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RESEARCH PAPERS

Improving the Germination and Growth of Basil Seed (*Ocimum basilicum* L.) under Salinity Stress Conditions with the Use of Priming Salicylic Acid and Potassium Nitrate

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Abstract—Salinity is one of the main environmental stresses affecting plant production. The technique of seed priming has been introduced as a means to improve germination and establishment under environmental stress. This study aimed to determine the best material for the priming of basil seed under salt-stress. Treatments included seed priming of basil (*Ocimum basilicum* L.) with salicylic acid (SA) at 0.5 mM and potassium nitrate (KNO_3) at 5% and salinity stress with potassium chloride (KCl) (50, 100, 150 mM), calcium chloride ($CaCl_2$) (50, 100, 150 mM) and the control (irrigation water) with and without priming. The results showed that salt stress decreased morphological traits such as leaf area, number of leaves, root length, and dry weight of roots compared to the control. The lowest and highest chlorophyll contents were observed in the KNO_3 × KCl (150 mM) and SA × KCl (100 mM) treatments, respectively. The highest amount of antioxidant compounds was obtained in the treatment with 150 mM $CaCl_2$ alone. It was found that the use of SA was more effective than KNO_3 and that the priming treatments were able to mitigate the negative effects of salt stress on the basil plant by accelerating germination and more uniform and rapid seedling growth, leading to better plant growth. As a result, priming seeds before planting under salt stress conditions led to an improvement in the growth indicators of the basil plant and can be mentioned as a strategy to increase plant efficiency under salt stress conditions.

Keywords: *Ocimum basilicum*, antioxidant, salicylic acid, salinity, seed priming, yield

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INTRODUCTION

Plants are exposed to various environmental fluctuations, including drought and salinity, which limit their growth. Salinity is one of the common environmental stresses in the world, which causes the reduction of agricultural products in the world [1]. Soil salinity is one of the main reasons for decreasing the yield in the cultivated area of agricultural products in the world [2].

Seed priming is a common strategy to increase the percentage, speed, and uniformity of seed germination and greening under adverse environmental conditions [3]. Under unfavorable conditions, the use of pretreatment of seeds using saline solutions, different osmotic potentials, the use of hormones, and hydropriming can increase the resistance to salinity stress in plants [4]. Basil (*Ocimum basilicum* L.) is the most economical species among the species of the genus *Ocimum* belonging to the mint family and is cultivated in almost all warm and temperate regions. Basil is used as a medicinal plant, spice, and fresh vegetable and has been introduced as a medicinal plant in most pharmacopeias [5, 6].

By using seed priming, seeds become physiologically able to better tolerate stress conditions, including salt stress. The mechanism of this tolerance is that under laboratory conditions and without stress, the seed goes through the stages of drinking water and the delay phase related to its germination and obtains the necessary metabolic preparation for stronger germination after planting. This is reflected in the acceleration of germination and faster establishment, better uniform green growth, and faster and more vigorous growth [3].

Potassium nitrate (KNO_3) (halopriming) is one of the most widely used substances for seed priming, and its positive effect is probably due to the reduction of growth inhibiting substances such as abscisic acid (ABA). Also, seed priming with salicylic acid (SA) (hormone priming) improved germination indicators

and increased the activity of alpha-amylase and protein enzymes and decreased the activity of catalase and peroxidase enzymes in saline and non-saline conditions [7].

Regarding high occurrence of salinity stress, a lot of attention attributed to this problem. Basil is also one of the plants faced with this issue and it is susceptible. So, finding the solution for decreasing it is also important. It was no references for seed priming of this plant with KNO_3 and SA. As a result, this study aimed to determine the best material for the priming of basil seed under salt-stress conditions and on improving physiological and biochemical characteristics and yield. Moreover, the most inhibiting salt among the salts used in laboratory conditions was introduced.

MATERIALS AND METHODS

Plant material and experimental design. This study was conducted in 2021–2022 in the research greenhouse of Ferdowsi University of Mashhad, Iran. This experiment was conducted as a factorial experimental design based on a completely randomized experimental design with 3 replicates. Basil seeds were soaked in salicylic acid (SA) at 0.5 mM and potassium nitrate (KNO_3) at 5% solution for 24 h before sowing and the untreated seeds were used as controls (no priming). Then the prepared seeds were planted in pots with garden soil. After germination of the seeds and after one month had passed from the time of sowing until the plants entered the four-leaf stage, salt stress was added to the plants. Potassium chloride (KCl) and calcium chloride ($CaCl_2$) were used for salt stress at concentrations of 50, 100, and 150 mM and irrigation water (control). The salt stress was added to the plants via the irrigation water, the type of treatment, and the stress level. To prevent plant shock, salinity treatments were applied gradually. The seedlings watered with saline solution for all 7 weeks After the seven-week application of salt stress, the investigated traits were measured.

Relative leaf water content (RWC). To measure the relative leaf water content, 10 slices with a diameter of 0.5 cm were taken from the width of the adult and young leaves with a punch and then weighed (FW). After weighing, they were placed in Petri dishes containing 10 mL of distilled water for 4 h. They were stored in the dark at a temperature of 0–4°C until the leaf cells swell completely, then they were weighed to obtain the full swelling weight (TW). After weighing, the samples were dried at 70°C and the dry weight (DW) of the slices was measured. The relative leaf water content was calculated using the following formula [8].

$$RWC = [(FW - DW)/(TW - DW)] \times 100.$$

Electrolyte leakage (EC). To determine the membrane stability of the leaf cells, the electrolyte leakage index was used [8]. In brief, leaf pieces with a size of 2 cm were prepared. After washing, the samples were placed in test tubes with 10 mL of distilled water and shaken with a vortex (160 rpm) for 17 to 18 h. At this stage, the electrical conductivity of the samples (E1) was measured using the JENWAY model conductivity meter. The tubes were placed in the autoclave at 121°C for 15 min. The electrical conductivity was measured at this stage after cooling the contents in the test tubes (E2). Finally, the leakage values of the electrolytes were calculated using the following equation.

$$EL = (E1/E2) \times 100.$$

Chlorophyll content of the leaves. The method of Dere et al. [9] was used to measure the amount of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl t), and carotenoids (Car). The absorbance values of the samples were measured separately at 663, 653 and 470 nm for Chl *a*, Chl *b*, and carotenoids using the model 2100 spectrophotometer, respectively. Finally, the amount of chlorophyll *a*, *b*, carotenoids, and total chlorophyll in mg/g of the sample weight is determined using the following formulas.

$$\text{Chl } a = 15/65\, A666 - 7/340\, A653,$$

$$\text{Chl } b = 05/27\, A653 - 11/21\, A666,$$

$$\text{Car} = 1000\, A470 - 2/860\, \text{Chl } a - 129/2\, \text{Chl } b/245,$$

$$\text{Chl t} = \text{Chl } a\, \text{Chl } b.$$

Phenolic compounds. The method of Khoddami et al. [10] was used to determine the amount of total phenol. 100 mg of the plant sample was extracted with 10 mL of a solvent (methanol or ethanol). Then 7.5% sodium carbonate (1.2 mL) and 10% Folin Cicalto (1.5 mL) were added. After 30 min in a dark room, the absorbance of the solution was measured at 765 nm.

Antioxidant capacity. In order to measure antioxidant activity [11], 100 mg of fresh leaf was completely homogenized in liquid nitrogen and extracted with 96% ethanol. Centrifugation was performed at 3500 rpm for 5 min to separate insoluble solids. An appropriate amount of the upper clear solution was mixed with 800 μL of half-molar DPPH solution and the light absorbance was read at a wavelength of 517 nm after keeping the samples in a dark room for 30 min.

Plant height root length, leaf area. The plant height was measured in two phases: after the application of stress and at the end of the experiment. Root length was also measured in two phases, after the application of stress and at the end of the experiment, using a digital caliper. At the end of the experiment, the leaf area of the plant was measured with a leaf area meter.

Fresh weight and dry weight of root and shoot. After the plants had been removed from the pot, the roots were separated from the aerial part of the plant and weighed on a balance. After the samples had been placed in the oven at 65°C for 72 h, their dry weight was measured.

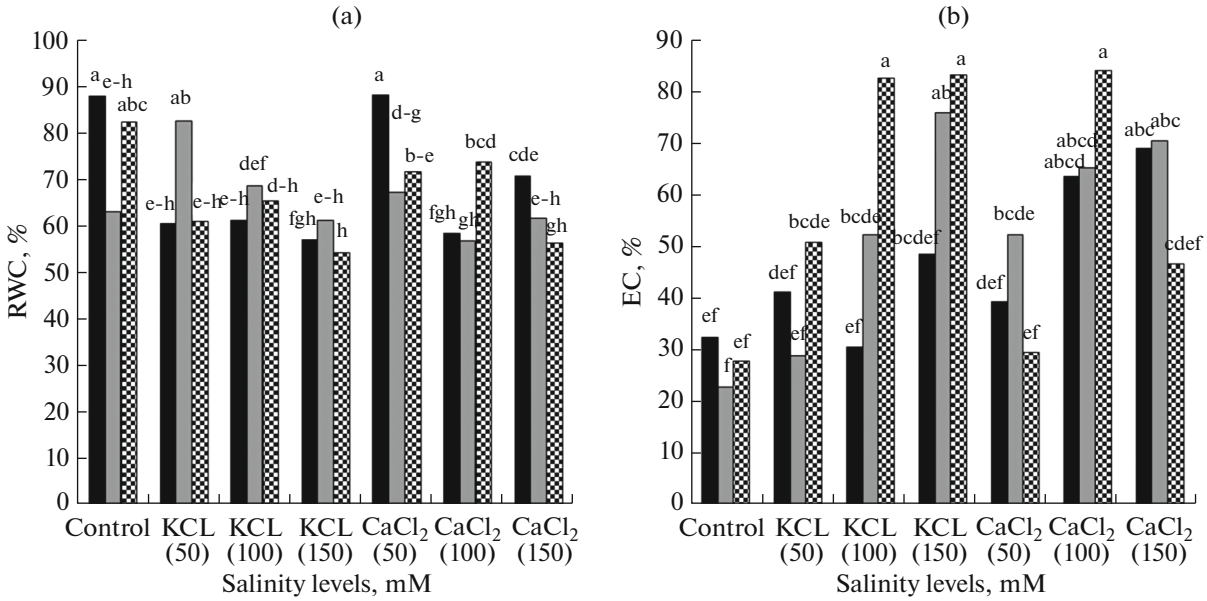

Fig. 1. Effects of seed priming with salicylic acid and potassium nitrate pretreatments on RWC (a) and EC (b) under salinity stress. Black columns—no priming; grey columns—priming with KNO_3; black and white columns—priming with SA.

Number of leaves and greenness index. The number of leaves per plant was counted at the end of the experiment.

Greenness index (Spad): The greenness index was measured using the SPAD 502 velocimeter (Konica-Minohta-Tokyo).

Statistical analysis. SAS software version 9.1 was used to analyze the variance. Comparison of mean traits was performed using Duncan's multiple range test at 5% probability level. The difference between the average values of the main and interaction effects was indicated in the figures as ± SE. Graphs and tables were created using Excel software and the information was displayed. R software was used to create clustering heat maps and correlation diagrams.

RESULTS

RWC and Electrolyte Leakage

The highest RWC of leaves was obtained in the treatment without priming × 50 mM $CaCl_2$, followed by the treatment with KNO_3 × 50 mM KCl. As shown in Fig. 1a, the RWC of leaves decreased for the two salts used in the experiment by increasing the salt content. Salinity stress with KCl at 50, 100 and 150 mM had higher decrease in RWC by 31, 30 and 40% than $CaCl_2$ in comparison with control without priming, respectively. Priming with KNO_3 had higher effect on increasing RWC than SA under salinity stress. Priming with KNO_3 alone led to increase of 30% in RWC.

With increasing stress, the EC value increased compared to the control treatment. The highest EC value of the leaves was measured in the SA primer in the treatments with 100 mM $CaCl_2$, 100, and 150 mM KCl. The lowest amount of EC was obtained in the control treatment alone (Fig. 1b). Salinity stress with $CaCl_2$ at 50, 100 and 150 mM increased EC by 39, 63 and 69% as compared to control without priming, respectively. Priming with KNO_3 had higher effect on decreasing RWC than SA under salinity stress.

Chlorophyll a, b, and Total Chlorophyll

The highest amount of chlorophyll a was observed in the control treatment (no salt stress) and priming with SA and no priming (Fig. 2a). The lowest amount of chlorophyll a was due to salt stress caused by the application of 150 mM KCl under both priming and non-priming conditions.

On the other hand, priming the seeds reduced the negative effects of stress on chlorophyll b content. The highest amount of chlorophyll b was obtained under salt stress conditions with 50 mM KNO_3 alone, followed by 100 mM KCl × SA (Fig. 2b).

The lowest and highest total chlorophyll were observed when priming with KNO_3 under 150 mM KCl stress and priming with SA under KCl stress (100 mM), respectively. The application of SA × $CaCl_2$ (50 mM) increased the amount of chlorophyll b compared to the control alone (Fig. 2b). Chl *a* decreased by 19, 65 and 76% in treatments with KCl at 50, 100 and 150 mM and by 41, 56 and 66% by treatments with $CaCl_2$ at the same concentrations in comparison with control without priming.

The decrease in Chl *b* was lower in priming with SA under salinity stress by KCl at 100 and 150 mM by 10 and 44% as compared to control without priming.

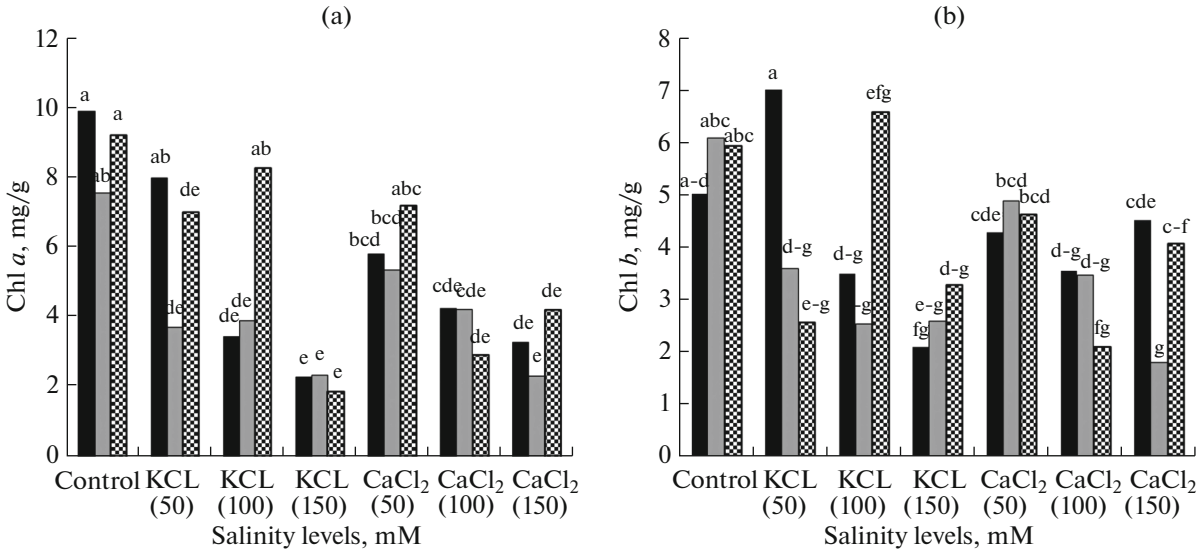

Fig. 2. Effects of seed priming with salicylic acid and potassium nitrate pretreatments on Chl *a* (a) and Chl *b* (b) under salinity stress. Black columns—no priming; grey columns—priming with KNO_3; black and white columns—priming with SA.

Total Chl decreased by 53 and 70% in treatments with KCl at 100 and 150 mM and by 32, 47 and 47% by treatments with $CaCl_2$ at the same concentrations in comparison with control without priming. Although, salinity stress with KNO_3 and $CaCl_2$ treatments had lower decrease in priming with KNO_3 and SA but this decrease was higher in KNO_3 along with salinity stresses.

Carotenoids and Antioxidant Capacity

The amount of leaf carotenoids was influenced by the interaction of salt stress and priming (Fig. 3b) the 150 mM KCl treatment had the highest carotenoid content. Priming with SA in KCl (100 mM and $CaCl_2$ (150 mM) also had higher carotenoid content than other treatments.

Our findings showed that the amount of antioxidant compounds increased under the influence of salt stress in the plant. Treatment with $CaCl_2$ 150 mM showed the highest antioxidant activity. Priming SA under stress with KCl 150 mM, $CaCl_2$ 100 and 150 mM, and KNO_3 100 and 150 mM had higher antioxidant activity than other treatments (Fig. 4a). Salinity stress with KCl at 50, 100 and 150 had higher increase in Antioxidant activity in comparison with control without priming, respectively. Priming with KNO_3 had higher antioxidant activity than SA under salinity stress.

Phenolic Content

The amount of plant phenol was affected by salt stress and priming (Fig. 4b). The $CaCl_2$ 100 mM and KCl 150 mM treatments also had higher phenol levels than other treatments. The results indicate that the amount of phenolic compounds in the plant increased when salt stress was applied compared to the control treatment (no salt application). In contrast, priming the seeds with SA and KNO_3 decreased the amount of phenolic compounds under salt stress conditions. Regarding phenol content of leaf, all of the treatments increased by increasing salinity stress with $CaCl_2$ and KNO_3 with or without priming treatments except for KCl and $CaCl_2$ at 50 mM with SA priming that they decreased as compared to control without priming, respectively.

Leaf Area

Plant leaf area was affected by salt stress and seed priming (Table 1). The results show that high salt stress caused by both salts used led to a decrease in the leaf area of the plant, although the decrease in leaf area was more pronounced at high concentrations of salt stress. The highest leaf area (783.80 cm) was recorded in the KNO_3 × without stress, and the lowest leaf area was obtained in the treatment without priming × 150 mM $CaCl_2$. Salinity stress with $CaCl_2$ at 50, 100 and 150 had higher decrease in leaf area by 35, 61 and 74% than in comparison with control without priming, respectively. Priming with SA had higher effect on increasing this trait than KNO_3 under salinity stress. Priming with KNO_3 alone led to increase of 30% in leaf area. Salinity stress with $CaCl_2$ and KCl at 50, 100 and 150 decreased leaf area in comparison with control without priming. However, priming with SA and KNO_3 led to decrease of 24 and 16% in plant height.

Plant Height

The highest plant height was achieved in the condition without salt stress and with SA and KNO_3 as a

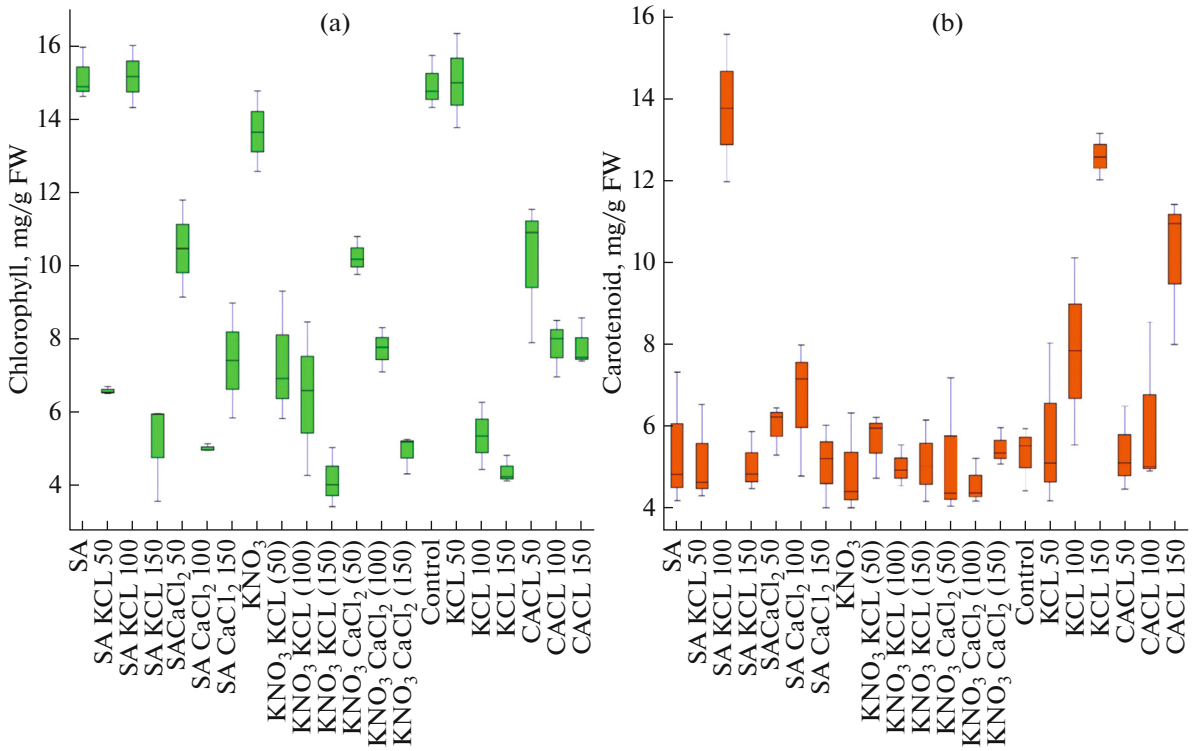

Fig. 3. Effects of seed priming with salicylic acid and potassium nitrate pretreatments on Total chlorophyll (a) and carotenoid (b) under salinity stress.

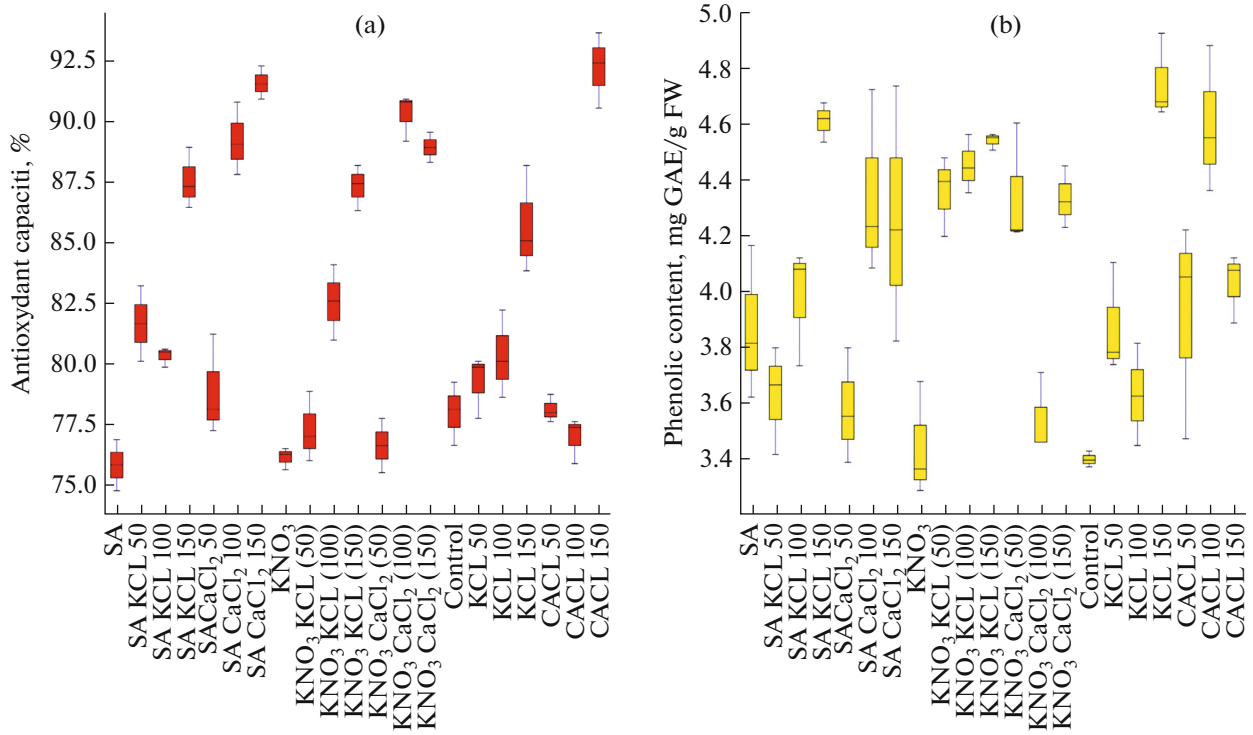

Fig. 4. Effects of seed priming with salicylic acid and potassium nitrate pretreatments on antioxidant capacity and phenolic compound under salinity stress.

Table 1. Effects of seed priming with salicylic acid (SA) and potassium nitrate (KNO_3) pretreatments on morphological traits under salinity stress

Treatments		Plant Height, cm	Yield of Fresh Leaf, %	Yield of Dry Leaf, %	Yield of Fresh Stem, %	Yield of Dry Stem, %	Yield of Fresh Shoot, %	Yield of Dry Shoot, %	Yield of Dry Root, %	Leaf Area, cm^2
No priming	Control	10.33ef	1.41d–h	0.149e–h	0.736d–h	0.223defg	2.15c–f	0.223d–g	0.323a	484.55bc
	KCL (50)	10ef	1.28e–h	0.098g–j	0.543g–j	0.211defg	1.826e–h	0.211d–g	0.113d–g	355.65c–g
	KCL (100)	6.66ij	1.06ghi	0.113f–i	0.44ij	0.152efg	1.506fgh	0.152efg	0.065g	283.58e–i
	KCL (150)	6.33j	0.08j	0.14e–h	0.116k	0.212defg	0.202i	0.212d–g	0.112d–g	178.64hij
	$CaCl_2$ (50)	9fgh	1.48d–h	0.105ghi	0.68e–i	0.194defg	2.16c–f	0.194d–g	0.113d–g	312.703d–h
	$CaCl_2$ (100)	7ij	0.32ij	0.045ij	0.8c–g	0.07g	1.12ghi	0.07g	0.096efg	187.903hij
	$CaCl_2$ (150)	6.66ij	1.22fgh	0.017j	0.613f–j	0.1fg	1.836e–h	0.1fg	0.072fg	123.463j
	Control	16.66a	1.85c–g	0.252bcd	0.87b–f	0.633a	2.72cde	0.633a	0.188b–f	783.803a
Priming with KNO_3	KCL (50)	12.66bcd	2.19bcd	0.176d–g	0.806b–g	0.27cdef	3.003bc	0.273c–f	0.24abc	296.77d–i
	KCL (100)	10ef	2.15b–e	0.192def	0.756d–g	0.289cde	2.91ncd	0.289cde	0.126c–g	234.91f–j
	KCL (150)	7.66hij	1.56d–h	0.098g–j	0.49hij	0.153efg	2.053d–g	0.153efg	0.089fg	212.823g–j
	$CaCl_2$ (50)	11.33de	1.98b–f	0.214cde	0.936b–e	0.345cd	2.916bcd	0.345cd	0.207a–e	369.736c–f
	$CaCl_2$ (100)	9.33fd	1.54d–h	0.143e–g	0.68e–i	0.266edef	2.22c–f	0.266c–f	0.186b–f	402.513cde
	$CaCl_2$ (150)	8ghi	0.99ghi	0.178d–g	0.553g–i	0.27cdef	1.546fgh	0.27c–f	0.115d–g	383.02c–f
	Control	16.33a	3.93a	0.349a	1.421a	0.58ab	4.814a	0.58ab	0.174a	573.17b
Priming with SA	KCL (50)	13.66b	2.84ab	0.367a	0.97bcd	0.551ab	3.813b	0.551ab	0.313ab	564.856b
	KCL (100)	10.33ef	2.72abc	0.302ab	1.066b	0.446bc	3.786b	0.446bc	0.284a–d	238.006f–j
	KCL (150)	10ef	1.61d–g	0.077hij	0.8bc	0.214defg	2.413c–f	0.214d–g	0.223ab	147.046ij
	$CaCl_2$ (50)	13.33bc	2.78ab	0.346a	1.056c–g	0.538ab	3.84b	0.538ab	0.278fg	556.89b
	CaC_{12} (100)	12cd	3.11a	0.296abc	0.586g–j	0.415bc	3.703b	0.415bc	0.086fg	440.716bcd
	$CaCl_2$ (150)	9.33fg	0.72hij	0.215cde	0.373jk	0.273edef	1.093hi	0.273c–f	0.172b–g	238.853f–j

Mean value followed by the same letters in each column are not significantly different $P < 0.05$ (Duncan's multi-range test)

primer (Table 1). The lowest plant height (6.66 cm) was also recorded in the treatment without priming and 150 mM KCl. At all salinity levels, priming the seeds before sowing resulted in greater plant height than without priming.

Root Length

Salt stress had a negative effect on the root length of the plants and led to a reduction in root length compared to the control condition (no application of salt stress). The maximum root length (24.33 cm) was obtained in the no priming × control (no stress) treatment (Table 2). The lowest root length (4 cm) was obtained in the no priming × 150 mM $CaCl_2$ treatment.

Yield of Fresh Weight and Dry Weight of the Root

Priming the basil seeds with KNO_3 and SA resulted in an increase in the fresh weight of the root compared to the control treatment (no priming). Statistically, no difference was observed between the priming treatments with SA and KNO_3 (Table 1). According to Table 1, all types of stress resulted in a decrease in root dry weight compared to the control (no salt stress). With increasing salt stress, the root dry weight decreased.

Yield of Fresh Weight and Dry Weight of the Leaf

The fresh leaf yield was affected by salinity stress and showed a decreasing trend (Table 1). The lowest value of leaf yield was observed in the no-priming treatment × 150 mM KCl. The highest leaf yield was recorded in the SA × control treatment (no application of salt stress), as well as in the SA× 100 mM $CaCl_2$ treatment. In general, priming with SA and KNO_3 increased the leaf yield under salt stress conditions (Table 1). The highest amount of leaf dry yield was recorded in the treatments with SA× 50 mM of KCl (0.36%), SA× control (no application of salt stress) (0.34%), and SA × 100 mM of $CaCl_2$ (0.34%) (Table 1).

Yield of Fresh Weight and Dry Weight of the Shoot

With an increase in salinity stress, the yield of shoots showed a decreasing trend (Table 1). The highest yield (4.81%) of fresh shoots was obtained in the SA × control treatment (without salinity stress). The dry shoot yield was affected by salinity stress and showed a decreasing trend. With increasing levels of salinity stress, the dry yield of shoots decreased compared to that with of the control (no salinity stress). In contrast, seed priming before planting at high stress levels led to an increase in shoot dry yield compared to that of the control (no priming), which shows the positive effect of priming under stressful conditions (Table 1).

Number of Leaves

The application of salt stress to the basil plant resulted in a decrease in the number of leaves on the plant (Table 2). The highest and lowest number of leaves per plant was obtained when treated with SA (alone) and 150 mM KCl, respectively. At high stress levels (concentrations of 100 and 150 mM) in both salts, the plants whose seeds were primed with SA had more leaves than the plants without priming (control).

Greenness Index (Spad)

The highest leaf green index was obtained in the control treatment (no salt stress) and the SA priming treatment. With the application of KCl, the leaf green index was reduced at all stress levels, and SA priming reduced the negative effects of salt stress (Table 2).

Dry Weight ratio of Root to Shoot

The root-to-shoot dry weight ratio was affected by salinity stress and priming. In this way, this ratio showed a decreasing trend with increasing salinity stress levels, which indicates a greater decrease in the dry weight of the shoot compared to the dry weight of the root under the influence of salt stress (Table 2). In contrast, seed priming showed a decreasing trend compared with the control condition (no priming).

Root to Shoot Fresh Weight Ratio

The ratio of fresh weight of roots to shoots was affected by the interaction effect of the experimental treatments and showed a decreasing trend (Table 2). In the salinity treatment caused by the application of calcium chloride, seed priming led to adjustment of the negative effects of salinity stress. The decrease in fresh and dry weight, and stem and root length obtained from this study, in general, indicate a decrease in plant growth with increasing salinity.

The Ratio of the Length of the Stem to the Root

The root-to-stem length ratio was also affected by salinity stress and priming. Salt stress led to a decrease in the ratio of root to stem length in basil plants, indicating a negative effect of salinity stress on plant root length (Table 2). Stem and root length are the most important specific parameters of the effects of environmental stress, especially salinity and drought stress, because the root is in direct contact with the soil and absorbs water and salts from the soil, and the stem transfers it to other parts of the plant., therefore, the longitudinal changes in these two parameters (stem and root) are considered an important sign of the response of plants to salinity stress.

Table 2. Effects of seed priming with salicylic acid(SA) and potassium nitrate (KNO_3) pretreatments on morphological traits under salinity stress

Treatments		Root Length, cm	Fresh Weight Root to Shoot Ratio	Dry Weight Root to Shoot Ratio	Root to Shoot Length Ratio	Stem Moisture, %	Leaf Moisture, %	Shoot Moisture, %	Stem Dry Matter	Shoot Dry Matter	Number of Leaves	Chl (SPAD unit)	Leaf Dry Matter, %
No priming	Control	24.333a	0.295b–e	1.43a	2.357a	89.4ab	89.755a	89.531abc	10.24c	8.66b–e	30.23bcd	10.59cd	10.468d
	KCL (50)	20.66abc	0.313b–e	0.605b–e	2.049ab	88.13ab	69.077ab	84.146bcd	30.92bc	7.33c–f	20.33hi	11.86cd	15.853bcd
	KCL (100)	9ijk	0.393bcd	0.48cde	1.365cde	89.42ab	87.488a	90.005abc	12.51c	4.33ghi	15.46jk	10.57cd	9.995d
	KCL (150)	10g–i	1.906a	0.542cde	1.587bcd	59.61d	33.455c	24.985f	66.54a	3.33i	12.7kl	163.857a	103.319a
	$CaCl_2$ (50)	14.66d–g	0.185de	0.552b–e	1.622bc	92.91ab	86.61a	91.101abc	13.39c	7d–g	26.93def	7.08cd	8.899d
	$CaCl_2$ (100)	9.16hij	0.29b–e	1.39a	1.309cde	84.3abc	96.812a	93.705a	3.18c	5.33f–i	21.76gh	15.69bcd	6.249d
	$CaCl_2$ (150)	4k	0.144e	0.762bcd	0.611f	98.56a	86.517a	94.567a	13.42c	5f–i	17.7ij	1.43d	5.432d
Priming with KNO_3	Control	17.66cde	0.244de	0.335de	1.064def	86.42ab	46.094bc	74.455e	53.9ab	11.33b	33.23ab	13.57cd	25.544b
	KCL (50)	16.5c–f	0.229de	0.849bc	1.305cde	91.29ab	87.03a	90.134abc	12.96c	9.33bcd	24.36fg	8.7cd	9.866d
	KCL (100)	13.33d–i	0.238de	0.469cde	1.333cde	90.51ab	87.137a	89.878abc	12.86c	6.33e–h	18.73hij	9.48cd	10.122d
	KCL (150)	12.5e–i	0.212de	0.596b–e	1.646bc	93.54ab	88.835a	92.39ab	11.16c	5f–i	17.7ij	6.45cd	7.608d
	$CaCl_2$ (50)	14.5d–h	0.245de	0.59b–e	1.289cde	88.62ab	85.963a	88.014abc	14.03c	10bc	28.96cde	11.37cd	11.985bcd
	$CaCl_2$ (100)	11.83f–j	0.317b–e	0.722bcd	1.287cde	90.67ab	81.85a	87.893abc	18.14c	6.66d–h	18.73hij	9.32cd	12.107bcd
	$CaCl_2$ (150)	10g–j	0.528b	0.422cde	1.257cde	80.34bc	83.275a	81.875cde	16.72c	4hi	18.6hij	19.65bc	18.124bcd
Priming with SA	Control	23.66ab	0.159de	0.346de	1.467cde	89.37ab	82.493a	87.898abc	17.56c	16.88a	35.4a	10.62cd	12.101bcd
	KCL (50)	21abc	0.181de	0.588be	1.559bcd	88.91ab	80.163a	85.357abc	16.93c	6.88b–e	31.96abc	13.27cd	14.643bcd
	KCL (100)	18.33bcd	0.254cde	0.644be	1.766bc	88.914ab	86.405a	88.253abc	13.59c	8.33cde	29cde	cd	11.756bcd
	KCL (150)	16.33c–f	0.286b–e	1.017ab	1.633bcd	95.18a	83.067a	91.1abc	16.93c	7.33c–f	25.83ef	4.81cd	8.89d
	$CaCl_2$ (50)	21.16abc	0.291b–e	0.544b–e	1.589bcd	86.57ab	81.91a	85.393abc	18.08c	9.33bcd	28.46de	13.42bcd	14.606bcd
	$CaCl_2$ (100)	6.83jk	0.1e	0.206e	0.598f	90.46ab	79.675a	88.69abc	20.32c	7d–g	29.9bcd	9.53cd	11.309cd
	$CaCl_2$ (150)	8.5ijk	0.512bc	0.5b–e	0.916ef	69.89cd	84.522a	75.006de	15.47c	5f–i	11.83l	30.108b	24.993bc

Mean value followed by the same letters in each column are not significantly different $p < 0.05$ (Duncan's multi-range test)

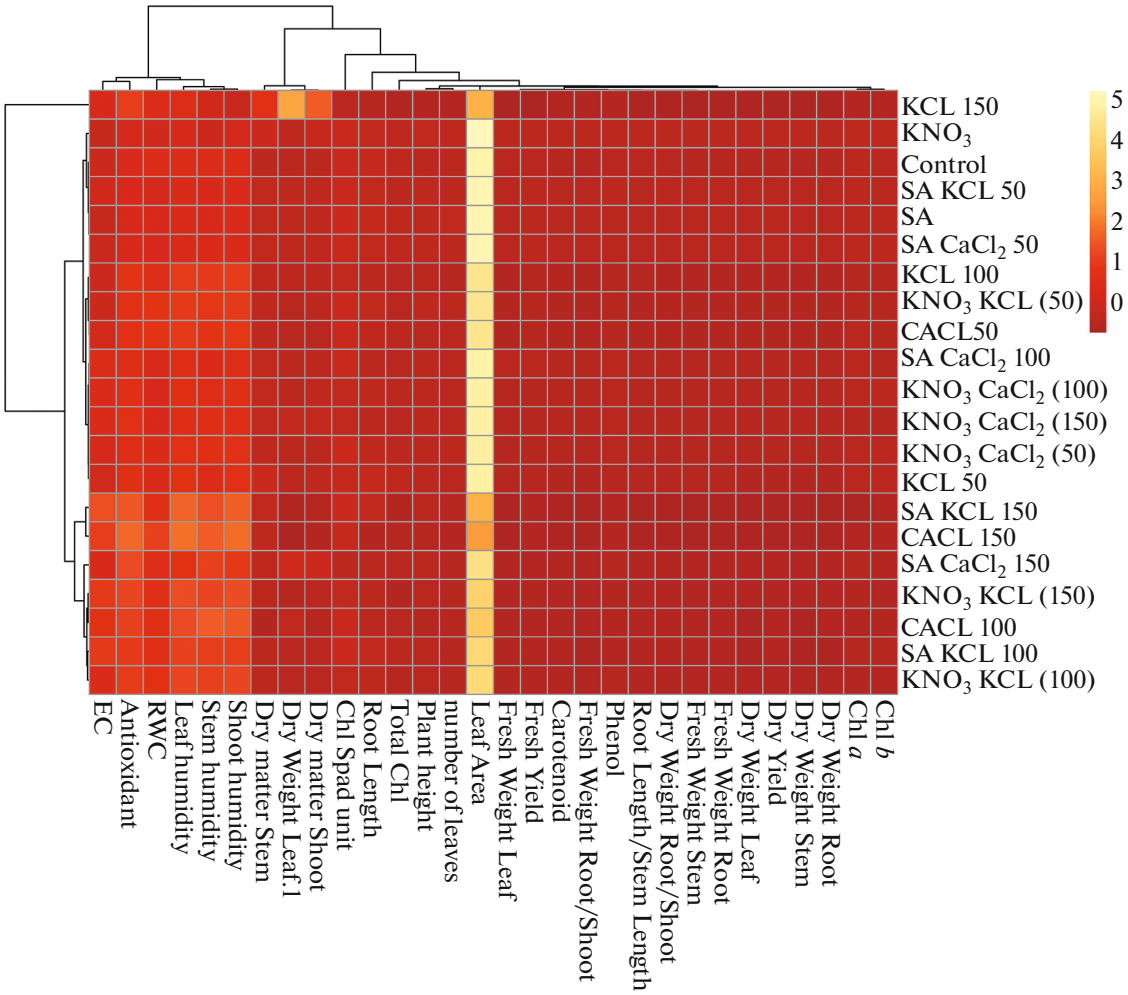

Fig. 5. Cluster analysis of basil based on morphological and chemical properties under salinity stress using seed priming with salicylic acid and potassium nitrate pretreatments.

Multivariate Analysis and Correlation of Quantitative and Qualitative

A "hierarchical agglomerative cluster assessment" was used to group the treatments based on increasing dissimilarity. Cluster I included 150 mM of KCl, which had high values for parameters of leaf area and dry matter shoot (Fig. 5). Cluster II included KNO_3 through $KNO_3 \times CaCl_2$ (50 mM) was characterized by plants with medium to low EC, Antioxidant capacity, RWC, leaf, stem and shoot humidity and very low dry weight leaf, dry matter shoot, plant height, number of leaves and total chlorophyll. The third cluster, which included SA × KCl (150 mM) and KNO_3 × KCl (100 mM), showed medium values for EC, Antioxidant capacity, RWC, and leaf, stem, and shoot humidity. Within this cluster, the two treatments had the lowest values of the remaining traits.

All traits had a negative correlation with phenol and antioxidant activity, except for EC. Chlorophyll a and total chlorophyll contents were positively correlated with root length ($r = 0.75$) and ($r = 0.69$), respectively (Fig. 6).

DISCUSSION

Our results showed that priming basil seeds with salicylic acid and nitrate through a positive effect on the antioxidant system and improving physiological traits can be an effective solution for crop production under salt stress conditions, although salicylic acid was more effective compared to potassium nitrate.

A decrease in the RWC of plant leaves under salt stress indicates a lack of water in the plant. This may be due to the creation of an osmotic potential that prevents water uptake by the roots and consequently a decrease in water in the plant [12]. The reason for the superiority of primed seeds compared to non-primed seeds in different plant species can be deduced from the fact that, firstly, the pretreatment of seeds accelerates the development of two of the three germination phases, i.e. shortens the time of metabolism. The

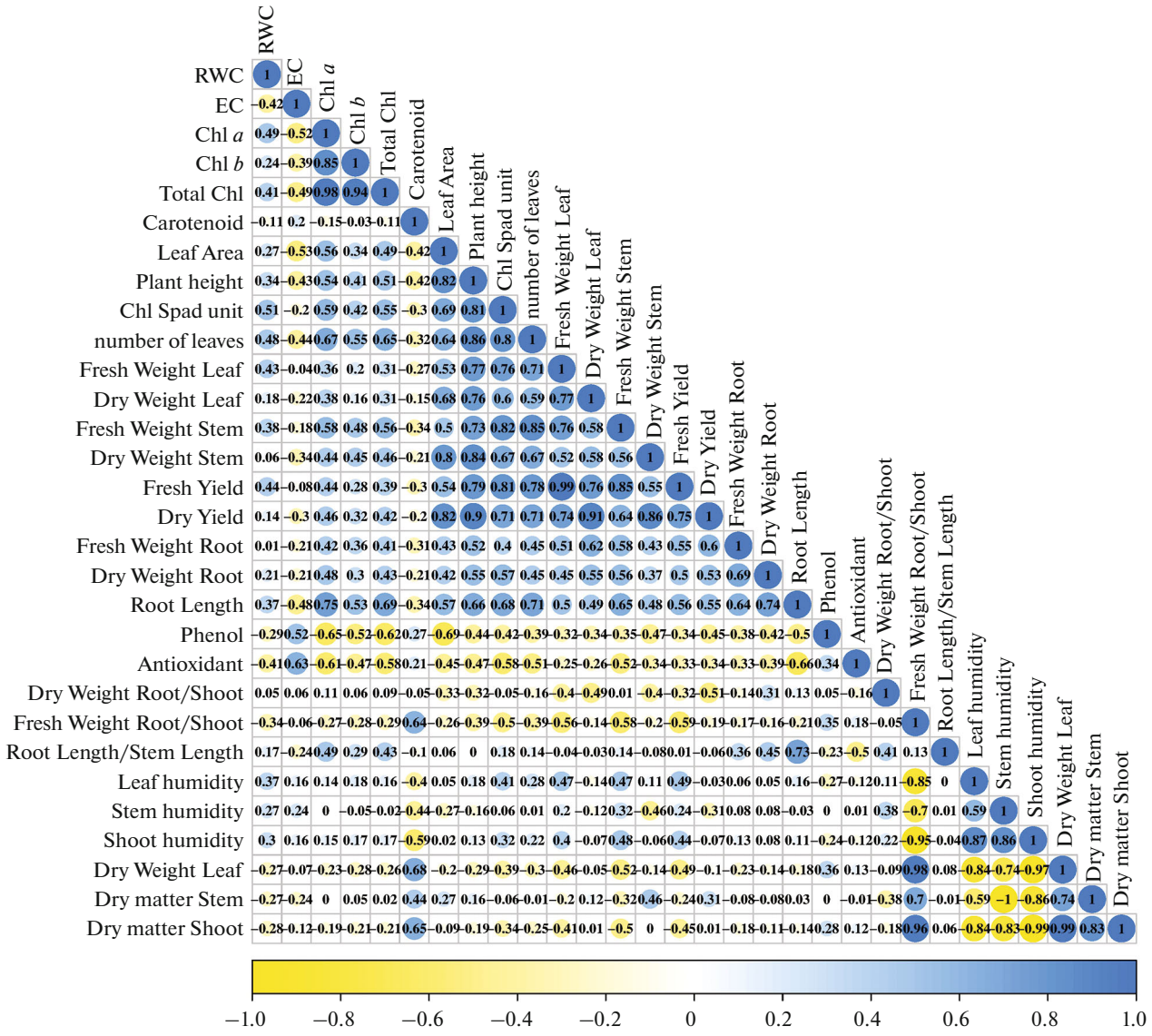

Fig. 6. Correlation plots of basil based on morphological and chemical properties.

maintenance of cell membrane integrity under stress conditions is indicative of the presence of control mechanisms in tolerance to post-wear. In general, stress increases the oxidation of lipids and ultimately decreases the stability index of the cell membrane in various plants [13, 14].

Chlorophyll content was influenced by the interaction effect of salinity stress and priming. The decrease in chlorophyll concentration is one of the most important factors affecting the photosynthetic capacity of the plant, and the increase in salinity leads to poor efficiency of the leaves in carrying out photosynthesis and exacerbation of stress damage [15]. One of the most important reasons for the reduction of chlorophylls is their destruction by active oxygen [6, 16]. Other reasons for the improvement of plant growth parameters under the influence of SA treatment are the photosynthetic apparatus, the amount of photosynthesis, the activity of the enzyme Rubisco, the amount of photosynthetic pigments, stomatal conductance, the antioxidant defense system, the reduction of oxidative stress and leaky ions, the increase of biomembrane correlations and plant mineral nutrition [17, 18].

Oxidative stress caused by salt stress in plant tissues is reduced by the activity of carotenoids in both enzymatic and non-enzymatic antioxidant systems. Carotenoids are one of the most important and essential pigments of the antioxidant system in plants, but they are very sensitive to oxidative degradation. Protection against damage by active oxygen radicals at this point is a vital measure for chloroplasts. At this point, beta-carotene plays a role not only as an auxiliary pigment but also as an effective antioxidant to protect photochemical processes and their stability [19]. As a result

of salt stress, secondary stresses such as oxidative stress can also occur, whereby the production and accumulation of active radicals leads to the oxidation of proteins and lipids and thus to cell death [20, 21].

According our findings, the highest content of phenolic compounds was obtained in the treatment without priming and 150 mM KNO_3. Under salt stress conditions, more phenolic compounds are produced, and the results of the present study also indicate an increase in the production of phenolic compounds under salt stress. Neelam et al. [20] explained the reason for the increase in phenolic content in plants under stress is that plants under stress use special defense mechanisms such as increasing the concentration of total phenolics against oxidative stress.

The results showed that the use of SA for priming was more effective than KNO_3. Salt stress reduces plant growth and height by reducing cell growth (reduction in cell division and reduction in cell size) at the vegetative growth stage. SA has been reported to increase cell division in the meristem of wheat seedlings and improve plant growth. It is also reported that this substance alters the hormonal balance in the plant and stimulates the production of auxin and cytokinin. This shows that priming seeds leads to stronger seedlings [22, 23]. KNO_3 probably increases the photosensitivity of germinating seeds and acts as a complementary factor of phytochrome and increases seed germination [24].

Salinity stress decreased the yield of fresh shoots compared that with of the control (no salinity stress). Excessive consumption of energy for the production of some organic substances that stabilize osmotic balance is carried out by absorbing ions, which is considered another factor in reducing the weight of aerial organs [25]. Seed priming before cultivation with KNO_3 and SA led to an increase in dry leaf yield compared with the control (no priming) under salt stress conditions. Avarseji, et al. [26] reported that the dry weight and leaf length of saffron decreased significantly with increasing salinity stress intensity. According to the aforementioned cases, the efficiency of the plant in absorbing water decreases, and subsequently the growth of the plant decreases. In an experiment conducted on basil plants, it was found that the number and area of its leaves decreased with increasing salinity [27]. Seeds treated with SA germinated faster and had better establishment. This is because the most sensitive stage of plants to soil salinity is the stage of germination and establishment, as a result, the seeds that have been treated with SA passed this stage better and entered the next stages with more seeds that could better tolerate salinity. Probably, salicylic acid improves plant growth and performance by altering hormone balance, particularly the increase in auxin and cytokinin hormones [28].

The results of this study showed that salt stress leads to a decrease in basil plant growth. It was also found that with increasing stress levels, the decreasing trend in the measured traits in this plant was faster and greater. The negative effects of stress on these traits were maximal at high salt concentrations (150 mM). In contrast, seed priming had positive effects on plant growth indices under stressful conditions. According to the results obtained from this research, seed priming before sowing led to an improvement in the growth indices measured in basil plants under salinity stress conditions caused by $CaCl_2$ and KCl salts. In this study, basil seed priming with SA and KNO_3 led to faster and more uniform seed germination. Finally, the resulting seedlings grew faster and more than the control condition (no priming), and subsequently performed better under stress conditions. This is known as saltiness. It was also found that priming with salicylic acid led to better performance than priming with potassium nitrate in most of the measured traits.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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