

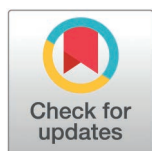
RESEARCH ARTICLE

Enhancement of plant growth in lentil (*Lens culinaris*) under salinity stress by exogenous application or seed priming with salicylic acid and hydrogen peroxide

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Abstract

This study was conducted in order to test the effect of seed pretreatment or exogenous application through the rooting medium of 0.1 mM Salicylic Acid (SA) and 0.1 mM hydrogen peroxide (H_2O_2) on growth, nutritional behavior and some biochemical parameters (photosynthetic pigments, gas exchange parameters, oxidative stress indicators and antioxidant enzymes activities) of lentil plants (*Lens culinaris*) under 75mM salt stress. Our results demonstrated that salt stress noticeably reduced shoot and root DWs by 39.01 and 42.81%, respectively, as compared to controls. This reduction was associated with a significant decrease in all photosynthetic parameters, including Chlorophyll (Chl) and carotenoid (Car), net assimilation of photosynthesis (A), stomatal conductance (g_s), transpiration (E) and internal CO_2 level (C_i), an accumulation of Na^+ and Cl^- and a decrease of K^+ and Ca^{2+} concentrations in plant shoots and roots. In addition, relative to control plants, salt stress remarkably increased the malondialdehyde MDA and H_2O_2 contents especially in roots and increased GPOX and SOD activities, especially in plant shoots. Both methods of SA and H_2O_2 application recovered the plant growth, enhanced shoot and root DWs (increase of 67.65 and 82.36% in shoots and roots, respectively, as compared to salt-stressed plants) and increased all parameters that were reduced by NaCl treatment. Nevertheless, the most prominent effects of SA and H_2O_2 on plant growth were obtained with the seed priming method. Thus, SA and H_2O_2 applications, especially the H_2O_2 seed priming method, induced the antioxidant system, improved the membrane stability and ameliorated the gas exchange parameters. As compared to salt plant stressed, Na^+ and Cl^- contents were significantly decreased and K^+ and Ca^{2+} were significantly increased in shoots and roots following SA and H_2O_2 applications,

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especially with the H₂O₂ seed priming method. Similarly, this method was more efficient in alleviating the adverse effects of salt stress on all photosynthetic pigment contents and measured gas exchange parameters. Compared to salt stressed plants, it significantly decreased the H₂O₂ and MDA contents and further stimulated GPOX and SOD activities. Our results indicated that the seed priming method, particularly with H₂O₂, could be recommended for obtaining better growth of lentil seedlings under salt-affected soil conditions.

Introduction

Salt stress is considered as an abiotic stress that severely affects crop production, especially in arid and semi-arid areas, due to scarcity of rains and increased evapotranspiration [1]. Salt stress affected, approximately, 33% of irrigated crop lands, and by 2050, this could exceed 50% [2].

Salt stress disturbs growth and developmental processes by influencing several physiological and biochemical plant's processes [3]. These influences can occur in two phases; the first one is related to the high salt concentration in root zones (osmotic effect), whereas the second is related to high accumulation of salt ions in plant tissues (toxic effect) [4]. Moreover, it is well established that plants exposed to salt stress showed a production of high amounts of reactive oxygen species (ROS) resulting thereby in the deactivation of various important cellular and metabolic processes in plants [3,5]. In line with this, several studies showed that salt stress results in alteration in the ultra-structural cell components, decrease in the amount of chlorophyll pigments and in enzymatic activities, disturbance of the stomatal conductance and the photosynthesis machinery limiting thereby the development and productivity of crops [3,6].

Lentils (*Lens culinaris*) are considered as one of the most important legumes due to their numerous benefits [7,8]. In addition to their high-quality source of vitamins, micronutrients, dietary fibers, oligosaccharides, fatty acids and vitamins, lentils are considered as an essential crop due to their high protein contents [9–11]. Despite their ability to grow and produce in some marginal environments, such as extremely cold temperatures and drought, lentils are generally vulnerable to salt stress during all plant's life stages, which may reduce their yield up to 50% [12].

Nowadays, to alleviate water scarcity, the irrigation of crop plants with saline water has become necessary, especially in semi-arid and arid areas. Thus, it is extremely important to identify new strategies that minimize the deleterious effects of the use of saline waters on plants [13]. An alternative strategy that can be used is the application of growth regulators, fertilizers, osmoprotectants and antioxidants [14–17]. During the last two decades, salicylic acid (SA) has been the focus of intensive research as a plant growth regulator with various roles in many plants' biological processes [18,19]. Besides its important role in plant growth and development, SA has emerged as secondary metabolite involved in the regulation of respiration, photosynthesis and enzyme biosynthesis [19]. Furthermore, several previous

studies showed that SA acts in the regulation of the plant resistance response to different abiotic stresses, particularly salt stress [20,21]. The ability of SA to mitigate the adverse effects of salt stress has been demonstrated in many crops such as cabbage [22], cowpea [23], strawberries [24] and alfalfa [25]. As already reported, this SA-mediated tolerance can be achieved through the regulation of a wide range of vital physiological and biochemical processes such as water relations, stomatal conductance, photosynthesis, ion homeostasis, cell membrane permeability as well as the regulation of the osmotic adjustment system and the activation of the antioxidant enzymes to counter the deleterious effects of ROS [19,26].

On another hand, H_2O_2 , as a signaling molecule, plays an important role in plant developmental processes [27–29]. Several previous studies showed that H_2O_2 is involved in the regulation of various biological processes, such as increase of Ca^{2+} concentration in plants and the synthesis of osmolytes such as proline, resulting in osmotic adjustment [30,31]. Furthermore, apart from its involvement in the general plant's growth and metabolism, H_2O_2 is involved in the modulation of many fundamental functions in plants under marginal soil conditions, mainly salt-affected soils [28,32,33]. For instance, it has been shown that exogenous application of H_2O_2 alleviated the negative effects of salt stress on photosynthesis and production components in bell pepper plants [30]. Recently, Nobrega et al. [33] showed that the application of H_2O_2 reduced the effects of salt stress on several photosynthesis parameters of cotton plants under salt stress.

As described above, several studies and arguments demonstrated the ability of SA and H_2O_2 to regulate the plant tolerance-responses to salt stress. Nevertheless, the SA and H_2O_2 -mediated tolerance depends on several parameters like the salt stress concentration, duration of stress application, plant species, the plant growth stage as well as the method of application of these two chemical agents and the applied dose [19]. Despite SA and H_2O_2 may be applied to plants as seed priming or through exogenous supplementation, data regarding to the efficiency of these methods under salt stress conditions are scarce. Therefore, this study was conducted in order to evaluate the effect of seed pretreatment or exogenous application through the rooting medium of SA and H_2O_2 on growth, mineral nutrition, photosynthetic pigments, gas exchange parameters, oxidative stress indicators and antioxidant enzymes' activities of lentil plants under salt stress. This is one of the few works released to compare the effectiveness of exogenous application or seed pretreatment with SA and H_2O_2 on salt stressed plants, especially on *Lens culinaris*.

Materials and methods

Growth conditions and experimental designs

Seeds of lentil cultivar Ncir were sterilized by soaking in 3.6% sodium hypochlorite solution, followed by washing with sterile distilled water. In a first set of experiment, seeds were unprimed (control) or primed by soaking for 3 hours in 0.1 mM salicylic acid (SA) or 0.1 mM hydrogen peroxide (H_2O_2). Unprimed and primed seeds were then germinated in Petri plates (15 seeds per Petri plate) double lined with moistened filter paper in the dark at 25°C. Ten days after germination, seedlings obtained from unprimed and primed seeds were grown hydroponically in a glasshouse at 26°C/20°C day/night temperature, 70–80% relative humidity, a 16 h photoperiod and using a nutrient solution with the following composition: KH_2PO_4 (0.36 mM), K_2SO_4 (0.7 mM), $MgSO_4 \cdot 7H_2O$ (1 mM), $CaCl_2 \cdot 2H_2O$ (1.65 mM), Urée (4 mM), Sequestrène (1.26 mM), $MnSO_4 \cdot H_2O$ (6.6 mM), $ZnSO_4 \cdot 7H_2O$ (1.55 mM), $CuSO_4 \cdot 7H_2O$ (1.56 mM), H_3BO_3 (4 mM), $CaSO_4 \cdot 7H_2O$ (0.12 mM), Na_2MoO_4 (0.12 mM) [34]. Salt treatment was applied 30 days after seedling transplantation by adding 75 mM NaCl in the culture medium. The concentrations of 75 mM NaCl-salinity, 0.1 mM SA and 0.1 mM H_2O_2 used in this work were selected on the basis of previous published experiments [28,32,35–37]. In a second set of experiment, SA (0.1 mM), H_2O_2 (0.1 mM) and NaCl (75 mM) were exogenously applied through the rooting medium to thirty-day-old seedlings obtained from unprimed seeds. The experiment was conducted in a randomized complete block design with three replications. Treatment conditions followed in the two sets of experiments are shown below.

Denotation of the used treatment

Treatment	Denotation
Control (without NaCl and chemical agent treatments)	Control
75 mM NaCl-salinity (without chemical agent treatment)	NaCl
Seeds primed with 0.1 mM SA (without NaCl treatment)	SA/SP
Seeds primed with 0.1 mM H ₂ O ₂ (without NaCl treatment)	H ₂ O ₂ /SP
Exogenous application of 0.1 mM SA (without NaCl treatment)	SA/EA
Exogenous application of 0.1 mM H ₂ O ₂ (without NaCl treatment)	H ₂ O ₂ /EA
Seeds primed with 0.1 mM SA (with 75 mM NaCl treatment)	NaCl+SA/SP
Seeds primed with 0.1 mM H ₂ O ₂ (with 75 mM NaCl treatment)	NaCl+H ₂ O ₂ /SP
Exogenous application of 0.1 mM SA (with 75 mM NaCl treatment)	NaCl+SA/EA
Exogenous application of 0.1 mM H ₂ O ₂ (with 75 mM NaCl treatment)	NaCl+H ₂ O ₂ /EA

Plant harvest and dry weight determination

Thirty days after treatment applications, roots and shoots were harvested and fresh and dry weights were immediately determined. Dry weights were obtained after drying plant material at 80°C for 3 days.

Mineral analysis

Dried tissues were digested with 7% nitric acid (HNO₃) for mineral analysis. Na⁺, K⁺ and Ca²⁺ ions were analyzed using a spectrophotometer (Eppendorf Geratebau Netherler). However, Cl⁻ ions were quantified using a Chloridometer (Haake, Buchler instruments Inc., New Jersey, USA).

Gas-exchange parameters measurement and photosynthetic pigment contents

Measurements of gas-exchange parameters (net CO₂ rate (*A*), stomatal conductance (*g_s*), transpiration rate (*E*) and intern CO₂ content (*C_i*)) were determined at the end of the experiment using a portable gas exchange system (LCPro+, Bio-Scientific, Great Amwell, Herts, UK). Measurements were released on mature leaves cultivated at 1200 μmol m⁻² s⁻¹ PAR, 400 μmol mol⁻¹ CO₂ concentrations and 29 ± 2°C leaf temperature. Measurements were done on acclimated leaves (15 min in leaf chamber) between 10:00 am and 2:00 pm.

The leaf chlorophyll and carotenoid concentrations were determined and estimated following the methods of Arnon [38] and McKinney [39], respectively. Extracts were prepared using 100 mg FW in 80% chilled acetone.

Determination of H₂O₂ and malondialdehyde MDA contents

The method of Velikova et al. [40] was used to determine the H₂O₂ content. Extract absorbance was read at 390 nm. A standard curve of H₂O₂ was used in order to estimate contents.

The thiobarbituric acid (TBA) method [41] was used for lipid peroxidation estimation. Extracts absorbances were recorded at 532 nm (specific) and 600 nm (non-specific).

Determination of protein content and antioxidant enzymes activities

Tissue samples were homogenized in 50 mM KH₂PO₄/K₂HPO₄, pH 7.8, and then centrifuged for 15 at 12,000g min at 4°C. The Bradford method [42] was used in order to estimate the tissue protein concentration. Guaiacol peroxidase GPOX activity was measured at 470 nm according to Anderson et al. [43]. However, Superoxide dismutase SOD activity was evaluated at 560 nm according to Lee et al. [44].

Statistical analysis

Differences between treatments for all measurements were analyzed using the SPSS software (Version 15.0 for Windows). Comparison was carried out at $P=0.05$ using Duncan's multiple-range test. All measurements were carried out in triplicate.

Results

Shoot and root dry weights under salt stress and different SA and H₂O₂ treatments

The impacts of SA and H₂O₂, applied either through the rooting medium or seed priming, on the DW production of lentil plants under salt stress are displayed in Fig 1. Results showed that salt stress significantly reduced shoot and root DWs by 39.01 and 42.81%, respectively, as compared to controls. Nevertheless, independently of their method of application, SA and H₂O₂ treatments alleviated the decrease in the growth of the salt-stressed plants. With the SA and H₂O₂ priming method, shoot and root DWs were increased by 29.34% and 38.37% as well as by 67.65% and 82.36%, respectively, compared to plants treated only with NaCl. Similarly, shoot and root DWs were increased by 12.24 and 30.85% as well as 34.03% and 39.92% respectively, following exogenous application through the rooting medium of SA and H₂O₂. It is noteworthy that, as compared to the exogenous application, the seed priming method was more efficient in alleviating the negative effects of salt stress on dry matter production of lentil plants, particularly with H₂O₂ (increase of 67.65 and 82.36% in shoots and roots, respectively, as compared to salt-stressed plants). Under non saline conditions, SA and H₂O₂, applied either through seed priming or the rooting medium, did not affect root DW. However, a slight decrease was observed in shoot DW of plants obtained from SA and H₂O₂-primed seeds, relative to unprimed ones (Fig 1).

Mineral nutrition in lentil plants under salt stress and different SA and H₂O₂ treatments

As expected, salt treatment increased Na⁺ and Cl⁻ and decreased the K⁺ and Ca²⁺ concentrations in shoots and roots of lentil plants (Table 1). Although SA and H₂O₂, applied either through seed priming or rooting medium, noticeably decreased shoot and root salt ions, obtained results showed that K⁺ and Ca²⁺ concentrations depended on the plant organ and on the used chemical agent as well as its manner of application. As for root Ca²⁺, shoot K⁺ concentration was significantly increased by the both methods of H₂O₂ treatment. However, no obvious effects were observed following SA treatment, whatever its method of application. In roots, K⁺ concentration was significantly increased following SA and H₂O₂ treatments, independently of their method of application. In salt-stressed lentil plants obtained from seeds primed with SA or H₂O₂, shoot Ca²⁺ concentration was increased by 22.09 and 30.33%, respectively, relative to plants treated only by NaCl. Contrarily to the exogenous application of H₂O₂, for which we noted an increase of 16.47%, no obvious effects were

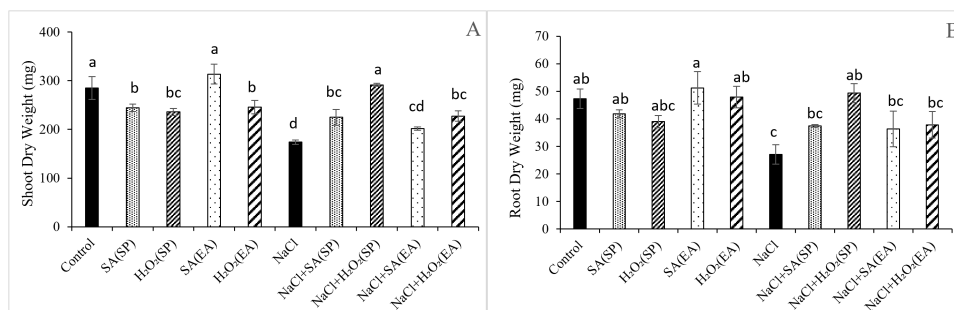


Fig 1. Effect of 0.1mM salicylic acid (SA) or hydrogen peroxide (H₂O₂) and salt treatment (0 and 75mM NaCl) on shoots (A) and roots (B) DW of lentil plants. SA and H₂O₂ are applied either by seed's priming (SP) or added in the culture medium (EA). Bars are means $\pm$ SE. Values with the same letter per parameter are not significantly different according to Duncan test ($p=0.05$), $n=3$.

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Table 1. Effect of 0.1mM salicylic acid (SA) or hydrogen peroxide (H₂O₂) and salt treatment (0 and 75 mM NaCl) on Na⁺, K⁺, Ca²⁺ and Cl⁻ levels in meq. g⁻¹DW in shoots and roots of lentil plants. SA and H₂O₂ are applied either by seed's priming (SP) or added in the culture medium (EA).

	Shoots				Roots			
	Na ⁺	K ⁺	Ca ²⁺	Cl ⁻	Na ⁺	K ⁺	Ca ²⁺	Cl ⁻
Control	1,07 ef*	21,14 d	4,60 ab	0,65 d	1,12 de	21,14 d	4,37 a	0,47 d
SA (SP)	0,99 f	32,23 a	3,36 cd	0,70 d	1,15 de	27,45 a	3,37 bc	0,37 e
H ₂ O ₂ (SP)	0,97 f	25,20 c	4,72 a	0,68 d	1,14 de	24,50 c	4,52 a	0,38 e
SA (EA)	1,02 ef	21,42 d	3,07 de	0,66 d	1,14 de	19,47 e	3,73 b	0,46 d
H ₂ O ₂ (EA)	1,20 e	27,45 b	4,39 b	0,66 d	1,07 e	26,12 b	4,50 a	0,42 de
NaCl	2,39 a	13,71 g	2,67 f	1,41 a	1,60 a	6,05 h	2,97 c	0,85 a
NaCl+ SA(SP)	1,66 c	14,53 g	3,26 cd	1,29 b	1,23 cd	7,52 g	3,41 bc	0,65 c
NaCl+ H ₂ O ₂ (SP)	2,00 b	17,53 e	3,48 c	1,20 c	1,35 b	8,25 fg	3,82 b	0,75 b
NaCl+ SA(EA)	1,91 b	14,15 g	2,84 ef	1,19 c	1,35 b	7,50 g	3,41 bc	0,68 bc
NaCl+ H ₂ O ₂ (EA)	1,46 d	16,03 f	3,11 de	1,17 c	1,32 bc	8,97 f	3,81 b	0,63 c

Values with the same letter per parameter are not significantly different according to Duncan test ($p=0.05$), $n=3$.

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observed in shoot Ca²⁺ concentration when SA was applied through the rooting medium. Furthermore, among all applied treatments, the most prominent effect on root and shoot K⁺ and Ca²⁺ was obtained with the seed priming method, especially with H₂O₂ (Table 1). Under non-saline conditions, SA applied either through the rooting medium or seed priming, significantly increased K⁺ and Ca²⁺ contents, in shoots and roots. However, no obvious effects were observed following treatments with H₂O₂, irrespective of its method of application (Table 1).

Gas exchange parameters in lentil plants under salt stress and different SA and H₂O₂ treatments

Salt stress significantly decreased all photosynthetic parameters of lentil plants (Table 2). As compared to controls, net assimilation of photosynthesis (*A*), stomatal conductance (*gs*), transpiration (*E*) and internal CO₂ level (*Ci*), were reduced by 59.58, 83.33, 78.19 and 63.07%, respectively. Nevertheless, all analyzed photosynthetic parameters were

Table 2. Effect of 0.1mM salicylic acid (SA) or hydrogen peroxide (H₂O₂) and salt treatment (0 and 75 mM NaCl) on photosynthetic assimilation (*A*), stomatal conductance (*gs*), transpiration (*E*) and intern CO₂ content (*Ci*) in lentil leaves. SA and H₂O₂ are applied either by seed's priming (SP) or added in the culture medium (EA).

	<i>A</i> ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	<i>Gs</i> ($\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	<i>E</i> ($\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	<i>Ci</i> ($\mu\text{mol}\cdot\text{mol}^{-1}$)
Control	13,56 a	0,18 a	2,11 a	371,00 cd
SA (SP)	10,05 c	0,16 b	1,32 c	416,33bc
H ₂ O ₂ (SP)	13,47 a	0,15 c	1,43 c	331,67 de
SA(EA)	14,65 a	0,18 a	1,86 b	369,67 cd
H ₂ O ₂ (EA)	9,99 c	0,14 c	1,34 c	451,67 ab
NaCl	5,48 e	0,03 g	0,46 f	137,00 f
NaCl+ SA(SP)	11,74 b	0,11 d	1,36 c	346,33 d
NaCl+ H ₂ O ₂ (SP)	11,27bc	0,05 f	0,54ef	476,00 a
NaCl+ SA(EA)	8,30 d	0,08 e	0,95 d	289,33 e
NaCl+ H ₂ O ₂ (EA)	8,47 d	0,05 f	0,66 e	424,00 b

Values with the same letter per parameter are not significantly different according to Duncan test ($p=0.05$), $n=3$

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noticeably enhanced by SA and H₂O₂, irrespective of their method of application. In fact, *A*, *gs*, *E* and *Ci* were increased by 114.23, 266.66, 195.65% and 152.79% as well as 105.65, 66.66, 17.39 and 247.44%, in salt-stressed plants obtained from seeds primed with SA and H₂O₂, respectively, as compared to those from unprimed seeds. Similarly, *A*, *gs*, *E* and *Ci* were enhanced by 51.4, 166.66, 106.52 and 111.1% as well as 54.56, 66.66, 43.47 and 209.49%, in salt-stressed plants exogenously treated with SA and H₂O₂, respectively. It is noteworthy that the most prominent effects on *A*, *gs*, *E* and *Ci* of salt-stressed plants were obtained following the seed priming method, especially with H₂O₂. With some exceptions, SA and H₂O₂ did not affect all measured photosynthetic parameters, under non-saline conditions (Table 2).

Photosynthetic pigment contents in lentil plants under salt stress and different SA and H₂O₂ treatments

Data regarding photosynthetic pigment contents in salt-stressed lentil plants following treatments with SA and H₂O₂ are shown in Table 3. Obtained findings showed that Chl a and Chl b as well as Chl (a + b) and carotenoids were reduced by 27.59, 22.32, 20.81 and 45.38%, respectively in salt-stressed as compared to control plants. Contrarily to Ch (a + b) for which a significant increase was observed only following the H₂O₂ primed seeds treatment, Ch a content of salt-stressed plants was noticeably increased by 24.97, 36.77, 26.2 and 22.7% following the application of SA and H₂O₂ as seed priming and through the rooting medium, respectively. Except the H₂O₂ primed seeds treatment for which a significant increase was observed, all other treatments did not affect Chl b contents. Carotenoid content of salt-stressed plants was increased by 46.24, 32.51 and 29.57%, following the application of SA as seed priming and following the application of SA and H₂O₂ either as seed priming or through the rooting medium, respectively; whereas no obvious effect was observed with the H₂O₂ primed seeds treatment (Table 3).

H₂O₂ and MDA contents in lentil plants under salt stress and different SA and H₂O₂ treatments

Results relative to MDA and H₂O₂ contents, as oxidative damage markers, are shown in Figs 2 and 3. Salt stress induced the generation of H₂O₂ in roots of lentil plants by 83.10%, causing an increase of 42.73% in lipid peroxidation. Although no obvious effect of salt stress was observed in shoot H₂O₂ content, MDA concentration was increased by 39.64%, as compared to control. Whatever its method of application, SA was efficient in decreasing the concentrations of H₂O₂ both in shoots in roots of salt-stressed plants (Fig 2). Interestingly, MDA contents were significantly decreased

Table 3. Effect of 0.1mM salicylic acid (SA) or hydrogen peroxide (H₂O₂) and salt treatment (0 and 75 mM NaCl) on Chlorophyll a (Chla), Chlorophyll B (Chlb), Carotenoids and Chlorophyll a and b (Chla+b) content in µg.g⁻¹ FW in shoots of lentil plants. SA and H₂O₂ are applied either by seed's priming (SP) or added in the culture medium (EA).

	Chla	Chlb	Carotenoids	Chla+b
Control	366,56 ab	168,72 abc	15,60 b	535,28 ab
SA (SP)	310,82 d	143,25 cd	18,30 a	454,08 c
H ₂ O ₂ (SP)	315,02 d	147,18 cd	13,75bc	462,20 c
SA (EA)	384,46 a	178,07 ab	13,80bc	562,53 a
H ₂ O ₂ (EA)	302,28 d	145,31 cd	13,85bc	447,59 c
NaCl	265,40 e	131,05 d	8,52 e	423,86 c
NaCl + SA(SP)	331,69bcd	151,93bcd	12,46 cd	483,62bc
NaCl + H ₂ O ₂ (SP)	363,00 abc	182,22 a	8,70 e	545,22 b
NaCl + SA(EA)	334,96bcd	158,46abcd	11,29 d	466,01 c
NaCl + H ₂ O ₂ (EA)	325,66 cd	156,28abcd	11,04 d	481,94bc

Values with the same letter per parameter are not significantly different according to Duncan test ($p = 0.05$), $n = 3$

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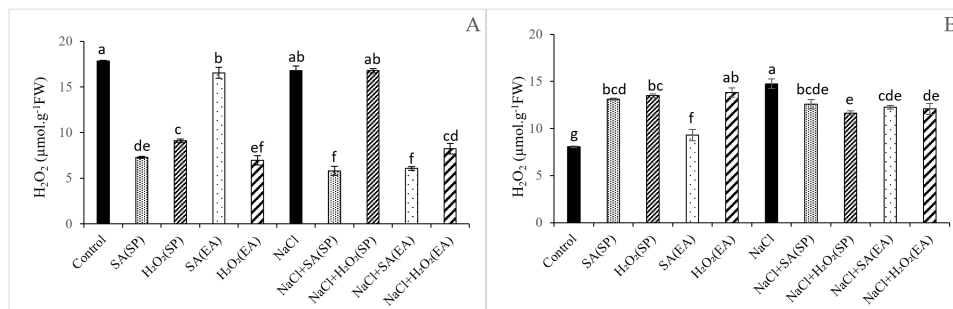


Fig 2. Effect of 0.1mM salicylic acid (SA) or hydrogen peroxide (H₂O₂) and salt treatment (0 and 75mM NaCl) on hydrogen peroxide (H₂O₂) content in μmol.g⁻¹ FW in shoots (A) and roots (B) of lentil plants. SA and H₂O₂ are applied either by seed's priming (SP) or added in the culture medium (EA). Bars are means ± SE. Values with the same letter per parameter are not significantly different according to Duncan test ($p=0.05$), $n=3$.

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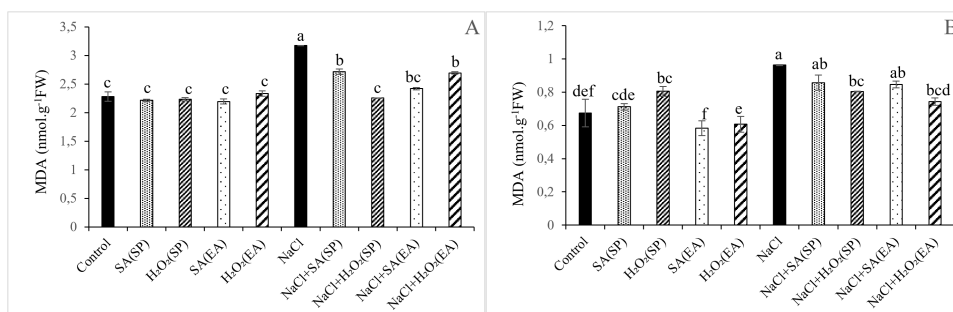


Fig 3. Effect of 0.1mM salicylic acid (SA) or hydrogen peroxide (H₂O₂) and salt treatment (0 and 75mM NaCl) on malondialdehyde (MDA) level in shoots (A) and roots (B) of Lentil plants. SA and H₂O₂ are applied either by seed's priming (SP) or added in the culture medium (EA). Bars are means ± SE. Values with the same letter per parameter are not significantly different according to Duncan test ($p=0.05$), $n=3$.

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in shoots and remained unchanged in roots. It is noteworthy that exogenous application of H₂O₂ to salt-stressed plants significantly decreased shoots and roots H₂O₂ contents. However, with the seed priming method, H₂O₂ content was significantly reduced in roots and remained unchanged in shoots, as compared to plants treated only with NaCl. Concerning lipid peroxidation, our findings showed that MDA contents were noticeably decreased in salt-stressed plants following SA and H₂O₂ treatments, independently of their method of application. In roots, MDA contents were significantly decreased by exogenous application and seeds priming with H₂O₂. Nevertheless, SA had no obvious effects on root MDA contents (Fig 3). Under non-saline conditions, H₂O₂ contents were significantly decreased and increased in shoots and roots, respectively, following SA and H₂O₂ treatments, independently of their method of application. Except in roots under the seed priming method, the MDA contents were unaffected by SA and H₂O₂ treatments both in roots and shoots (Fig 3).

Activities of enzymatic antioxidant of lentil plants under salt stress and different SA and H₂O₂ treatments

Data regarding the antioxidant enzymes activities SOD and GPOX under the different treatments are given in Figs 4 and 5. Except for root SOD activities for which no obvious effects were observed, salt stress remarkably increased shoot and root GPOX and shoot SOD activities, relative to controls. The effects of SA and H₂O₂ treatments on GPOX and SOD activities depended on the plant organ and on the method of application of the chemical agent. Irrespective of the method of their application, SA and H₂O₂ treatments sharply increased GPOX activities in roots of salt-stressed plants (by 130.02

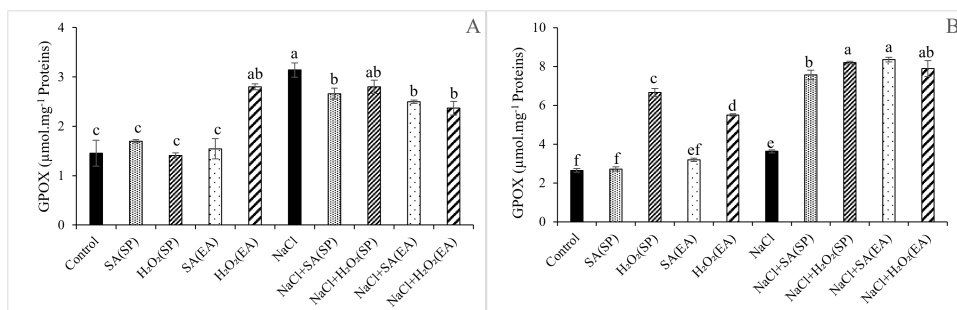


Fig 4. Effect of 0.1mM salicylic acid (SA) or hydrogen peroxide (H₂O₂) and salt treatment (0 and 75mM NaCl) on in the guaicolperoxydase activity (GPOX) in shoots (A) and roots (B) of lentil plants. SA and H₂O₂ are applied either by seed's priming (SP) or added in the culture medium (EA). Bars are means $\pm$ SE. Values with the same letter per parameter are not significantly different according to Duncan test ($p=0.05$), $n=3$.

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and 125.89%, respectively). However, no obvious effects were observed in shoots. Similarly, shoot SOD activities were not affected by H₂O₂ treatment. Nevertheless, significant decreases were observed following SA treatment, whatever its method of application. All treatments noticeably increased root SOD activities, relative to those of salt-stressed plants, with the H₂O₂/SP and H₂O₂/EA treatments being more efficient (Fig 5).

Discussion

Salinity is a major abiotic stress that negatively affects plant growth and development through the disruption of different physiological and biochemical mechanisms [6]. In this work, we observed significant reduction in biomass production of lentil plants by a 75mM NaCl treatment (Fig. 1). Similar reductions in plant growth in response to salinity have been recorded in other plant species such as *Helianthus annuus* [45], *Hordeum vulgare* [46] and *Triticum durum* [47]. The observed reduction in lentil plants growth following salt application could be related to the nutritional imbalances, resulting in the reduction of several physiological activities [48]. Treatment of the salt-stressed lentil plants with SA and H₂O₂ recovered the plant growth and enhanced shoot and root DW (Fig. 1). This result on SA-ameliorating effects is consistent with those obtained in other legumes [49,50], wheat [51,52], sunflower [53], sugarcane [54] and tomato [55]. The application of H₂O₂ increased the DW of salt-stressed lentil plants, demonstrating its beneficial role in alleviating the negative effects of salinity. In pea plants, Dito et al. [56] attributed this amelioration effect of H₂O₂ to its action as a signaling molecule, and its involvement in the increase of the tolerance of plants to salinity through the regulation of various metabolic processes.

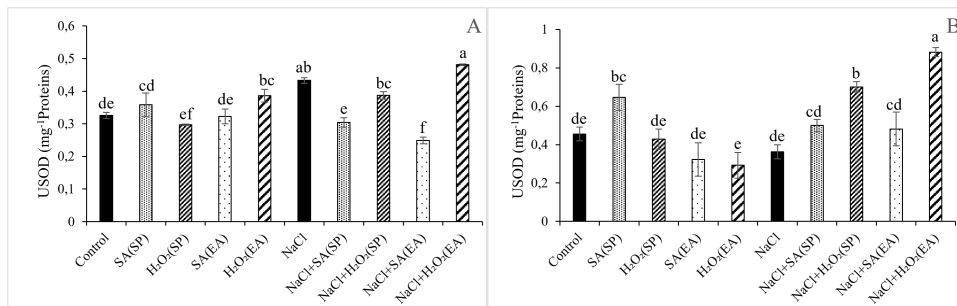


Fig 5. Effect of 0.1mM salicylic acid (SA) or hydrogen peroxide (H₂O₂) and salt treatment (0 and 75mM NaCl) on the superoxide dismutase activity (SOD) in shoots (A) and roots (B) of lentil plants. SA and H₂O₂ are applied either by seed's priming (SP) or added in the culture medium (EA). Bars are means $\pm$ SE. Values with the same letter per parameter are not significantly different according to Duncan test ($p=0.05$), $n=3$.

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It is worth pointing out that, in this work, the most prominent effects of SA and H₂O₂ on plant growth were obtained with the seed priming method, indicating the efficiency of this method in the mitigation of the adverse effects of salt stress on lentil growth. This could be linked to the induction of the antioxidant system (Figs 4 and 5), improvement of the membrane stability (Fig. 3) and amelioration of the gas exchange parameters (Table 2).

It is well known that salt stress disturbs ions uptake by plants, especially the K⁺ content which adversely affects the K⁺/Na⁺ ratio in plant tissues [57,58]. As already noted for many plant species [59–62], our results showed that salt increases Na⁺ and decreases K⁺ contents in leaves and roots of lentil plants, and hence reduce the K⁺/Na⁺ ratio. SA and H₂O₂ applications, especially the H₂O₂ seed priming method, significantly decreased the Na⁺ and Cl⁻ contents and increased the K⁺ and Ca²⁺ contents in shoots and roots (Table 1). The involvement of SA application in the increase of nutrient uptake under salt stress has been indicated by several studies [18,63,64]. This may be explained by the protective effects of SA on cell membrane and root nutrient uptake systems [63]. Using H₂O₂ as seed pretreatment, Wahid et al. [65] showed that H₂O₂-treated wheat seedlings showed higher tissue K⁺, Ca²⁺, NO₃⁻ and PO₄³⁻ levels and induced K⁺/Na⁺ ratio. As K⁺/Na⁺ ratio in plants under salt stress is considered as one of the important selection criteria for salt tolerance [66], we can suggest that H₂O₂ treatment, particularly via the seed priming method, enhances the tolerance of lentil plants to salt stress. Likewise, the maintenance of Ca²⁺ transport under salinity constitutes another important indicator of salinity tolerance [67], making plants less sensitive to osmotic and ionic disturbances. In our study, this scenario seems to have occurred, especially following seed pretreatment with H₂O₂, since we observed significant increase in Ca²⁺ content in response to this treatment (Table 1).

As already mentioned for many plant species such as cotton [68] and sunflower [69], results of the current study indicated a marked reduction in all measured gas exchange parameters in plants subjected to salt stress (Table 2), reinforcing the deleterious effects of this constraint on lentil. The stomatal limitation caused by salinity affected the *g*_s and thus reduced transpiration and C_i concentration. This could be linked to stomata closure and considered as a defense mechanism against water loss, which in turn decreases transpiration and CO₂ assimilation [33,70,71]. SA and H₂O₂, regardless of the way of their application, mitigated the negative effects of salt stress on all measured gas exchange parameters. The beneficial effects of H₂O₂ on gas exchange parameters under salinity were reported in many plant species. For instance, Wahid et al. [65] indicated that treatment with H₂O₂ increased the photosynthetic capacity of salt-affected wheat seedlings through the improvement of the *g*_s and the internal CO₂ concentration. In bell pepper, Aragao et al. [30] observed that 15 μM H₂O₂ promoted the *g*_s, CO₂ assimilation, and carboxylation efficiency of salt stressed plants. It is worth pointing out that the beneficial effects of H₂O₂ on all gas exchange parameters depended on the method of application of this molecule, with the most prominent effects being obtained with the seed priming method. Similarly, the beneficial effects of SA on gas exchange parameters were reported in many plant species. Fariduddin et al. [72] found that, in *Brassica juncea*, photosynthetic parameters were induced following the exogenous application of SA. However, non-stomatal factors, such as the PSII efficiency, the Rubisco activity, and the ATP and NADPH production might also affect photosynthetic parameters under stressful conditions [73]. The observed ameliorated effects of SA on photosynthesis were related to induced effects on the pigment contents and Rubisco activity [74].

It is well known that photosynthetic pigments play an important role in light interception and energy transduction during photosynthesis [75]. As already indicated in several plant species [23,25,46,47], results of our study showed that salinity significantly reduced the concentrations of photosynthetic pigments in lentil plants (Table 3). The reduction of leaf chlorophyll under salinity could be due to increments of the chlorophyllase enzyme and to decline in chlorophyll biosynthesis [23]. In addition, during salinity stress, the high ROS production in cells causes oxidation and degradation of photosynthetic pigments [76]. Although SA and H₂O₂ significantly increased photosynthetic pigments of salt-stressed lentil plants, our findings showed that, as compared to the exogenous application, the seed priming method was more efficient in alleviating the negative effects of salinity on all photosynthetic pigment contents, especially following H₂O₂ application (Table 3). The reduction of the harmful effects of salinity following SA and H₂O₂ treatments could be achieved through the inhibition

of chlorophyll oxidase enzymes, promotion of the activities of enzymes related to chlorophyll biosynthesis as well as decrease in the ROS contents and induction of the antioxidant defense systems [20,77].

In plants, MDA content serves as an indicator of membrane injury and physiological disorders [78]. Consistently with those in *Ocimum basilicum* [79], in Fennel [80], in wheat and barley [46,47], findings of this study showed that salinity provoked an oxidative stress indicated by the excessive generation of H_2O_2 and MDA (Figs 2 and 3), leading to a decline in the stability of the cell membranes [77]. Nevertheless, the application of 0.1 mM SA or H_2O_2 under saline conditions significantly decreased the H_2O_2 and MDA contents, especially in shoots with the seed priming treatment (Figs 2 and 3). The lower MDA and H_2O_2 contents observed in salt-stressed lentil plants following the application SA and H_2O_2 could be due to an efficient reduction in ROS damage and to a greater membrane protection, preventing thereby the unsaturated fatty acid damage and the electrolyte leakage [19,79]. It is now well documented that, to overcome the harmful effects of oxidative stress, plants activate the non-enzymatic and enzymatic antioxidant system [81]. POXs, CAT and SOD are the most important enzymes involved in oxidants detoxification [73]. Our study showed that salinity induced a significant increase in GPOX and SOD activities, especially in leaves, compared to control plants (Figs 4 and 5). This increase in antioxidants activities has been suggested as protection mechanism against salt-induced ROS as mentioned in cotton [82] and tomato [83]. The application of SA or H_2O_2 either as a seed pre-treatment or as an external application in the growing medium further stimulated GPOX and SOD activities. This result is in agreement with that published by Aazami et al. [3] in tomato; and seems to be directly linked to the reduction in MDA contents. In line with this, Xu et al. [19] and Shi et al. [84] showed that SA treatment mitigated the oxidative damage of *Saponaria officinalis* and *Cucumis sativa* seedlings, respectively by enhancing SOD, CAT and GPX activities, reducing thereby the lipid peroxidation levels compared to salt-stressed plants. Similar results were observed by AzevedoNeto et al. [85] in salt-stressed maize plants following the addition of H_2O_2 to the nutrient. As reported above, the enhancement of the antioxidant defense system in response to SA and H_2O_2 treatments has been reported in many crop plants under salt-affected conditions. Interestingly, results of this study showed that such an effect was more pronounced with the seed priming as compared to the root application method. The over activation of enzyme antioxidant system (Figs 4 and 5) and the subsequent reduction in ROS-induced membrane damage (Fig 3) in lentil plants following seed priming treatment are indication of the effectiveness of this treatment method in the mitigation in the salt-induced oxidative stress and, hence, in the amelioration of lentil plants growth under salt stress conditions.

In conclusion, our study demonstrated that salinity (i) decreases the growth and carbon assimilation in lentil plants, (ii) increased membrane alteration, lipid peroxidation and disrupted the uptake of essential ions. SA and H_2O_2 efficiently reduced the deleterious effects of salinity by inducing the growth and photosynthesis of lentil plants, by increasing the chlorophyll content and enhancing the plants antioxidant enzyme mechanisms, thus alleviating the membrane oxidative damage. Furthermore, our results indicate that the seed priming method, particularly with H_2O_2 , is more efficient in alleviating the negative effects of salinity on lentil plants. This strategy could be recommended for obtaining better growth of lentil seedlings under salt stress. Therefore, the seed priming with H_2O_2 may be suggested as a potential strategy to alleviate the effects of salt stress in lentil plants.

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References

1. Zia A, Munsif F, Jamal A, et al. Morpho-physiological attributes of different maize (*Zea mays* L.) genotypes under varying salt stress conditions. *Gesunde pflanz.* 2022; 74: 661. <https://doi.org/10.1007/s10343-022-00641-2>
2. Gopalakrishnan T, Kumar L. Modeling and mapping of soil salinity and its impact on paddy lands in Jaffna Peninsula, Sri Lanka. *Sustain.* 2020;12:1. <https://doi.org/10.3390/su12208317>
3. Aazami MA, Rasouli F, Ebrahimzadeh A. Oxidative damage, antioxidant mechanism and gene expression in tomato responding to salinity stress under in vitro conditions and application of iron and zinc oxide nanoparticles on callus induction and plant regeneration. *BMC Plant Biol.* 2021;21(1):597. <https://doi.org/10.1186/s12870-021-03379-7> PMID: 34915853
4. Munns R, Tester M. Mechanisms of salinity tolerance. *Annu Rev Plant Biol.* 2008;59:651–81. <https://doi.org/10.1146/annurev.arplant.59.032607.092911> PMID: 18444910
5. Ahanger MA, Qin C, Begum N, Maodong Q, Dong XX, El-Esawi M, et al. Nitrogen availability prevents oxidative effects of salinity on wheat growth and photosynthesis by up-regulating the antioxidants and osmolytes metabolism, and secondary metabolite accumulation. *BMC Plant Biol.* 2019;19(1):479. <https://doi.org/10.1186/s12870-019-2085-3> PMID: 31703619
6. Zhao S, Zhang Q, Liu M, Zhou H, Ma C, Wang P. Regulation of Plant Responses to Salt Stress. *Int J Mol Sci.* 2021;22(9):4609. <https://doi.org/10.3390/ijms22094609> PMID: 33924753
7. Benmoussa S, Nouairi I, Rajhi I, Rezgui S, Manai K, Taamali W, et al. Growth performance and nitrogen fixing efficiency of faba bean (*Vicia faba* L.) genotypes in symbiosis with rhizobia under combined salinity and hypoxia stresses. *Agronomy.* 2022;12:606.
8. Yasir TA, Khan A, Skalicky M, Wasaya A, Rehmani MIA, Sarwar N, et al. Exogenous sodium nitroprusside mitigates salt stress in lentil (*Lens culinaris* Medik.) by affecting the growth, yield, and biochemical properties. *Molecules.* 2021;26(9):2576. <https://doi.org/10.3390/molecules26092576> PMID: 33925107; PMCID: PMC8125612.
9. Faris MA, Takruri HR, Issa AY. Role of lentils (*Lens culinaris* L.) in human health and nutrition: a review. *Mediterr J Nutr Metab.* 2013;6(1):3–16.
10. Zhang B, Deng Z, Tang Y, Chen P, Liu R, Ramdath DD, et al. Fatty acid, carotenoid and tocopherol compositions of 20 Canadian lentil cultivars and synergistic contribution to antioxidant activities. *Food Chem.* 2014;161:296–304. <https://doi.org/10.1016/j.foodchem.2014.04.014> PMID: 24837953
11. Rajhi I, Baccouri B, Rajhi F, Hammami J, Abbas Z, Mhadhbi H, et al. HS-SPME-GC-MS combined with chemometrics to assess the impact of germination, dehulling, and milling on flavor attributes of brown and green lentils (*Lens culinaris* subsp. *culinaris*). *S Afr J Bot.* 2022;150:1102–10.
12. Golezani KG, Yengabad FM. Physiological responses of lentil (*Lens culinaris* Medik.) to salinity. *Int J Agric Crop Sci.* 2012;4:531–5.
13. Ellouzi H, Oueslati S, Hessini K, Rabhi M, Abdely C. Seed-priming with H₂O₂ alleviates subsequent salt stress by preventing ROS production and amplifying antioxidant defense in cauliflower seeds and seedlings. *Scientia Horticulturae.* 2021;288:110360. <https://doi.org/10.1016/j.scienta.2021.110360>
14. Rademacher W. Plant growth regulators: Backgrounds and uses in plant production. *J Plant Growth Regul.* 2015;34:845–72. <https://doi.org/10.1007/s00344-015-9541-6>
15. Ahmad F, Kamal A, Singh A, Ashfaq F, Alamri S, Siddiqui MH. Salicylic Acid Modulates Antioxidant System, Defense Metabolites, and Expression of Salt Transporter Genes in *Pisum sativum* Under Salinity Stress. *J Plant Growth Regul.* 2020;41(5):1905–18. <https://doi.org/10.1007/s00344-020-10271-5>
16. Kumar P, Choudhary M, Halder T, Prakash NR, Singh V, V VT, et al. Salinity stress tolerance and omics approaches: revisiting the progress and achievements in major cereal crops. *Heredity (Edinb).* 2022;128(6):497–518. <https://doi.org/10.1038/s41437-022-00516-2> PMID: 35249098
17. Baghour M, Akodad M, Dariouche A. Gibberellic acid and indole acetic acid improves salt tolerance in transgenic tomato overexpressing LeNHX4 antiporter. *Gesunde Pflanz.* 2023;75:687. <https://doi.org/10.1007/s10343-022-00734-y>
18. Wasti S, Mimouni H, Smi S, Zid E, Ben Ahmed H. Enhanced salt tolerance of tomatoes by exogenous salicylic acid applied through rooting medium. *Omics.* 2012;16(4):200–7. <https://doi.org/10.1089/omi.2011.0071> PMID: 22416884
19. Xu L, Chen H, Zhang T, Deng Y, Yan J, Wang L. Salicylic Acid Improves the Salt Tolerance Capacity of *Saponaria officinalis* by Modulating Its Photosynthetic Rate, Osmoprotectants, Antioxidant Levels, and Ion Homeostasis. *Agronomy.* 2022;12(6):1443. <https://doi.org/10.3390/agronomy12061443>
20. Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: a review. *Environ Exp Bot.* 2010;68:14–25.

21. Wang Z, Dong S, Teng K, Chang Z, Zhang X. Exogenous salicylic acid optimizes photosynthesis, antioxidant metabolism, and gene expression in Perennial ryegrass subjected to salt stress. *Agronomy*. 2022;12(8):1920. <https://doi.org/10.3390/agronomy12081920>
22. Linić I, Mlinarić S, Brkljačić L, Pavlović I, Smolko A, Salopek-Sondi B. Ferulic acid and salicylic acid foliar treatments reduce short-term salt stress in chinese cabbage by increasing phenolic compounds accumulation and photosynthetic performance. *Plants (Basel)*. 2021;10(11):2346. <https://doi.org/10.3390/plants10112346> PMID: 34834709
23. El-Taher AM, Abd El-Raouf HS, Osman NA, Azoz SN, Omar MA, Elkelish A, et al. Effect of Salt Stress and Foliar Application of Salicylic Acid on Morphological, Biochemical, Anatomical, and Productivity Characteristics of Cowpea (*Vigna unguiculata* L.) *Plants (Basel)*. 2021;11(1):115. <https://doi.org/10.3390/plants11010115> PMID: 35009118
24. Roshdy A, Alebidi A, Almutairi KF, Al-Obeed RS, Elsabagh AS. The effect of salicylic acid on the performances of salt stressed strawberry plants, enzymes activity, and salt tolerance index. *Agronomy*. 2021;11:775.
25. Horchani F, Mabrouk L, Borgi MA, Abbes Z. Foliar spray or root application: which method of salicylic acid treatment is more efficient in alleviating the adverse effects of salt stress on the growth of alfalfa plants, *Medicago sativa* L.?. *Gesunde Pflanzen*. 2023;75:1–16.
26. Jahan MS, Wang Y, Shu S, Zhong M, Chen Z, Wu J, et al. Exogenous salicylic acid increases the heat tolerance in tomato (*Solanum lycopersicum* L.) by enhancing photosynthesis efficiency and improving antioxidant defense system through scavenging of reactive oxygen species. *Sci Hortic*. 2019;247:421–9.
27. Wojtyła Ł, Lechowska K, Kubala S, Garnczarska M. Different Modes of Hydrogen Peroxide Action During Seed Germination. *Front Plant Sci*. 2016;7:66. <https://doi.org/10.3389/fpls.2016.00066> PMID: 26870076
28. Bouallègue A, Souissi F, Nouairi I, Souibgui M, Abbes Z, Mhadhbi H. physiological and biochemical changes modulated by seeds' priming of lentil (*Lens culinaris* L.) under salt stress at germination stage. *Acta Scient Polon Hort Cult*. 2019;18(5):27–38. <https://doi.org/10.24326/asphc.2019.5.3>
29. Tania SS, Rhaman MS, Rauf F, Rahaman MdM, Kabir MH, Hoque MdA, et al. Alleviation of Salt-Inhibited Germination and Seedling Growth of Kidney Bean by Seed Priming and Exogenous Application of Salicylic Acid (SA) and Hydrogen Peroxide (H₂O₂). *Seeds*. 2022;1(2):87–98. <https://doi.org/10.3390/seeds1020008>
30. Aragão J, Lima GS de, Lima VLA de, Silva AAR da, Capitulino JD, Caetano EJM, et al. Effect of Hydrogen Peroxide Application on Salt Stress Mitigation in Bell Pepper (*Capsicum annuum* L.). *Plants (Basel)*. 2023;12(16):2981. <https://doi.org/10.3390/plants12162981> PMID: 37631192
31. Bouazzi A, Bouallegue A, K h a r r a t M, Abbes Z, Horchani F. Seed priming with gallic acid and hydrogen peroxide as a smart approach to mitigate salt stress in faba bean (*Vicia faba* L.) at the germination stage. *Russ J Plant Physiol*. 2024;104.
32. Bouallègue A, Souissi F, Nouairi I, Souibgui M, Abbes Z, Mhadhbi H. Salicylic acid and hydrogen peroxide pretreatments alleviate salt stress in faba bean (*Vicia faba*) seeds during germination. *Seed Sci Technol*. 2017. <https://doi.org/10.15258/sst.2017.45.3.07>
33. Nóbrega JS, Gomes VR, Soares LA d A, Lima GS d, Silva AAR d, Gheyi HR, et al. Hydrogen peroxide alleviates salt stress effects on gas exchange, growth, and production of naturally colored cotton. *Plants*. 2024;13:390. <https://doi.org/10.3390/plants13030390>
34. Vadez V, Rodier F, Payre H, Drevon JJ. Nodule permeability to O₂ and nitrogenase linked respiration in bean landraces varying in the tolerance of N₂ fixation to P deficiency. *Plant Physiol Biochem*. 1996;34:871–8.
35. Stevens J, Seneratna T, Sivasithamparam K. Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. Roma): associated changes in gas exchange, water relations and membrane stabilization. *Plant grow Regul*. 2006;49:77–83.
36. Wahid A, Sehar S, Perveen M, Gelani S, Basra SMA, Farooq M. Seed pretreatment with hydrogen peroxide improves heat tolerance in maize at germination and seedling growth stages. *Seed Sci Technol*. 2008;36(3):633–45. <https://doi.org/10.15258/sst.2008.36.3.13>
37. Dallali H, Maalej EM, Boughanmi NG, Haouala R. Salicylic acid priming in *Hedysarum carnosum* and *Hedysarum coronarium* reinforces NaCl tolerance at germination and the seedling growth stage. *Aust J Crop Sci*. 2012;63:407–14.
38. Arnon DI. Copper enzymes in isolated chloroplasts. polyphenoloxidase in beta vulgaris. *Plant Physiol*. 1949;24(1):1–15. <https://doi.org/10.1104/pp.24.1.1> PMID: 16654194
39. McKinney G. Absorption of light by chlorophyll solutions. *J Biol Chem*. 1941;140:315–22.
40. Velikova V, Yordanov I, Edreva A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Sci*. 2000;151:59–66.
41. Zhao SJ, Xu CC, Zhou Q, Meng QW. Improvements of the method for measurement of malondialdehyde in plant tissue. *Plant Physiol Com*. 1994;30:207–10.
42. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72:248–54. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3) PMID: 942051
43. Anderson MD, Prasad TK, Stewart CR. Changes in Isozyme Profiles of Catalase, Peroxidase, and Glutathione Reductase during Acclimation to Chilling in Mesocotyls of Maize Seedlings. *Plant Physiol*. 1995;109(4):1247–57. <https://doi.org/10.1104/pp.109.4.1247> PMID: 12228666
44. Lee DH, Kim YS, Lee CB. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *J Plant Physiol*. 2001;158:737–45.
45. Lalarukh I, Shahbaz M. Response of antioxidants and lipid peroxidation to exogenous application of alpha-tocopherol in sunflower (*Helianthus annuus* L.) under salt stress. *Pak J Bot*. 2020;52:75–83.
46. Horchani F, Bouallegue A, Mabrouk L, Namsi A, Abbes Z N. Nitrate reductase regulation in wheat seedlings by exogenous nitrate: A possible role in tolerance to salt stress. *J Plant Nutr Soil Sci*. 2023;186:633–46.

47. Horchani F, Bouallegue A, Namsi A, Abbes Z. Exogenous application of ascorbic acid mitigates the adverse effects of salt stress in two contrasting barley cultivars through modulation of physio-biochemical attributes, K⁺/Na⁺ homeostasis, osmoregulation and antioxidant defense system. *Russ J Plant Physiol.* 2024;70:1–14.
48. Ma L, Liu X, Lv W, Yang Y. Molecular Mechanisms of Plant Responses to Salt Stress. *Front Plant Sci.* 2022;13:934877. <https://doi.org/10.3389/fpls.2022.934877> PMID: 35832230
49. Stanton ES. The ability of salicylic acid to reduce the damaging effects of salt water stress on *Phaseolus vulgaris*. USA: California State University; 2004.
50. Gutierrez-Coronado MA, Trejo-Lopez C, S K A. Effect of salicylic acid on the growth of roots and shoots in soybean. *Plant Physiol Biochem.* 1998;36(8):563.
51. Iqbal M, Ashraf M, Jamil A, Shafiq UR. Does seed priming induce changes in the levels of some endogenous plant hormones in exaploidy wheat plant under salt stress. *J Integr Plant Biol.* 2006;48(2):181–9.
52. Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA, Fatkhutdinova DR. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.* 2003;164(3):317–22.
53. Ebrahimian E, Bybordi A. Effect of salinity, salicylic acid, silicium and ascorbic acid on lipid peroxidation, antioxidant enzyme activity and fatty acid content of sunflower. *Afr J Agri Res.* 2012;7(25):3685–94.
54. Ashraf MA, Rasheed R, Hussain I, Iqbal M, Haider MZ, Parveen S, et al. Hydrogen peroxide modulates antioxidant system and nutrient relation in maize (*Zea mays* L.) under water-deficit conditions. *Archives Agronomy Soil Sci.* 2014;61(4):507–23. <https://doi.org/10.1080/03650340.2014.938644>
55. Tari I, Csiszár J, Horváth E, Poór P, Takács Z, Szepesi Á. The alleviation of the adverse effects of salt stress in the tomato plant by salicylic acid shows a time-and organ-specific antioxidant response. *Acta Biol.* 2015;57:21–30.
56. Dito S, Gadallah M. Hydrogen peroxide supplementation relieves the deleterious effects of cadmium on photosynthetic pigments and oxidative stress and improves the growth, yield and quality of pods in pea plants (*Pisum sativum* L.). *Acta Physiol Plant.* 2019;41:113.
57. Munns R. Comparative physiology of salt and water stress. *Plant Cell Environ.* 2002;25(2):239–50. <https://doi.org/10.1046/j.0016-8025.2001.00808.x> PMID: 11841667
58. Munns R, Tester M. Mechanisms of salinity tolerance. *Annu Rev Plant Biol.* 2008;59:651–81. <https://doi.org/10.1146/annurev.arplant.59.032607.092911> PMID: 18444910
59. Sarker U, Oba S. The Response of Salinity Stress-Induced A. tricolor to Growth, Anatomy, Physiology, Non-Enzymatic and Enzymatic Antioxidants. *Front Plant Sci.* 2020;11:559876. <https://doi.org/10.3389/fpls.2020.559876> PMID: 33178233
60. Silva EN, Silveira JAG, Rodrigues CRF, Viégas RA. Physiological adjustment to salt stress in *Jatropha curcas* is associated with accumulation of salt ions, transport and selectivity of K⁺, osmotic adjustment and K⁺/Na⁺ homeostasis. *Plant Biol (Stuttg).* 2015;17(5):1023–9. <https://doi.org/10.1111/plb.12337> PMID: 25865670
61. Ashrafi E, Razmjoo J, Zahedi M. Effect of salt stress on Growth and Ion accumulation of alfalfa (*Medicago sativa* L.) cultivars. *J Plant Nutrition.* 2018;41(7):818–31. <https://doi.org/10.1080/01904167.2018.1426017>
62. Cornacchione MV, Suarez DL. Evaluation of Alfalfa (*Medicago sativa* L.) Populations' Response to Salinity Stress. *Crop Science.* 2017;57(1):137–50. <https://doi.org/10.2135/cropsci.2016.05.0371>
63. Gunes A, Inal A, Alpaslan M, Eraslan F, Bagci EG, Cicek N. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J Plant Physiol.* 2007;164(6):728–36. <https://doi.org/10.1016/j.jplph.2005.12.009> PMID: 16690163
64. Mimouni H, Wasti S, Manaa A, Gharbi E, Chalh A, Vandoorne B, et al. Does Salicylic Acid (SA) Improve Tolerance to Salt Stress in Plants? A Study of SA Effects On Tomato Plant Growth, Water Dynamics, Photosynthesis, and Biochemical Parameters. *Omics.* 2016;20(3):180–90. <https://doi.org/10.1089/omi.2015.0161> PMID: 26909467
65. Wahid A, Perveen M, Gelani S, Basra SMA. Pretreatment of seed with H₂O₂ improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J Plant Physiol.* 2007;164(3):283–94. <https://doi.org/10.1016/j.jplph.2006.01.005> PMID: 16545492
66. Ashraf M, Harris PJC. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* 2004;166(1):3–16.
67. Unno H, Maeda Y, Yamamoto S, Okamoto M, Takenaga H. Relationships between salt tolerance and Ca²⁺ retention among plant species. *Plant Nutr.* 2002;73:715–8.
68. Hamani AKM, Wang G, Soothar MK, Shen X, Gao Y, Qiu R, et al. Responses of leaf gas exchange attributes, photosynthetic pigments and antioxidant enzymes in NaCl-stressed cotton (*Gossypium hirsutum* L.) seedlings to exogenous glycine betaine and salicylic acid. *BMC Plant Biol.* 2020;20(1):434. <https://doi.org/10.1186/s12870-020-02624-9> PMID: 32957907
69. Rodrigues VS, Sousa GG, Gomes SP, Soares SC, Silva Júnior FB, Freire MHC, et al. Gas exchange and growth of sunflower subjected to saline stress and mineral and organic fertilization. *Rev Bras Eng Agríc Amb.* 2022;11:840–7.
70. Roşca M, Mihalache G, Stoleru V. Tomato responses to salinity stress: From morphological traits to genetic changes. *Front Plant Sci.* 2023;14:1118383. <https://doi.org/10.3389/fpls.2023.1118383> PMID: 36909434
71. Lima GS, Pinheiro FWA, Souza WBB, Soares LAA, Gheyi HR, Nobre RG, et al. Physiological indices of sour passion fruit under brackish water irrigation strategies and potassium fertilization. *Rev Bras Eng Agríc Amb.* 2023;27:383–92.

72. Fariduddin Q, Hayat S, Ahmad A. Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. *Photosynthetica*. 2003;41:281–4.
73. Lawlor DW, Cornic G. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ*. 2002;25(2):275–94. <https://doi.org/10.1046/j.0016-8025.2001.00814.x> PMID: 11841670
74. Khodary SEA. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. *Inter J Agric Biol*. 2004;6(1):5–8.
75. Shao Q, Wang H, Guo H, Zhou A, Huang Y, Sun Y, et al. Effects of shade treatments on photosynthetic characteristics, chloroplast ultrastructure, and physiology of *Anoectochilus roxburghii*. *PLoS One*. 2014;9(2):e85996. <https://doi.org/10.1371/journal.pone.0085996> PMID: 24516523
76. Schütz M, Fangmeier A. Growth and yield responses of spring wheat (*Triticum aestivum* L. cv. Minaret) to elevated CO₂ and water limitation. *Environ Pollut*. 2001;114(2):187–94. [https://doi.org/10.1016/s0269-7491\(00\)00215-3](https://doi.org/10.1016/s0269-7491(00)00215-3) PMID: 11504341
77. Jaleel CA, Sankar B, Sridharan R, Panneerselvam R. Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus*. *Turk J Biol*. 2009;32(2):79–83.
78. Zhou H, Shi H, Yang Y, Feng X, Chen X, Xiao F, et al. Insights into plant salt stress signaling and tolerance. *J Genet Genomics*. 2024;51(1):16–34. <https://doi.org/10.1016/j.jgg.2023.08.007> PMID: 37647984
79. Gohari G, Alavi Z, Esfandiari E, Panahirad S, Hajihoseinlou S, Fotopoulos V. Interaction between hydrogen peroxide and sodium nitroprusside following chemical priming of *Ocimum basilicum* L. against salt stress. *Physiol Plant*. 2020;168(2):361–73. <https://doi.org/10.1111/ppl.13020> PMID: 31433490
80. Sanaz Adalatizadeh-Aghdam, Toorchi M, Zarei M. Fennel (*Foeniculum vulgare* Mill) Plants Responses to Salicylic Acid Foliar Application as Chemical Priming Agent under Salt Stress. *Biol Bull Russ Acad Sci*. 2021;48(S3):S45–53. <https://doi.org/10.1134/s1062359022010034>
81. Ma X, Zheng J, Zhang X, Hu Q, Qian R. Salicylic Acid Alleviates the Adverse Effects of Salt Stress on *Dianthus superbus* (Caryophyllaceae) by Activating Photosynthesis, Protecting Morphological Structure, and Enhancing the Antioxidant System. *Front Plant Sci*. 2017;8:600. <https://doi.org/10.3389/fpls.2017.00600> PMID: 28484476
82. Meloni DA, Oliva MA, Martinez CA, Cambraia J. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ Exp Bot*. 2003;49:69–76.
83. Herbette S, Menn AL, Rousselle P, Ameglio T, Faltin Z, Branlard G, et al. Modification of photosynthetic regulation in tomato overexpressing glutathione peroxidase. *Biochim Biophys Acta*. 2005;1724(1–2):108–18. <https://doi.org/10.1016/j.bbagen.2005.04.018> PMID: 15921856
84. Shi QH, Bao ZY, Zhu ZJ, Ying QS, Qian QQ. Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. *Plant Growth Regul*. 2006;48:127–35.
85. de Azevedo Neto AD, Prisco JT, Enéas-Filho J, Medeiros J-VR, Gomes-Filho E. Hydrogen peroxide pre-treatment induces salt-stress acclimation in maize plants. *J Plant Physiol*. 2005;162(10):1114–22. <https://doi.org/10.1016/j.jplph.2005.01.007> PMID: 16255169