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

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Abstract: Soil salinity poses a major threat to plant growth and food security. Seed germination are more sensitive to salinity stress. In this study, sorghum, wheat, tomato and peanut were used to analyze and evaluate (using the membership function method) the salt tolerance at the germination with three exogenous substances - *Conyza Canadensis* water extract (CCE), ascorbic acid (AsA), and gibberellin (GA_3). At the germination stage, the germination rate, the germination energy and the germination vigor index are measured and the membership function values (MFVs) are calculated. After salt-tolerant analysis and evaluation, the sequence of salt tolerance is sorghum ≈ wheat > tomato > peanut. AsA and GA_3 can significantly improve the salt tolerance of crops($P<0.05$). Interestingly, CCE can also improve the salt tolerance in germination stage (sorghum, wheat and tomato) ($P<0.05$). Therefore, *Conyza Canadensis* has shown ecological benefits in improving the salt tolerance of some crops.

1. Introduction

Soil salinization, one of the world's major environmental problems, has been aggravated by the current warming climate [1]. Soil salinization has severely affected soil quality, land ecological processes, plant and animal growth and development, resulting in lower crop yields.The adverse effects of soil salinization on crop productivity add to food security problems.Although the management of salinized land continues, the area of salinized land continues to increase year-on-year. Therefore, it is important to strengthen the development and utilization of salt-tolerant plants for the adjustment of the farming industry, the improvement of the ecological environment, the increase of the income of farmers, the increase of crop yields and the promotion of sustainable development of regional agriculture.

The study and evaluation of plant salt tolerance can be carried out from various stages in the life history of the plant. The seed germination of plants are important periods for studying plant salt-tolerance [2-5]. A large amount of metal ions accumulated in the plant body cause toxicity to the plant, thus affecting the physiological and biochemical metabolism process of the plant, limiting the growth and slow development of the plant, and ultimately reducing the biomass [2]. The negative effects of saline soil environments on plants, especially on the growth and development of crops, severely affect the development of agriculture and are detrimental to the development of global food security.

Fortunately, the addition of some exogenous substances will improve the salt tolerance of plants to varying degrees [3,4]. The accumulation and scavenging ability of reactive oxygen species (ROS) in plants under salt stress are affected by a variety of endogenous and exogenous substances. Therefore, the application of exogenous substances to improve plant salt tolerance has attracted a lot of attention. Of course, different exogenous substances have different effects on plants. Some exogenous substances have positive effects [3,4,6,7], and some have negative effects [7,8]. In this study, ascorbic acid (AsA) and gibberellin (GA_3), which have typically positive effects on plant salt-tolerant, were selected. In addition, a plant whose effects on plant salt-tolerant have not been widely studied -- *Conyza canadensis* (taking *Conyza canadensis* water extract (CCE) as an example) was selected as the exogenous substance for the study. *Conyza canadensis*, an annual herb, is widely distributed throughout temperate regions. There is an agricultural disused area within the study area which is currently in the early stages of secondary succession. *Conyza canadensis* is the dominant species in this abandoned land. Several studies have classified the herb as an invasive plant, and as a result, more attention has been paid to the negative impact of *Conyza canadensis* has on ecosystem balance. For example, *Conyza canadensis* inhibit seed germination and growth of certain weeds, and enhance its allelopathic properties under salt stress [9]. Based on such relevant results, some

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*Corresponding author's e-mail: ripplelj@126.com scholars have been exploring and developing biological herbicides by using *Conyza canadensis* extract [10], or even directly using *Conyza canadensis* compost to prepare biological fertilizers that can simultaneously control grasses [11].

At the same time, some scholars have found that the extracts of *Conyza canadensis* contain antibacterial compounds. These compounds have antifungal activity against *Candida albicans*, *Ralstonia solanacearum*, etc., and can also be used in the control of fruit and vegetable post-harvest diseases [12]. So what is the effect on crops when CCE is used in agriculture as a herbicide? Does CCE show a positive or negative effect on crops under conditions where soil salinization is severe in the study area? In order to understand the influence of CCE on crops and maximize the development and utilization of salt-tolerant crops, combined with previous studies and the above questions, four widely planted crops (sorghum, wheat, tomato, peanut) were selected in this study. The salt tolerance of seeds was analyzed and evaluated under the influence of three different exogenous substances.

2. Materials and Methods

2.1. Plant Material

Four crops, sorghum, wheat, tomato and peanut, were used in the study. Crop seeds are purchased from seed stations,Zibo.

Full-grain, uniform-sized seeds were selected for each crop, sterilized with 75% ethanol for 1-2 minutes, and immediately washed several times with clean water. After soaking the seeds in clean water for 24 hours, blot them with filter paper.Then the seeds were evenly spread in a glass culture vessel with high temperature disinfection and filter paper, and incubated in a constant temperature incubator at 25°C.

2.2. Seed Germination

A total of 5 treatments were set up in the experiment (Table 1).

Conyza canadensis water extract was used in this study. The samples were one-month old *Conyza canadensis* seedings, which were collected from the abandoned agricultural field in the study area (Shandong Agriculture and Engineering University, Zibo, Shandong) in April.

CCE preparation: Wash off the plant seedlings with water and blot them with filter paper. The above-ground parts of plant were cut into small pieces for grinding (*Conyza canadensis* (fresh weight, g): deionized water (ml) = 1:10). After the grinding homogenate was filtered with gauze and centrifuged for 7 min (1700 RPM/min), the supernatant was taken as the mother liquor of CCE (100g/L) and stored at 4°C [13].

In the experiment, the mother liquor of CCE was diluted with distilled water to obtain the culture solution 2 (30g/L) in Table 1.

Table 1. Test processing settings

Treatment	Culture Solution 1	Culture Solution 2	Culture Solution 1/Culture Solution 2（V/V）
CK	Deionized Water		-
T1	Salt-NaCl, 0.1mol/L	Deionized Water	1/1
T2	Salt-NaCl, 0.1mol/L	AsA, 50mg/L	1/1
T3	Salt-NaCl, 0.1mol/L	GA3, 50mg/L	1/1
T4	Salt-NaCl, 0.1mol/L	CCE, 30g/L	1/1

Four crops were treated with three treatments of 50 seeds per replicate. During the seed germination stage, 20 ml of culture solution was quantitatively applied to each petri dish daily, and the culture solution was added at irregular times throughout the day according to the water consumption of the seed. A seed was considered germinated when the radicle emerged through the seed coat. The germination of the seed is regularly observed and recorded every day. The experiment of germination stage was 7 days.

To analysis the salt tolerance of crop seeds at the germination stage, germination rate (GR) (Equation 1), germination energy (GE) (Equation 2), and germination vigor index (GVI) (Equation 3) were determined [5]. These parameters were calculated using the following equation:

$$\mathrm{GR} = \frac{G_7}{\mathrm{N}} \times 100\% \tag{1}$$

$$\mathrm{GE} = \frac{\mathrm{G}_4}{\mathrm{N}} \times 100\% \tag{2}$$

$$\mathrm{GVI} = \sum \frac{\mathrm{G_T}}{\mathrm{T}} \tag{3}$$

In these equations, T is the number of days, G_T is the total number of seeds germinated on day T, G_4 and G_7 are the total number of seeds germinated on the 4th and 7th days, respectively,and N is the total number of seeds.

2.3. Evaluation of Salt Tolerance

The salt resistance was evaluated using the membership function method of fuzzy mathematics. The method calculates the membership function values (MFVs) (Equation 4) of each measured indicator in each treatment condition. In order to comprehensively assess the crops salt-tolerant, the mean values of the membership functions of each of the measured indicators were obtained for different treatment conditions [8,10]. The higher the mean value, the stronger the salt tolerance. The membership function value for salt tolerance is calculated as follows.

$$X(\mu) = \frac{X\text{-}X_{MIN}}{X_{MAX} - X_{MIN}} \tag{4}$$

Here, $X(\mu)$ is the membership function value of the μth indicator (E.g. GR, GE, etc), X is the measured value of an indicator, and X_{MAX} and X_{MIN} represent the maximum and minimum measured value of the indicator, respectively.

2.4. Statistical analysis

Data were expressed as the means ± SE (standard error). Statistical analyses of significant differences were performed using SPSS Version 16.0 software, One-Way ANOVA, Duncan test ($P<0.05$).

3. Results

3.1. Effects of different treatments on salt tolerance of four crops at germination stage

Table 2 shows the germination rate (GR) results for the four crops under different treatment conditions. As can be seen from Table 2, under single salt solution stress (Table 1, T1), GR of the four crops were significantly decreased compared with the control ($p<0.05$). GR of tomato and peanut were reduced by more than half (64.71% and 64.86%, respectively). The seed germination of four crops showed different characteristics after the addition of exogenous substances. After adding AsA (Table 1, T2), the GR of four crops was significantly higher than that under single salt stress, and there was no significant difference between sorghum, wheat and tomato and the control. The GR of tomatoes can be as high as 208.35% under single salt stress after AsA treatment. After adding GA_3 (Table 1, T3), the GR of sorghum, wheat and tomato was significantly higher than that of single salt stress ($p<0.05$), the GR of sorghum and wheat was not significantly different from that of the control, and the GR of tomato was even higher than that of the control (up to 1.35 times) and showed significant difference ($p<0.05$). Peanuts with GA_3 added had a higher GR than those under single salt stress, but no significant difference was observed. The GR of sorghum, wheat and tomatoes after CCE was added (Table 1, T4) was significantly higher than those under single salt stress. However, the GR of the peanuts was slightly lower than that of the single salt stress, but the difference was not significant.

As a result, the GR of crop seeds under salt stress was improved by three thexogenous substances, except for peanut treated with CCE.

Table 2 Effects of different exogenous substances on the germination rate of four crops under salt stress

Crops	Germination Rate / %				
	CK	T1	T2	T3	T4
Sorghum	95.33±4.16a	78.00±2.00b	95.33±5.03a	93.33±4.16a	89.33±6.43a
Wheat	93.33±4.36a	80.95±1.65b	89.52±1.65ac	86.67±4.36abc	84.76±4.36bc
Tomato	56.67±7.64a	20.00±8.66b	61.67±7.64a	76.67±7.64c	36.67±2.89d
Peanut	82.22±10.18a	28.89±10.18b	55.56±10.18c	37.78±3.85b	26.67±11.55b

*Error bars represent standard errors of the germination rate (GR) with 3 replicates measurements. The same letter in each line represents no significant difference (Duncan test, $P < 0.05$, $n = 3$). Same below.

Table 3 shows the germination energy (GE) results for the four crops under different treatment. As can be seen from Table 3, the GE of the four crops decreased significantly ($p<0.05$) under single salt . The GE of tomato and peanut decreased more significantly (83.33% for tomato and 75.00% for peanut than that of sorghum and wheat, which was consistent with the results of GR in Table 2. After the application of the three exogenous substance, salt damage to seeds in sorghum, wheat, and tomato was significantly mitigated, and the GE of the three crops was able to achieve no significant difference from the control. But for peanuts, the GE was lower after adding GA_3 and CCE than at single salt stress, except for AsA, which significantly improved GE at salt stress.

Table 3 Effects of different exogenous substances on the germination energy of four crops under salt stress

Crops	Germination Energy/%				
	CK	T1	T2	T3	T4
Sorghum	94.67±5.03a	76.67±3.06b	94.00±6.00a	91.33±4.16a	88.67±7.57a
Wheat	89.52±5.95a	77.14±2.86b	86.67±1.65a	82.86±5.71ab	81.90±4.36ab
Tomato	40.00±10.00a	6.67±2.89b	33.33±7.64a	35.00±13.23a	23.33±7.64a
Peanut	71.11±13.88a	17.78±3.85b	35.56±7.7c	7.78±3.85b	13.33±6.67b

Table 4 shows the germination vigor index (GVI) results for the four crops under different treatment. As can be seen from Table 4, the GVI of four crops under single salt stress was lower than that of the control (decreased by 20.52% for sorghum, 14.73% for wheat, 74.32% for tomato and 69.26% for peanut). After adding AsA, the GVI of the four crops was significantly higher than that under single salt stress ($p<0.05$), and the GVI of sorghum, wheat and tomato was no different from that of the control. After applying GA_3, sorghum, wheat and tomato achieved no difference in GVI with respect to control but peanut. After adding CCE, the GVI of tomato was significantly higher than that of single salt ($p<0.05$), but lower than that of control ($p<0.05$). The GVI of sorghum and wheat increased slightly, but not significantly, compared to the case under single salt stress. Peanut has a lower GVI than single salt stress, but the difference is not significant.

Table 4 Effects of different exogenous substances on the germination vigor index of four crops under salt stress

Crops	Germination Vigor Index				
	CK	T1	T2	T3	T4
Sorghum	45.56±1.42a	36.21±0.28b	44.30±2.77a	43.44±1.60a	39.18±2.15b
Wheat	41.22±2.86a	35.15±1.42a	42.21±1.29a	39.08±5.22a	36.19±6.40a
Tomato	7.71±1.31a	1.98±0.82b	7.86±1.22a	9.14±1.42a	4.41±0.76c
Peanut	11.94±0.35a	3.67±0.95b	6.66±0.89c	4.37±0.79b	2.99±1.16b

3.2. Evaluation of the effect of different treatments on the salt tolerance of crops at the germination

Table 5 shows the membership function values (MFVs) and their mean values with respect to seed germination for the four crops under different treatment conditions. The GR, GE and GVI of the seeds are all positively correlated with salt tolerance, so the MFVs are calculated using Eq.4. The MFVs for each index were calculated for different treatment conditions and then the mean MFV was obtained. The larger the value, the stronger the salt tolerance. From the perspective of species, according to the mean value of membership function, under single salt treatment, the order was wheat ≈ sorghum > peanut > tomato. After adding three exogenous substances, all the order was sorghum > wheat > tomato > peanut.From the perspective of treatment conditions, after the addition of exogenous substances under salt stress, the mean MFVs of sorghum, wheat and peanut under each treatment was ranked as T2 > T3 > T4, but the mean MFV of tomato was T3 > T2 > T4.

From the exogenous substances point of view, the addition of AsA and GA_3 under salt stress increased the mean MFVs of the four crops, and CCE showed a positive effects on sorghum, wheat and tomato, but not on peanut. After the addition of the CCE, the MFVs and the calculated mean MFVs for each index of the peanut are lower than the values at the single salt stress.

Table 5. Membership function values (MFVs) and salt tolerance evaluation of four crop germination indicators under salt stress by different exogenous substances

Crops	Treatment	MFV of GR	MFV of GE	MFV of GVI	Mean MFV
Sorghum	T1	0.756	0.754	0.757	0.756
	T2	0.948	0.937	0.931	0.939
	T3	0.926	0.909	0.913	0.916
	T4	0.881	0.881	0.821	0.861
Wheat	T1	0.788	0.759	0.734	0.761
	T2	0.884	0.860	0.886	0.876
	T3	0.852	0.820	0.819	0.830
	T4	0.831	0.810	0.757	0.799
Tomato	T1	0.111	0.018	0.020	0.050
	T2	0.574	0.298	0.147	0.340
	T3	0.741	0.316	0.174	0.410
	T4	0.296	0.193	0.072	0.187
Peanut	T1	0.210	0.135	0.057	0.134
	T2	0.506	0.322	0.121	0.316
	T3	0.309	0.135	0.072	0.172
	T4	0.185	0.088	0.042	0.105

4. Discussion

Soil salinization has severely impacted current agricultural production and food security. A toxic buildup of sodium ions in the cell's cytoplasm can cause an imbalance of ions in the plant. Therefore, the accumulation of salt in soil will inhibit plant growth and reduce the absorption capacity of water and nutrients, leading to osmotic stress or water shortage [2]. In order to adapt to the effects of salt stress, the regulation of reactive oxygen metabolism balance, hormones etc. in crops plays an important role [4].

The most typical period of plant resistance to adversity is the seed germination stage. The seeds'response to salt at the germination stage partly reflects the overall salt tolerance of the plant [4]. The results showed that the seeds of the four crops showed symptoms of salt damage under single salt stress. According to the analysis and evaluation by membership function method, the salt resistance of four crops was sorghum ≈ wheat > peanut > tomato. Through the analysis of interspecific differences, the salt tolerance of group A (sorghum and wheat) was significantly higher than that of group B (peanut and tomato) ($P<0.05$).

Ascorbic acid (AsA) is a typical antioxidant, which can remove free radicals in cells by regulating REDOX reaction and energy metabolism in plants [3], so as to make plants grow naturally. In this study, it was found that AsA could improve significantly seed germination ability of four crops ($P<0.05$), which was consistent with the results of El-Beltagi et al. [3]. AsA promotes salt resistance in sorghum, wheat and tomato seeds more oblivious than in peanuts. Adding appropriate concentration of GA_3 can promote the regulation of seed germination and improve the plants salt-tolerant [14]. In this study, the same results were found, that is, GA_3 had a greater effect on sorghum and tomato, and could significantly improve the salt tolerance of seeds ($P<0.05$) but the peanut. The difference may be due to the crop variety or application concentration of gibberellin. Some studies have shown that excessive gibberellin concentration may inhibit seed germination ability [6].

Allelochemicals are secondary metabolites released by plants into the environment, which after entering the environment can alter the microenvironment and thus affect the growth and development of surrounding plants. Many allelopathic substances can significantly inhibit the germination of plant seeds, such as *Solidago canadensis*, ginger and other water extracts, etc. [8]. However, some allelopathic substances can promote seed germination of other plants, such as soybean, wheat and other plant extracts [7]. *Conyza canadensis*, the species of interest in this study, is the dominant species found in abandoned land in field surveys, and is readily available. Due to its characteristics of non-native species, rapid propagation, etc., the influence of allelochemicals on plants is mainly reflected in negative effects at present [9]. However, *Conyza canadensis* is classified as a pioneer plant, have shown that can enrich Mn, Cd, uranium and other pollutants in ecological restoration [15]. At the same time, an interesting result was discovered in this study. Through the determination of seed germination ability of four crops and the overall evaluation of membership function, it was found that the addition of CCE under salt stress could significantly improve the salt damage resistance ability of sorghum, wheat and tomato ($P<0.05$), which reflected the positive effect of CCE on some plants' resistance.

Some scholars have made attempts under the condition of applying CCE alone, but have shown negative allopathic effects. For example, when the CCE concentration is higher than 25g/L, the GR and growth of six crops (tomato, radish, corn, wheat, mung bean, and millet) are restricted [16]. These results indicate the limitations of CCE on plant growth. However, the treatment T4 used in this study was a mixture of salt solution and CCE. The actual concentration of CCE applied was only 15g/L. Under such conditions, improved salt tolerance of crops can be observed, for example, the GR and GE of sorghum can even achieve no significant difference from the control. These results suggest that CCE has a positive effect on improving crop resistance. Therefore, CCE should be considered for further ecological studies.

5. Conclusions

In this study, the membership function was used to comprehensively evaluate the salt tolerance of four crops under salt stress supplemented with CCE, AsA and GA_3. The salt tolerance of the four crops at the germination stage was sorghum ≈ wheat > tomato > peanut. AsA and GA_3 have been shown to significantly improve the salt tolerance in four crop seeds. And a good result is that the salt-tolerant at germination stage of sorghum, wheat and tomato could be improved by CCE.

In this study, *Conyza canadensis* allelopathic substances were shown to have a positive effect on seed germination, opening a new avenue to discuss the ecological value of *Conyza canadensis*. Combined with the ability of alleloids to remove other weeds [9], the dual role of *Conyza canadensis* (increasing crop resistance + suppressing weeds) could boost agriculture in saline-alkali areas, if more crops with increased salt tolerance due to CCE can be identified or the range of concentrations of extracts with positive effects of CCE on crop salt-tolerant may be explored. Therefore, the assessment of the ecological value of *Conyza canadensis* may require a more extensive discussion and analysis.

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