Journal of the Korean Society of Grassland and Forage Science 39(3): 153~157, 2019
https://doi.org/10.5333/KGFS.2019.39.3.153
ISSN 2287-5824 (Print)
ISSN 2287-5832 (Online)

Research Article

Salicylic Acid Counteracts Aluminum Stress-induced Growth and Biomass Yield Reduction in Medicago sativa L.

Md. Atikur Rahman, Sang-Hoon Lee, Yowook Song, Hee Jung Ji, Ki-Yong Kim, Gi Jun Choi and Ki-Won Lee*

Grassland & Forages Division, National Institute of Animal Science, Rural Development Administration, Cheonan, 330-801, Republic of Korea

ABSTRACT

Salicylic acid (SA) is an essential plant growth regulator that functions as a signaling molecule in plants. The purpose of this study was to clarify how the exogenous application of SA counteracts aluminum stress-induced growth and biomass yield reduction in alfalfa exposed to aluminum (Al) stress. Two-week-old alfalfa seedlings were exposed to a combination of $AlCl_3$ (0 μM, 50 μM and 100 μM, respectively) and SA (0.1 mM) for 72 hours. We observed, Al stress-induced plant growth inhibition and forage yield reduction are Al stress-dependent manner. A significant reduction of plant height (42.0-52.9%), leaf relative water content (13.0-21.4%), root length (35.4-48.7%), shoot fresh weight (31.2-25.9%), root fresh weight (15.4-23.3%), shoot dry weight (12.7-22.2%), roots dry weight (47.3 -53.5%), were observed in alfalfa. In contrast, SA alleviated the Al-stress and enhanced growth and biomass yield in alfalfa. This study provides useful information concerning the role of SA that counteracts aluminum stress-induced growth and yield reduction in alfalfa.
(**Key words**: Salicylic acid, Aluminum stress, Alfalfa, Growth, Forage yield)

Ⅰ. INTRODUCTION

Aluminum (Al) toxicity is one of the critical limiting factors that inhibit plant growth and productivity globally (Jaiswal et al., 2018). Al is the most abundant metal on earth; it ubiquitously distributed as the third most abundant element in the earth's crust that comprising 7-8% of its mass after oxygen and silicon (Bojórquez-Quintal et al., 2017). Though the specific biological function of Al is still unknown (Singh et al., 2017), but it can be toxic for living organisms (Rahman et al., 2018). There is a potential correlation among the soil acidity, aluminum solubility and its phytotoxicity. When soil pH drops down below at 5.0 Al is solubilized into $[Al(H_2O)_6]^{3+}$, usually referred soluble form of Al as Al^{3+} (Kochian et al., 2015). Al-toxicity not only influences on growth and productivity but also alters molecular alterations in plants (Rahman et al., 2014). The initial symptom of Al toxicity is the inhibition of root elongation, while the toxicity turns into more severe it leads to plasma membrane injury, generation of reactive oxygen species (ROS) and lipid peroxidation in roots (Liu et al., 2017).

Plant growth regulator and/or signaling molecules played pivotal role in physiological and molecular alterations in plants (Lee at al., 2019). Salicylic acid (SA) is a phenolic compound plays pivotal role as signaling molecules that modulate plant growth and development along with enhances abiotic-abiotic stress tolerances (Liu et al., 2017). Alfalfa is world leading forage crop. As a legume forage, alfalfa is capable of fixing atmospheric nitrogen (135-605 kg/ha year) and those have pivotal role in biomass production (Putnam et al., 2001). The quality and yield of forage biomass are important to the farmer in terms of livestock production. Therefore, it is imperative to clarify how Al-stress negatively affects on plant growth along with its biomass yield.

A wide range of molecular and physiological and biochemical studies have been performed in response to salt stress (Rahman et al., 2015), heat-cold (Li et al., 2013; Lee et al., 2017a,b), water-deficit (Rahman et al., 2016) stresses in alfalfa. Subsequently, cold-heat stress tolerant DnaJ-like candidate gene has been characterized from alfalfa (Lee et al., 2018). Despite of these above progresses in alfalfa, it has not been performed SA-mediated Al-stress alleviate strategy in alfalfa, along with negative impact of Al-stress on alfalfa growth and forage yield. Therefore,

*Corresponding author: Ki-Won Lee, National Institute of Animal Science, Cheonan 31000, Korea.
Tel: +82-41-580-6757, E-mail: kiwon@korea.kr

the aim of this study was to determine to clarify how SA counteracts aluminum stress-induced growth and biomass yield reduction in alfalfa.

Ⅱ. MATERIALS AND METHODS

1. Alfalfa growth and treatments

Medicago sativa L. seeds were grown on plastic sieve containing hydroponic system. Total 1-gram seeds was weighted and placed to plastic chamber, and covered by black plastic cover then moved to dark for 48 h. The germination box was uncovered after 48 h and kept it at light condition. After 7 day-old alfalfa seedlings were placed with half strength of Hoagland solution (Phytotech lab). The nutrient solution was changed after every 2 days interval. Alfalfa seedlings were grown for 14 days in a controlled growth chamber at 25°C, with a photoperiod 16-h light and 8-h dark with intensity of 350 μmol m^{-2} s^{-1} irradiance, and humidity was maintained at 60-70%. Two-week-old alfalfa seedlings were exposed to four different levels of aluminum chloride ($AlCl_3.5H_2O$; Sigma) and sodium salicylate ($C_7H_5NaO_3$; Sigma) treatment for 72 h. Note, the experiment consisted of six treatments viz. (a) aluminum (Al) 0 μM or control, (b) Al 50 μM, (c) Al 100 μM, (d) Al 50 μM+ salicylic acid (SA) 0.1 mM, (e) Al 100 μM+ SA 0.1 mM, (f) Al 0 μM+ SA 0.1 mM. After 72 h of treatment, seedlings from different treatments were harvested separately, and data were collected following standard methodologies.

2. Measurement of growth and biomass yield in alfalfa

Alfalfa seedling height was determined after 72 h of treatment. Plant height was determined from the root tip to leaf tip of 10 randomly selected seedlings where mean value was considered as in centimeter (cm) basis. Root length was recorded from the root tip root-shoot transition zoon of same seedling. Fresh weight (FW) and dry weight (DW) of roots and shoots were taken as gram per plant (g/plant) basis, respectively.

3. Determination of leaf relative water content (RWC%) of alfalfa

Five randomly selected trifoliate alfalfa leaves were weighed (FW), and submerged into deionized water in a petri dish for 12 h. Then, the water was removed from the leaves with tissue paper, followed by the leaves were weighed and recorded as turgid weights (TW). The leaves were kept at 80 °C for 48 h for achieving DW. Finally, RWC (%) was calculated using the following formula used previously by Rahman et al (2015):
$RWC\ (\%) = [(FW-DW)/(TW-DW)] \times 100$

4. Statistical analysis

All results related to plant growth and biomass yield were analyzed using the analysis of variance (ANOVA), and Duncan's multiple range test (DMRT) to assess statistically significant difference at $P<0.05$. Results of individual index were represented as mean ±S.E. of at least five independent replications.

Ⅲ. RESULTS AND DISCUSSION

Aluminum (Al) stress significantly influenced on growth and biomass yield in alfalfa (Figs. 1, 2 and 3). In this study, we also found that salicylic acid (SA) counteracts aluminum stress-induced growth and biomass yield reduction in alfalfa. Exposure alfalfa seedlings with Al stresses (Al 50 and100 μM) exhibited significant inhibition of the plant growth. The height was reduced significantly by 42.03% and 52.90% at Al 50 and 100 μM, respectively, compared with the control (Fig. 2A), whereas only 6.52% and 21.01% growth reduction observed while used SA 0.1 mM with those same concentrations (Al 50 and100 μM), respectively. These results indicate that SA counteracts the Al-stress induced growth inhibition in alfalfa. Relative

Fig. 1. Morphological changes of alfalfa plants by aluminum (Al) stress. Photo was taken after 72 h of plant exposure to Al with or without salicylic acid (SA).

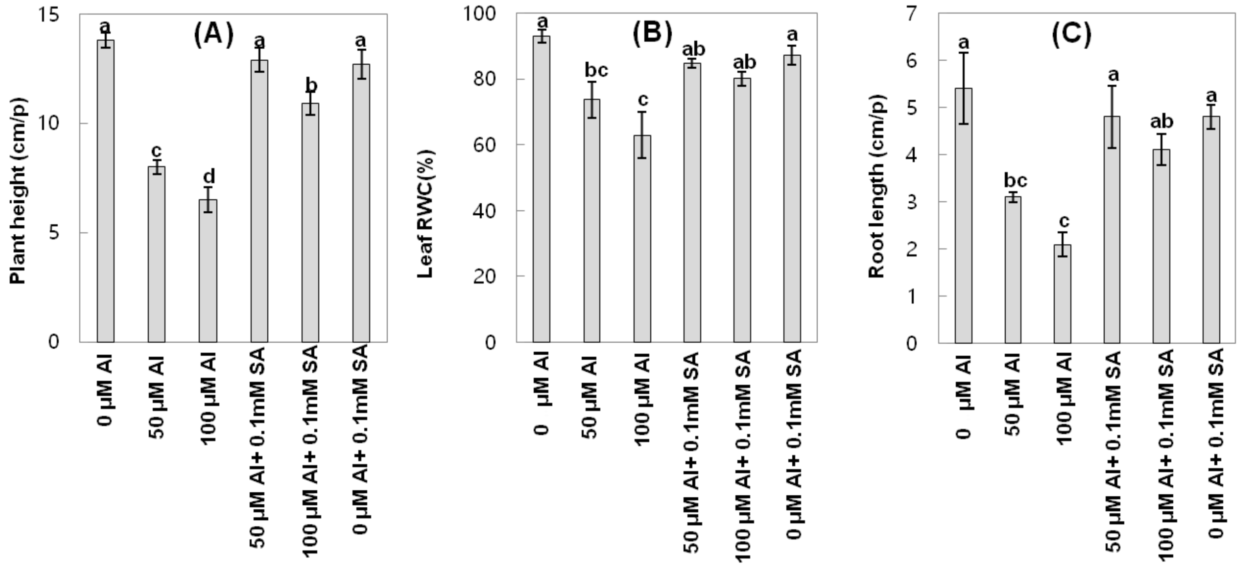

Fig. 2. Salicylic acid (SA) counteracts aluminum (Al) stress-induced growth and biomass yield reduction in alfalfa. Al stress and SA-mediated regulation of plant height (A), leaf relative water content (RWC%) (B), root length (C). Data were represented as mean ±SE of at least five independent replications. The different letters above the bar represent statistically significant ($P<0.05$) differences among the treatment groups.

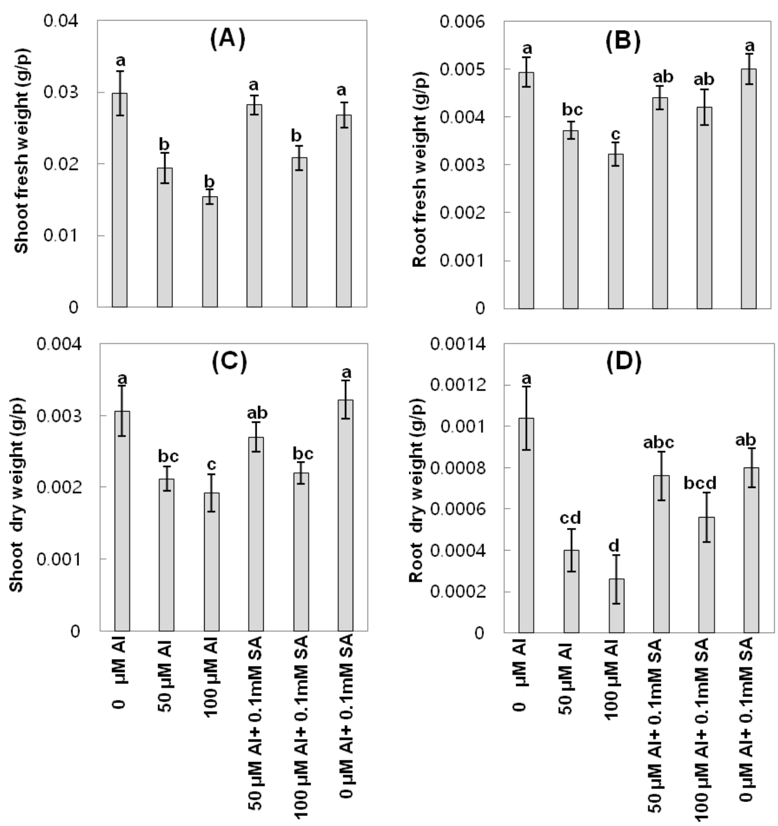

Fig. 3. Salicylic acid (SA) counteracts aluminum (Al) stress-induced growth and biomass yield reduction in alfalfa. Al stress and SA-mediated regulation of shoot fresh weight (A), root fresh weight (B), shoot dry weight (C), and root dry weight (D), Data were represented as mean ±SE of at least five independent replications. The different letters above the bar represent statistically significant ($P<0.05$) differences among the treatment groups.

water content was declined in alfalfa leaves by 13.08% and 21.42% at Al 50 and100 μM, respectively, compared to Al 50 μM +SA 0.1 mM and Al 100 μM +SA 0.1 mM, repectively (Fig. 2B). Root growth inhibited by 35.42% and 48.78% under Al stresses compared to the combined treatments (Al 50 μM +SA 0.1 mM and Al 100 μM +SA 0.1 mM), respectively (Fig. 2C). Al-stress also hampered the biomass yield of alfalfa seedlings. The shoot fresh weight reduced by 31.21% and 25.96% at Al 50 μM and Al 100 μM compared to same concentration with SA 0.1 mM (Fig. 3A). Similarly, root fresh weight reduced 15.45% at Al 50 μM, and 23.33% at Al 100 μM compared to the SA treatments, respectively (Fig. 3B). In case of shoot dry weight, no significant difference has been observed between the Al 50 and100 μM. In contrast, significant differences have been found while added SA 0.1 mM with those same treatments, respectively (Fig. 3C). Root dry weight declined by 47.37% and 53.57% competed to the treatments of SA 0.0 mM with 50 and100 μM Al, respectively (Fig. 3D).

Several reports have been documented that crop growth and productivity hampered by aluminum stress (Yang et al., 2013; Rahman et al., 2018). In the present study, we observed the negative effects of Al stress inhibit the growth and biomass yield in alfalfa. A significant decrease of alfalfa growth in terms of seedling height, RWC (%), root length, fresh weight and dry weight of both shoots and roots were observed, whereas SA alleviates Al-stress in alfalfa associated with growth and biomass yield. Several studies have been reported that SA alleviates Al-stress in plants (Pandey et al., 2013; Liu et al., 2017). These findings provide support to the role of SA in response to Al stress in plants. A significant reduction in root elongation was reported under Al-toxicity due to the high level of H^+ toxicity in several crops legumes (Jaiswal et al., 2018; Rahman et al., 2018). In addition, the reduction of plant growth and biomass yield might be due to the decrease of root cell division and development. However, this study would be helpful for determining negative impact of Al-stress along with role of SA in Al-toxicity alleviation in plants.

Ⅳ. Conclusion

Alfalfa growth and forage yield significantly inhibited by aluminum stress wherein salicylic acid (SA) counteracts aluminum stress-induced growth and biomass yield reduction in alfalfa. This study would be useful concerning the impact of SA for the alleviation of Al-stress induced growth and forage yield inhibition in alfalfa.

Ⅴ. ACKNOWLEDGMENTS

This work was supported by the “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01193504)” and Postdoctoral Fellowship Program of 2019, National Institute of Animal Science, Rural Development Administration, Republic of Korea

Ⅵ. REFERENCES

Bojórquez-Quintal, E., Escalante-Magaña, C., Echevarría-Machado, I. and Martínez-Estévez, M. 2017. Aluminum, a friend or foe of higher plants in acid soils. Frontier of Plant Science. 8:1767.

Jaiswal, S.K., Naamala, J. and Dakora, F.D. 2018. Nature and mechanisms of aluminium toxicity, tolerance and amelioration in symbiotic legumes and rhizobia. Biology and Fertility of Soils. 54:309-318.

Kochian, L.V., Piñeros, M.A., Liu, J. and Magalhaes, J.V. 2015. Plant adaptation to aid soils: The molecular basis for crop aluminum resistance. Annual Review of Plant Biology. 66:571-598.

Lee, K-.W., Rahman, M.A., Lee, S.-H., Kim, Y.-G., Lee, D.-G., Jones, C.S. and Lee, B.-H. 2019. Nitric oxide-induced proteomic analysis in rice leaves. Plant Biotechnology Reports. 13:375-387.

Lee, K.-W., Rahman, M.A., Kim, K.-Y., Choi, G.J., Cha, J.-Y., Cheong, M.S., Shohael, A.M., Jones, C. and Lee, S.-H. 2018. Overexpression of the alfalfa DnaJ-like protein (*MsDJLP*) gene enhances tolerance to chilling and heat stresses in transgenic tobacco plants. Turkish Journal of Biology. 42:12-22.

Lee, K.W., Rahman, M.A., Choi, G.J., Kim, K.Y., Ji, H.C., Hwang, T.Y. and Lee, S.H. 2017a. Expression of small heat shock protein23 enhanced heat stress tolerance in transgenic alfalfa plants. Journal of Animal and Plant Sciences. 27:1238-1244.

Lee, K.W., Rahman, M.A., Choi, G.J., Kim, K.Y., Ji, H.C., Kim, J.G. and Lee, S.H. 2017b Identification of cold and heat-induced differentially expressed genes in alfalfa (*Medicago sativa* L.) leaves. Journal of Animal and Plant Sciences. 27:1231-1237.

Li, W., Wei, Z., Qiao, Z., Wu, Z., Cheng, L., and Wang, Y. 2013. Proteomics analysis of alfalfa response to heat stress. PLoS ONE. 8:e82725.

Liu, N., Song, F., Zhu, X., You, J., Yang, Z., and Li, X. 2017. Salicylic acid alleviates aluminum toxicity in soybean roots through modulation of reactive oxygen species metabolism. Frontiers in Chemistry. 5:96.

Pandey, P., Srivastava, R.K. and Dubey, R.S. 2013. Salicylic acid alleviates aluminum toxicity in rice seedlings better than magnesium and calcium by reducing aluminum uptake, suppressing oxidative damage and increasing antioxidative defense. Ecotoxicology. 22:656-670.

Putnam, D., Russelle, M., Orloff, S., Kuhn, J., Fitzhugh, L., Godfrey, L., Kiess, A. and Long, R. 2001. Alfalfa, wildlife and the environment. In: The Importance and Benefit of Alfalfa in the 21st Century, California Alfalfa and Forage Association. pp. 1-24.

Rahman, M.A., Kim, Y.-G. and Lee, B.-H. 2014. Proteomic response of alfalfa subjected to aluminum (Al) stress at low pH soil. Journal of the Korean Society of Grassland and Forage Science. 34:262-268.

Rahman, M.A., Alam, I., Kim, Y.-G., Ahn, N.-Y., Heo, S.-H., Lee, D.-G., Liu, G. and Lee, B.-H. 2015. Screening for salt-responsive proteins in two contrasting alfalfa cultivars using a comparative proteome approach. Plant Physiology and Biochemistry. 89:112-122.

Rahman, M.A., Kim Y.-G., Alam, I., Liu, G., Lee, H., Lee, J.J. and Lee, B.-H. 2016. Proteome analysis of alfalfa roots in response to water deficit stress. Journal of Integrative Agriculture. 15:1275-1285.

Rahman, M.A., Lee, S.-H., Ji, H.C., Kabir, A.H., Jones, C.S. and Lee, K.-W. 2018. Importance of mineral nutrition for mitigating aluminum toxicity in plants on acidic soils: current status and opportunities. International Journal of Molecular Sciences. 19:3073.

Singh, S., Tripathi, D.K., Singh, S., Sharma, S., Dubey, N.K., Chauhan, D.K., Vaculík, M. 2017. Toxicity of aluminium on various levels of plant cells and organism: A review. Environmental and Experimental Botany. 137:177-193.

Yang, Z.-B., Rao, I.M. and Horst, W.J. 2013. Interaction of aluminium and drought stress on root growth and crop yield on acid soils. Plant and Soil. 372:3-25.

(Received : August 21, 2019 | Revised : September 10, 2019 | Accepted : September 10, 2019)