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The effect of seed priming with allantoin and serotonin on germination and growth indices of bitter melon under salt stress

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ABSTRACT

Purpose: Salinity stress is one of the most important limiting factors affecting all plant processes, from seed germination to seedling development and plant growth. Seed priming is the most promising seed technique which quickens the germination process and enhances stand establishment. Therefore, seed priming with allantoin and serotonin was performed to investigate their effects on germination and growth of bitter melon seeds under salt. **Research method**: The experiment was conducted as a factorial experiment in a completely randomized design with three replications. The first factor consisted of three concentrations of salinity (0, 50 and 100 mM NaCl) and the second factor included seed priming (48 h) with modulators: control (distilled water), allantoin (1000 µM), serotonin (100 µM) and allantoin + serotonin. After 48 h of applying the priming treatments, the seeds were dried and placed in 9 cm diameter petri dishes lined with filter paper, moistened with the prepared salt solutions, and allowed to germinate at 25 °C. Germination percentage, seed vigor and selected growth characteristics including length, fresh and dry weight of seedling, shoot and root of bitter melon were evaluated. **Findings:** The results revealed that seed germination and seedling growth of bitter melon decreased in line with increasing salt concentrations: on the other hand, seed priming with allantoin (1000 µM) and serotonin (100 µM) alleviated this negative effect of salt stress. It was concluded that allantoin and serotonin, as seed priming agents, positively influenced bitter melon germination under salt stress. These findings may also be applicable to other plants and probably the observed stress tolerance might occur throughout the plant growth period. **Research limitations**: No limitations were identified. **Originality/Value:** Given the medicinal value of bitter melon, solving its germination problem, especially under salt stress conditions, will be of great help to those interested in cultivating this plant.

Keywords:
Allantoin, Germination percentage, Priming, Seed vigor, Serotonin

INTRODUCTION

Bitter melon or bitter gourd (*Momordica charantia* L.) is a tropical and semi-tropical annual vegetable from the Cucurbitaceae family, with distinctive nutritional and medicinal properties (Sheikhalipour et al., 2022). Medicinal impacts of bitter melon could be identified as prevention and treatment of different diseases including cancer, attributed to its antimicrobial and antioxidant properties (Sheikhalipour et al., 2023). Nutritionally high in vitamin C, fiber, iron (Fe), calcium (Ca), proteins, and carbohydrates (Bara et al., 2025), bitter melon also contains bioactive compounds including insulin-like peptides and triterpenoids (quarantin, momordicin) with demonstrated antidiabetic effects (Gayathry & John, 2022). Bitter melon is cultivated around the world and, like other plant species, is affected by salinity (Ostaci et al., 2024). Consequently, developing effective strategies to meliorate bitter melon seed germination and growth under saline environments could be of great importance.

According to literature, more than 20% of the world's agricultural lands and 33% of irrigated land are exposed to salinity stress (Mustafa et al., 2019). Salinity stress, as an important abiotic stress, has negative effects on the quantity and quality of agricultural products (Aydin, 2024). Salinity stress, through a decrease in soil osmotic potential and the accumulation of sodium (Na^+) and chlorine (Cl^-) ions, causes destruction of the plasma membrane and the structure of proteins and enzymes, as well as disruption of photosynthesis and ionic imbalance, which ultimately leads to reduced and stop plant growth (Wang et al., 2024). As the utmost imperative environmental stress, salinity has adverse effects on seed germination, the main and most important phase of life cycle of a plant where success directly determines subsequent plant establishment and quality (Biswas et al., 2023). Though salinity affects all phases of plant growth and development, its impact is particular severe during germination (Mwando et al., 2021), ascribed mostly to decreased germination percentage and rate and stopped seedling growth (Ahmed et al., 2014). Bitter gourd requires well-drained sandy to sandy loam soils for optimum growth, development, and germination, while its growth is retarded in extreme saline conditions. It is very sensitive to salinity stress, which imposes devastating limits on its productivity. Thus, the impact of soil salinization on the economics of bitter gourd yield deserves scientific inquiry (Ali et al., 2025).

To deal with salt stress, various methods have been utilized, among which seed priming is very effective (Zulfiqar et al., 2024). Seed priming method increases germination rate and leads to faster and more uniform emergence of seedlings especially in inappropriate conditions, such as salinity, thereby resulting in improved yield. Priming stimulates germination by inducing metabolic changes in seeds that promote rapid, uniform emergence and stress tolerance (Paul et al., 2024). Notably it improves seed germination percentage, germination indices, growth characteristics, and seedling establishment (Alam et al., 2023a). In tomato (*Solanum lycopersicum* L.), the results showed that seed priming, especially with PEG6000, significantly positively affected tomato root growth under salt stress (Habibi et al., 2025). Seed priming using indole-3-acetic acid (IAA) significantly improved maize (*Zea mays* L.) salt response and reflected by the increased seed germination dynamics, early seedling establishment (Ellouzi et al., 2024).

Allantoin is indeed a nitrogen-containing compound and a crucial intermediate in purine metabolism, particularly in plants. Allantoin accumulates in higher contents in plant cells under various stress like drought (Navarro-Morillo et al., 2024), salinity (Yu et al., 2024) and heavy metals (Alam et al., 2023b), which indicates its potential role in plant protection against adverse conditions. Serotonin also known as 5-hydroxytryptamine (5-HT), is an indoleamine with antioxidant properties that is found in different parts of the plant such as roots, stems, leaves, fruits and seeds (Mishra & Sarkar, 2023). Serotonin plays a crucial role in various plant activities, influencing growth, development, and responses to stress (Akcay & Okudan, 2023). It acts as a regulator, modulating gene expression and interacting with phytohormones to influence root and shoot growth, flowering, and overall plant architecture. Additionally, serotonin helps plants adapt to environmental stressors, such as salinity, drought, and pathogens, through its involvement in antioxidant defense mechanisms and other stress-responsive pathway (He et al, 2021). Thanks to its antioxidant activity, serotonin eliminates reactive oxygen species (ROS) production and accumulation in plant organs (Araz et al., 2021). Under salinity stress conditions, serotonin prevents oxidative damage by removing ROS and regulating osmotic pressure (Liu et al., 2021).

Taking into account the mentioned facts, we hypothesized that allantoin and serotonin could improve parameters related to seed germination of bitter melon under salinity stress. So this research, first-time, examines the separate and combined effects of allantoin and serotonin on seed germination and seedling growth of bitter melon in saline environments.

MATERIALS AND METHODS

This research was conducted in 2025 at the Plant Physiology Laboratory of Razi University, and the effects of salinity and seed priming with allantoin and/or serotonin on seed germination and seedling growth of bitter melon were investigated in a factorial experiment in a completely randomized design with three replications. The first factor consisted of three salinity levels (0, 50 and 100 mM NaCl) (Sheikhalipour et al., 2022) and the second factor included seed priming with modulator: control (distilled water), allantoin (1000 µM) (Raihan et al., 2023), serotonin (100 µM) (Liu et al., 2021; Gavyar et al., 2024) and allantoin+serotonin. For each experimental unit (petri dish), 8 seeds were selected and sterilized. First, the seeds were placed in 70% alcohol for 5 min for disinfection and then in 2% sodium hypochlorite (NaClO) solution for 5 min and finally seeds were washed 3 times with distilled water. In order to perform priming, bitter melon seeds were placed in solutions containing allantoin and serotonin for 48 hours according to the desired treatments. Then, the seeds were dried and transferred to 9 cm Petri dishes and finally, saline treatments were applied and 10 ml of each saline treatment was added to the Petri dishes (Shamsaddin Saied, 2023). Distilled water was used in the control treatments. To maintain humidity, the Petri dishes were closed with parafilm and placed in a germinator at a temperature of 25 °C. Germinated seeds were counted daily at a specific time until the end of day 21. During counting, seeds with a root length of 2 mm or more were considered germinated seeds (Ellis & Robert, 1981).

At the end of the experiment (day 21), the percentage of seed germination and some morphological traits including length, fresh and dry weight of the seedling (stem and root) were measured. To measure dry weight, stem and root samples were placed in an oven at 70°C for 48 hours.

Germination percentage calculated according to the following formula (1):

$$GP = \frac{n}{N} \times 100 \quad (1)$$

GP: Germination percentage, n: The number of germinated seeds, N: Total number of seeds

Seedling vigor index was calculated based on length (Seed Vigor Length Index) (VI) and seedling weight (Seed Vigor Weight Index) (VII) was assayed based on the following equations (2 and 3) (Alizadeh & Isvand, 2004):

$$\text{Seed Vigor Length Index }(cm)(VI) = \frac{\text{Germination percentage} \times \text{Seedling length}}{100} \quad (2)$$

$$\text{Seed Vigor Weight Index }(mg)(VII) = \frac{\text{Germination percentage} \times \text{Seedling weigh}}{100} \quad (3)$$

Statistical analysis

The data obtained from the measurements were analyzed using SAS (9.1) statistical software and the mean data were compared at a 5% error level (P < 0.05) using Duncan's test.

RESULTS

The results of the analysis of variance of the effect of different levels of salinity and seed priming on germination percentage and seed vigor of bitter melon showed that salinity, seed priming, and the interaction between salinity and seed priming had a significant effect at the probability level of p≤0.01 on germination percentage and seed vigor (Table 1).

Table 1. Results of variance analysis of germination percentage and seed vigor index of bitter melon under salt stress and seed priming conditions.

		Mean square		
(S.O.V)	df	GP	VI	VII
Salt stress (S)	2	4040.79**	86.09**	0.001859**
Priming (P)	3	606.19**	13.21**	0.000241**
S×P	6	59.31**	2.41**	0.000023**
Error	24	17.36	0.18	0.000003
C. V (%)	-	6.21	9.58	6.98

**: Significant at the probability level 1 %, respectively.

Table 2. Results of comparing the average germination percentage and seed vigor of bitter melon under salt stress and seed priming conditions.

Treatment	GP (%)	VI (cm)	VII (mg)
S_1P_1	75.00±0.00[c]	5.51±0.06[c]	0.031±0.002[d]
S_1P_2	83.33±2.40[b]	6.47±0.20[b]	0.036±0.011[c]
S_1P_3	87.50±0.00[b]	7.14±0.11[b]	0.040±0.004[b]
S_1P_4	100.00±0.00[a]	10.54±0.32[a]	0.050±0.007[a]
S_2P_1	62.50±0.00[d]	3.47±0.03[e]	0.021±0.002[ef]
S_2P_2	62.50±0.00[d]	3.56±0.00[e]	0.023±0.001[e]
S_2P_3	62.50±0.00[d]	3.92±0.03[de]	0.024±0.001[e]
S_2P_4	70.83±2.40[c]	4.63±0.19[d]	0.029±0.009[d]
S_3P_1	37.50±0.00[g]	1.10±0.04[g]	0.010±0.000[h]
S_3P_2	45.83±2.40[f]	1.64±0.11[g]	0.013±0.008[g]
S_3P_3	54.16±2.40[e]	2.59±0.16[f]	0.016±0.007[g]
S_3P_4	62.50±0.00[d]	3.29±0.00[ef]	0.020±0.002[f]

In each column, different letters demonstrate that means are significantly different at p ≤ 0.05 (Duncan's multiple range tests). Values express mean ± SE (n = 3). S_1, S_2 and S_3 are 0, 50 and 100 mM NaCl; P_1, P_2, P_3 and P_4 are control, serotonin (100 µM), allantoin (1000 µM) and serotonin+ allantoin.

According to the results (Table 2), the highest percentage of germination (100%) was observed in seed priming with allantoin + serotonin under non-stress conditions. The lowest germination percentage (37.50%) was observed in 100 mM salinity without the application of any seed priming agents. Likewise, the highest and lowest seed vigor was achieved by seed priming with allantoin + serotonin under non-stress condition and 100 mM salinity without the application of any seed priming agents, respectively. According to the results obtained, VI and VII had a positive and significant correlation with GP, VI and VII (Fig. 1).

According to the analysis of variance (Table 3), salinity stress, seed priming agents and the interaction of salinity stress and seed priming agents had a significant effect at $p \leq 0.01$ on seedling and root length, seedling, stem and root fresh weight, and root dry weight.

A shown in Table 4, in contrast to seed priming salt stress had a negative effect on seedling and root length, shoot fresh weight and root fresh and dry weights. Under 50 and 100 mM NaCl, seed priming with allantoin and serotonin improved the growth characteristics of bitter melon seedlings. According to the obtained results, the maximum length of seedling (10.54 cm), root (7.31 cm), shoot fresh weight (0.53 mg), root fresh (0.612 mg) and dry (0.0140 mg) weights were observed in allantoin (1000 µM) + serotonin (100 µM) treated control plants (Table 4).

Based on Table 5, the highest and lowest shoot length, fresh weight and dry weight of seedlings were observed in the control and 100 mM NaCl treatments, respectively. Seed priming with allantoin and serotonin had a positive effect on the length of the shoot, the fresh weight and the dry weight of the bitter melon seedlings, and the highest amounts of the traits were observed in allantoin (1000 µM) + serotonin (100 µM) treated plants under non-stress condition as compared to control. The increase in the fresh weight of the seedling could be attributed to the increase in the root and stem weight (Fig. 1).

Table 3. Results of variance analysis of some growth characteristics of the bitter melon seedling under salt stress and seed priming conditions.

		Mean square								
(S.O.V)	df	Seedling Length	Shoot Length	Root Length	Seedling Fresh Weight	Shoot Fresh Weight	Root Fresh Weight	Seedling Dry Weight	Shoot Dry Weight	Root Dry Weight
Salt stress (S)	2	56.13**	9.19**	40.90**	0.11**	0.133**	0.18**	0.0040**	0.006^{ns}	0.0001**
Priming (P)	3	8.13**	0.84**	7.16**	0.011**	0.019**	0.06**	0.0009*	0.002^{ns}	0.00002**
S×P	6	1.04**	0.02^{ns}	1.12**	0.0005^{ns}	0.003**	0.05**	0.0021^{ns}	0.001^{ns}	0.00001**
Error	24	0.13	0.01	0.11	0.0002	0.0008	0.005	0.00020	0.0009	0.000003
C.V (%)	-	5.91	3.53	10.09	3.10	7.70	17.84	13.37	18.29	14.30

*, ** and ns: significant at the probability level of 5, 1 % and non- significant, respectively.

Table 4. Results of comparing the average some morphological characteristics of bitter melon under salt stress and seed priming conditions.

Treatment	Seedling Length (cm)	Root Length (cm)	Shoot Fresh Weight (mg)	Root Fresh Weight (mg)	Root Dry Weight (mg)
S_1P_1	7.35±0.08^{c}	3.91±0.05^{c}	0.42±0.0007^{cd}	0.094±0.0021^{c}	0.0051±1.92^{bcd}
S_1P_2	7.76b±0.02^{c}	4.15±0.01^{c}	0.47±0.0015^{bc}	0.106±0.0015^{c}	0.0059±7.40^{bc}
S_1P_3	8.16±0.12^{b}	5.56±0.34^{b}	0.49±0.0020^{ab}	0.286±0.0513^{b}	0.0077±0.00^{b}
S_1P_4	10.54±0.32^{a}	7.31±0.00^{a}	0.53±0.0007^{a}	0.612±0.0728^{a}	0.0140±0.00^{a}
S_2P_1	5.55±0.06^{f}	2.60±0.04^{fg}	0.37±0.004^{e}	0.067±0.000^{c}	0.0037±3.84^{cd}
S_2P_2	5.70±0.01^{ef}	2.92±0.04^{ef}	0.38±0.000^{de}	0.070±0.000^{c}	0.0037±3.84^{cd}
S_2P_3	6.27±0.06^{de}	3.24±0.03^{de}	0.39±0.002^{de}	0.074±0.001^{c}	0.0040±1.92^{bcd}
S_2P_4	6.53±0.07^{d}	3.67±0.06^{cd}	0.41±0.003^{de}	0.083±0.000^{c}	0.0044$^{b±1.92cd}$
S_3P_1	2.94±0.11^{i}	0.78±0.02^{h}	0.14±0.03^{g}	0.041±0.00^{c}	0.0016±0.00^{d}
S_3P_2	3.57±0.06^{h}	1.16±0.10^{h}	0.30±0.00^{f}	0.047±0.00^{c}	0.0024±1.54^{cd}
S_3P_3	4.78±0.13^{g}	2.04±0.08^{g}	0.31±0.00^{f}	0.052±0.00^{c}	0.0024±0.00^{cd}
S_3P_4	5.27±0.00^{fg}	2.24±0.00^{f}	0.31±0.00^{f}	0.064±0.00^{c}	0.0029±0.00^{cd}

In each column, different letters demonstrate that means are significantly different at $p \leq 0.05$ (Duncan's multiple range tests). Values express mean ± SE (n = 3).
S_1, S_2 and S_3 are 0, 50 and 100 mM NaCl; P_1, P_2, P_3 and P_4 are control, serotonin (100 µM), allantoin (1000 µM) and serotonin+ allantoin.

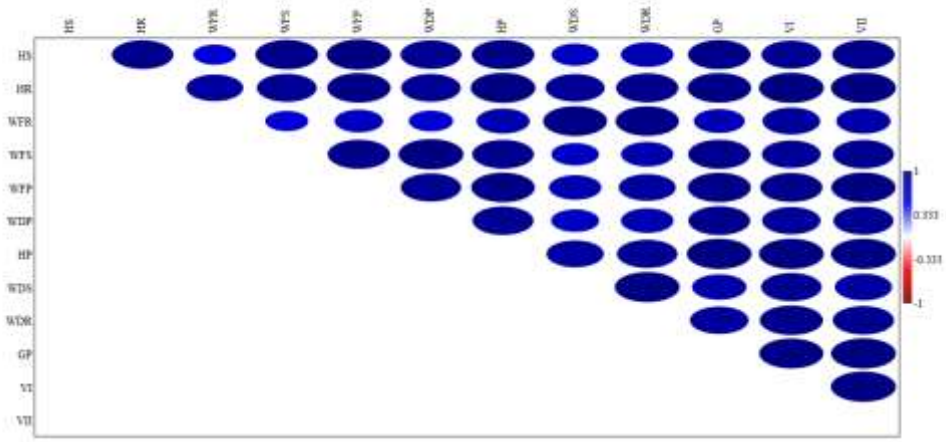

Fig. 1. Correlation coefficient between the test traits
HS: Length of stem, HR: Length of root, WFR: Root fresh weight, WFS: Shoot fresh weight, WFP: Plant fresh weight, WDP: Plant dry weight, HP: Length of plant, WDS: Shoot dry weight, WDR: Root dry weight, 10: GP, 11: VI, 12: VII

Table 5. Results of comparing the average shoot length, seedling fresh weight and seedling dry weight of bitter melon under salt stress and seed priming conditions.

	Shoot Length (cm)	Seedling Fresh Weight (mg)	Seedling Dry Weight (mg)
		Salt Stress (mM)	
S_1	4.03 ± 0.02^{a}	0.57 ± 0.00^{a}	0.12 ± 0.00^{a}
S_2	3.36 ± 0.01^{b}	0.48 ± 0.00^{b}	0.10 ± 0.00^{b}
S_3	2.30 ± 0.03^{c}	0.37 ± 0.00^{c}	0.087 ± 0.00^{c}
		Moderator (µM)	
P_1	2.87 ± 0.09^{d}	0.43 ± 0.008^{d}	0.092 ± 0.003^{b}
P_2	3.10 ± 0.08^{c}	0.46 ± 0.008^{c}	0.106 ± 0.001^{a}
P_3	3.36 ± 0.08^{b}	0.48 ± 0.009^{b}	0.111 ± 0.001^{a}
P_4	3.58 ± 0.07^{a}	0.51 ± 0.011^{a}	0.115 ± 0.001^{a}

In each column, different letters demonstrate that means are significantly different at $p \leq 0.05$ (Duncan's multiple range tests). Values express mean ± SE (n = 3). S_1, S_2 and S_3 are 0, 50 and 100 mM NaCl; P_1, P_2, P_3 and P_4 are control, serotonin (100 µM), allantoin (1000 µM) and serotonin+ allantoin.

According to the results of examining the correlation coefficients of the traits (Fig. 1), a positive and significant correlation was observed between the traits. Height of seedling has a positive correlation with shoot and root fresh weight. Germination percentage has a positive and significant relationship with seed vigor index. Seedling vigor had a positive correlation with height of seedling and weight.

Principal component analysis

Scree plot is used as a visual tool to assess the importance of each principal component. It represents the proportion of the total variance explained by each principal component in descending order (Fig. 2).

Tables 6 and 7 reveals that each principal component is separately loaded with various traits under investigation known as factor loadings. Factor loadings represent the relationship between the original variables and the principal components. These values indicate how strongly each variable contributes to or correlates with a specific principal component. Confirmation of grouping based on principal components also showed that the first and second two components contributed (91.09% and 6.98%) to the confirmation of groupings, respectively.

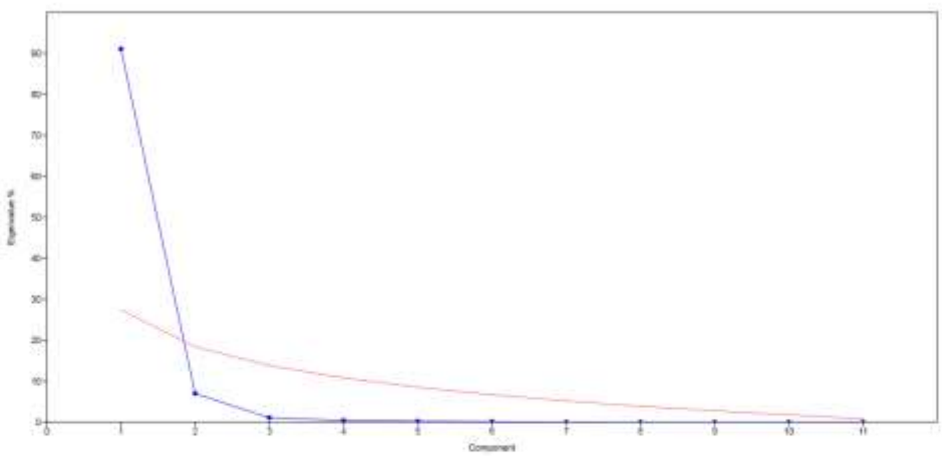

Fig. 2. Scree plot between Eigen value and principal components.

Table 6. Eigen values and variability percentage of PC1 and PC2 principal components.

Particulars	Eigen value	Variability (%)
PC1	10.93	91.09
PC2	0.83	6.98

Table 7. Factor loadings for traits under study.

Characters	PC1	PC2
Length of stem	0.284	-0.325
Length of root	0.300	0.030
Root fresh weight	0.258	0.550
Shoot fresh weight	0.283	-0.337
Plant fresh weight	0.293	-0.187
Plant dry weight	0.281	-0.300
Length of plant	0.300	-0.060
Shoot dry weight	0.272	0.456
Root dry weight	0.287	0.336
Germination percentage	0.297	-0.129
VI	0.300	0.081
VII	0.300	-0.040

PCA provided a comprehensive overview of the effects of biostimulants growth on germination and growth characteristics under NaCl stress (Fig. 3). The biplot (Fig. 3) typically displays the first two principal components (PC1 and PC2) as the axes, as these components capture the most of the variability. In grouping the studied treatments based on principal component analysis, the grouping results showed that the studied treatments were placed in separate groups based on the studied traits (Fig. 4). The results of this grouping showed that, based on the principal components, traits such as HR, VI, WDR, WDS and WFR were included in the S_1P_4 treatment groups. The WFS, HS, WDP, WFP, GP and VII were in treatment group S_1P_1, S_1P_2, S_1P_3 and S_2P_4. (Fig. 4).

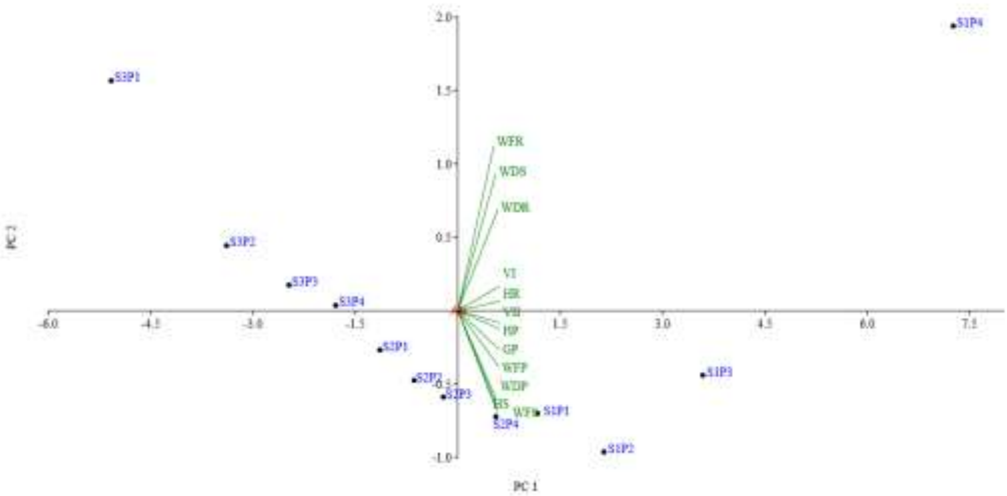

Fig. 3. Two-dimensional diagram of principal component analysis in grouping treatments and studied traits in bitter melon plant. HS: Length of stem, HR: Length of root, WFR: Root fresh weight, WFS: Shoot fresh weight, WFP: Plant fresh weight, WDP: Plant dry weight, HP: Length of plant, WDS: Shoot dry weight, WDR: Root dry weight, Germination percentage: GP.

DISCUSSION

Salt stress significantly impacts agricultural productivity in arid and semi-arid regions, posing a major threat to food security. Seed germination is indeed considered the most vulnerable stage in a plant's life cycle due to the severe impact of salt stress. Salt stress can significantly inhibit seed sprouting and hinder the establishment of seedlings, ultimately affecting plant growth and yield. Subsequently, rapid seed germination and seedling establishment are vital for plants survival particularly under salinity stress (Wang et al., 2011). Poor germination in saline soils causes poor establishment and low seedling production (Zhang et al., 2022a). Germination percentage and seed vigor of bitter melon seed were decreased by the application of 50 and 100 mM NaCl. A similar conclusion was drawn by Amerian and Khosravi (2021). Salt stress increased the concentration of ABA hormone in seeds that prevented seed germination (Chen et al., 2021). A decrease in the percentage of seed germination with increasing salinity stress has been reported in different plant species, including carrot (*Daucus carota* L.), radish (*Raphanus sativus* L. var. radicula), black radish (*Raphanus sativus* L.), and red beet (*Beta vulgaris* L.) (Şavkan & Çandar, 2024) and *Scutellaria baicalensis* (Wang et al., 2024). Salinity stress affects seed germination in two main ways: by causing osmotic stress, reducing water uptake for the mobilization of nutrients required for germination, and toxicity of salty ions to the embryo (Wang et al., 2024). With the increase in salinity levels that leads to negative osmotic potential of water, the embryo of seeds difficultly absorb water, and as a result, a decrease in germination and seedling vigor happens, as observed in the current study.

According to the results, salinity stress significantly reduced seed germination and seedling growth of bitter melon, regardless of whether the seeds were primed with allantoin and serotonin, and it is consistent with the results of Amerian et al. (2023) that salt stress has a negative effect on germination and plant growth. Zhang et al. (2022b) reported that the root growth is particularly sensitive to high salt concentrations in the growth media. This is because roots are the first plant organ to encounter and respond to salinity in the soil. High salt concentrations reduce water availability for plants, causing osmotic stress and inhibiting root growth. Additionally, excessive salt uptake can lead to ionic toxicity, further affecting root development. That is the important reason for rapid reduction or prevention of root length increase under salinity. Increase in NaCl concentrations (0 to16 ds/m) decreased the shoot and root length of the quinoa (*Chenopodium quinoa* L.) (Amerian et al., 2023). In germinating seeds, saline environments lead to reduced shoot length and inhibit the development of embryonic tissues due to the stress caused by NaCl, which can be either osmotic or ionic in nature (El-Badri et al., 2020). Salinity decreased water potential of germination medium due to increased toxicity rate (Ali et al., 2021), which might have declined shoot length.

Salt stress during germination can indeed lead to the production of ROS and damage to cell structures and components, potentially inhibiting plant height growth. Salt stress caused a significant decrease in fresh and dry weight of shoots of bitter melon (Irik and Bikmaz, 2024). Under salinity stress conditions, a decrease in dry weight of various plant tissues is observed due to increased metabolic energy expenditure and decreased photosynthesis, associated with salt adaptation (Ambede et al., 2012) as reported in sorghum (*Sorghum bicolor* L. Moench) (Ahmed et al., 2024). Salt stress reduces O_2 availability leading to deprivation of plants from an energy source and accumulation of high levels of ethylene which is associated with reduced root growth. (Atta et al., 2023), that may be the reason for the decrease in dry weight of the plant of bitter melon root in this study. The reduction in dry weight of the plant under salinity condition may be attributed to osmotic imbalance and effects of toxic ions (Cl^- and Na^+) (Ludwiczak et al., 2021). In addition, disruption in the movement and transfer of nutrients from the cotyledon to the developing embryo could be another reason for the reduction of seedling length (Amerian et al., 2023). According to the results of this study, a decreasing trend in the fresh and dry weight of seedlings, shoots, and roots was observed with increasing salinity levels in bitter melon seedling. Excessive consumption of energy to control and reduce the effect of salinity could be one of the main causes of this decrease. With the increase in salinity stress, probably water absorption by the seed was disturbed, hormones values and the activity and production of enzymes was reduced; consequently, the growth of the seedling (shoot and root) was reduced.

Seed priming before sowing is considered an effective method to increase seed germination and seedling growth under unfavorable environmental conditions (Amerian et al., 2023). The results of many studies have shown that primed seeds have better germination and growth under abiotic stress conditions than unprimed seeds (Wang et al., 2024). Under salt stress conditions, priming bitter melon seeds with serotonin and allantoin increased the percentage of seed germination and seedling growth, indicating the positive effect of seed priming on plant response to abiotic stresses. Shreevastav et al. (2023) found that seed priming increase speed of emergence, seedling vigor index, and root and shoot length in Bitter Gourd. The introduction of stimuli to seeds during priming with water resulted in a number of connected biochemical changes, including the production and activity of enzymes, production of germination and growth stimulants, changes in the metabolism of germination inhibitors healing cell damages, and enhancement of germination (Devika et al., 2021). Similar results in previous reports have been suggested that allantoin and serotonin have many positive effects on the seed germination percentage, morphological and metabolism in various plant species (Araz et al., 2021; Ying et al., 2022). Allantoin plays various roles in plant response to environmental stresses. For example, application of allantoin increases plant tolerance to saline conditions (Ying et al., 2022). Allantoin exerts its function through triggering the expression of PER1, 1-CYS PEROXIREDOXIN (PER1) is a seed-specific antioxidant that enhances seed longevity through scavenging ROS over-accumulation, subsequently initiating antioxidant enzyme system to scavenge ROS level, and ultimately enhances seed germination under stress (Ying et al., 2022).

As seen in the current study, serotonin, through its function as a plant growth regulator with cytokinin-like activity, increased germination and morphological characteristics of bitter melon and is involved in various developmental processes such as root and shoot formation, cell division and differentiation, germination, somatic embryogenesis, and senescence (Roychoudhury, 2021). Serotonin can also increase tolerance to salt stress by detoxifying ROS and influencing the activity of enzymes and antioxidant compounds. According to research conducted in rapeseed, serotonin reduced the effects of salt stress by scavenging ROS, regulating osmotic pressure, and increasing growth (Liu et al., 2021). Consequently, allantoin and serotonin, as seed priming agents, probably accelerated the initial growth of seedlings by increasing water absorption during germination.

CONCLUSIONS

In the present study, the effect of seed priming with allantoin and serotonin on seed germination and seedling growth of bitter melon under salt stress was investigated. The results revealed that seed germination and seedling growth of bitter melon decreased in line with increasing salt concentrations: on the other hand, seed priming with allantoin (1000 µM) and serotonin (100 µM) alleviated this negative effect of salt stress. The germination of bitter melon seeds decreased in parallel with increasing salt concentrations, and this negative effect of salt stress decreased with seed priming. Although it has been known that allantoin and serotonin are involved in various metabolic, physiological and biochemical processes in plants as well as protective effect against various stressors, their effects on salinity abiotic stress were not well known. In this study, it was concluded that allantoin and serotonin could alleviate the effects of salinity in bitter melon seeds, individually and better in combination. To the best of our knowledge, any studies has been assayed the application of allantoin and serotonin in salt stressed bitter melon and consequently the current findings could be used as a reference for future studies. Seed priming with serotonin and allantoin caused significantly enhanced seed germination percentage and seedling growth of bitter melon under salt stress, certificating the positive roles of serotonin and allantoin seed priming agents in plant response to salinity and probably other environmental stressors.

Conflict of interest

The authors declare that there are no conflicts of interest

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