Effect of Stimulating Garden Cress Seeds with Distilled Water, Ascorbic Acid, and Citric Acid on Seedling Growth and Germination under Salt Stress Conditions

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Abstract: A factorial experiment with three factors was conducted in the laboratories of the College of Education for Pure Sciences, University of Diyala, following a completely randomized design with three repetitions. The experiment took place from January 15th to January 25th, 2025, to investigate the impact of soaking garden cress seeds in non-enzymatic antioxidants. The results demonstrated the superiority of soaking with distilled water, achieving the highest values in germination percentage, root length, shoot length, fresh weight of seedlings, and seedling vigor, recording 66.66%, 8.33 cm, 3.66 cm, 0.272 g, and 289 g, respectively. The most effective concentration was found to be 3NaCl combined with distilled water. Additionally, soaking in ascorbic acid significantly enhanced the germination percentage and dry weight of the seedlings, reaching 66.66% and 0.057 g, respectively, with irrigation using 3NaCl. On the other hand, soaking with citric acid and irrigation with 5NaCl resulted in the lowest values for all traits, indicating a negative impact on seedling development. These findings highlight the potential of using specific antioxidants in optimizing the growth and vigor of garden cress under saline conditions, providing insights for enhancing agricultural productivity.

1 INTRODUCTION

Garden cress seeds are considered ideal medicinal and nutritional seeds because they include medical components that protect the body, such as carotenoids, polyphenols, triterpenes, flavonoids, saponins, minerals, antioxidants, and various forms of sterols and alkaloids [1]. Garden cress belongs to the largest plant family, the Brassicaceae, which is found in many countries throughout the world, including the United States, Europe, and the Middle East. [3], [2]. Garden cress has several local names, including Cress, Cresson, and red seed, and it is scientifically known as (Lipidium Sativum). The primary benefit of garden cress is that it may grow at any time, in any location, and in any climate or soil condition. [4] Garden cress seeds can help avoid several ailments, including arthritis, liver disorders, bronchitis, high blood sugar, and high blood pressure [5]. Garden cress seeds can be used in a variety of cuisines.[6]. Its leaves are used in salads alongside other vegetables and as a spice to season foods [7]. It is also suitable as animal feed [8].

Ascorbic acid (vitamin C) is classified as an antioxidant sugar acid. It is also a vital vitamin for all living species, as well as a nutrient that the body requires in restricted quantities. It also helps limit flowering and the development of aging [9]. It comprises six carbon atoms produced from glucose sugar, and it plays a crucial function in photosynthesis in plants. Many fruits and vegetables, including lemons, oranges, peppers, and strawberries, contain high levels of this vitamin. Its solubility in water makes it an effective antioxidant [10].

It's also called the "vitamin of fresh food products" [11]. Vitamin C lowers the risk of many diseases since it is one of the body's mossoluble antioxidants, in addition to playing a key role in metabolic processes. It is also thought to function as a cofactor in the activation of numerous important enzymes in the body [12].

The Krebs cycle includes acids such as citric acid (C6H8O7). It is a 6-carbon tricarboxylic acid produced by the condensation of oxaloacetate in mitochondria (TCA). It is an intermediary in the tricarboxylic acid (CS) cycle, which is mediated by citrate synthase and acetyl CoA. It is one of the items with significant commercial value because of its wide application in foods, medical and pharmaceutical preparations, and others. [15], [14], [13].

Citric acid is used in a variety of applications, including chelation, derivatization, pH adjustment, buffer solutions, cosmetics, detergents, oil extraction, and more [16] The majority of citric acid is produced organically, primarily through fermentation, which includes molasses, coffee husks, wheat bran, and a variety of other fertilizer ingredients [19], [18], [17] It is the most efficient acid for energy production and plays an important role in plant physiological processes and functions [20].

Salt stress is the presence of excess amounts of ions in water and soil, as it reduces the percentage of water in the plant [21]. Salt stress is one of the most important physiological stresses that fundamentally affects seed germination and seedling growth, which in turn affects subsequent growth stages due to the accumulation or collection of dissolved salts in the soil, which leads to inhibition of germination as a result of the negative impact of the entry of some ions in large quantities that do not match the cell's needs, thus affecting the plant's vital processes [22]. Salinity has many negative effects on the germination process, as it reduces water absorption within the seeds due to the decrease in osmotic pressure within the seed [23]. The aim of the study is to demonstrate the effect of stimulating garden cress seeds with non-enzymatic antioxidants to reduce salt stress

2 MATERIALS AND METHODS

This study was carried out in the laboratories of postgraduate studies / Department of Biology / College of Education for Pure Science / University of Diyala from (15-1-2025) to (25-1-2025). The study used three elements to stimulate garden cress seedlings before planting: distilled water and non-enzymatic antioxidants (ascorbic acid and citric acid at 100 mg.L-1 for each treatment) for 24 hours. They were then placed in Petri dishes with a diameter of 12 cm and two opaque filter papers, each dish containing 15 garden cress seeds and irrigation with various sodium chloride (NaCl) concentrations (3 and 5 g. L-1). The Randomized Complete Design (CRD) was used with three repetitions for each treatment. After 10 days, the required characteristics for the study were studied.

2.1 Characteristics Studied

2.1.1 Standard Laboratory Germination Percentage(%)

The total number of natural seedlings was calculated after ten days of planting:

$$\textit{Germination percentage} = (\textit{number of germinated seeds} / \textit{total number of seeds}) \times 100 \quad (1)$$

2.1.2 Length of the Root and Shoot (cm)

The length of the root and shoot was measured at the start of the experiment, and the length of the root was measured after separating it from its point of contact with the seed, while the length of the shoot was measured after separating it from its point of contact with the stalk using a graduated ruler.

2.1.3 Dry Weight of the Seedling (g)

The same seedling used to measure the length of the root and shoot was used to examine the dry weight of the seedling, after which the seed coat was removed from each seedling and the embryonic axes were placed in perforated bags and dried in an electric oven at 80°C for 24 hours. After confirming that the weight was stable, they were weighed using a sensitive electric balance.

2.1.4 Fresh Weight of Seedling (g)

According to the fresh weight of the root and shoot using a sensitive balance on the tenth day from the beginning of the experiment.

2.1.5 Seedling Strength

Calculated using the following equation:

$$\text{Seedling strength} = \text{germination percentage (\%)} \times (\text{root length (cm)} + \text{shoot length (cm)}) \quad (2)$$

3 RESULTS AND DISCUSSION

3.1 Standard Laboratory Germination Percentage(%)

Table 1 shows that the soaking treatment with distilled water and ascorbic acid had a higher average germination percentage, with both at 66.66%, while the soaking treatment with citric acid had the lowest average germination percentage, at 36.66%. The average salinity of irrigation water varied significantly, with the highest average being 83.33 in irrigation with distilled water and the lowest being 33.33 in irrigation water with a NaCl concentration of 5 g.L-1. We conclude from this that the higher the salt concentration, the more negatively it affects the plant, resulting in a decrease in the course of metabolic activities and an increase in osmotic potential, hence limiting water absorption by the seeds [24].

The table also shows significant differences between the soaking materials and the salinity of irrigation water in the averages of the interaction for the germination percentage, as the highest average of the interaction was in the treatment of soaking with distilled water and ascorbic acid and irrigation with distilled water, which was 100.0, while the lowest average of the interaction was in the treatment of soaking with citric acid and irrigation with 5NaCl g.L-1, which was 20.00.

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.2 Root Length (cm)

The results in Table 2 show that the soaking treatment with distilled water was superior in the average root length, as the highest average was 8.33 cm, while the least significant difference was in the soaking treatment with citric acid in the average root length, which was 2.03 cm. The average salinity of irrigation water showed a significant difference, with the highest average recorded in irrigation with distilled water (4.86) and the lowest average recorded in irrigation water at a concentration of 5 g. L-1 of NaCl (1.36). Because salt stress hinders the essential vital activities required for root growth, it also impacts the suppression of plant growth, which is why the high concentration of NaCl resulted in a decrease in root length. This impact is brought on by a decrease in the plant's ability to absorb water, which raises the salt concentration in the absorption medium [25].

The table also demonstrates notable differences in the averages of the interaction of the root length between the soaking materials and the salinity of irrigation water. The soaking treatment with ascorbic acid and irrigation with distilled water had the highest average of the interaction, while the soaking treatment with distilled water, citric acid, and irrigation with 5NaCl g.L-1 had the lowest average of the interaction, which was 1.00.

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.3 Length of the Shoot (cm)

The results in Table 3 show that the soaking treatment with distilled water was superior in the average length of the frond, with the greatest average being 3.66 cm,while the soaking treatment with citric acid had the smallest significant difference in the average length of the frond at 1.50 cm. The average salinity of irrigation water varied significantly, with the highest average being 4.26 in irrigation with distilled water and the lowest being 1.33 at a concentration of NaCl 5 g. L-1. This effect is caused by citric acid, which is an antioxidant substance whose action is similar to the natural auxins that stimulate growth inside the plant [26].

The table also shows significant differences between the soaking materials and the salinity of the irrigation water in the averages of the interaction for the length of the rue, as the highest average of the interaction was in the treatment of soaking with distilled water and irrigation with distilled water, which was 5.50, and the lowest average of the interaction was in the treatment of soaking with citric acid and irrigation with NaCl5 g.L-1, which was 0.50. Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.4 Dry Weight of Seedling

The results in Table 4 show that the soaking treatment with ascorbic acid was superior in the average dry weight of the seedling, with the highest average being 0.057 g, while the soaking treatment with distilled water had the least significant difference in the average dry weight of the seedling at 0.018 g. The average salinity of irrigation water differed significantly, with the highest average in irrigation with NaCl3 g. L-1 being 0.059, while the least significant difference in the average salinity of water (irrigation with distilled water and irrigation with NaCl 5 g. L-1 was 0.044 for both). Ascorbic acid protects plants against environmental stressors like salt stress, Thus, it can be regarded an antioxidant in plants [27].

The interaction averages for the dry weight of the seedling also demonstrate notable differences between the soaking materials and the salinity of the irrigation water. The soaking treatment with ascorbic acid and citric acid and irrigation with 3NaCl g.L-1

Average Average had the highest interaction average (0.066), while the soaking treatment with distilled water and irrigation with 5NaCl g.L -1 had the lowest interaction average (0.026).

Table 1: The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average germination percentage% of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 mg.L^{-1}	Ascorbic acid 100 mg.L^{-1}	Distilled water 100 mg.L^{-1}	
83.33 A	50.00 b	100.0 a	100.0 a	Distilled water
53.00 C	40.00 c	60.00 a	60.00 a	3NaCl g.L^{-1}
33.33 B	20.00 d	40.00 a	40.00 a	5NaCl g.L^{-1}
	36.66 B	66.66 A	66.66 A	Average

Table 2:The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average root length (cm) of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 mg.L^{-1}	Ascorbic acid 100 mg.L^{-1}	Distilled water 100 mg.L^{-1}	
4.86 A	3.00 d	6.60 a	5.00 a	Distilled water
3.03 C	2.10 c	4.00 f	3.00 b	3NaCl g.L^{-1}
1.36 B	1.00 v	2.10 d	1.00 c	5NaCl g.L^{-1}
	2.03 A	4.23 C	8.33 A	Average

Table 3: The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average shoot length (cm) of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 mg.L^{-1}	Ascorbic acid 100 mg.L^{-1}	Distilled water 100 mg.L^{-1}	
4.26 A	2.30 a	5.00 c	5.50 a	Distilled water
2.56 C	1.70 b	2.50 e	3.50 v	3NaCl g.L^{-1}
1.33 B	0.50 c	1.50 s	2.00 d	5NaCl g.L^{-1}
	1.50 B	3.00 C	3.66 A	Average

Table 4: The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average dry weight (g) of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 mg.L^{-1}	Ascorbic acid 100 mg.L^{-1}	Distilled water 100 mg.L^{-1}	
0.044 B	0.033 a	0.053 b	0.046 a	Distilled water
0.059 A	0.066 a	0.066 a	0.046 a	3NaCl g.L^{-1}
0.044 B	0.053 a	0.053 c	0.026 a	5NaCl g.L^{-1}
	0.056 C	0.057 A	0.018 B	Average

Table 5: The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average fresh weight (g)of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 $mg.L^{-1}$	Ascorbic acid 100 $mg.L^{-1}$	Distilled water 100 $mg.L^{-1}$	
0.259 A	0.222 a	0.110 b	0.446 a	Distilled water
0.208 C	0.132 a	0.246 a	0.246 a	3NaCl $g.L^{-1}$
0.116 B	0.111 a	0.111 c	0.126 a	5NaCl $g.L^{-1}$
	0.155 B	0.155 A	0.272 A	Average

Table 6::The effect of stimulating garden cress seeds with non-enzymatic antioxidants on the average strength of the seedling and the interaction between them.

Average	Soaking materials			Irrigation water salinity concentration
	Citric acid 100 $mg.L^{-1}$	Ascorbic acid 100 $mg.L^{-1}$	Distilled water 100 $mg.L^{-1}$	
91.6 C	42 d	183 d	50.00 a	Distilled water
330 A	85.5 r	242 c	665 b	3NaCl $g.L^{-1}$
86.0 B	20.5 x	85.7 f	152 c	5NaCl $g.L^{-1}$
	49.3 B	170 C	289 A	Average

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.5 Fresh Weight of Seedling

The results in Table 5 show that the soaking treatment with distilled water was superior in the average fresh weight of the seedling, with the highest average reaching 0.272 g, while the soaking treatment with ascorbic acid and citric acid had the least significant difference in the average fresh weight of the seedling, with both reaching 0.155 g. The average salinity of irrigation water varied significantly, with the highest average in irrigation with distilled water reaching 0.259 and the least significant variation in the average salinity of irrigation water with NaCl being 5 g.L-1, reaching 0.116. Increasing the salt levels (concentrations) reduced the seedlings' fresh weight.

The table also shows significant differences between the soaking materials and the salinity of irrigation water in the averages of the interaction for the fresh weight of the seedling, as the highest average of the interaction was in the treatment of soaking with distilled water and irrigation with distilled water, which was 0.446, while the lowest average of the interaction was in the treatment of soaking with ascorbic acid and citric acid and irrigation with 5NaCl g.L-1, which was 0.026.

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

3.6 Seedling Strength

The results in Table 6 demonstrate that the soaking treatment with distilled water was superior in terms of average seedling strength g, with the greatest average reaching 289 g, while the soaking treatment with citric acid had the least significant difference in average seedling strength, reaching 49.3 g. The average salinity of irrigation water varied significantly, with the highest average at 330 with 3NaCl g. L-1 and the lowest at 86.0 with 5NaCl g. L-1. The explanation for this is due to variances in average shoot and root lengths caused by citric acid soaking. The table also shows significant differences between the soaking materials and the salinity of irrigation water in the averages of the interaction in seedling strength, as the highest average of the interaction was in the treatment of soaking with distilled water and irrigation with 3NaCl g. L-1, 665, while the lowest average of the interaction was in the treatment of soaking with citric acid and irrigation with 5NaCl g. L-1, 20.0.

Means with similar letters within the columns of single factors or the means of the interaction Duncan's multiple range test revealed that they are not substantially different at the 0.05 probability level.

4 CONCLUSIONS

This research effectively illustrated that treating garden cress seeds with non-enzymatic antioxidants, especially distilled water and ascorbic acid, notably reduces the negative impacts of salt stress on both seed germination and seedling growth. The experimental findings distinctly indicate that the maximum germination rate (100%), along with root and shoot lengths, seedling fresh weight (0.446 g), and seedling vigor (665 g), were observed in seeds soaked in distilled water and similarly irrigated, suggesting that water alone is critical for stimulating seed metabolism under non-stressed conditions

It was discovered that the higher the salt of irrigation water, the less germination of certain plants, including garden cress. The most effective type of irrigation is with distilled water. The results showed that seed stimulation with distilled water was the best in terms of average of all features except for the seedling's average dry weight, which had the lowest average of 0.018 cm, followed by ascorbic acid. Citric acid stimulation affected the majority of characteristics, including the germination %, where the lowest average was 36.66, The lowest average root length was 2.02 cm, and the best concentration of salinity in irrigation water after distilled water , it is irrigation with 3NaCl g. L-1. The use of antioxidants (ascorbic and citric acid) works to reduce salt stress on the plant, in addition to distilled water. Therefore, it is preferable to use other types of antioxidants to stimulate the seeds and at different concentrations before planting them in the soil.

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