Journal of Engineering and Science Research 1 (2): 43-46, 2017
e-ISSN 2289-7127

DOI: 10.26666/rmp.jesr.2017.2.8

Effect of Aluminium on the Growth of Nodal Explants of *Lobelia chinensis*

Thong Weng Hing & Pang Wei Wei
INTI International University, 71800 Nilai, Negeri Sembilan, Malaysia
Metta Space Continuous Learning Center

Abstract: Being the most abundant element on earth, aluminium has become one of the major restricting factors that affect the growth and development of plants. The first target of aluminium toxicity is the root apex which would in turn affect the overall growth of the plants. In this study, the effect of various concentrations of aluminium (Al) on the growth of *Lobelia chinensis* was determined. Nodal explants of *L. chinensis* were cultured on MS medium containing various concentrations of aluminium chloride (AlCl3) (0, 1.0, 2.5, 5.0, 7.5, 10.0 and 20 µM) with 1250.0 µM ion phosphate and pH was adjusted to 4.6. It was observed that both the growth of shoot and root decreased with the increasing concentrations of Al.

Key words: *aluminium, nodal explants, root, shoot, Lobelia chinensis*

INTRODUCTION

Aluminium (Al) is the most abundant metal in the earth's crust [1], comprising about 7% of its mass [2, 3]. Hence, the potential for soils to be Al toxic is considerable. In acidic soil, Al could exist as Al^{3+}, $Al(OH)^{+}$ and $Al(OH)^{2+}$ which are deteriorate to plants [1, 4]. Many plant species are sensitive to Al even at micromolar concentration, thus, Al toxicity is a major factor restricting plant production on acidic soils [1, 4, 5, 6, 7], currently destroying more than 40% of agricultural land around the world [8, 9, 10].

It was reported that Al affects the zone of root apex and eventually the growth of the root [11]. [12] reported that the roots of *in vitro* wheat plants were found to exhibit reduced root growth, accumulating more Al^{3+} and reactive oxygen species. Aluminium accumulation was found chiefly in the root apex (epidermis cell and cortex) [1]. [13] observed that the elongation of roots exposed to Al^{3+} was inhibited within an hour(s). This was due to inhibition of cell elongation and cell division in addition to reduced water and nutrient uptake by the root cells [14, 15, 16]. [17] found that increasing Al^{3+} concentration and exposure time reduced the rate of callus growth and frequency of non-embryogenic cells of *Cynodon dactylon*. It was observed that exposure to 0.8 mM Al^{3+} for 2 weeks resulted in an 88% reduction in the meristematic cell number of *Cynodon dactylon.* Similarly, the fresh weight of *Nicotiana plumbaginifolia* callus tissues was found decreased with the elevated concentrations of $Al_2(SO4)_3$ [18, 19]. Al toxicity was found to depress the cells growth of *Medicago sativa* [20] and the growth of protocorm-like bodies of banana [21] as well as caused the death of the non aluminium-tolerant cell line of *Citrus* species [22]. [23] noticed that there was a significant reduction of the frequency of embryoid induction and root regrowth rate of *Triticum aestivum* on induction media containing aluminium. Al toxicity also affected pollen germination as well as pollen tube elongation [24].

The Al tolerance strategies of plants can be separated into those involved in exclusion of Al from the root apex and mechanisms that allow the plant to tolerate Al within cells [7]. Many Al tolerant plants exclude Al from roots by excretion of organic acids such as citrate, malate or oxalate from the roots that chelate Al [6, 7, 25, 26, 27, 28], excretion of ion H^{+} [17, 29, 30], excretion of phosphate that precipitate Al [31], excretion of mucilage [32, 33] and protein [34] that bind Al. On the other hand, internal mechanisms of Al tolerance include reduction of accumulation of lipid peroxides through a higher proline and carbohydrate content related to osmoregulation and membrane stabilization [35], internal Al detoxification by organic acid anions and enhanced scavenging of free oxygen radicals, [7], protection of root apices by development of root border cells [36], acceleration of senescence of root tips [1], programed cell death under Al stress [1, 37] immobilization of Al at cell wall [17], as well as selective permeability of the plasma membrane as a barrier to movement of Al into the cytosol [38].

Corresponding Author: name, affilation, address, no.phone

RESEARCH OBJECTIVE

The aim of this study was to determine the effect of various concentration of Al on the growth of *Lobelia chinensis.*

MATERIAL AND METHOD

Plant Material

Plants of *L. chinensis* used in the present research were obtained from Air Itam, Pulau Pinang and grown in the glasshouse.

Establishment of Aseptic Explants

Surface sterilization procedures were carried out to obtain aseptic nodal explants of *L. chinensis.* Explants of *L. chinensis* obtained from the glasshouse were directly washed under running tap water for 20 to 30 min in order to remove all the attached dust or small particles, followed by washing several times with detergent. Then the explants were immersed in absolute alcohol for 20 to 30 s under aseptic condition. Lastly, the explants were subsequently surface sterilized with Clorox® 10% with five drops of Tween 20 as a wetting agent for 10 min continuous stirring and rinsed three times with sterile distilled water. The disinfected explants were aseptically excised to 4-6 mm segments and cultured in vials 2.5 cm in diameter containing 10.0 mL of MS (Murashige and Skoog, 1962) medium. All cultures were incubated at 25±2°C under a 16 hours photoperiod provided by cool-white fluorescent lamps and 8 hours of darkness.

Effect of Al on Multiple Shoot Formation

Experiments were carried out to investigate the effect of Al on multiple shoot formation of *L. chinensis.* Nodal explants of *L. chinensis* were cultured on MS medium containing various concentrations of aluminium chloride ($AlCl_3$) (0, 1.0, 2.5, 5.0, 7.5, 10.0 and 20 µM) with 1250.0 µM ion phosphate and pH was adjusted to 4.6. The number of shoot and root regenerated was recorded for a period of eight weeks.

DATA ANALYSIS

All experiments were performed using completely randomized design. Analysis was performed using Statistical Analysis System (SAS) (SAS Institute Inc., Cary, NC, 1985). Statistical differences were tested by Duncan's multiple range tests for the means. Standard deviations were also used to compare mean differences. The significance level was set up at $p<0.05$.

RESEARCH FINDINGS

The results showed that the regeneration rate of nodal segments of *L. chinensis* was 100.0% when cultured in all the nutrient medium. The control treatment achieved the highest mean number of multiple shoot, 3.4 shoot per explant at the end of eighth week. Among different concentrations of $AlCl_3$, 1.0 µM $AlCl_3$ induced the highest mean of multiple shoot followed by 2.5 µM $AlCl_3$. At the end of eighth week, the mean of shoots formed per explant was 2.7 and 2.6 shoots, respectively. The mean number of shoot formed decreased with the elevated concentrations of Al. The lowest number of shoot produced was 1.1 shoots at 20.0 µM $AlCl_3$ (Figure 1 & 2).

In this study, the roots regenerated were short in medium supplemented with $AlCl_3$. The mean number of roots formed decreased with the increasing concentrations of $AlCl_3$. The maximum mean number of roots produced per explant, 8.3 roots, was induced in MS medium without any Al, followed by 4.3 roots produced in MS medium containing 1.0 µM $AlCl_3$. In 2.5 µM $AlCl_3$ supplemental MS medium, the roots number reduced to 3.4 roots and it further decreased to 1.8 roots at 7.5 µM $AlCl_3$. However, no root was formed at 10.0 and 20.0 µM $AlCl_3$ (Figure 1 & 2).

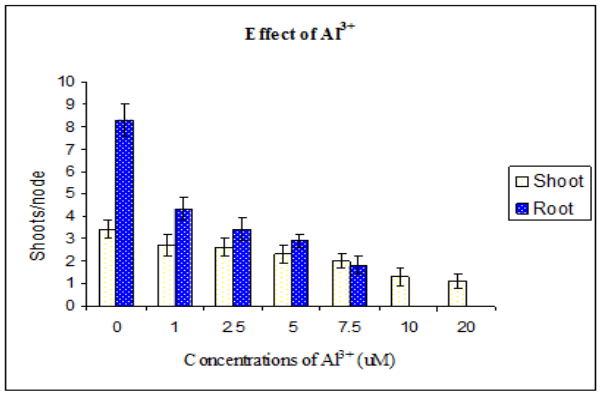

Figure 1. Effect of various concentrations of Al^{3+} on shoot and root proliferation of nodal segments of *L. chinensis* after eight weeks of culture. Means with different letters differ significantly at $P \leq 0.05$ by DMRT. Vertical bars represent standard deviations of the means

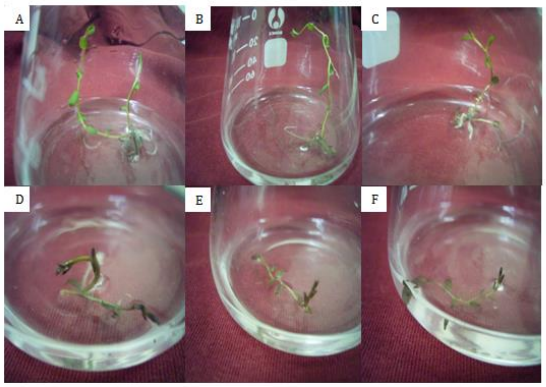

Figure 2. Plantlets of *L. chinensis* produced at different concentrations of $AlCl_3$ after eight weeks of culture. (A) 1.0 µM (B) 2.5 µM (C) 5.0 µM (D) 7.5 µM (E) 10.0 µM (F) 20.0 µM.

DISCUSSIONS

In this study, it was showed that $AlCl_3$ containing medium reduced the production and growth of shoot and root of *L. chinensis.* This was agreed with the findings of [1] on peanut and [12] and [23] on *in vitro* wheat plants. Accumulation of Al occurred at root meristems and as a result inhibition of root elongation was the first sign of Al toxicity [7, 36, 39]. It was reported that inhibition of root elongation of Leucaena leucocephala [28] and peanut [40] achieved at 30 µM and 100 µM Al respectively. The reduced growth of roots might be caused by the Al toxicity which affect the plasma membrane of the root cells at the root apex, inhibit cell division and cell extension [5]. Similarly, it was reported that exposure to Al caused stunting of the primary root and inhibition of lateral root formation which reduced yield and crop quality [15, 41]. It was reported Al induced lower concentration of endogenous nitric oxide which inhibit the elongation of roots [30]. In addition, it was observed that low concentration of Al was detrimental to the growth of cell cultures of *Medicago sativa* [20], tobacco [18, 19] and carrot [42]. Al toxicity could cause the death of the non aluminium-tolerant cell line of *Citrus* species [22].

CONCLUSION

In conclusion, both the growth of shoot and root of *Lobelia chinensis* decreased with the increasing concentrations of Al. The growth of *L. chinensis* was significantly inhibited by the addition of higher concentrations of Al in the culture media. Further research could be carried out to find out the mechanisms involved in the detoxification of Al in this plant.

REFERENCES

[1] Huang, W.J., Oo, T.L., He, H.Y., Wang, A.Q., Zhan, J., Li, C.Z., Wei, S.Q., He, L.F., 2014. Aluminum induces rapidly mitochondria-dependent programmed cell death in Al-sensitive peanut root tips. Bot Stud. 55(1):67. doi: 10.1186/s40529-014-0067-1.

[2] Mistrik, I., Tamas, L., Huttova, J., 2000. Quantitative changes in maize membrane proteins induced by aluminium. Biologia Plantarum 43(1): 85-91.

[3] Sivaguru, M., Fujiwara, T., Samaj, J., Baluska, F., Yang, Z., Osaka, H., Maeda, T., Mori, T., Volkmann, D., Matsumoto, H., 2000. Aluminium-induced (1,3-β-d-glucan) inhibits cell-to-cell trafficking of molecules through plasmodesmata. A new mechanism of aluminum toxicity in plants. Plant Physiology 124(3): 991-1005.

[4] Ryan, P.R., Tyerman, S.D., Sasaki, T., Furuichi, T., Yamamoto, Y., Zhang, W.H., Delhaize, E., 2011. The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. J Exp Bot. 62(1):9-20. doi: 10.1093/jxb/erq272.

[5] Mossor-Pietraszewska, T., 2001. Effect of aluminium on plant growth and metabolism. Acta Biochim Pol. 2001;48(3):673-86.

[6] Deng, W., Luo, K., Li, Z., Yang, Y., Hu, N., Wu, Y., 2009. Overexpression of Citrus junos mitochondrial citrate synthase gene in Nicotiana benthamiana confers aluminum tolerance. Planta. 230(2):355-65. doi: 10.1007/s00425-009-0945-z.

[7] Inostroza-Blancheteau, C., Rengel, Z., Alberdi, M., de la Luz Mora, M., Aquea, F., Arce-Johnson, P., Reyes-Díaz, M., 2012. Molecular and physiological strategies to increase aluminum resistance in plants. Mol Biol Rep. 39(3):2069-79. doi: 10.1007/s11033-011-0954-4.

[8] Taylor, G.J., McDonald-Stephens, J.L., Hunter, D.B., Bertsch, P.M., Elmore, D., Rengel, Z., Reid, R.J., 2000. Direct measurement of aluminum uptake and distribution in single cells of *Chara corallina*. Plant Physiology 123: 987-996.

[9] Kidd, P.S., Proctor, J., 2001. Why plants grow poorly on very acid soils: are ecologists missing the obvious? Journal of Experimental Botany 52: 791–799.

[10] Kochian, L.V., Hoekenga, O.A., Pineros, M.A., 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annual eview of Plant Biology 55: 459–493.

[11]Panda, S.K., Matsumoto, H., 2007. Molecular physiology of aluminum toxicity and tolerance in plants. Bot Rev. 2007;73:326–347. doi: 10.1663/0006-8101

[12] Darkó, É., Ambrus, H., Stefanovits-Bányai, É., Fodor, J., Bakos, F. and Barnabás, B., 2004. Aluminium toxicity, Al tolerance and oxidative stress in an Al-sensitive wheat genotype and in Al-tolerant lines developed by *in vitro* microspore selection. Plant Science 166 (3): 583-591.

[13] Matsumoto, H., 2000. Cell biology of aluminum toxicity and tolerance in higher plants. International Review of Cytology 200: 1–46.

[14] Ciamporova, M., 2002. Morphological and structural responses of plant roots to aluminium at organ, tissue and cellular levels. Biology of Plant 45: 161–171.

[15] Samac, D.A., Tesfaye, M., 2003. Plant improvement for tolerance to aluminum in acid soils–a review. *Plant Cel, Tissue and Organ Culture* 75: 189–207.

[16] Ahn, S.J., Rengel, Z., Matsumoto, H., 2004. Aluminum-induced plasma membrane surface potential and H^+-ATPase activity in near-isogenic wheat lines differing in tolerance to aluminum. New Phytologist 162: 71-79.

[17] Ramgareeb, S., Cooke, J.A. and Watt, M.P., 2004. Responses of meristematic callus cells of two *Cynodon dactylon* genotypes to aluminium. *Journal of Plant Physiology* 161(11): 1245-1258.

[18] Conner, A.J. and Meredith, C.P. 1985. Strategies for the selection and characterization of aluminum-resistant variants from cell cultures of *Nicotiana plumbaginifolia*. Planta 166: 466-473.

[19] Szöke, E., Kiss, A.S., Kursinszki, L., Petri, G., 1992. Inhibition of aluminium toxicity by magnesium and citric acid in tobacco tissue culture. Magnesium Research 5(2): 83-7.

[20] Parrot, W.A., Bouton, J.H., 1990. Aluminum Tolerance in Alfalfa as Expressed in Tissue Culture. Crop Science 30: 387-389.

[21] Matsumoto, K., Yamaguchi, H., 1990. Selection of aluminium-tolerant variants from irradiated protocorm-like bodies in banana. Tropical Agriculture (Trinidad) 67(3): 229-232.

[22] Toan, N.B., Ve, N.B., Pierre, C.D., 2004. Tissue culture approaches for the selection of aluminium-tolerant Citrus species in the Mekong Delta of Vietnam. Journal of Horticultural Science and Biotechnology 79: 911-916.

[23] Karsai, I., Bedö, Z., 1998. Relationship between anther culture response and aluminium tolerance in wheat (*Triticum aestivum* L.). Euphytica 100(1-3): 249-252.

[24] Ma, J.F., Taketa, S., Yang, Z.M., 2000. Aluminum tolerance genes on the short arm of chromosome 3R are linked to organic acid release in triticale. Plant Physiology 122: 687-694.

[25] Ma, J.F., Hiradate, S., Matsumoto, H., 1998. High aluminum resistance in buckwheat. *Plant Physiology*. 117: 753-759.

[26] Barceló, J., Poschenrieder, C., 2002. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. Environmental and Experimental Botany 48(1): 75-92.

[27] Ma, J.F., Furukawa, J., 2003. Recent progress in the research of external Al detoxification in higher plants: a minireview. J Inorg Biochem. 97(1):46-51.

[28] Osawa, H, Kojima, K., 2006. Citrate-release-mediated aluminum resistance is coupled to the inducible expression of mitochondrial citrate synthase gene in Paraserianthes falcataria. Tree Physiol. 26(5):565-74.

[29] Degenhardt, J., Larsen, P.B., Howell, S.H. and Kochian, L., 1998. Aluminum resistance in the Arabidopsis mutant *alr*-104 is caused by an aluminum increase in rhizosphere pH. Plant Physiology 117: 19–27.

[30] He, H., Zhan, J., He, L., Gu, M., 2012. Nitric oxide signaling in aluminum stress in plants. Protoplasma. 249:483–492. doi: 10.1007/s00709-011-0310-5.

[31] Tang, Y, Garvin, D.F., Kochian, L.V., Sorrells, M.E., Carver, B.F., 2002. Physiological genetics of aluminum tolerance in wheat cultivar Atlas 66. Crop Science 42: 1541–1546.

[32] Archambault, D.J., Zhang, G. and Taylor, G.J., 1996. Accumulation of Al in root mucilage of an Al-resistant and an Al-sensitive cultivar of wheat. Plant Physiology 112: 1471–1478.

[33] Li, X.F., Ma, J.F., Hiradate, S., Matsumoto, H., 2000. Mucilage strongly binds aluminum but does not prevent roots from aluminum injury in *Zea mays*. Physiologia Plantarum 108: 152–160.

[34] Basu, U., Good, A.G., Aung, T., Slaski, J., Basu, A., Briggs, K.G. and Taylor, G.J., 1999. A 23-kDa root exudate polypeptide co-segregates with aluminum resistance in *Triticum aestivum*. Physiology of Plant 106: 53–61.

[35] Giannakoula, A., Moustakas, M., Mylona, P., Papadakis, I., Yupsanis, T., 2008. Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. Journal of Plant Physiology 165(4): 385-396.

[36] Cai, M., Zhang, S., Xing, C., Wang, F., Ning, W., Lei, Z., 2011. Developmental characteristics and aluminum resistance of root border cells in rice seedlings. Plant Sci. 180(5):702-8. doi: 10.1016/j.plantsci.2011.01.017.

[37] Zhan, J., He, H.Y., Wang, T.J., Wang, A.Q., Li, C.Z., He, L.F., 2013. Aluminum-induced programmed cell death promoted by AhSAG, a senescence-associated gene in Arachis hypoganea L. Plant Sci. 210:108-17. doi: 10.1016/j.plantsci.2013.05.012.

[38] Archambault, D.J., Zhang, G.C., Taylor, G.J., 1997. Spatial variation in the kinetics of aluminium (Al) uptake in roots of wheat (*Triticum aestivum* L.) exhibiting differential resistance to Al-Evidence for metabolism-dependent exclusion of Al. *Journal of Plant Physiology* 151: 668–674.

[39] Garzón, T., Gunsé, B., Moreno, A.R., Tomos, A.D., Barceló, J., Poschenrieder, C., 2011. Aluminium-induced alteration of ion homeostasis in root tip vacuoles of two maize varieties differing in Al tolerance. Plant Science 180(5):709-715

[40] Huang, W., Yang, X., Yao, S., LwinOo, T., He, H., Wang, A., Li, C., He, L., 2014. Reactive oxygen species burst induced by aluminum stress triggers mitochondria-dependent programmed cell death in peanut root tip cells. Plant Physiol Biochem. 82:76-84. doi: 10.1016/j.plaphy.2014.03.037.

[41] Dubey, R.S., Pessarakali, M., 1995. Physiological mechanisms of nitrogen absorption and assimilation in plants under stressful conditions. In *Handbook of plant and crop physiology*, ed. M. Pessarakali, pp.605-618. USA: Marcel Dekker, Inc.

[42] Ojima, K., Ohira, K., 1983. Characterization of aluminium and magganese tolerant cell lines selected from carrot cell cultures. Plant Cell Physiology 24: 789-797.