fluorescence (F_m) was induced by white saturating flash. Ratio of F_v/F_m was used for characteristics potential photochemical efficiency of PSII. Microsoft Office Excel 2003 and Sigma Plot 12.0 version were used to the statistical analysis.

Results and discussion

Tóth et al. (2013) established that the dry weight of cucumber shoots and roots were lower in line with the increasing Al concentration. According their results bacteria fertilizer treatment can compensate the Al toxicity effect, thus producing higher dry weight result.

Root growth is the combination of cell division and elongation. Decrease of mitotic activity was reported as a consequence of Al exposure in root tips of several species as wheat (Li et al., 2008) and bean (Marienfeld et al., 2000). Some authors defended that inhibition of cell elongation was the primary mechanism leading to root growth inhibition (Zheng et al., 2005).

The root length was measured after 4h, 8h, 24h, 48 h and 72 h after Al-treatment. The root length decreased when 20 µM Al was applied. The root length was shorter with 50% after 4h, 55 % after 8h, and 89 % after 24 h of Al-treatment. The length of root did not changed after 24h, Al treatment had a very toxic effect on root growth. When bacteria fertilizer was added to the nutrient solution (Phy+20), the root growth was more intensive compared to 20+Phy treatment, when bacteria fertilizer was added at the same time with Al after 4 and 8h. The positive effect of bacteria treatment on root growth could not be detected (results are not shown). The dry weight of shoots and roots of mung bean can be seen in Figure 1 and Figure 2.

There is no significant difference in shoot dry weight. The lowest root dry weight was measured at 20 µM Al treatment compared to control.

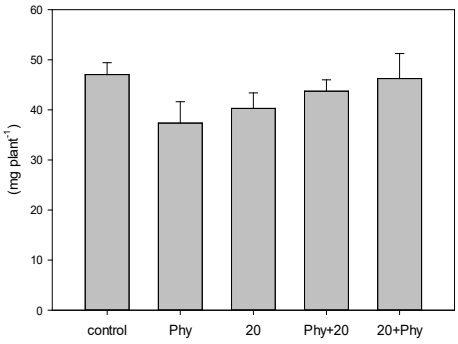

Figure 1: Effect of 20 µM Al and bacteria fertilizers on the dry weight of mung bean shoot (mg plant^{-1}) (n=60± S.D.)

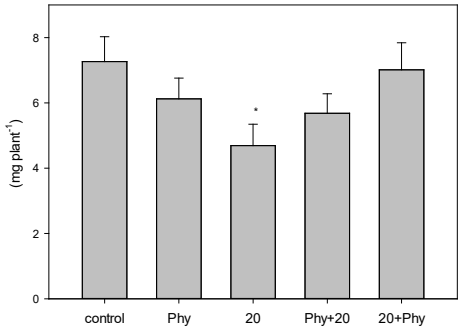

*Figure 2.: Effect of 20 µM Al and bacteria fertilizers on the dry weight of mung bean root (mg plant^{-1}) Significant difference compared to the control: *p<0.05*

The Soil-Plant Analyses Development (SPAD) unit of Minolta Camera Co. has developed the SPAD-502 chlorophyll meter (Minolta Camera Co., Japan), a hand-held, self-calibrating, convenient, and non-destructive lightweight device used to calculate the amount of chlorophyll present in plant leaves (Minolta, 1989; Yadava, 1985). This meter records optical density measurements at two wavelengths, converts them into digital signals, and then into a SPAD value (Minolta, 1989). Strong relationships between leaf N concentration and SPAD values were found e.g. in apple (Neilsen et al., 1995), in corn (Chapman and Barreto, 1997) and in faba bean (Abdelhamidg et al., 2003).

The relative chlorophyll content in the first foliar leaf of mung bean was significantly higher when bacteria fertilizer was added at the same the time with Al-treatment (20+Phy) compared to control value. 20 µM Al (20) treatment did not cause significant changes in SPAD-Units (Figure 3).

According to Veres et al. (2006) the chlorophyll content of the plants was higher when bacteria fertilizer was applied from the first day of the experiment than from the fourth day of the experiment.

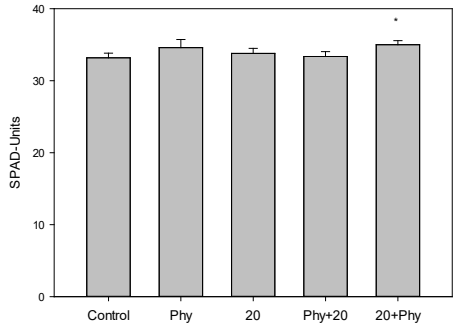

*Figure 3: Effect of 20 µM Al and bacteria fertilizers on the relative chlorophyll content (SPAD-Units) in the first foliar leaf of mung bean (n=60± S.E.) Significant difference compared to the control: *p<0.05 Treatments: control: 0 µM Al, Phy: bacteria fertilizer, 20: µM Al, Phy+20: bacteria treatment from the first day of the experiment, 20+Phy: bacteria treatment at the same time with Al treatment*

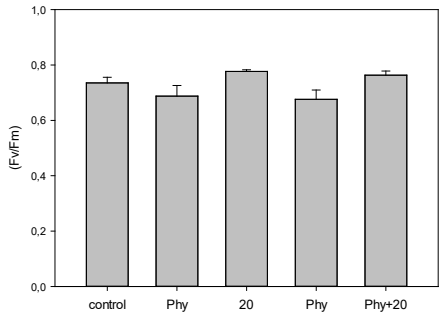

*Figure 4: Effect of 20 µM Al and bacteria fertilizers on the relative chlorophyll content (SPAD-Units) in the first foliar leaf of mung bean (n=60± S.E.) Significant difference compared to the control: *p<0.05 Treatments: control: 0 µM Al, Phy: bacteria fertilizer, 20: µM Al, Phy+20: bacteria treatment from the first day of the experiment, 20+Phy: bacteria treatment at the same time with Al treatment*

 DOI: 10.18380/SZIE.COLUM.2017.4.1.suppl

An Fv/Fm value in the range of 0.79 to 0.84 is the approximate optimal value for many plant species, with lowered values indicating plant stress (Maxwell and Johnson, 2000). The Fv/Fm value is lower than the average value, it is between 0.67-0.77. The lowest Fv/Fm value (0.67) was measured at Phy+20 treatments, the highest at 20 µM Al treatment. We assume that Al has not had any negative effect on chlorophyll content or on photosynthetic activity in short-term Al stress experiment. To investigate the decline of photosynthetic activity longer-time Al exposition needed.

Conclusions

Although some crops (e.g. tea (Matsumoto et al., 1976) are considered tolerant to high levels of exchangeable Al, for most crops it is a serious constraint. Species and genotypes within species greatly differ in their tolerance to Al. For most crops, fertilization and attempts of soil correction (e.g., liming) may not be enough per se to reduce Al toxicity (e.g., as the soil reaction remains strongly acid), and in most target countries these strategies may also be jeopardized by economical constrains. Therefore, it is imperative to fully understand the mechanisms that are used by the Al-tolerant species to cope Al toxicity, as well which genotypes, within the most resistant/tolerant cereal species, are more suitable to grow in acidic soils in order to increase world cereal production. Furthermore, the development of new cultivars (or the reinvestment in ancient genotypes from Al rich regions) with increased Al-tolerance is fundamental and economic solution to increase world food production.

References

Abdelhamidg M., Horiuchi T., Oba S. (2003): Evaluation of the SPAD value in faba bean (Vicia faba L.) leaves in relation to different fertilizer applications. Plant Production Science 6: 3, 185-189.

Aldoobie N. F., Beltagi M. S. (2013): Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris* L.) plants to heavy metal stress. African Journal of Biotechnology 12: 29, 4614-4622.

Arsintescu A., Neumann G., Petcu E., Stanciu D. (2001): Aspects of aluminum toxicity in sunflower. I. Aluminum stress induced in nutrient solutions. Romanian Agriculture Research 15: 43-49.

Bashan Y. (1998): Inoculants of plants growth-promoting bacteria for use on agriculture. Biotechnology Advances 729-770. http://dx.doi.org/10.1016/S0734-9750(98)00003-2

Carson K. C., Holliday S., Glenn A. R., Dilworth M. J. (1991): Siderophore and organic acid production in root nodule bacteria. Arch Microbiol 157:3, 264-271. DOI: 10.1007/BF00245160

Chapman S. C., Barreto H. J. (1997): Copper enzymes in isolated chloroplasts. Polyphenoloxides in Beta vulgaris. Plant Physiol 24: 241-249.

Delhaize E., Ryan P. R. (1995): Aluminum toxicity and tolerance in plants. Plant Physiol 107: 315-321.

Ma J. F., Ryan P. R., Delhaize E. (2001): Aluminum tolerance in plants and the complexing role of organic acids. Trends in Plant Science, 6: 273-278.

Marienfeld S., Schmohl N., Klein M., Schöder W. H., Kuhn A. J., Horst W. J. (2000): Localization of aluminum in root tips of Zea mays and *Vicia faba*. Journal of Plant Physiology 156: 5-6. 666-671. http://dx.doi.org/10.1016/S0176-1617(00)80229-1

Matsumoto H., Hirasawa E., Morimura S., Takahashi E. (1976): Localization of aluminum in the tea leaves. Plant Cell Physiol 17: 3, 627-631. DOI:https://doi.org/10.1093/oxfordjournals.pcp.a075318

Maxwell K., Johnson G. N. (2000): Chlorophyll fluorescence – a practical guide. Journal of Exp Bot 51:345, 659-668.

Mihailovic N., Dravic G., Vucinic Z. (2008): Effects of aluminum on photosynthetic performance in Al-sensitive and Al-tolerant maize inbred lines. Photosynthetica 46: 476-480.

Minolta (1989): SPAD-502 owner's manual. Industrial Meter Div. Minolta Corp. Ramsey N. J..

Mukhopadyay M., Bantawa P., Das A., Sarkar B., Bera B., Ghosh P., Mondal T. K. (2012): Changes of growth, photosynthesis and alteration of leaf antioxidative defence system of tea seedlings under aluminum stress. Biometals 25: 1141-1154. doi:10.1007/s10534-012-9576-0

Neilsen D., Hogue E. J., Neilsen G. H., Parchomchuck P. (1995): Using SPAD-502 values to assess the nitrogen status of apple trees. Hort Science 30: 508-512.

Kochian L. V., Hoekenge O. A., Pineros M. A. (2004): How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Ann Rev Plant Biol 55: 459-493.

Lazarevic B., Horvat T., Poljak M. (2014): Effect of acid aluminous soil on photosynthetic parameters of potato (Solanum tuberosum L.) Potato Res 57: 33-46.

Li Y., Yang G.., Luo L., Ke T., Zhang J., Li K. (2008): Aluminum sensitivity and tolerance in model and elite wheat varieties. Cereal Research Communication 36:2. 257-267. http://dx.doi.org/10.1556/CRC.36.2008.2.6

Pahnwar Q. A., Naher U. A., Radziah O, Shamshuddin J., Razi I. M. (2015): Eliminating aluminum toxicity in an acid sulfate soil for rice cultivation using plant growth promoting bacteria. Molecules 20: 3628-3646. Doi:10.33909/molecules20033628

Rout G., Samantaray S., Das P. (2001): Aluminum toxicity in plants: a review. Agricultural 21: 3-21.

Schreiber U., Schluwa U., Bilger W. (1986): Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynthetic Research 10: 51-62.

Tóth B., Lévai L., Kovács B., Varga M., Veres Sz. (2013): Compensation effect of bacterium containing fertilizer on the growth of *Cucumis sativus* L. under Al-stress conditions. Acta Biologica Hungarica 64: 1, 60-70.

Van A. F. V., Clijstres H. (1990): Effect of metal on enzymes activity in plant. P. C. E. 13: 195-206.

Yadava U. L. (1985): A rapid and non-destructive method to determine chlorophyll in intact leaves. Hort Science 21: 1449-1450.

Ying X. F., Liu P. (2005): Effect of aluminum stress on photosynthetic characters of soybean. Chinese Journal of Applied Ecology 16: 166-170.

Zheng S. J., Yang J. L. (2005): Target sites of aluminum toxicity. Biologia Plantarum 49: 3, 321-338. DOI: 10.1007/s10535-005-0001-1

 DOI: 10.18380/SZIE.COLUM.2017.4.1.suppl