Journal of the Korean Society of Grassland and Forage Science 41(3): 162~167, 2021
https://doi.org/10.5333/KGFS.2021.41.3.162
ISSN 2287-5824 (Print)
ISSN 2287-5832 (Online)

Research Article

Effect of Silicon Application on Growth Response of Alfalfa Seedlings Grown under Aluminum Stress in Pots

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ABSTRACT

Aluminum (Al) stress in acidic pH is known to decrease the growth and productivity of alfalfa. However, not much is known about how the application of silicon (Si) affects the Al stress response in alfalfa. This study was conducted to evaluate the effect of exogenous application of Si on the growth of alfalfa seedlings exposed to Al stress in pots. Alfalfa seedlings grown in pots for 2 weeks were treated either Al stress (pH 4.0, 0.2 mM Al) or Al stress + Si (1 mM) for 5 days, lengths and biomass of shoot and root, and chlorophyll and carotenoid contents in leaf tissues were analyzed respectively. Al stress treatment inhibited shoot and root growth, and decreased fresh and dry weights, and chlorophyll content in leaves, but increased carotenoid content. In contrast, when alfalfa seedlings treated with Al stress combined with Si, delayed growth caused by Al stress of shoot and root of alfalfa seedlings was restored, dry weight was increased and chlorophyll content of leaf tissue was increased, but carotenoid content was decreased. These results suggest that Si has a function of alleviating Al toxicity in alfalfa, of which it exhibits a mitigating effect by a function that overlaps with some of the intracellular functions of carotenoids.

(**Key words**: Acidic soil, Alfalfa, Aluminum stress, Silicon)

Ⅰ. INTRODUCTION

The total number of cattle raised in Korea has been steadily increasing from 3.088 million in 2015 to 3.637 million as of 2019, and the demand for forage is also increasing accordingly (Kim, 2021). As of 2019, the total demand for forage in Korea is 6.05 million tons, and the domestic production is 4.872 million tons, and the self-sufficiency rate for forage is 80.5%. However, rice straw, accounts for 44% of domestic forage, and imported forage amounts to 1.2 million tons (Kim, 2021). Therefore, in order to secure the competitiveness of livestock farms, it is necessary to increase the self-sufficiency rate of domestic quality forage feed.

Alfalfa (*Medicago sativa* L.) is a perennial legume with a high yield per unit area and has the advantage of being rich in nutrients compared to other grasses. However, acidic soil such as mountainous grasslands in Korea is a limiting factor for the growth and productivity of alfalfa (Choi and Chun, 1994). Acidic soil increases solubility of metallic ions in the soil. Among them, an increase in aluminum (Al) ions leads to a deficiency of essential nutrients in plants and inhibits seed germination, plant growth, and pollination, ultimately seriously reduces crop yields (Krstic et al., 2012).

Al is the third most abundant element in the earth's crust and exists in the form of insoluble Al_2O_3 in the soil. At pH<5.5, it is abundant in soil solution in the form of Al^{3+} ions (Siecińska and Nosalewicz 2016). In acidic soil, Al^{3+} is also known to be toxic even in trace concentrations, inhibiting root cell elongation and division and nutrient absorption (Siecińska and Nosalewicz 2016). It is known that alfalfa show growth inhibition at moderate acidic soil pH values of pH 5 - 5.5 (Yokota and Ojima, 1995). There is also a report that Al stress in alfalfa inhibits the movement and accumulation of auxin in the root tissue, thereby reducing the growth of alfalfa (Wang et al. 2016).

Silicon (Si) is the second most abundant element in the Earth's crust after oxygen and is considered a semi-essential element because of its many benefits to plants exposed to biotic and abiotic stresses (Deshmukh et al., 2017; Rasoolizadeh et al., 2018). Si improves water and nutrient absorption in roots (Eneji et al., 2008), and it has also been reported that Si reduces Al toxicity by binding with apoplastic Al (Wang et al., 2004). It

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is also known that the stabilized plasma membrane by Si helps in transporter or channel activation and has a positive effect on stress tolerance (Sheng and Chen, 2020). Studies on the effects of Si on biotic or abiotic-stress alleviation have also been identified in various crops such as maize (Wang et al., 2004), sorghum (Hodson and Sangster, 1993), rice (Singh et al., 2011) and ryegrass (Pontigo et al., 2017). It has been reported that Si improved alfalfa tolerance to heavy metal stress such as cadmium (Cd) (Kabir et al., 2016). However, studies on the alleviation effect of Si in alfalfa to Al stress have not been reported yet. Therefore, this study was conducted to analyze the effect of Si treatment on the Al stress response of alfalfa in alfalfa seedlings grown in acidic soil.

Ⅱ. MATERIALS AND METHODS

1. Growth of alfalfa

Alfalfa (vernal variety) seeds were sterilized by immersion in 1% sodium hypochlorite (NaOCl) for 15 minutes, washed 5 times with distilled water, and then germinated in the dark for 3 days. The uniformly germinated alfalfa seedlings were transplanted into pots filled with 700 ml of sand:Perlite 1:1 mixture soil. For transplanted pots, 1/4 × Hoagland solution (Phytotech Lab, USA) was applied every 2 days and cultivated for 2 weeks at 23°C, 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 16 hours light condition.

2. Al stress treatment and Si application

Two weeks-old alfalfa seedlings after germination were used to stress treatment. For stress treatment, each pot containing alfalfa seedlings was supplied 1/4 × Hoagland solution (pH 4.0) along with 0.2 mM Al ($AlCl_3 \cdot 6H_2O$, Sigma-Aldrich Co., USA), 1.0 mM Si ($Na_2SiO_3 \cdot 5H_2O$), and combined Al+Si. Each pot was fed with 200 ml solution every 2 days for 7 days. All experiments were repeated 3 times.

3. Measurement of plant length and biomass

After treatment, the roots of alfalfa seedlings were washed with distilled water, the water was removed and then separated into shoot and root tissues, and the length and fresh weight of each were measured. The dry weight of the root and shoot was measured after drying for 72 hours in a 65℃ dry oven (WiseVen, Daihan Scientific, Korea).

4. Analysis of chlorophyll and carotenoid

Twenty mg of leaf was immersed in 1 mL 95% EtOH and extracted for 20 minutes on a heat-plate at 80°C, and then absorbance at 470 nm, 648 nm, and 664 nm was measured using a spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japane). Chlorophyll a and b, and carotenoid contents were calculated according to the method described previously (Jeffrey and Humphrey, 1975).

Chl a = 13.36×664 nm -5.19×648 nm

Chl b = 27.43×648 nm -8.12×664 nm

Carotenoid = (1000×470 nm - 2.14×Chl a - 97.64×Chl b)/209

5. Statistical analysis

For statistical analysis, IBM SPSS statistics (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA) was used. Significant differences in shoot and root length, fresh and dry weight, and chlorophyll and carotenoid contents of alfalfa after treatment were verified at the significance level $P<0.05$ through independent sample t-test.

Ⅲ. RESULTS AND DISCUSSION

In order to investigate the response of alfalfa seedlings to Al stress and combined Al + Si treatments, 2 weeks old alfalfa seedlings grown in soil in pot were treated for 7 days (Fig. 1).

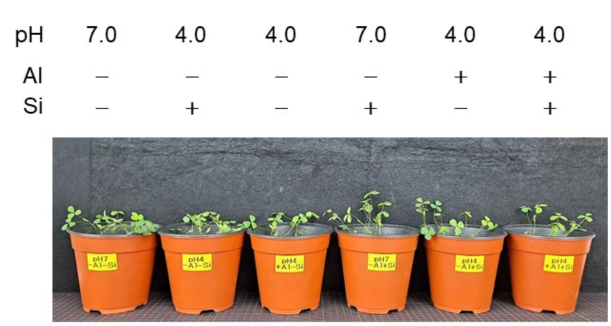

Fig. 1. Effects of aluminum stress on alfalfa plants. Alfalfa seedlings were grown in the pots. Two weeks-old seedlings were exposed to Al stress with or without 1.0 mM Si for 7 days at different pH (pH 4.0 or pH 7.0).

As a result of visual observation, the growth of alfalfa seedlings in pot grown at pH 7.0, it was observed that the above-ground shoots were slightly taller in the Si-treated group (Fig. 1). In acidic pH (pH 4.0), it was observed that the plant height decreased compared to the pH 7.0 group regardless of Al stress. In the Si treatment pot, growth was observed to be restored to a level similar to that of the control group.

On the other hand, the phenotypic differences and the lengths of shoots and roots of each seedling exposed to Al stress or Si treatment were measured and compared (Fig. 2). The length of the shoot was increased by Si treatment at pH 7.0, although there was no significance, and no change in length was observed at pH 4.0. However, in the case of roots, the length decreased in the pH 4.0 or Al stress treatment, and it was observed that the Al+Si combined treatment recovered as much as the length of the control group. These results indicate that, in alfalfa, Al stress at acidic pH causes damage to the growth of underground roots rather than above-ground shoots, and such root growth reduction caused by Al stress can be recovered by Si treatment. Similar results of which root growth was inhibited by Al stress treatment at low pH was reported in several other crops, including maize (Stass et al., 2006) and rice (Awasthi et al., 2017).

In addition, the results of the changes in the fresh and dry weights of shoots and roots after treatments with Al stress and Al+Si was shown in Fig. 3. In the case of shoot, the fresh weight was reduced by Al stress, and almost recovered by the Si treatment, but no change was observed in dry weight (Fig. 3A,C). On the other hand, although there was no significant difference, there was almost no Si mitigating effect on the reduction of root length (Fig. 2C) and fresh weight at acidic pH (pH 4.0), but a Si mitigating effect was observed at dry weight of root. These results suggest that Si exhibits a mitigation function through increasing root biomass rather than root growth in the acidic pH. Further studies, such as analysis using hydroponic systems, will be helpful to elucidate more precise mechanisms.

However, both fresh and dry weight were significantly reduced by Al stress in acidic pH, but were significantly restored to the control level by Al+Si combination treatment (Fig. 3B,D). These results suggest that Si treatment has a mechanism to alleviate the inhibition of alfalfa growth due to Al stress. Recently, similar results were reported by Vega et al. (2020), and in an experiment using barley, Si treatment alleviated Al toxicity and improved plant growth and tolerance to Al toxicity.

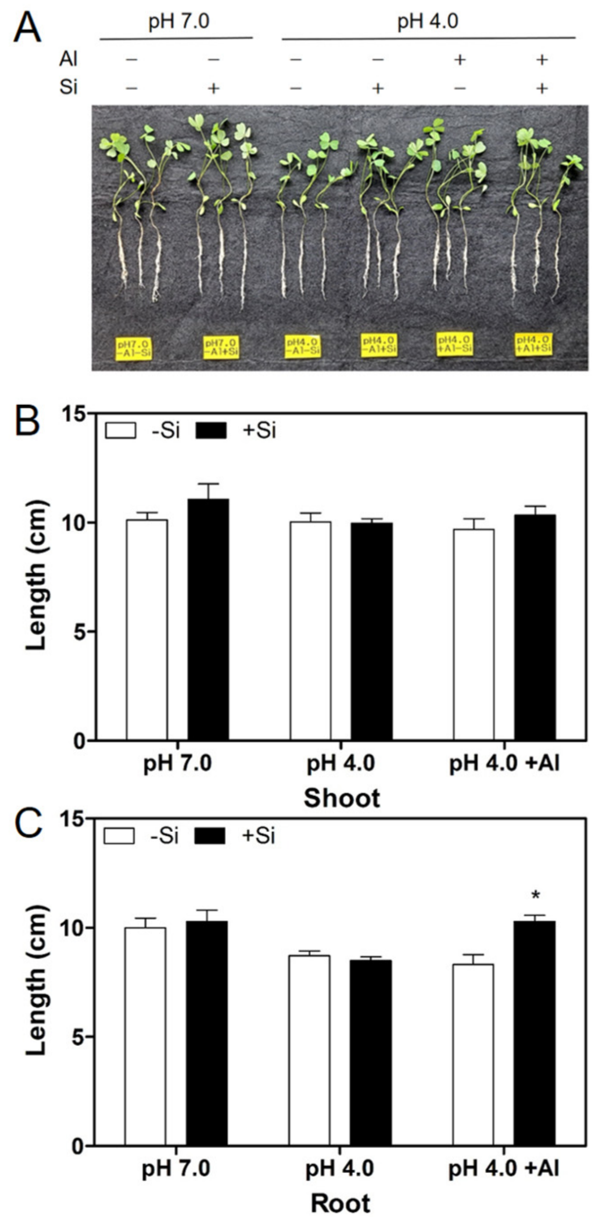

Fig. 2. Changes in shoot and root lengths of alfalfa seedlings grown in pots under Al stress with or without Si. Photograph of alfalfa seedlings after treatment (A), Shoot length (B), root length (C). Data are means±SE of three replicates. Bars with different dots indicate a significant difference at (*P*<0.05, *).

Changes in chlorophyll and carotenoid contents in leaves after treatment with Al stress and Al+Si are shown in Fig. 4.

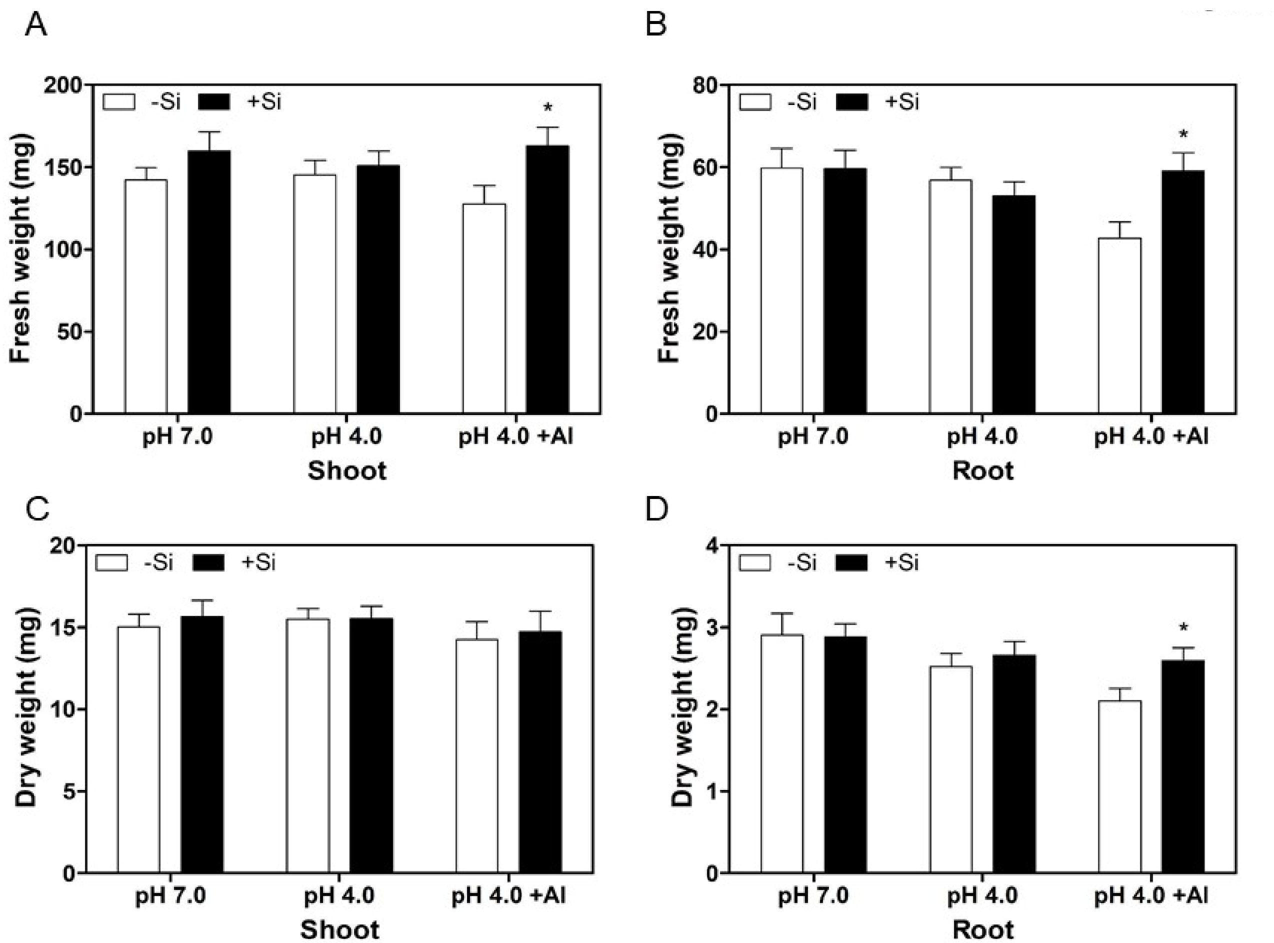

Fig. 3. Changes in fresh and dry weights of alfalfa seedlings grown in pots under Al stress with or without Si. Fresh weight of shoot (A) and root (B); Dry weight of shoot (C) and root (D). Data are means±SE of three replicates. Bars with different dots indicate a significant difference at ($P<0.05$, *).

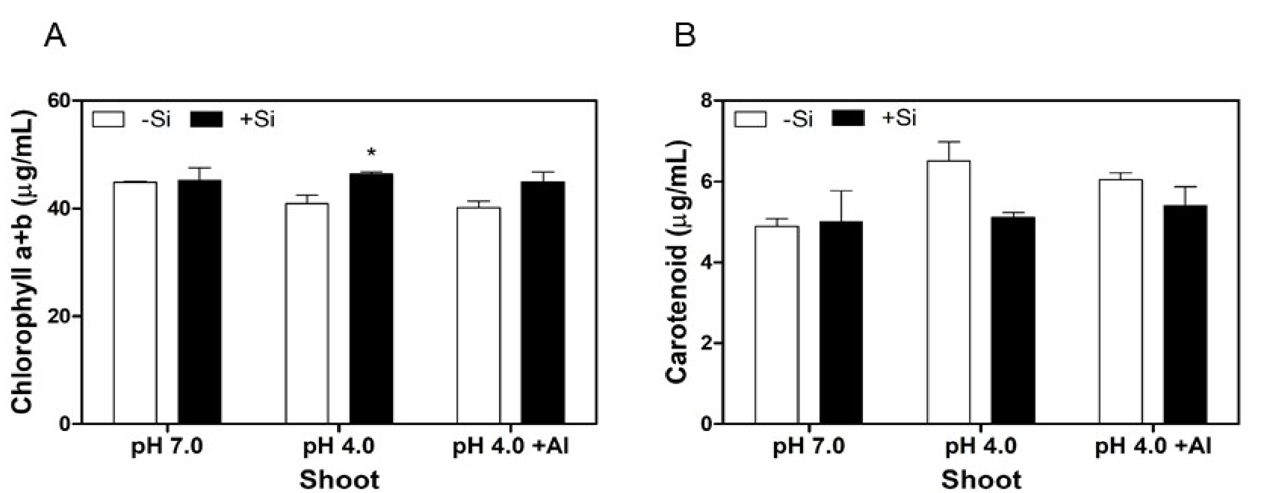

Fig. 4. Chlorophyll and carotenoid contents in alfalfa leaves after exposure to Al stress with or without Si. Chlorophyll a and b content (A), carotenoid content (B). Data are means±SE of three replicates. Bars with different dots indicate a significant difference at ($P<0.05$, *).

Chlorophyll content was decreased by Al stress in acidic pH and increased by Si treatment (Fig. 4A). On the other hand, carotenoid was increased by Al stress in acidic pH, and decreased to the control level by Si treatment (Fig. 4B). These results suggest that Al stress acidic pH may have damaged the chloroplast of alfalfa or damaged the machinery involved in chlorophyll biosynthesis. In addition, carotenoids are known to act as antioxidants against radicals and photochemical damage in plant cells (Sengar et al., 2008). The decrease in the increase of carotenoid content by Si treatment suggests that it is involved in the alleviation of intracellular reactive oxygen species toxicity caused by Al stress. Zengin (2013) reported that heavy metal stress (Cr, Co, Ni, Zn) in beans decreased chlorophyll content and increased carotenoid content to remove accumulated reactive oxygen species (ROS). Wei et al. (2021) reported similar results to this study that the treatment of Si in tomato maintained or increased photosynthetic pigment accumulation under Cd stress.

Ⅳ. CONCLUSION

As a result of investigating whether Si treatment can alleviate Al stress response in the growth of alfalfa, it was confirmed that Si treatment can improve the effect of suppressing Al stress-induced growth and reducing fresh and dry weight. Al stress showed a decrease in the chlorophyll content and an increase in the carotenoid content, which means the existence of a cell damage mechanism caused by Al toxicity. These results will be helpful in physio-biochemical and molecular biology studies to elucidate the function of Si related to the reduction of Al toxicity in alfalfa in the future.

Ⅴ. ACKNOWLEDGMENT

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2015R1D1A1A01060495)

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(Received : September 15, 2021 | Revised : September 17, 2021 | Accepted : September 22, 2021)