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Research Article

Extreme pH Reduced Vegetative Growth and Biomass Accumulation in Alfalfa

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

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

ABSTRACT

Soil acidity or alkalinity are serious limitations for crop production. The purpose of this study was to clarify the negative effects of extreme pH stress (low and high) on alfalfa vegetative growth (VG) and biomass accumulation (BA). Two-week-old alfalfa seedlings were exposed to different pH (4.0, 4.5, 7.0, 8.0 and 8.5, respectively) levels for 72 hours. Alfalfa grown at pH 4.0 and 8.5 significantly reduced VG and BA, wherein as neutral pH (7.0) comparably exhibited better growth and biomass yield. These results indicate that extreme acidic or alkaline level are critical limiting factors for growth and biomass yield in alfalfa.

(**Key words**: Extreme pH, Growth, Biomass yield, Alfalfa)

Ⅰ. INTRODUCTION

Extreme pH acidity and alkalinity severely hamper forage productivity that is the great challenges to the farmer for the livestock production globally. Soil pH is the potential index to express the acidity and alkalinity status in soils. The Soil Survey Division Staff (2017), United State Department of Agriculture (USDA) categorized total nine classes of soil based on pH level: ultra acidic (pH < 3.5), extremely acidic (3.5–4.4), very strongly acidic (4.5–5.0), strongly acidic (5.1–5.5), moderately acidic (5.6–6.0), slightly acidic (6.1–6.5), neutral (6.6–7.3), slightly alkaline (7.4–7.8), moderately alkaline (7.9–8.4), strongly alkaline (8.5–9.0), and very strongly alkaline (>9.0). In agricultural perspective, the optimal pH range is 5.5-7.0 is maintained for the most cultivable plants. However, grass crop prefers distinct pH levels, slightly acidic is preferred by your fine lawn grasses (e.g. bents and fescues) while broader leaved grasses (e.g. rye and meadow grasses) like a more pH at 6.5, the red clover prefers soil pH at 5.8-7.0 (Evans, 2010).

Soil pH influences by both acid-forming cations (AFCs) like (H^+), aluminum (Al^{3+}), and iron (Fe^{2+} or Fe^{3+}), and base-forming cations including calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+) and sodium (Na^+) (McCauley et al., 2009). AFCs like Al^{3+} reported to be toxic at pH below 4.5 that reduces plant growth and development (Rahman et al., 2018). Alfalfa is world leading forage crop. As a legume forage, alfalfa is capable to fix atmospheric nitrogen (135–605 kg/ha year) and those have pivotal role in biomass production (Putnam et al., 2001). A wide range of molecular and physiological and biochemical studies have been performed in response to aluminum (Rahman et al., 2014), heat (Li et al., 2013; Lee et al., 2017a,b), salt (Rahman et al., 2015), drought (Rahman et al., 2016) stresses in alfalfa. Subsequently, cold-heat induced DnaJ-like protein gene has been cloned from alfalfa (Lee et al., 2018). Despite of these above progresses of alfalfa there is yet to be disclosed the impact of extreme pH on vegetative growth and biomass accumulation in alfalfa. Therefore, the aim of this study was to determine the impact of critical pH level on vegetative growth, biomass accumulation and productivity of alfalfa.

Ⅱ. MATERIALS AND METHODS

1. Plant growth and treatments

Alfalfa (*Medicago sativa* L.) were grown on plastic sieve (with 6 chambers) containing hydroponic system. Total 1 gram seed was placed to each plastic chamber, and covered by black plastic cover then moved to dark for 3 days. Cover of germination box was opened after 3 days and kept at light condition. After

*Corresponding author: Md. Atikur Rahman, National Institute of Animal Science, Cheonan 31000, Korea.
Tel: +82-41-580-6767, E-mail: atikbt@korea.kr

7 days of germination, seedlings were grown with half strength of Hoagland salt mixture (Phytotech lab). The nutrient solution was changed after every 2 days interval. Alfalfa seedlings were grown for 2 weeks in a controlled growth chamber at 25°C, with a photoperiod 16/8 h light/dark cycle 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 acidic and alkaline pH viz. 4.0, 4.5, 8.0, and 8.5, along with a neutral pH (7.0) as a control, for 72 h. Note, the experiment consisted of four treatments viz. (a) extremely acidic (pH 4.0), (b) strongly acidic (pH 4.5), (c) neutral (pH 7.0) or control, and (d) moderate alkaline (pH 8.0), and strongly alkaline ((pH 8.5). After 72 h of extreme pH stress, seedlings from different treatments were harvested separately and data were collected following standard methodologies.

2. Determination of alfalfa growth and biomass accumulation

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

3. Measurement of chlorophyll content

Leaf chlorophyll (Chl) content was recorded by considering SPAD value using a portable diagnostic chlorophyll meter (SPAD-502, 2900P, Spectrum Technologies, Ireland). The quantification of the light intensity was programmed at peak wavelength: 650 nm (red LED). Third trifoliate leaf of alfalfa was considered for the reading of SPAD value of each treatment.

4. Statistical analysis

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

Ⅲ. RESULTS AND DISCUSSION

Extreme pH of the growing media influenced the vegetative growth and biomass accumulation of the alfalfa seedlings (Figs.1, 2 and 3). Upon exposure to extremely acidic (pH 4.0) -stress, the plant growth inhibited where height was reduced significantly by 53.70% compared with the control (Fig. 2A), whereas 65.43% reduction in root length resulted in extremely acidic (pH 4.0)-stress. The strongly alkaline (pH 8.5)-stress reduced the root length by 53.09% compared to the control (Fig. 2B). Extreme pH-stress again hampered the biomass accumulation of alfalfa seedlings. The shoot fresh weight significantly ($P<0.05$) reduced by 47.31% following strongly alkaline (pH 8.5) exposure, where it reduced 33.33% at extremely acidic (pH 4.0)-stress condition (Fig. 2C). Root fresh weight reduced 37.50% at extremely acidic (pH 4.0) but no significant difference has been observed among only 4 treatments group except control (Fig. 3A). Shoot dry weight reduced by 34.42% at extremely acidic (pH 4.0)-stress whereas reduced 40.91% at strongly alkaline (pH 8.5)-stress (Fig. 3B). The height reduction of root dry weight was observed at pH 4.0 that was 41.86% compared to control (pH 7.0). There was no significant difference for root dry weight (DW) among the 4 treatments except control (Fig. 3C). Extreme acidic (pH 4.0)-stress reduced the leaf chlorophyll (SPAD value) by 52.42%, and strongly alkaline (pH 8.5)-stress reduced by 64.5% compared to control plant

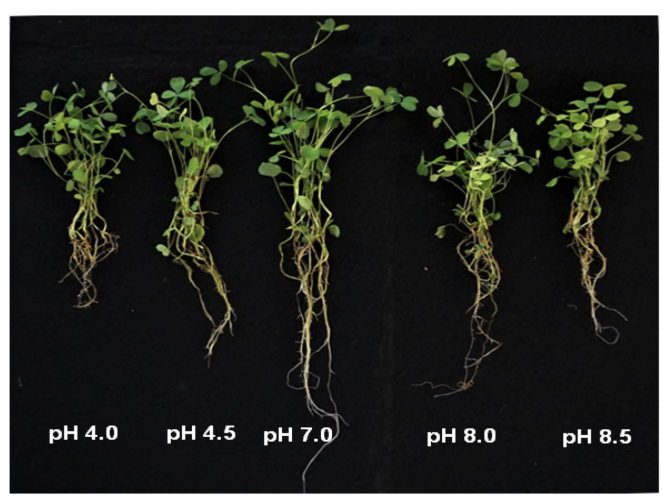

Fig. 1. Impact of different pH levels on alfalfa plant morphology. Photo was taken after 72 h of plant exposure to different pH levels.

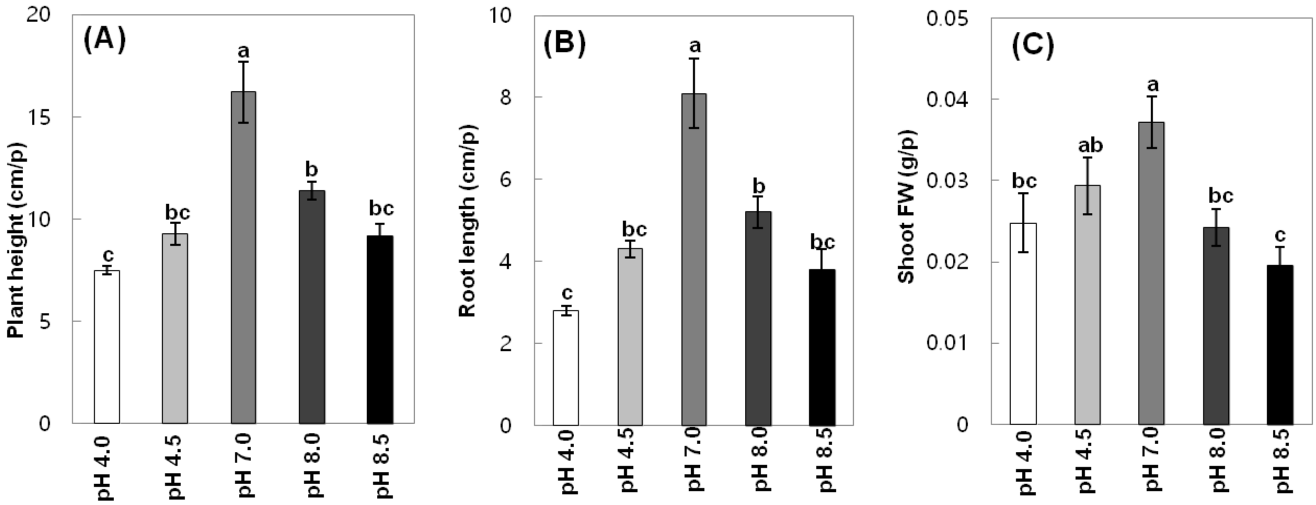

Fig. 2. Effects of extreme pH on alfalfa vegetative growth and biomass accumulation. Effects of extreme pH stress on plant height (A), root length (B), shoot fresh weight (C). Data were represented as mean ±SE of at least five independent replications. The different letters above the bar represent statistically significant ($P<0.05$) differences among the treatment groups.

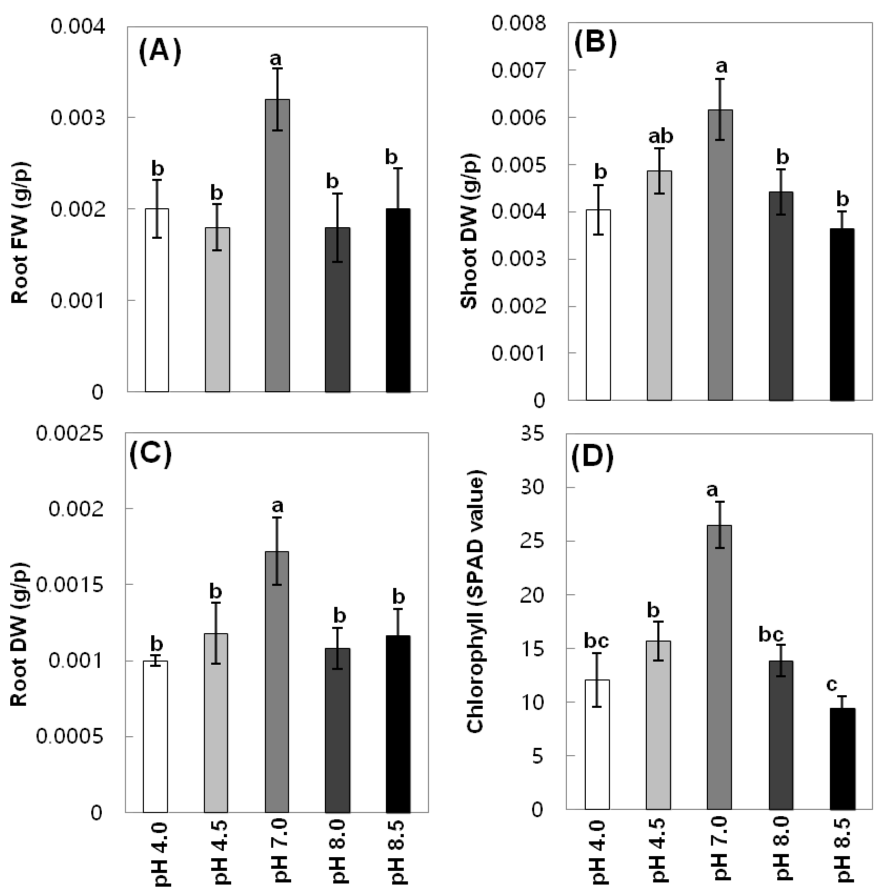

Fig. 3. Effects of extreme pH on alfalfa vegetative growth and biomass accumulation. Effects of extreme pH stress on root fresh weight (A), shoot dry weight (B), root dry weight (C), and chlorophyll content (D). Data were represented as mean ±SE of at least five independent replications. The different letters above the bar represent statistically significant ($P<0.05$) differences among the treatment groups.

(pH 7.0) (Fig. 3D). The highest pigment content (as leaf greenness) was significant reduced at pH 8.5. Control alfalfa plants maintained the better leaf greenness at pH 7.0. Previous several reports have been documented that crop productivity hampered by extreme pH (both low and high) (Kochian et al., 2015). In the present study we observed the negative effects of extreme pH both acidity and alkalinity-stress, on the alfalfa vegetative growth and biomass accumulation. Exposure to both acidity and alkalinity, a significant decrease of alfalfa growth and biomass accumulation were observed in terms of seedling height, root length, fresh weight, and dry weight of both shoots and roots, which corroborates with previous studies (Yang et al., 2009; Wang et al., 2001). A significant reduction in root elongation is reported under a low pH (pH 4.0) due to the higher H^+ toxicity in extremely acidic soil of crop plants such as tomato, barley etc. (Yang et al., 2009; Wang et al., 2001). This reduction in root growth might be due to the decrease of root cell division and development (Kochian et al., 2015). In addition, reduction of leaf chlorophyll by extreme pH was reported in rice plants (Suriyan, 2009). However, this study would be useful for determining critical pH level for alfalfa and its cultivation in distinct pH level in Korean soil.

Ⅳ. Conclusion

In this study, we observed alfalfa forage growth and biomass yield were significantly hampered following extreme pH (low and high) stress. In contrast, neutral pH level in medium led to provide better growth and forage biomass. This study provides important information concerning the impact of critical pH level on alfalfa growth and forage yield along with better strategy for the cultivation of alfalfa in Korean soils.

Ⅴ. ACKNOWLEDGMENTS

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