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PHYSIOLOGICAL AND BIOCHEMICALS CHANGES MODULATED BY SEEDS' PRIMING OF LENTIL (*Lens culinaris* L.) UNDER SALT STRESS AT GERMINATION STAGE

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ABSTRACT

Seed priming is one of the potential physiological approaches to enhance the seed germination under the salinity stress. The present study examined the role of two seed priming molecules: salicylic acid (SA) and hydrogen peroxide (H_2O_2), in enhancing the salt tolerance of lentil seeds at germination stage. Salinity stress caused significant decrease in germination percentage and primary root elongation. This decrease was associated with significant increase in lipid peroxidation and total lipid (TL) contents in embryonic axis. The catalase (CAT), guaiacol peroxydase (GPOX) and superoxide dismutase (SOD) activities remained unchanged or decreased significantly under the influence of salt stress, in both embryonic axis and cotyledons. Starch mobilization was not affected by the salt stress. The two priming treatments effectively alleviated the negative effects of salinity stress. SA and H_2O_2 applications after dose optimization resulted in a significant enhancement of germination percentage and primary root elongation. No significant changes in starch, soluble sugars contents and SOD activity were detected following SA and H_2O_2 treatments. Seed priming treatments triggered the activities of GPOX and CAT and caused the reduction in lipid peroxidation, especially in embryonic axis. TL content and especially the fatty acid C18:3 increased after SA applications. Better performance under salt stress of primed lentil seeds was associated with lower lipid peroxidation, and activation of enzymatic antioxidative defense system. Obtained results confirm the potential for using SA and H_2O_2 to improve germination and plant growth under salt stress conditions.

Key words: salt, seeds priming, Lens culinaris, antioxidant, fatty acids, germination, starch

INTRODUCTION

Plants in natural environments are subjected to a variety of stresses which have negative effects on their growth. Salinity is the major environmental stress and is a substantial constraint to crop production. The extent of damage caused by salt stress depends on the age and stage of plant growth [Läuchli and Grattan 2007]. Seed germination represents an important stage of vegetable development cycle as it is the first exchange interface with the environment. Several reports suggest that increased salt concentration cause delayed germination by retarding hydrolysis of storage compounds and by limiting their translocation from storage tissue to developing embryo axes [De Lacerda et al. 2003]. Salt stress reduce seed ger-

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mination, either by osmotic or by ionic effect [Duan et al. 2004]. The negative effect of salt stress on germination is a result of the occurrence oxidative stress caused by the increase of reactive oxygen species (ROS) in plants cells [Apel and Hirt 2004]. The increased level of ROS in plants affects chlorophyll degradation, lipid peroxidation as well as macromolecules and cellular structure damages [Amor et al. 2006, Harb et al. 2017]. For overcoming salt stress induced occurrence oxidative stress, plants have developed complex mechanisms that contribute to the adjustment to high salinity. To scavenge ROS, plants use specific mechanisms, which include activation of antioxidant enzymes including catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR) [Jaleel et al. 2008] and non-enzymatic antioxidants such as ascorbic acid, glutathione, tocopherols and carotenoids [Lee et al. 2001]. Recently, numerous studies have shown that plant growth regulators or signaling molecules such as Salicylic acid (SA) and hydrogen peroxide (H_2O_2) can significantly alleviate the negative effect of abiotic stresses on plant growth and production [Khan et al. 2015, Hossain et al. 2015].

SA is considered as a hormone like substance, which has been shown to act in the regulation of a number of physiological processes and also provide protection against biotic and abiotic stresses in plants. Ameliorative effects of SA have been well documented in inducing salt resistance in many crops [Joseph et al. 2010].

However, H_2O_2 is known to act as a signaling molecule, activating multiple defense mechanisms to various environmental stresses in plants. Many studies have shown that SA and H_2O_2 are effective in mitigating the negative effect of salinity in different plant species [Hossain et al. 2015].

The aim of this study was to assess the effect of different NaCl concentrations on biochemical and physiological parameters during germination of lentil seeds as well as the impact of SA and H_2O_2 on the alleviation of negative effect of salt stress. This was carried out on the basis of percentage of seed germination, primary root elongation, the level of lipid peroxidation and antioxidant enzymes activities as well as contents of sugar, starch, total lipids and fatty acids composition during the early stage of growth.

MATERIALS AND METHODS

Priming, stress application, germination and root elongation. Seeds of lentil (*Lens culinaris*) cv. Ncir used in this study were gently provided by Field Crops Laboratory, National Agricultural Research Institute of Tunisia (INRAT). Seeds were surface sterilized with a sodium hypochlorite solution (1%) for about 2 min and washed thoroughly three times with sterile distilled water. Lentil seeds were divided into three groups: the first group was soaked in distilled water, the second group was soaked in solutions containing several concentrations (0.1, 0.5 and 1 mM) of salicylic acid and the third group was soaked in several concentrations (0.05, 0.1 and 0.120 mM) of H_2O_2. Seeds were then placed to germinate in Petri dishes (90 mm diameter) containing a sheet of filter paper, saturated with distilled water (control) or NaCl solutions (75 and 150 mM NaCl) at 25°C in the dark. In each treatment, three replicates (each one contained 10 seeds) were used. The seeds were considered germinated when the primary root reached 2 mm, cumulative germination percentage was determined. Germinated seeds were transferred in squared dishes and primary root length was measured for 5 days. The most effective SA and H_2O_2 concentrations in inducing seed germination and primary root elongation were determined and will be used for the rest of this study. Cotyledons and embryonic axis from the selected treatments were separated, placed at –80°C and stored until analyses.

Determination of sugars content in cotyledons. The water-soluble sugars were determined by the anthrone sulphuric acid method of Yemm and Willis [1954]. Samples (25 mg each) of dried tissue powder were homogenized in mortar and pestle in a volume of 5 ml 80% (v/v) ethanol, incubated at 70°C and centrifuged at 3000 g for 30 min twice. The supernatant was stored at –20°C. The supernatant was added to the anthrone sulphuric acid and ethanol. The absorbance was measured at 640 nm using a calibration curve. The results are expressed in $mg \cdot g^{-1}$ DW.

Determination of starch content in cotyledons. Starch was extracted from dried and powdered samples of cotyledons of faba beans. The extraction was made by homogenization with KOH 1N, then neutralized with HCl 1N. The solution was incubated at 100°C for 15 min and centrifuged at 3000 g for

15 minutes. The supernatant was used for determination of total starch in presence of I_2/KI reactif at 580 nm using a standard curve. The results are expressed in mg·g^{-1} DW.

Determination of lipid peroxidation in embryonic axis and cotyledons. Lipid peroxidation level was measured as the content of malondialdehyde (MDA) using the thiobarbioturic method [Zhao et al. 1994]. 0.5 g tissue sample was homogenized in 1 ml 5% TCA. The homogenate was centrifuged at 10,000 g for 15 min. To 1 ml aliquot of the supernatant, 1ml of 20% TCA containing 0.5% TBA were added. The mixture was heated at 95°C for 15 min and cooled then immediately. The specific absorbance of extracts was recorded at 532 nm. Non-specific absorbance at 600 nm was measured and subtracted from the 532 nm readings. It was expressed as nmol of MDA formed using extinction coefficient of 155 mM^{-1} cm^{-1} and the results expressed as nmol (MDA) g^{-1} fresh weight.

Lipid extraction and fatty acids methylation in embryonic axis. Lipid was extracted according to the method of Bligh and Deyer [1959]. Fresh embryonic axis were fixed in boiling water for 5 min to denature phospholipases and then homogenized in chloroform: methanol mixture (1 : 1, v/v). The homogenate was centrifuged at 3000 g for 15 min. The lower chloroformic phase containing lipids was aspired and evaporated at 40°C under vacuum using a rotary evaporator or with nitrogen gas. The residue was immediately re-dissolved in 2 ml of toluene: ethanol mixture (4 : 1, v/v) for conservation. Fatty acids from total lipids were methylated by the method of Metcalfe et al. [1966]. Methyl esters of total fatty acids were separated and quantified with a Hewelett Packard chromatography model 4890D fitted with a 30 m × 0.25 mm × 0.25 µm film thickness fused silica capillary column (Innowax) coupled to a flame ionization detector were maintained at 230 and 250°C, respectively. Nitrogen was used as the carrier gas at 1 ml/min with split injector system (split ratio 1 : 100). For measuring the amount of fatty acids heptadecanoic acid (C17:0) was added as internal standard before methylation. Calculation of fatty acids quantities was done using an integrator HP model 3390 A.

Determination of total protein content and antioxidant enzyme activity in embryonic axis and cotyledons. Samples were homogenized in ice with a mortar and pestle in 50 mM potassium phosphate buffer (KH_2PO_4/K_2HPO_4) pH 7.8, containing 0.1 mM Na_2EDTA, 1 mM PMSF and 0.05 g of polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000 g for 15 min at 4°C. The supernatant was stored at –20°C for guaiacol peroxidase, catalase and superoxide dismutase (SOD) assays. Total protein concentration was determined according to the Bradford method [1976] using the Bio-Rad protein assay reagent.

Guaiacol peroxidase activity (GPOX, EC1.11.1.7) was determined by the oxidation of guaiacol in the presence of H_2O_2 [Anderson et al. 1995]. The increase in absorbance due to formation of tetraguaiacol was recorded at 470 nm. The activity of GPOX was expressed in µmol·mg^{-1} proteins.

Superoxide dismutase (SOD, EC1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Lee et al. [2001]. The reaction mixture contained 50 mM phosphate buffer (pH 7), 0.053 mM NBT, 10 mM methionine, 0.0053 mM riboflavin, and an appropriate aliquot of enzyme extract. The reaction was started by switching on the light and was allowed to run for 2 min. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition in the reduction of NBT as monitored at 560 nm. The activity of SOD was expressed in USOD·mg^{-1} proteins.

Catalase (CAT, EC1.11.1.6) activity was estimated by the decrease of absorbency at 240 nm for 1 min as a consequence of H_2O_2 consumption [Anderson et al. 1995] (extinction coefficient 36 mM·cm^{-1}). The assay mixture contained 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2, and 5 µl extract in a 1 ml volume. The activity of CAT was expressed in µmol·mg^{-1} proteins.

Statistical analysis. Statistical analyses were performed using the SPSS software (Version 15.0 for Windows). Analysis was based on Duncan's multiple ranges classification test at $P = 0.05$. Each experiment was replicated at least three times (n = 3).

RESULTS

The effect of seeds priming on salt induced changes of germination and primary root elongation: choice

and dose optimization of priming agents. Data regarding germination percentage of primed and non-primed lentil seeds under salt stress are shown in Table 1.

Exposure to salt stress in non-primed seeds considerably decreased the percentage of lentil germination by 27.56% at 150 mM NaCl. Primary root elongation also decreased consistently as the external salt concentration increased (Tab. 2). Priming with using 0.1 mM SA and 0.1 mM H_2O_2 were the most effective in alleviation salt-induced reduction of both germination percentage and primary root elongation. Significant amelioration of germination was observed at 75 mM NaCl for 0.1 mM SA primed seeds (25.6%) and at 150 mM NaCl for 0.1 mM H_2O_2 primed seeds (23%). 0.1 mM SA and 0.1 mM H_2O_2 applications also resulted in significant enhancement of primary root elongation since the first day for 75 mM NaCl and since the second day for 150 mM NaCl (Tabs 1 and 2).

The effect of seeds priming on salt induced changes in the level of starch and soluble sugar. No changes in the level of starch were observed in cotyledons of lentil seeds treated with 75 mM NaCl. On the other hand, when 150 mM NaCl was provided, the level of starch was reduced by 37.87% compared to the control (Fig. 1 A). Data in Figure 1 B revealed that salt stress did not affect significantly the level of soluble sugars in cotyledons of germinating lentil seeds. Under control conditions, SA did not affect the level of starch and soluble sugars in cotyledons. However, H_2O_2 reduced only the level of soluble sugars. Under salt conditions, application of SA and H_2O_2 reduced starch contents only at 75 mM NaCl, which result in a non-significant enhancement of the accumulation of soluble sugars in cotyledons of germinating lentil seeds. At 150 mM NaCl, SA and H_2O_2 treatments did not influence on starch and soluble sugars contents (Fig. 1).

The effect of seeds priming on salt induced changes in lipid peroxidation. The lipid peroxidation was measured in terms of malondialdehyde (MDA) contents, a major product of lipid peroxidation. The results (Fig. 2) revealed that, in embryonic axes of germinating lentil seeds, MDA was increased significantly by 29.7% as a result of salinity stress only at 150 mM NaCl. However, in cotyledons, no significant increase was observed. Under control conditions, SA and H_2O_2 did not affect MDA contents in embryonic axes. However, in cotyledons, only H_2O_2 treatment reduced the level of MDA. Under the influence of salt stress, application of SA and H_2O_2 induced a significant decrease of MDA level in embryonic axes at 150 mM NaCl. However, decreases in MDA in cotyledons were significant only at 75 mM NaCl following H_2O_2 application (Fig. 2).

The effect of seeds priming on salt induced changes in the amount of total lipid content and fatty acid composition. The level of total lipid (TL), evaluated from the amount of total fatty acids, was found to change in salt-treated seeds in a dose dependent manner (Fig. 3). Salt stress caused a significant increase in TL content by 41.90% and 113.00% at 75 and 150 mM NaCl, respectively (Fig. 3). Pre-treatment with SA and H_2O_2 significantly increased TL content in embryonic axes of germinating lentil seeds under control conditions. Under salt stress conditions, increases in TL content were only observed following SA application (about 95.30% and 63.83% in comparison with the salt controls, at 75 and 150mM NaCl, respectively). On the other hand, following H_2O_2 application, TL content was not affected at 150 mM NaCl, and decreased significantly at 75 mM NaCl (Fig. 3).

Results shown in Table 3 are expressed as a percentage of the major fatty acids in the total leaf fatty acids. The main difference in the fatty acid composition of embryonic axes of germinating lentil seeds between the control and NaCl treated seeds was a significant decrease in C16:0, C18:1 and a significant increase in C18:2. For the rest of fatty acids, no significant changes were observed (Tab. 3). SA application caused significant increases in C18:3 at 75 mM NaCl while C18:2 and C18:3 at 150 mM NaCl in comparison to control. SA application induced also significant decreases in C18:0 and C18:1 at 75 mM NaCl and C16:0 and C18:1 at 150 mM NaCl.

Concerning H_2O_2 application, the only significant increase was observed for C18:0 and C18:3 at 75 mM NaCl and for C18:1 at 150 mM NaCl. Significant decreases were noticed in C18:1 and C18:2 at 75 mM NaCl and C16:0 and C18:3 at 150 mM NaCl. The rest of treatments do not show significant changes in comparison to the control (Tab. 3).

The effect of seeds priming on salt induced changes in the activity of antioxidant enzymes. Under the influence of salt stress, the activity of CAT decreased significantly at 75 mM NaCl in cotyledons and at 150

Table 1. Effect of different levels of NaCl, salicylic acid (SA) and hydrogen peroxide (H_2O_2) on germination of *Lens culinaris* seeds

	Germination (%)						
	C	SA (mM)			H_2O_2 (mM)		
		0.1	0.5	1	0.05	0.1	0.120
0	99 ^{a}	99 ^{a}	93 ^{ab}	81 ^{cdefg}	95 ^{ab}	100 ^{a}	90 ^{abc}
75	72 ^{fgh}	90 ^{abc}	78 ^{defg}	82 ^{cdef}	79 ^{defg}	79 ^{defg}	76 ^{efg}
150	71 ^{gh}	83 ^{cde}	86 ^{bcde}	53 ^{i}	63 ^{h}	87 ^{bcd}	75 ^{efg}

Values with the same letter are not significantly different according to Duncan test ($p = 0.05$), n = 36

Table 2. Effect of different levels of NaCl, salicylic acid (SA) and hydrogen peroxide (H_2O_2) on primary root elongation of germinating *Lens culinaris* seeds

NaCl (mM)	Treatment		Root length (cm)				
			Day 1	Day 2	Day 3	Day 4	Day 5
0	C		1.20 ^{abc}	1.84 ^{abc}	3.16 ^{bc}	4.46 ^{b}	5.52 ^{c}
	SA (mM)	0.1	1.26 ^{a}	2.16 ^{a}	3.66 ^{a}	5.06 ^{a}	7.10 ^{a}
		0.5	1.21 ^{ab}	1.98 ^{abc}	3.13 ^{bc}	5.32 ^{a}	6.29 ^{b}
		1	0.33 ^{gh}	1.06 ^{fgh}	1.51 ^{ghij}	2.88 ^{ef}	5.28 ^{cd}
	H_2O_2 (mM)	0.05	1.01 ^{bcd}	2.15 ^{a}	2.87 ^{cd}	3.97 ^{c}	5.12 ^{cd}
		0.1	0.70 ^{ef}	1.47 ^{de}	3.05 ^{bc}	4.00 ^{bc}	5.00 ^{cd}
		0.12	1.20 ^{abc}	2.10 ^{ab}	3.28 ^{ab}	5.27 ^{a}	7.30 ^{ab}
75	C		0.27 ^{h}	0.60 ^{i}	1.57 ^{ghij}	2.46 ^{fg}	3.73 ^{fgh}
	SA (mM)	0.1	0.62 ^{ef}	1.65 ^{cd}	2.54 ^{def}	3.78 ^{cd}	5.22 ^{cd}
		0.5	0.58 ^{f}	1.20 ^{efg}	1.82 ^{g}	2.67 ^{f}	4.14 ^{efgh}
		1	0.86 ^{de}	1.68 ^{cd}	2.26 ^{f}	3.78 ^{cd}	4.68 ^{de}
	H_2O_2 (mM)	0.05	0.54 ^{fg}	1.33 ^{ef}	1.74 ^{gh}	2.40f ^{gh}	4.40 ^{def}
		0.1	0.86 ^{de}	1.82 ^{bc}	2.64 ^{de}	3.36 ^{de}	4.85 ^{cde}
		0.12	0.60 ^{f}	1.68 ^{cd}	2.40 ^{ef}	3.36 ^{de}	4.19 ^{efg}
150	C		0.48 ^{fgh}	0.78 ^{hi}	1.24 ^{jk}	1.58 ^{i}	1.95 ^{j}
	SA (mM)	0.1	0.97 ^{cd}	1.73 ^{cd}	2.33 ^{ef}	2.67 ^{f}	3.57 ^{gh}
		0.5	0.48 ^{fgh}	0.80 ^{hi}	1.40 ^{hij}	1.80 ^{i}	2.65 ^{ij}
		1	0.32 ^{gh}	0.67 ^{i}	0.96 ^{k}	1.93 ^{hi}	2.72 ^{ij}
	H_2O_2(mM)	0.05	0.52 ^{fgh}	0.68 ^{i}	1.37 ^{ij}	1.74 ^{i}	2.09 ^{j}
		0.1	0.69 ^{ef}	1.37 ^{ef}	1.71 ^{ghi}	2.42 ^{fgh}	3.38 ^{hi}
		0.12	0.60 ^{f}	0.89 ^{ghi}	1.26 ^{jk}	2.01 ^{ghi}	2.75 ^{ij}

Values with the same letter per parameter are not significantly different according to Duncan test ($p = 0.05$), n = 10

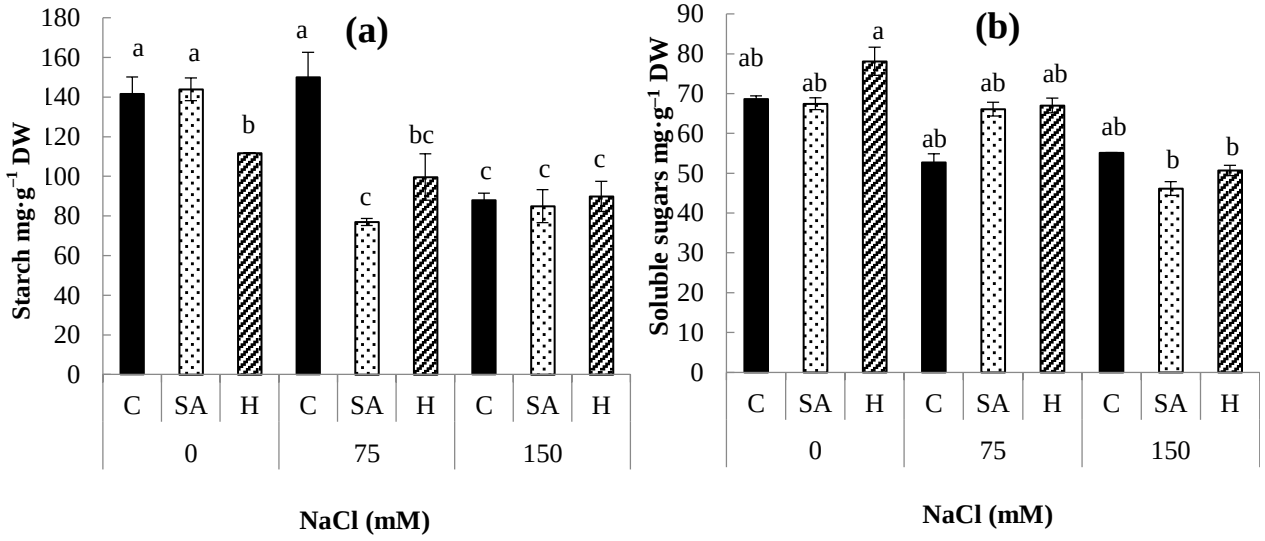

Fig. 1. Effect of different levels of NaCl, salicylic acid (SA, 0.1 mM) and hydrogen peroxide (H, 0.1 mM) on starch (a) and soluble sugars (b) (mg·g^{-1} DW) of cotyledons of germinating *Lens culinaris* seeds. Bars are means ± SE. Values with the same letter per parameter are not significantly different according to Duncan test (p = 0.05), n = 9 (a) and n = 6 (b)

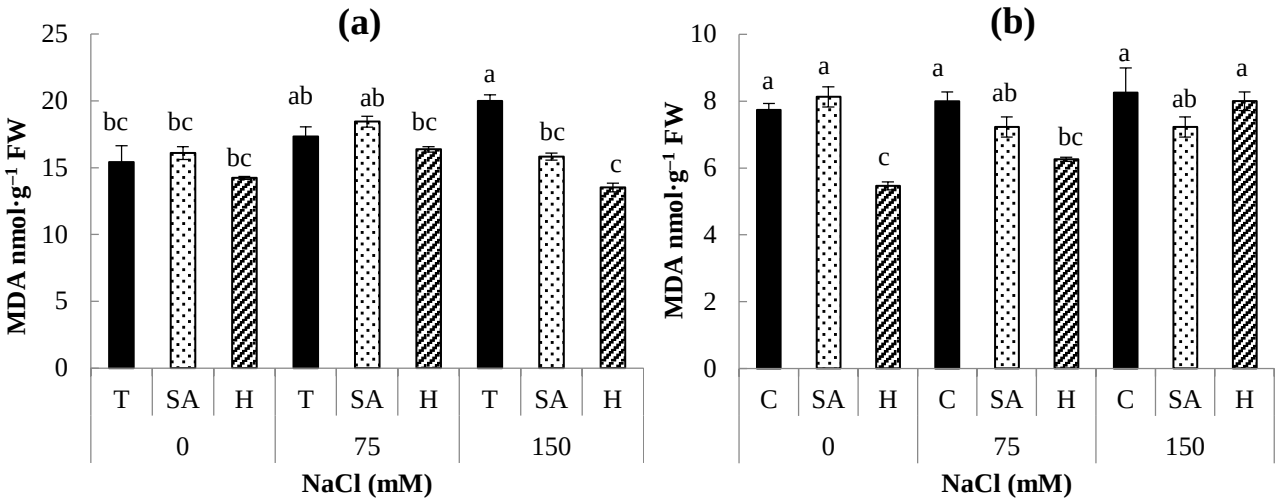

Fig. 2. Effect of different levels of NaCl, salicylic acid (SA, 0.1mM) and hydrogen peroxide (H, 0.1 mM) on malondialdehyde MDA content (nmol·g^{-1} FW) in embryonic axes (a) and cotyledons (b) of germinating *Lens culinaris* seeds. Bars are means ± SE. Values with the same letter per parameter are not significantly different according to Duncan test (p = 0.05), n = 6

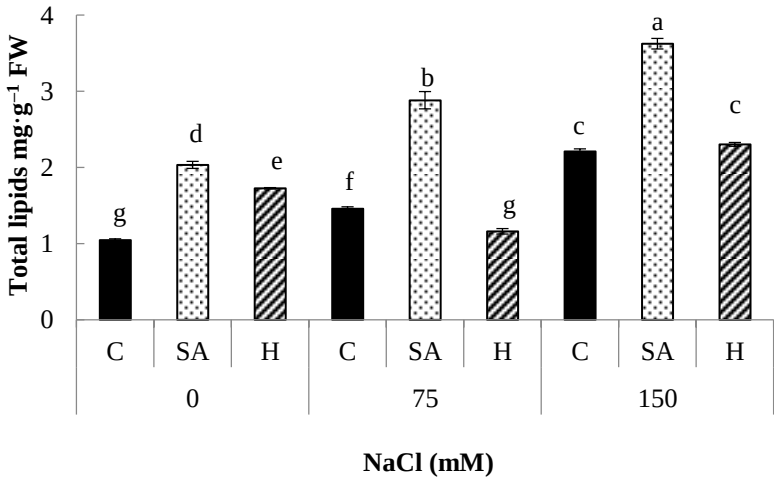

Fig. 3. Effect of different levels of NaCl, salicylic acid (SA, 0.1 mM) and hydrogen peroxide (H, 0.1 mM) on total lipids (mg·g^{-1} FW) in embryonic axes of germinating *Lens culinaris* seeds. Bars are means ± SE. Values with the same letter are not significantly different according to Duncan test (p = 0.05), n = 3

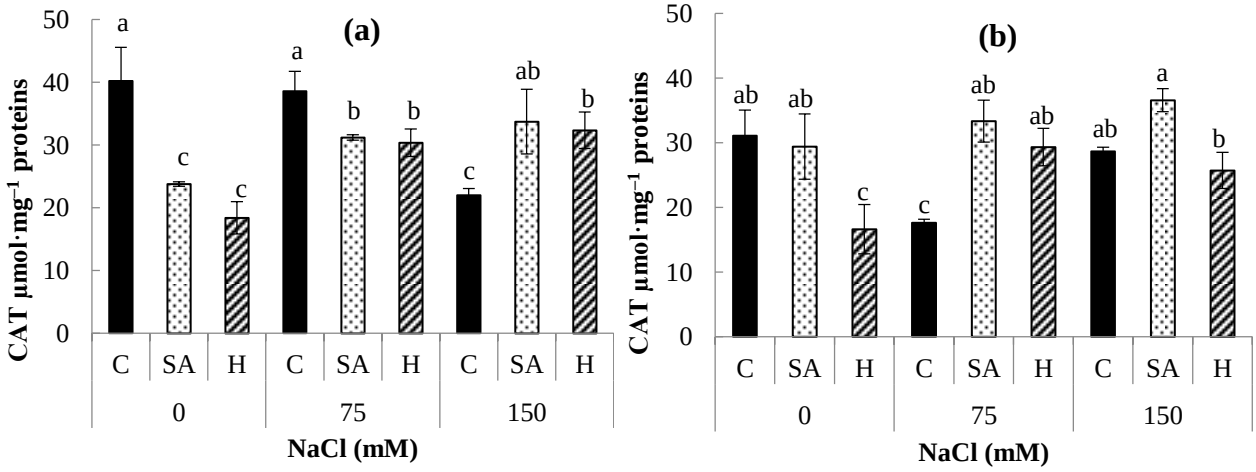

Fig. 4. Effect of different levels of NaCl and salicylic acid (SA, 0.1 mM) and hydrogen peroxide (H, 0.1 mM) on CAT activity (µmol·mg^{-1} proteins) in embryonic axes (a) and cotyledons (b) of germinating *Lens culinaris* seeds. Bars are means ± SE. Values with the same letter per parameter are not significantly different according to Duncan test (p = 0.05), n = 6

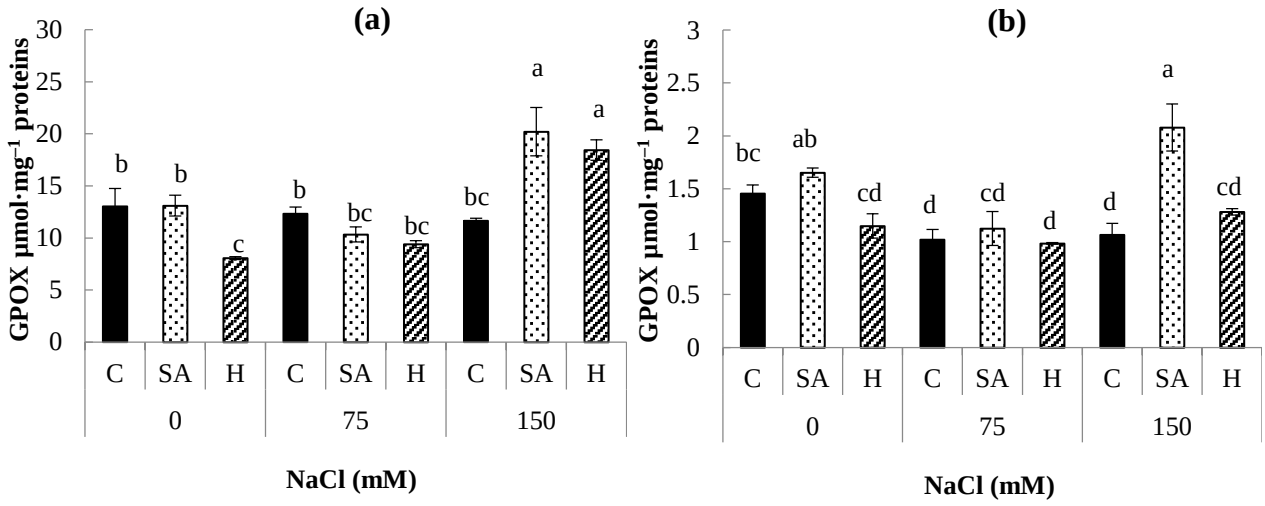

Fig. 5. Effect of different levels of NaCl, salicylic acid (SA, 0.1 mM) and hydrogen peroxide (H, 0.1 mM) on GPOX activity (µmol·mg^{-1} proteins) in embryonic axis (a) and cotyledons (b) of germinating *Lens culinaris* seeds. Bars are means ± SE. Values with the same letter per parameter are not significantly different according to Duncan test (p = 0.05), n = 3

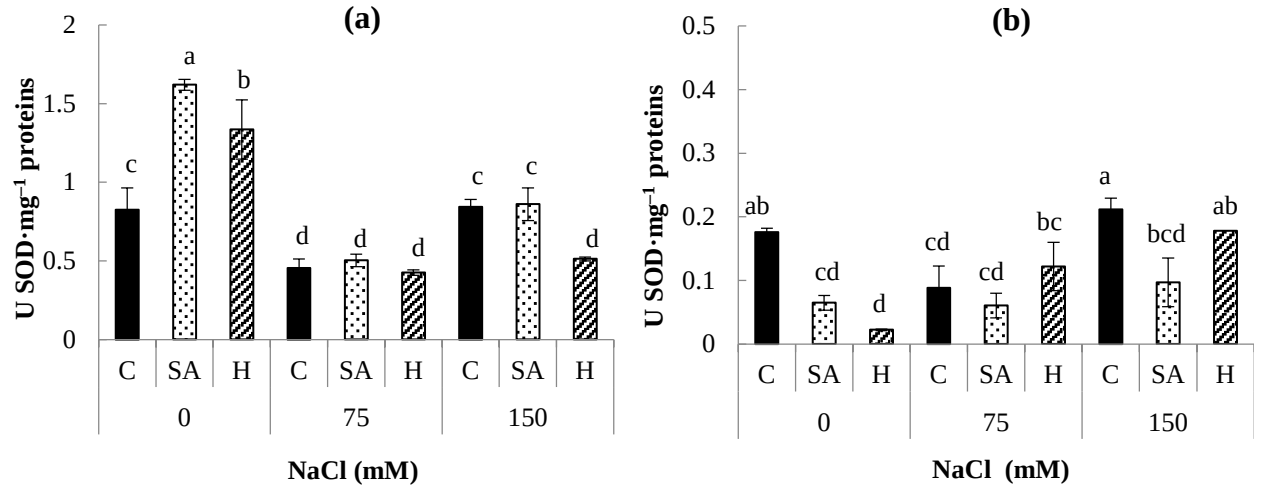

Fig. 6. Effect of different levels of NaCl, salicylic acid (SA, 0.1 mM) and hydrogen peroxide (H, 0.1 mM) on SOD activity (USOD·mg^{-1} proteins) in embryonic axis (a) and cotyledons (b) of germinating *Lens culinaris* seeds. Bars are means ± SE. Values with the same letter per parameter are not significantly different according to Duncan test (p = 0.05), n = 3

Table 3. Effect of different levels of NaCl, salicylic acid (SA, 0.1 mM) and hydrogen peroxide (H_2O_2, 0.1 mM) on the percentage (%) of fatty acids in embryonic axes of germinating *Lens culinaris* seeds

NaCl (mM)	Treatment	C16:0	C18:0	C18:1	C18:2	C18:3
0	C	35.97 ^{a}	9.72 ^{ab}	8.22 ^{b}	38.25 ^{d}	7.83 ^{d}
	SA	31.75 ^{b}	6.51 ^{c}	6.95 ^{bc}	43.29 ^{b}	11.49 ^{c}
	H_2O_2	34.59 ^{a}	9.16 ^{b}	5.66 ^{cd}	39.03 ^{d}	11.55 ^{c}
75	C	28.29 ^{c}	8.33 ^{b}	6.72 ^{c}	47.84 ^{a}	8.83 ^{d}
	SA	28.27 ^{c}	6.32 ^{c}	4.77 ^{de}	47.61 ^{a}	13.03 ^{b}
	H_2O_2	29.31 ^{c}	11.37 ^{a}	3.98 ^{ef}	39.58 ^{cd}	15.76 ^{a}
150	C	31.68 ^{b}	8.76 ^{b}	6.75 ^{c}	44.58 ^{b}	8.23 ^{d}
	SA	27.42 ^{cd}	9.46 ^{b}	3.21 ^{f}	48.59 ^{a}	11.32 ^{c}
	H_2O_2	25.29 ^{d}	9.84 ^{ab}	16.27 ^{a}	42.21 ^{bc}	6.39 ^{e}

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

mM NaCl in embryonic axes. However, the activity of SOD decreased only at 75 mM NaCl in both embryonic axes and cotyledons of germinating lentil seeds. For GPOX activity, significant decreases were only observed in cotyledons (Figs 4, 5 and 6).

Under non-saline conditions, application of SA and H_2O_2 caused the increase of SOD activity but only in embryonic axes. Seed priming with SA and H_2O_2 caused an increase in the activity of CAT in both embryonic axes and cotyledons under salt stress conditions. The activity of this enzyme was higher by 53.27% and 47.00% in embryonic axes at 150 mM NaCl, while 89.27% and 66.46% in cotyledons at 75 mM NaCl of seeds primed with SA and H_2O_2, respectively.

Significant increases of GPOX activity were only observed at 150 mM NaCl in embryonic axes following SA and H_2O_2 applications by 73.68% and 58.60% respectively, and in cotyledons following SA application (2-fold higher). On the other hand, seed priming with SA and H_2O_2 do not cause significant increases in SOD activity in both embryonic axes and cotyledons of germinating lentil seeds (Fig. 6).

DISCUSSION

In the present work, results showed a decrease in germination and root elongation of lentil seeds with increasing salinity. Inhibition of germination due to salinity has been reported by Alyari et al. [2004] and Bouallègue et al. [2017]. Thakur and Sharma [2005] have reported that decrease in germination particularly under salt stress may be related to the development of an osmotically enforced dormancy in seeds. In *Brassica napus*, salinity higher than 50 mM NaCl cause a retardation of seed germination and a severe reduction in the lengths of seedling primary roots [Ben Miled et al. 2000]. In other cases, it has been reported that salinity delayed germination of several species without reducing the final germination percentage [Ayers and Westcot 1985]. This is not observed in this study, where the final percentage of germination of lentil seeds was severely reduced (27.56%) by high level of salt (150 mM NaCl).

Salicylic acid (SA) and hydrogen peroxide H_2O_2 treatments (0.1 mM) had beneficial effects on germination percentage under salt stress conditions. The two priming treatments did not only alleviate the inhibitory effect of salt stress on germination percentage, but also induced primary root elongation. Similar results were observed in many other plant species [Shakirova et al. 2003]. It has also been shown that SA increases the degree of growth of primary root cells both by division and expansion of meristem cells [Janda et al. 2007].

In the present work, little changes in starch and soluble sugars contents were detected in cotyledons under salt stress conditions. Similar results were shown by Corchete and Guerra [1986]. Seeds priming with SA and H_2O_2 reduced starch contents especially at 75 mM NaCl but without significant enhancement of the accumulation of soluble sugars in cotyledons of germinating lentil seeds treated with 75 and 150 mM NaCl. These results could be probably explained by a non-significant effect of salinity and SA and H_2O_2 pretreatments on amylase activity. On the other hand, it has been reported that salt limits starch mobilization in various species, as a result of inhibition of different enzymatic activities such as amylases [Bouallègue et al. 2017]. The hydrolysis of starch into sugars induce osmotic adjustment and plays an important role in an adaptive response to salt stress conditions [Giorgini and Sudat 1990]. A correlation between amylase activity, soluble sugars and starch in cotyledons of many species was observed by several studies [Monerri et al. 1986].

In this study, salt stress caused the increase of MDA content in embryonic axes and cotyledons of germinating lentil seeds. Many reports indicate that salt stress induce oxidative stress leading to lipid peroxidation, disruption of membrane damage and permeability in plant cells [Jaleel et al. 2008]. Membrane injury under salt stress is related to increased production of high toxic ROS [Hernandez et al. 2000]. Here presented results have shown that seed priming with SA and H_2O_2 caused significant decrease of MDA level especially in embryonic axes at 150 mM NaCl. Reduction of MDA levels in response to SA and H_2O_2 treatments might be due to induction of antioxidant responses that enhance scavenging of harmful free radicals and protect the plant from the oxidative damage [Bouallègue et al. 2017].

The higher activity of antioxidant enzymes in salt stressed seeds treated with SA and H_2O_2 is an indication of plant ability to diminish the level of ROS produced under salt stress conditions. This suggested that salt tolerance could be induced by enhancing antioxidant capacity of plants.

The results presented here have shown that activity of CAT and GPOX remained unchanged or decreased under salt stress as compared to unstressed conditions. However, SA and H_2O_2 applications caused increases in CAT and GPOX activities. Catalase and peroxidase are oxygen scavenging enzymes, which remove toxic substrates during development, which are otherwise lethal. Decreased CAT and GPOX activities in stressed germinating seeds might have enhanced H_2O_2 accumulation, which could result in the formation of hydroxyl radicals which are known to damage biological structures [Gill and Tuteja 2010]. The higher activities of CAT and GPOX in primed seeds might have resulted in increased oxidation of harmful substrates, leading to improved seed germination. On the other hand, SOD activity, which catalysis the conversion of the superoxide anion to H_2O_2, do not show significant increase following seed priming in both embryonic axes and cotyledons of germinating lentil seeds.

Salt was found to induce increase in TL content (Tab. 3). Contrarily, TL content was found to be significantly higher only in the salt exposed SA-pretreated seeds than in the salt-treated controls indicating that lipid synthesis increased after SA applications. This increase can induce an augmentation of the vacuolar membrane allowing to participate in the induction of salt tolerance and ion detoxification by controlling cellular salt homeostasis [Kim et al. 2007]. On the other hand, the increase of the tri-unsaturated fatty acids (C18:3) observed under the influence of especially SA lead to increase the fluidity of lipid membranes and probably influence their permeability. This is correlated with reduced MDA content and enhanced antioxidant activities. In fact, fatty acids of lipid membranes affect bilayer properties regulating the membrane fluidity and permeability, and enzymes activities [Quartacci et al. 2001]. Globally, these results exhibit the beneficial effect of SA and H_2O_2 pretreatment on lipid metabolism.

CONCLUSIONS

It may be concluded that SA and H_2O_2 pretreatments significantly decreased negative effect of salt stress on germination of lentil seeds. This was mainly linked with better anti-oxidative defense system and reduced lipid peroxidation that may help plants to avoid the damaging effect of salt stress. Therefore, SA and H_2O_2 could be used as potential molecules to improve germination and plant growth under salt stress. However, further studies are required to unravel the mechanism of SA and H_2O_2 action in alleviating salt stress in germinating seeds.

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