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Research Article

Citric Acid Reduces Alkaline Stress-induced Chlorosis, Oxidative Stress, and Photosynthetic Disturbance by Regulating Growth Performance, Antioxidant Activity and ROS Scavenging in Alfalfa

Ki-Won Lee, Sang-Hoon Lee, Yowook Song, Hyung Soo Park, Jae Hoon Woo, Bo Ram Choi, Eun A Lim and Md Atikur Rahman*

Grassland & Forages Division, National Institute of Animal Science, Rural Development Administration, Cheonan 31000, Republic of Korea

ABSTRACT

Pollution of agricultural soil by alkaline salts, such as Na_2CO_3, is a critical and long-lasting problem in cultivable land. The aim of the study was to examine the putative role of citric acid (CA) in alleviating Na_2CO_3-stress in alfalfa. In this study, Na_2CO_3 significantly induced leaf chlorosis, inhibited plant growth and photosynthesis related parameters, increased hydrogen peroxide (H_2O_2) and reduced major antioxidant enzymes (SOD ,CAD, APX) in alfalfa. However, the presence of CA these negative effects of Na_2CO_3.stress largely recovered. Interestingly, expression of antioxidant and ion transporter genes (*Fe-SOD, CAT, APX, DHAR and NHX1*) involved in Reactive oxygen species (ROS) homeostasis and oxidative stress tolerance in alfalfa. These findings suggest that CA-mediated Na_2CO_3 stress alleviation is an ecofriendly approach that would be useful to local farmer for alfalfa and other forage crop cultivation in alkaline soils.

(Key words: Citric acid, alkaline, Photosynthetic disturbance, ROS, Alfalfa)

Ⅰ. INTRODUCTION

Soil contamination by alkaline or other salts is a long lasting agricultural problem globally (Hassani et al., 2020). High level of alkaline salt such as Na_2CO_3 or $NaHCO_3$ induces the alkaline stress, where as other neutral salts including NaCl and $NaSO_4$ lead to induce saline stress and effect on chemical properties in soils (Irakoze et al., 2021). High level of alkaline salts in growth medium reduces K^+ content and enhances the accumulation of Na^+ that greatly effects on plant growth and development (Zang and Mu, 2009). Moreover, the impairment of mineral imbalance due to alkaline stress induces chlorosis and photosynthetic disturbance in plants (Wu et al., 2014). Excess Na^+ of alkaline salt negatively impacts at cellular levels by inducing ionic stress, osmotic stress and water deficit, which lead to induce generation of ROS (H_2O_2, O_2^- and $H^•$) (Peng et al., 2008).

This generation of these excess ROS due to excess Na^+ can oxidize ultrastructure of cells, which leads to oxidative stress, lipid peroxidation and cellular injury in plants (Rahman et al., 2015). Fortunately, plants have evolved an antioxidant system by which oxidative stress is comprised. Several enzymatic components such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydro-ascorbate reductase (MDHAR) play pivotal role in ROS homeostasis and antioxidant defense in plants (Haque et al., 2021). Moreover, ionic homeostasis (K^+ and Na^+) is the strategy of plants by protecting ion toxicity induced cellular injury in plants. Interestingly several Na^+/H^+ antiporter like *NHX* involved in Na^+ sequestration and osmotic adjustment or the initial resistance to Na^+ uptake by extruding Na^+ to the external environment (Assaha et al., 2017).

Recent reports suggest that excess alkaline or neutral salts (Na_2CO_3, $NaHCO_3$, NaCl, and $NaSO_4$) induces toxicity at physiologically level and cellular levels that severely restricts plant growth and development of global agriculture (Fang et al.,

*Corresponding author: Md Atikur Rahman, Grassland & Forages Division, National Institute of Animal Science, Rural Development Administration, Cheonan 31000, Republic of Korea, Tel: +82-41-580-6746, Fax: +82-41-580-6779, E-mail: atikbt@korea.kr

2021). Ionomic and metabolomic alteration found to be induced in maize seedlings due to neutral salt or alkaline salt stresses, which significantly reduced plant nutrient availability, photosynthesis, N metabolism, and suppressed sugars and amino acids production (Guo et al., 2017). Moreover, previous reports also suggest that growth, development and nutrient accumulation in legumes plants also affected by salt and alkali stresses, respectively. Study in *Lotus tenuis* indicates that neutral and alkaline salts detrimentally effect on growth, nutrient accumulation and root morphology (Paz et al., 2012). It has been reported that salt and alkali stresses greatly induces the inhibition of germination, growth and photosynthetic disturbance in alfalfa (Li et al., 2010). Therefore, development of alkaline tolerant alfalfa verity or mitigation of alkaline stress by environment friendly strategy is essential for sustainable alfalfa production.

Alfalfa is a potential forage legume with a promising source of protein, cultivating as hay crops for sustainable agriculture globally (Kulkarni et al., 2018). Cultivation of legume crops in saline and alkaline soils, significantly reduces germination rate and radicle elongation, which greatly impairs plant growth, photosynthesis and ultimate productivity. Therefore, we explored citric acid (CA)-mediated alkaline stress alleviation in alfalfa plants as cost effective and environmental friendly approach. CA is an organic acid, essential for plant growth and development, involved in abiotic stress tolerance in plants (Tahjib-Ul-Arif et al., 2021). In this study, the supplementation of CA significantly recovered the negative effects of Na_2CO_3-stress. Furthermore, induced the expression of antioxidant and ion transporter genes (*Fe-SOD, CAT, APX, DHAR and NHX1*), which regulated the ROS homeostasis and oxidative stress tolerance in alfalfa.

Ⅱ. MATERIALS AND METHODS

1. Plant growth and alkaline treatment

Alfalfa (*Medicago sativa* L.) seeds were surface sterilized using 70% ethanol for 1 min, and rinse properly by DEPC treated water. Plant seeds were moved in a plastic tray for 3 days followed by alfalfa seedling were transferred and grown in Hoagland (Hoagland and Arnon, 1950) nutrient basal salt containing medium. The plants were treated with four groups: control (normal nutrient solution); Na_2CO_3 (5.0 mM); Na_2CO_3 (5.0 mM) and citric acid (CA, 250 μM). Seedlings were treated individually for each treatment group in a plastic container and cultivated in a controlled environment with light and dark (14 h/10 h) photoperiod (550–560 μmol s^{-1} per μA) at 25°C. After two weeks alfalfa plants were harvested for further analyses. Following treatments, root and shoot were separated then washed with deionized water properly to remove excess alkaline salt, and the samples were kept at -80°C until further analysis.

2. Plant growth and physiological parameters

Following treatments alfalfa growth and its physiological parameters were measured. Root and shoot length were measured using a metric scale (cm), and weight (g) was measured using electronic balance. The chlorophyll score of young alfalfa leaves was measured using SPAD meter (Minolta, Japan). The maximum yield of photosystem II (Fv/Fm) was measured using a portable fluorometer temperature (PAM 200, Effeltrich, Germany), plants were adapted for 20 minutes at dark condition prior to take reading.

3. Determination of hydrogen peroxide

Hydrogen peroxide (H_2O_2) was measured using the protocol used previously (Rahman et al. 2016). Briefly, 100 mg of plant sample was homogenized with potassium phosphate buffer (50 mM, pH 7.0). The mixture was centrifuged for 15 min at 13,000 rpm then supernatant (0.6 mL) was taken into a new eppendorf tube and added 0.6 mL of 20% H_2SO_4 containing titanium chloride (TiCl). The solution was centrifuged at 13,000 rpm for 15 min. Finally, supernatant (1 ml) was taken and read at 410 nm using a spectrophotometer (UV-1650PC, Shimadzu, Japan).

4. Determination of antioxidant enzyme activity

Antioxidant enzyme activities in alfalfa tissue were determined potassium phosphate buffer (KP-buffer, pH 7.0) following the protocol used previously (Kabir et al., 2021). Shortly, 100 mg of grinded tissue was mixed in 0.5 mL of 100 mM (KP-buffer, pH 7.0), and vortex well. Then the mixture was centrifuged at 12,000 rpm for 15 min, and this extract was considered for further enzymatic analyses. Superoxide dismutase (SOD) activity was

measured using a solution mixture containing of 100 μL extract, 0.1 mM EDTA, 50 mM $NaHCO_3$ (pH 9.8) and 0.6 mM epinephrine. The drenochrome was confirmed by exposing the solution at 475 nm. The catalase (CAT) activity was determined using a mixture containing 100 mM KP-buffer (pH 7.0), 6% H_2O_2 and 100 μL plant extract, and the solution was read at 240 nm (extinction co-efficient 0.036 mM^{-1} cm^{-1}) whereas time interval between 30s-60s. The activity of ascorbate peroxidase (APX) was measured according to the protocol used previously (Rahman et al., 2020). The reaction buffer for APX consisted of 100 μL of sample extract, 0.1 mM EDTA, 50 mM KP-buffer, 0.1 mM H_2O_2, and 0.5 mM ascorbic acid. The 1 mL supernatant was taken into new tube and absorbance was read at 290 nm. Finally the activity of APX was calculated with extinction co-efficient (2.8 mM^{-1} cm^{-1}).

5. Analysis gene expression

Total RNA was extracted from the alfalfa tissue using traditional mini kit (QIAGEN, Germany). Shortly, 100 mg of grinded plant tissue was mixed with extraction buffer containing 2M Dithiothreitol (DTT), and centrifuged at 13,000 rpm for 2 min. Total RNA was obtained after several washing steps, and final RNA was collected by adding 40-45 μL RNase-free water. The quantification of RNA carried out using a micro-volume UV/Vis spectrophotometer (UVIS Drop-99, Taiwan). cDNA synthesis was performed with 1 μg of total RNA using cDNA synthesis kit (Bio-Rad, USA). Gene expression was conducted by CFX96 Real Time system (BIORAD, USA) for the expression of target genes using gene specific primers: *Actin* Forward- TTCTCACCACACTTCTCGCC, *Actin* Reverse-CCAGCCTTCACCATTCCAGT; *FeSOD* Forward-GAGTACCA TTGGGGAAAGCA, *FeSOD* Reverse- CCATACCTGTGCTGCATTGT; *CAT* Forward- CCAAGTCCCACATTCAGGAG, *CAT* Reverse-ACTGCTTTCCCAGCCTTGTT; *APX* Forward- GAAATGCG CTCCTCTTATGC, *APX* Reverse- TGTTAGCACCATGAGCAAGC, *DHAR* Forward- GTGTTGCTGACACTGGAGGA, *DHAR* Reverse-CCAGCTGTAGCCTTTTCAGG. Total 20 μL reaction mixture consisted of SYBR Green (10 μL), template cDNA (2 μL), 10 μM forward and reverse primer (0.8 μL) each, rest of DEPC treated water. The amplification was carried out following the conditions: 95 °C for 30 sec, followed by 40 cycles at 95 °C for 5 sec, 60 °C for 30 sec. *MsActin* used as internal control, whereas the gene expression were calculated using the $dd^{-\Delta Ct}$ method (Livak and Schmittgen 2001)

6. Statistical analysis

All physiological and molecular parameters were statistically analyzed using one way analysis of variance (ANOVA). The significant differences ($P \leq 0.05$) were measured using Tukey test. The software GraphPad Prism 8.4.3 was used for graphical analyses. Al least three independent replications were considered for the analysis.

Ⅲ. RESULTS AND DISCUSSION

1. Alkaline stress-induced chlorosis, photosynthetic disturbance, and growth inhibition were recovered by citric acid (CA)

Alkaline (Na_2CO_3) stress significantly changes the morphological and physiological features (Fig. 1). In this study, alkaline stress significantly reduced SPAD value (Fig. 2a), and Fv/Fm (Fig. 2b), which indicating the induction of leaf chlorosis and photosynthetic disturbance in alfalfa. Furthermore, root-shoot length and root-shoot fresh weight were significantly reduced in response to alkaline stress (Fig. 2c-f). However, these above negative impact of alkaline stress were largely recovered after

Fig. 1. Alkaline stress-induced morphological changes in alfalfa exposed to different growth conditions: control (Normal nutrient solution); Na_2CO_3 (5.0 mM); Na_2CO_3 (5.0 mM) and citric acid (CA, 250 μM); and CA (250 μM).

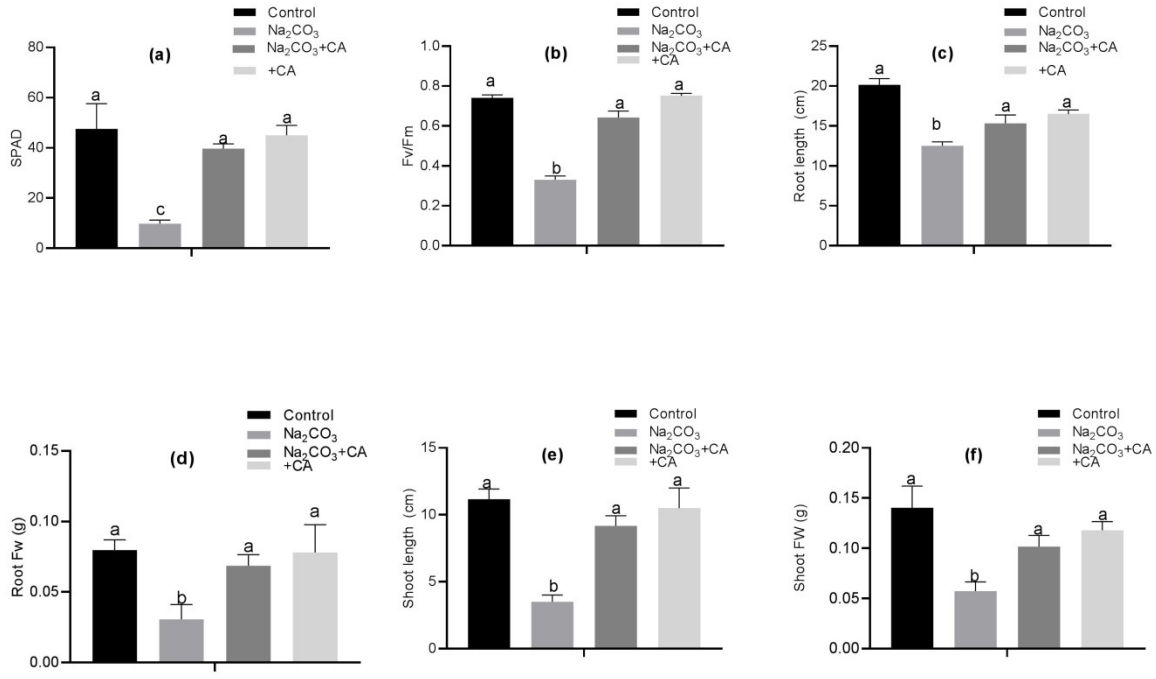

Fig. 2. SPAD (a), Fv/Fm (b), root length (c), and root fresh weight (d), shoot length (e), and shoot fresh weight (d) in alfalfa exposed to different growth conditions: control (Normal nutrient solution); Na_2CO_3 (5.0 mM); Na_2CO_3 (5.0 mM) and citric acid (CA, 250 μM); and CA (250 μM). Different letters above the error bar indicate significant differences ($P < 0.05$) among means ±SD of treatments (n=3).

supplementation of CA, indicating that CA is associated with physiological improvement as well as alkaline stress alleviation in alfalfa. Our study supported by the previous study in other legume and other crops where CA was actively involved in abiotic stress tolerance in plants (Tahjib-Ul-Arif et al., 2021). This finding may useful to the local farmer for development of new alfalfa cultivar in alkaline soils along with plant abiotic stress tolerance for sustainable agriculture.

2. Citric acid (CA) induced the activity of major antioxidant enzymes and reduced ROS

In this current study, we observed alkaline stress significantly increased ROS (H_2O_2) activity, whereas it reduced after addition of CA to alkaline stress (Fig 3a). Major antioxidants (SOD, CAT, and APX) were reduced in response to alkaline stress (Na_2CO_3) but the activity was recovered while CA supplemented with alkaline stress (Na_2CO_3+CA) (Fig. 3b-d). These results indicating that Na_2CO_3 was toxic for plant survival that also impaired in antioxidant homeostasis. In contrast, antioxidant activities mostly induced by CA, which suggest that CA is associated with induction of antioxidant activity as well as alkaline stress alleviation in alfalfa. Therefore, CA is an important component of alkaline stress tolerance in alfalfa. Our study related to CA-mediated abiotic stress alleviation would be exciting and promising as very little study has been reported (Tahjib-Ul-Arif et al., 2021; Sun and Hong, 2011).

3. The expression of antioxidant genes was linked to changes in corresponding enzyme activities

An intimate link between antioxidant enzyme activities and alkaline-induced stress was reported in halophyte *Leymus chinensis* (Sun and Hong, 2011). In this study, we observed ROS scavenging related gene expression (*SOD, CAT,* and *APX*) related to altering the corresponding enzyme activities (Fig. 4a-c). So, it is clear that a possible interaction exist between the response of major ROS scavenging enzymes and alkaline stress. SOD enzyme involved in detoxification of superoxide formed during photosynthetic electron transport and function in ROS metabolism (Pilon et al., 2011). The expression of *DHAR* significantly induced under alkaline stress

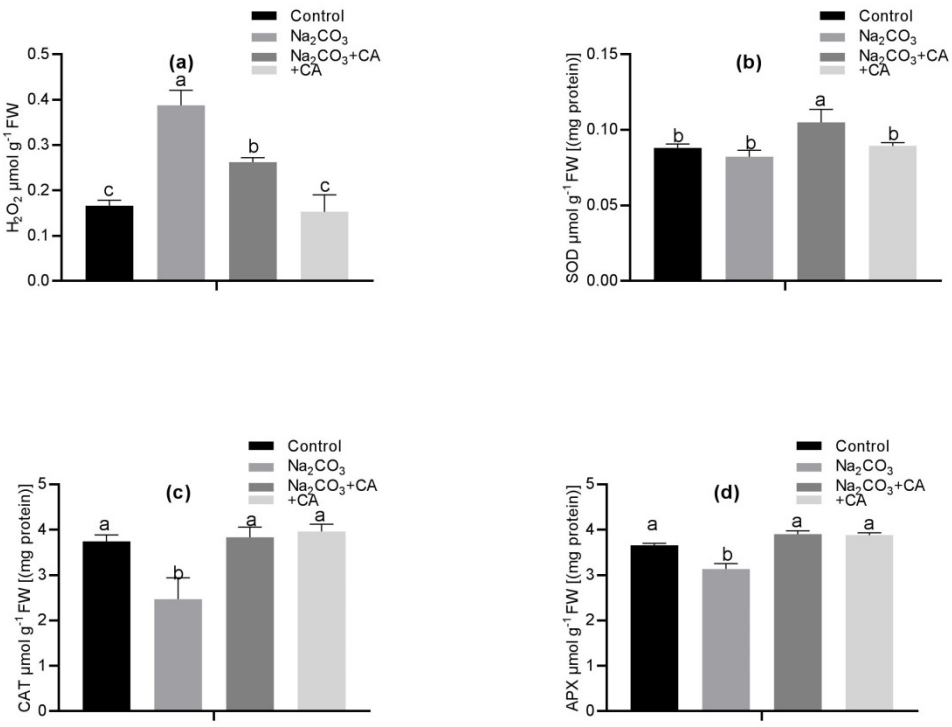

Fig. 3. H_2O_2 (a), SOD (b), CAT (c), and APX (d) enzyme activity in alfalfa exposed to different growth conditions: control (Normal nutrient solution); Na_2CO_3 (5.0 mM); Na_2CO_3 (5.0 mM) and citric acid (CA, 250 μM); and CA (250 μM). Different letters above the error bar indicate significant differences (P < 0.05) among means ±SD of treatments (n=3).

as well as the supplementation of CA with alkaline stress (Na_2CO_3+CA) (Fig. 4d). The expression is slightly higher compared to alkaline stress, indication that relation of alkaline stress and antioxidant activity where CA played a pivotal role as antioxidant inducer as well as stress alleviator.

4. The expression of the genes *NHX1* and *P5CS1* was influenced by alkaline stress.

The expression of *NHX1* induced in response to alkaline stress compared with or without CA supplementation (Fig. 5a). The expression of *P5CS1* transcript was significantly unregulated in response to alkaline stress (Fig. 5b). However, the expression was consistent to *NHX1*. Study indicates that Na^+/H^+ antiporter like *NHX* associated with Na^+ sequestration and osmotic adjustment or the inhibition of Na^+ uptake by extruding Na^+ to the external environment (Assaha et al., 2017), whereas *pyrroline-5-carboxylate synthetase (P5CS)* involved in proline biosynthesis from glutamate (Funck et al., 2020). In this study, the response of *NHX1* related to the present of alkaline salt where it may involve either alkaline salt sequestration or osmotic adjustment in alfalfa. As a consequence of oxidative stress the response of *P5CS1* indicates that a major contributor to alkaline stress-induced proline accumulation in alfalfa. Furthermore, up regulation of *P5CS1* suggests that intermediate response of proline accumulation that may require in oxidative stress alleviation in alfalfa during alkaline stress.

Ⅳ. CONCLUSION

This study provides shed light of CA-mediated alkaline stress alleviation in alfalfa. Alkaline stress-induced chlorosis, growth inhibition, photosynthetic disturbance, and oxidative stress in plants. Alkaline stress-induced chlorosis and physiological impairment are the particular events when plants are grown in alkaline/neutral salt containing environments. In this study, alkaline stress showed negative impacts on root-shoot length, leaf greenness, maximum quantum yield PSII, increased H_2O_2 accumulation in alfalfa. Interestingly, these negative impacts of

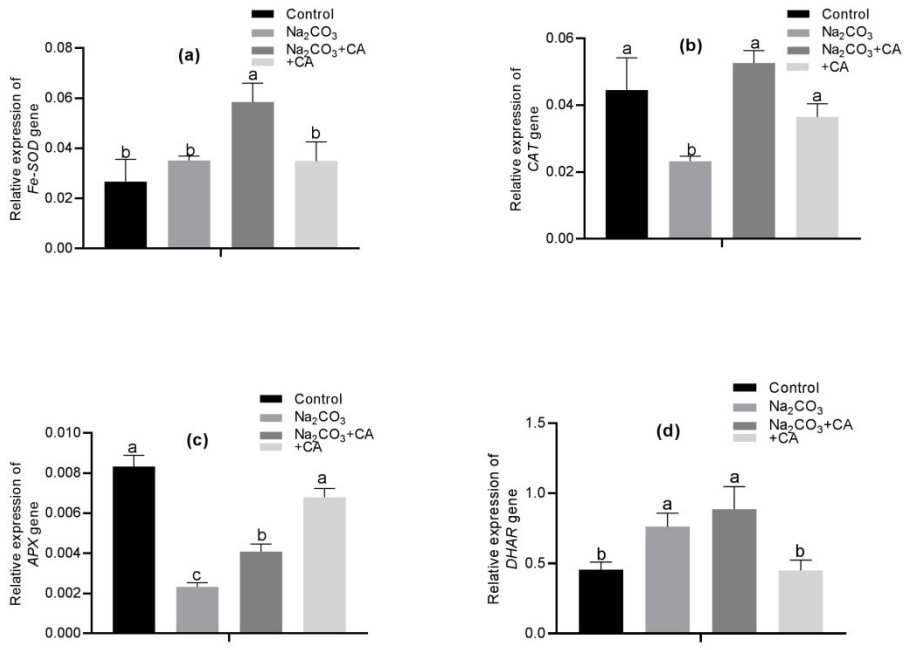

Fig. 4. *Fe-SOD* (a), *CAT* (b), *APX* (c), and *DHAR* (e) candidate gene expression in alfalfa exposed to different growth conditions: control (Normal nutrient solution); Na_2CO_3 (5.0 mM); Na_2CO_3 (5.0 mM) and citric acid (CA, 250 μM); and CA (250 μM). Different letters above the error bar indicate significant differences ($P < 0.05$) among means ±SD of treatments (n=3).

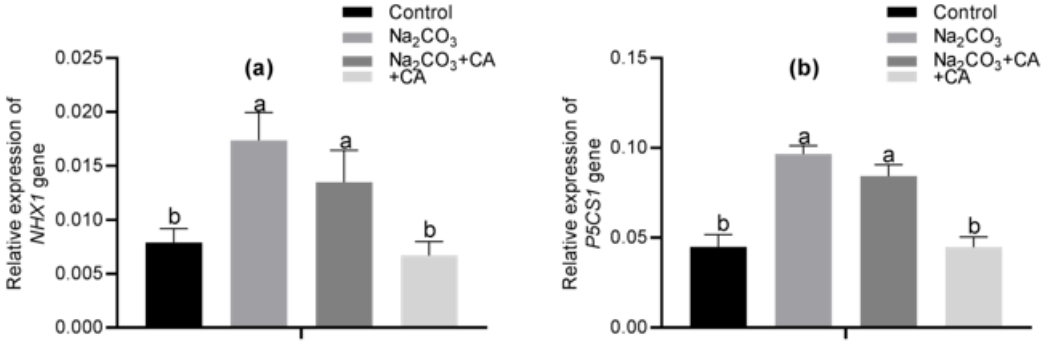

Fig. 5. *NHX1* (a) and *P5CS1* (b) candidate gene expression in alfalfa exposed to different growth conditions: control (Normal nutrient solution); Na_2CO_3 (5.0 mM); Na_2CO_3 (5.0 mM) and citric acid (CA, 250 μM); and CA (250 μM). Different letters above the error bar indicate significant differences ($P < 0.05$) among means ±SD of treatments (n=3).

alkaline stress were largely recovered after exogenous application of CA. Expression of Na^+/H^+ antiporter and proline biosynthesis genes under alkaline response suggested that these potential candidates were associated with salt sequestration or osmotic adjustment. Furthermore, CA-induced antioxidant genes along with their corresponding enzyme activities indicate that CA-induced antioxidant enzymes are involved in alleviation of alkaline stress-induced physiological disturbance, and ROS-induced cellular injury as well as oxidative stress in alfalfa plants.

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Ⅵ. REFERENCES

Assaha, D.V.M., Ueda, A., Saneoka, H., Al-Yahyai, R. and Yaish, M.W. 2017. The role of Na+ and K+ transporters in salt stress adaptation in glycophytes. Frontiers of Plant Science. 8:509.

Fang, S., Hou, X. and Liang, X. 2021. Response mechanisms of plants under saline-alkali stress. Frontiers of Plant Science. 12:1049.

Funck, D., Baumgarten, L., Stift, M., Von Wirén, N. and Schönemann, L. 2020. Differential contribution of *P5CS* isoforms to stress tolerance in Arabidopsis. Frontiers in Plant Science. 11:1483.

Guo, R., Shi, L., Yan, C., Zhong, X., Gu, F., Liu, Q., Xia, X. and Li, H. 2017. Ionomic and metabolic responses to neutral salt or alkaline salt stresses in maize (*Zea mays* L.) seedlings. BMC Plant Biology. 17(1):41.

Haque, A.F.M.M., Tasnim, J., El-Shehawi, A.M., Rahman, M.A., Parvez, M.S., Ahmed, M.B. and Kabir, A.H. 2021. The Cd-induced morphological and photosynthetic disruption is related to the reduced Fe status and increased oxidative injuries in sugar beet. Plant Physiology and Biochemistry. 166:448-458.

Hassani, A., Azapagic, A. and Shokri, N. 2020. Predicting long-term dynamics of soil salinity and sodicity on a global scale. Proceedings of the National Academy of Sciences of the United State of America. 117(52):33017-33027.

Hoagland, D.R. and Arnon, D.I. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station. 347:32.

Irakoze, W., Prodjinoto, H., Nijimbere, S., Bizimana, J.B., Bigirimana, J., Rufyikiri, G. and Lutts, S. 2021. NaCl- and Na2SO4-induced salinity differentially affect clay soil chemical properties and yield components of two rice cultivars (*Oryza sativa* L.) in Burundi. Agronomy. 11(3):571.

Kabir, A.H., Das, U., Rahman, M.A. and Lee, K.W. 2021. Silicon induces metallochaperone-driven cadmium binding to the cell wall and restores redox status through elevated glutathione in Cd-stressed sugar beet. Physiologia Plantarum. 173(1):352-368.

Kulkarni, K.P., Tayade, R., Asekova, S., Song, J.T., Shannon, J.G. and Lee, J.D. 2018. Harnessing the potential of forage legumes, alfalfa, soybean, and cowpea for sustainable agriculture and global food security. Frontiers of Plant Science. 9:1314.

Li, R., Shi, F., Fukuda, K. and Yang, Y. 2010. Effects of salt and alkali stresses on germination, growth, photosynthesis and ion accumulation in alfalfa (*Medicago sativa* L.). Soil Science and Plant Nutrition. 56(5):725-733.

Livak, K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 25:402-408.

Paz, R.C., Rocco, R.A., Reinoso, H., Menéndez, A.B., Pieckenstain, F.L. and Ruiz, O.A. 2012. Comparative study of alkaline, saline, and mixed saline-alkaline stresses with regard to their effects on growth, nutrient accumulation, and root morphology of *Lotus tenuis*. Journal of Plant Growth Regulation. 31(3):448-459.

Peng, Y.L., Gao, Z.W., Gao, Y., Liu, G.F., Sheng, L.X. and Wang, D.L. 2008. Eco-physiological characteristics of alfalfa seedlings in response to various mixed salt-alkaline stresses. Journal of Integrative Plant Biology. 50(1):29-39.

Pilon, M., Ravet, K. and Tapken, W. 2011. The biogenesis and physiological function of chloroplast superoxide dismutases. Biochimica and Biophysica Acta (BBA)- Bioenergetics. 1807:989-998.

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(6):1275-1285.

Rahman, M.A., Parvin, M., Das, U., Ela, E.J., Lee, S.H., Lee, K.W. and Kabir, A.H. 2020. Arbuscular mycorrhizal symbiosis mitigates iron (Fe)-deficiency retardation in alfalfa (*Medicago sativa* L.) through the enhancement of Fe accumulation and sulfur-assisted antioxidant defense. International Journal of Molecular Science. 21:2219.

Sun, Y.L. and Hong, S.K. 2011. Effects of citric acid as an important component of the responses to saline and alkaline stress in the halophyte *Leymus chinensis* (Trin.). Plant Growth Regulation. 64(2):129-139.

Tahjib-Ul-Arif, M., Zahan, M.I., Karim, M.M., Imran, S., Hunter, C.T., Islam, M.S., Mia, M.A., Hannan, M.A., Rhaman, M.S., Hossain, M.A., Brestic, M., Skalicky, M. and Murata, Y. 2021. Citric acid-mediated abiotic stress tolerance in plants. International Journal of Molecular Sciences. 22(13):7235.

Wu, Z.H., Yang, C.W. and Yang, M.Y. 2014. Photosynthesis, photosystem II efficiency, amino acid metabolism and ion distribution in rice (*Oryza sativa* L.) in response to alkaline stress. Photosynthetica. 52:157-160.

Zhang, J.T. and Mu, C.S. 2009. Effects of saline and alkaline stresses on the germination, growth, photosynthesis, ionic balance and anti-oxidant system in an alkali-tolerant leguminous forage *Lathyrus quinquenervius*. Soil Science and Plant Nutrition. 55(5):685-697.

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