Exploration of ecological benefits of *Conyza Canadensis* — analysis and evaluation of the effects of *Conyza Canadensis* water extract, ascorbic acid, and gibberellin on the salt tolerance to two crops at seedling stage

Qiuyue He[1], Tiangang Xu[2], Dongming Wang[2], Wenyuan Li[1], Chunjuan Gong[1], Jing Li[1,*]

[1]School of Resource and Environmental Engineering, Shandong Agriculture and Engineering University, Jinan, Shandong, 250100, China
[2]Zibo Wellcell Biotechnology Co., LTD, Zibo, Shandong, 256302, Chhina

*Corresponding author E-mail: ripplelj@126.com

Abstract. This study aimed to investigate the effects of various exogenous additives on crop salt tolerance, utilizing sorghum and wheat as model plants. We measured soluble protein content, malondialdehyde (MDA) content, and peroxidase (POD) activity to elucidate the response characteristics of these crops to different exogenous substances: ascorbic acid, gibberellin and *Conyza canadensis* water extract (CCE). Additionally, we employed the membership function method to analyze and evaluate the impact of these three additives on salt-tolerant in seedling stages. Under conditions of salt stress, all three exogenous substances significantly enhanced soluble protein and POD levels ($P<0.05$) while effectively reducing MDA content. Following the analysis and evaluation of salt-tolerant, the hierarchy of crop salt-tolerant after the application of three exogenous substances is as follows: wheat > sorghum. Although the extent of improvement varied among treatments, all three exogenous substances markedly increased salt-tolerant in both crop seedlings ($P<0.05$). Therefore, from the perspective of resource recycling, the application of CCE in improving crop salt-tolerant would be more conducive to agricultural input reduction and current support for carbon emission reduction. From these points of view, *Conyza canadensis* is of great ecological value.

Keywords: salt stress; *Conyza Canadensis*; ascorbic acid; gibberellin

1 Introduction

Soil salinization has become a typical representative of the current global environmental issues. As a major agricultural country, China has a wide distribution of saline-alkali land, which has seriously impacted agricultural development. The high concentration of salt in saline-alkali soil reduces the soil water potential, resulting in physiological water shortage for plants. At the same time, the accumulation of a large number of

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metal ions in plants inhibits the physiological and biochemical metabolic processes of plants, eventually causing plant death [1].

Enhancing the salt resistance of plants has emerged as a crucial factor influencing crop yields. To counter salt stress, plants have generated various salt-resistant mechanisms. Osmotic adjustment, ion equilibrium, and the balance of reactive oxygen species metabolism are the main mechanisms for plant salt-tolerant. Moreover, numerous studies have discovered that the addition of exogenous substances has the ability to promote and enhance the salt resistance of plants. For instance, the addition of hormones, resistant substances, etc. Ascorbic acid (AsA), also known as vitamin C, is a kind of antioxidant. AsA can eliminate free radicals within cells. The addition of exogenous ascorbic acid can accelerate the cell division process of plant cells, promote cell elongation, and facilitate the synthesis of plant cell walls by promoting the synthesis of substances such as pectin, sugar, and protein, thereby maintaining the normal growth of plants and enhancing their stress resistance [2]. Gibberellin is a significant member of the six endogenous hormones in plants. It can promote seed germination and the growth of stems and leaves of plants. The exogenous addition of gibberellin can also enhance the activity of antioxidant enzymes in plants and accumulate osmotic adjustment substances, effectively mitigating the influence of external stress on plants [3].

Meanwhile, allelochemicals released by plants, either indirectly or directly to the outside world, can also exert influences on the development of agricultural plants. For instance, the extracts from the fresh leaves of walnuts inhibits the germination of wheat and cucumber seeds, but promotes that of mung bean seeds [4]; allelopathy of Actinidia chinensis leaf extract can can facilitate the germination of garden cosmos, ryegrass and alfalfa, but have inhibitory effects on white clover [5]. In our earlier research, we discovered that *Conyza canadensis* water extract (CCE) primarily exhibited inhibitory allelopathic effects on the germination of crop seeds; however, at lower concentrations, CCE was observed to promote both plant height and fresh weight in lettuce and wheatgrass. [6]; in the research on the influence of CCE on the salt-tolerant of crops, it was discovered that low CCE concentration can increase the germination rate of corn, sorghum, wheat, tomato, etc. under salt stress [7]. However, how CCE affects the salt-tolerant of crops during the seedling stage has not been further investigated. Additionally, from the perspective of the development and utilization of waste resources, conducting related research on the resource utilization of the widely distributed *Conyza canadensis* is also a highly valuable matter.

Therefore, in this study, sorghum and wheat were selected as the research subjects. Under salt stress conditions, the effects of CCE, gibberellin (GA_3), and AsA on the seedling stage of sorghum and wheat were analyzed to understand their influences on the salt-tolerant of crops and to compare the magnitudes of the effects of CCE, GA_3, and AsA on the salt tolerance in crops. Thus, more data support is provided for the resource utilization of *Conyza canadensis*, and it is even more expected to open up a broader space for the resource utilization of *Conyza canadensis*.

2 Materials and Methods

2.1 Plant Material

Sorghum and wheat were used in the study, and the seeds of them were purchased from seed stations.

2.2 Design and Measurement

Full-grain, uniform-sized seeds were selected and disinfect them using 75% ethanol. Subsequently, rinse with distilled water and allow the seeds to soak for 24 hours prior to planting. After the seeds germinate, select wheat/sorghum plants with similar growth status and transplant them into pots (15 cm × 12 cm × 10 cm) with 5 seeds per hole, with four holes per pot and five plants per hole. Each treatment was established with three replicates. A total of five treatments were designed (see Table 1). Starting from the fourth day after transplanting, the treatment solution was applied, with 80 ml per day. Throughout the cultivation period, irrigation was adjusted as necessary based on soil moisture content. Physiological indicators were measured on days 7, 9, and 11 following transplantations.

Seedling leaves were randomly sampled (3~5 replicates) to determine the soluble protein content, peroxidase (POD) activity content and malondialdehyde (MDA). Soluble protein content was determined by Biuret method, POD activity was determined by guaiacol method, and MDA content was determined by Thiobarbituric Acid method [8].

Table 1. Experimental design

Treatments	Culture Solution
CK	Deionized Water
T1	V(NaCl, 0.1mol/L)/V(Deionized Water)=1:1
T2	V(NaCl, 0.1mol/L)/V(AsA, 50mg/L)=1:1
T3	V(NaCl, 0.1mol/L)/V(GA_3, 50mg/L)=1:1
T4	V(NaCl, 0.1mol/L)/V(CCE, 30g/L)=1:1

2.3 Evaluation of Salt Tolerance

Salt tolerance was assessed through the membership function approach derived from fuzzy mathematics, which determines membership function values (MFVs) for each indicator across different treatments. To comprehensively evaluate the salt tolerance of crops, average MFV values were calculated across various treatments [9]. A higher average value signifies enhanced salt resistance.

The MFV for salt-tolerant is determined using the following formulas. Formula 1 is applied when the measured index is positively correlated with salt tolerance, and Formula 2 is used when the correlation is negative.

$$X(\mu) = \frac{X - X_{MIN}}{X_{MAX} - X_{MIN}} \quad (1)$$

$$X(\mu) = 1 - \frac{X - X_{MIN}}{X_{MAX} - X_{MIN}} \quad (2)$$

Here, X(μ) is the MFV of the μth indicator, X is the measured value of an indicator, and X_{MAX} and X_{MIN} represent the maximum and minimum value of the indicator, respectively.

2.4 Statistical Analysis

Data are presented as means ± SE (standard error). Statistical analyses for significant differences were conducted by SPSS Version 16.0, employing One-Way ANOVA and Duncan's test ($P < 0.05$).

3 Results

3.1 Impact of Various Treatments on the Salt Tolerance of Sorghum and Wheat During the Seedling Stage

Figure 1 shows the changes in the soluble protein content of wheat and sorghum seedlings on days 7, 9 and 11 of culture after transplantation to the pot. The figure shows that the soluble protein content of the treatment except for the control, essentially increases gradually as the stress time is extended.

The 11th day of seedling transfer into the pot was the last day of crop physiological index measurements. We analyzed the differential significance of the data measured on day 11 to understand the salt tolerance and adaptability of the crops under different treatment conditions after a certain amount of salt stress. The data showed that the soluble protein of two crops under different treatments was significantly higher than that of the control ($P<0.05$). Meanwhile, after the addition of three exogenous substances, the soluble protein levels in the two crops were notably greater compared to those observed under individual salt stress ($P<0.05$).

Figure 2 shows the changes in malondialdehyde (MDA) content of wheat and sorghum seedlings on days 7, 9 and 11 of culture after planting. The figure shows that, for both crops, the trends in MDA content for each treatment condition are generally consistent with the trends in soluble protein content as the stress time is extended. Analysis of data from day 11 showed that MDA content were significantly higher in the two crops treated with a individual salt compared to controls and other treatments. In addition, with the addition of three exogenous substances, the MDA content in sorghum was higher than in the control, but significantly lower than in the individual salt stress ($P<0.05$). The MDA levels in wheat were significantly lower than in the control after the addition of either AsA or GA_3, and could also be reached no significant difference from the control after the addition of CCE.

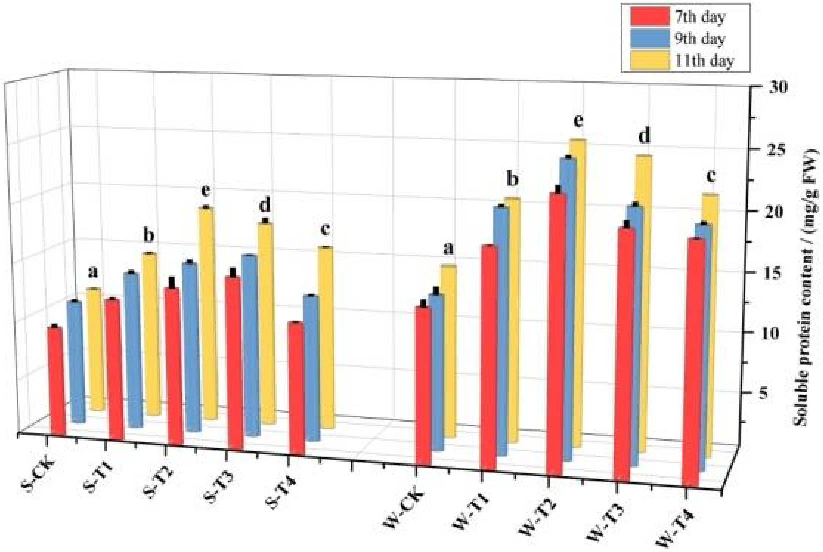

Fig. 1. Responses of soluble proteins of sorghum (S-CK, S-T1, S-T2, S-T3, S-T4) and wheat (W-CK, W-T1, W-T2, W-T3, W-T4) seedlings to different treatments (Table 1). The culture medium was watered daily with 80ml per pot, and soluble protein was measured on the 7th, 9th and 11th days after planting. The treatment (CK, T1, T2, T3 and T4) is shown in Table 1. Error bars represent standard errors of soluble protein content with 3~5 replicates measurements. The same letter represents no significant difference in the 11th day's soluble protein content of crops (Duncan test, $P < 0.05$, $n = 3\sim5$). The same below.

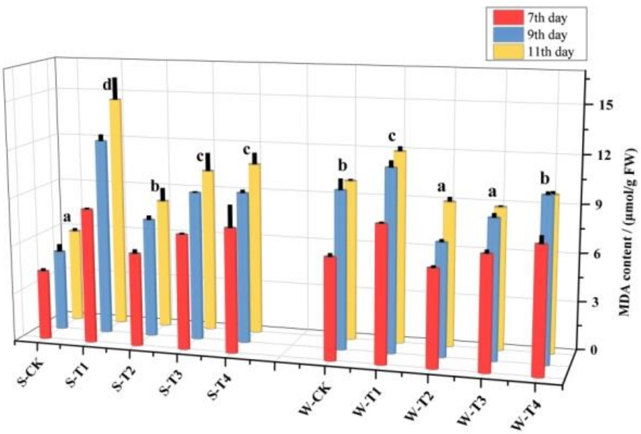

Fig. 2. Responses of malondialdehyde (MDA) of sorghum (S-CK, S-T1, S-T2, S-T3, S-T4) and wheat (W-CK, W-T1, W-T2, W-T3, W-T4) seedlings to different treatments (Table 1).

Figure 3 shows the changes in POD activity of wheat and sorghum seedlings on days 7, 9 and 11 of culture after planting. This figure shows that the overall POD activity is higher for wheat than for sorghum. For both crops, the trends in POD activity for each treatment condition were generally consistent with the trends in the soluble protein and MDA content as the stress time is extended. According to the analysis of the data on the 11th day, the POD activity in the two crops was markedly elevated compared to the control across all treatment condition ($P<0.05$). After the addition of three exogenous substances under salt stress, POD activity in both crops was significantly greater across all treatment conditions compared to that observed with individual salt stress, except for W-T4 (the CCE added in wheat).

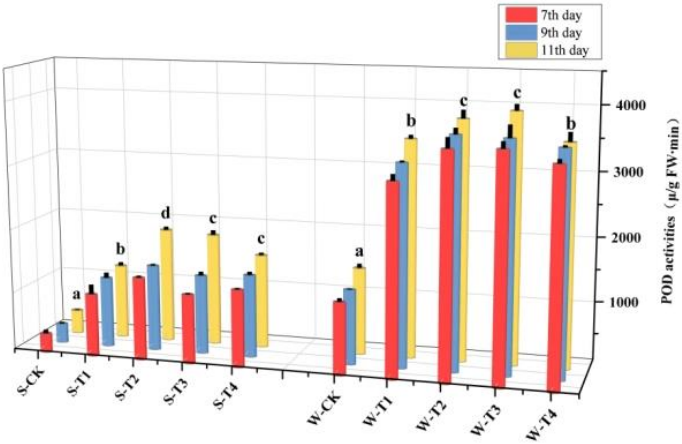

Fig. 3. Responses of peroxidase (POD) activity of sorghum (S-CK, S-T1, S-T2, S-T3, S-T4) and wheat (W-CK, W-T1, W-T2, W-T3, W-T4) seedlings to different treatments (Table 1).

3.2 Evaluation of How Various Treatments Influence the Salt Tolerance of Crops

Table 2 shows the MFVs and their mean MFVs for the physiological indicators of wheat and sorghum at the seedling stage under different treatment conditions. Under stress, plants improve their tolerance by increasing the amount of soluble proteins and POD, and thus are positively correlated with salt tolerance. Therefore, Eq.1 is used to calculate the MFV. MDA represents the damage of plant cell membrane when stress occurs, the higher the MDA content, the more severely damaged to the cell membrane system. This MDA is negatively correlated with salt tolerance and the MFV is obtained using Eq.2.

In terms of species, Table 2 shows that wheat has higher mean MFVs than sorghum under each treatment. The average MFVs for both crops following the application of three exogenous substances are greater than those observed under individual salt stress. Under each treatment, the resistance is ranked of MFVs of wheat and sorghum as T2>T3>T4>T1.

Table 2. Membership function values (MFVs) of various indicators of sorghum and wheat seedlings under different treatments and their salt tolerance evaluation

Crops	Treat-ments	MDA MFV	Soluble Protein MFV	POD MFV	Mean MFV
Sorghum	T1	0.133	0.235	0.221	0.197
	T2	0.757	0.519	0.395	0.557
	T3	0.554	0.445	0.385	0.461
	T4	0.500	0.325	0.307	0.377
Wheat	T1	0.384	0.651	0.841	0.625
	T2	0.671	0.988	0.933	0.864
	T3	0.687	0.913	0.972	0.857
	T4	0.599	0.715	0.852	0.722

* The treatment (T1, T2, T3 and T4) is shown in Table 1.

4 Discussion

Soil salinization has a significant impact on current agricultural production and food security. The accumulation of salts in the soil reduces the plant's ability to absorb water and nutrients, leading to osmotic stress or water deficiency, thus inhibiting plant growth. Ascorbic acid (AsA) is a typical antioxidant that regulates the plant's oxidation-reduction reactions and energy metabolism to remove free radicals from the cell, allowing the plant to grow normally. Gibberellin can promote cell growth and division, thereby promoting seedling growth and development. Allelopathic substances can change the micro-environment after entering the environment, thus affecting the growth and development of surrounding plants.

The soluble proteins, MDA and POD, commonly used in resistance studies, were selected as indices to evaluate salt tolerance in seedling experiments. Soluble proteins are key indicators of plants' osmotic adaptability and serve as important markers for stress responses [10]. Under stress conditions, plants adjust the content of soluble substances to change osmotic pressure, maintaining cell membrane stability and ensuring normal enzyme activity for water intake. MDA is a primary product of membrane lipid peroxidation and its level reflects the extent of cellular damage [10]. Higher MDA levels indicate greater damage to the cell membrane system. POD is an antioxidant enzyme present in plant tissues that reduces peroxide damage by catalyzing their decomposition, thereby enhancing stress resistance [10]. Under stress conditions, increased POD synthesis accelerates the removal of reactive oxygen species and helps maintain oxidative balance while reducing plant damage.

As shown in Figs. 1-3, the gradual increase in the content/activity of the three indices for sorghum and wheat with prolonged stress time indicates that both crops exhibit sustained resistance and can maintain normal survival during the study period. To assess seedling adaptability to stress, data from day 11 of seedling culture revealed that soluble protein content and POD activity in sorghum and wheat were significantly higher than those of the control under individual salt stress, further confirming their inherent salt resistance. Following the addition of three exogenous substances, both soluble protein content and POD activity exceeded levels observed under individual salt stress, as did their membership function values. MDA analysis revealed that the MDA levels were considerably elevated under individual salt stress in comparison to the control; however, adding these exogenous substances markedly reduced MDA levels in both crops under salt stress, suggesting they effectively alleviate membrane damage caused by salinity. Therefore, these three exogenous substances, including CCE, enhance salt resistance in sorghum and wheat at the seedling stage.

From the perspective of species, combined with the evaluation results of the two crops (Table 2), we found that exogenous substances were better at improving the salt tolerance of wheat than sorghum.

Regarding the impact of three exogenous substances on enhancing crop salt resistance, CCE was found to be less effective than AsA and GA_3; however, it still significantly boosted the salt tolerance of both sorghum and wheat ($P<0.05$). It is noted that when CCE concentration exceeds 25g/L, plant growth can be adversely affected [11,12]. These findings highlight the constraints posed by CCE on plant development. In this study, treatment T4 involved a combination of salt solution and CCE, with an actual application concentration of only 15g/L under salt stress conditions. At this level of CCE concentration combined with salt stress, improvements in crop salt tolerance were evident. Therefore, CCE, which can improve crop salt tolerance, should be considered for further ecological studies.

5 Conclusions

This study employed the membership function to evaluate the salt-tolerant of two crops under salt stress, supplemented with CCE, AsA, and GA_3. The results indicated that wheat exhibited greater salt tolerance than sorghum. AsA, GA_3 and CCE were found to significantly enhance the salt resistance in seedlings of sorghum and wheat.

The allelopathic compounds from *Conyza canadensi*s positively influence seedling salt tolerance, providing a new perspective on the ecological significance of this species. Considering the practical application, as a pioneer species/invasive alien species widely distributed and ubiquitous, the collection and extraction of the extract (using water extract) of *Conyza canadensis* are relatively easy to operate, without excessive human and material resources and financial investment. Therefore, for the three exogenous inputs to agricultural production, the application of CCE in improving crop salt-tolerant would be more conducive to agricultural input reduction and current support for carbon emission reduction. From these points of view, *Conyza canadensis* is of great ecological value.

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References

1. Moez H, Chantal E, Mariama N, et al. New Insights on Plant Salt Tolerance Mechanisms and Their Potential Use for Breeding. Frontiers in Plant Science, 2016, 7:1787. DOI: 10.3389/fpls.2016.01787
2. Zhou F., Xu D., Xiong S.,et al.Inhibition of wound healing in fresh-cut potatoes by ascorbic acid is associated with control of the levels of reactive oxygen species and the AsA-GSH cycle. Scientia horticulturae, 2024:323.
3. Zhu K., Liu H.J., Li B. Study on the Alleviating Effects of Exogenous Gibberellin on Alfalfa Seedlings under Salt Stress. Journal of Yunnan Agricultural University (Natural Science). 2022,37(6):926–931.
4. Zhang F.Y., Zhai M.Z., Jia C.X., et al. Allelopathic Study on Volatile Oil from Fresh Walnut Leaf. Journal of Northwest Forestry University. 2005,20(2):144-146.
5. Lu Y.P., Huang G.H., Gao Z., et al. Allelopathy of *Actinidia chinensis* leaf extract on four grass species and identification of its allelopathy substances. 2023,51(22):237-244.
6. Wang X.Q., Chen J.H., Li W.D., et al. Allelopathic Effects of *Conyza canadensis* Water Extract on Seed Germination and Seedling Growth. Asian Journal of Agricultural and Horticultural Research. 2023,10(4):81-87.
7. Wang Q.Q., Li M.H., Cao X.T., et at. Exploration of ecological benefits of *Conyza Canadensis* — analysis and evaluation of the effects of Conyza Canadensis water extract, ascorbic acid, and gibberellin on the salt tolerance to several crops at seed germination. E3S Web of Conferences. 2024,536.
8. Wang X.K. Principles and Techniques of Plant Physiologic Biochemical Experiment (2nd Edition). Higher Education Press, 2006.
9. Xie Y., Liu X., Amee M., Yu H., Huang Y., Li X., Chen L., Fu J., Sun X. Evaluation of Salt Tolerance in Italian Ryegrass at Different Developmental Stages. Agronomy, 2021, 11, 1487.
10. Ren Z.X., Shi J.N., He J.X., Wang Y., Fan X.F., Li R.Z., et al. Effects of Salt Stress on Growth and Physiological Characteristics of *Carex leucochlora*. Acta Agrestia Sinica, 2022,6,14.
11. Hu G., Zhang Z.H. Aqueous Tissue Extracts of Conyza canadensis Inhibit the Germination and Shoot Growth of Three Native Herbs with No Autotoxic Effects. Planta Daninha, 2013, 31(4):805-811.
12. Shaukat S.S., Nadia M., Siddiqui I.A. Allelopathic Responses of *Conyza canadensis* (L.) Cronquist: A Cosmopolitan Weed. Asian Journal of Plant Sciences, 2003, 14(14).