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Antioxidant enzymes and physiological traits of *Vicia faba* L. as affected by salicylic acid under salt stress

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Abstract

Salt stress causes a number of changes in plant metabolism. However, salicylic acid (SA) is one of plant growth regulator involved in various physiological processes. The effects of salt stress and seeds soaked in SA on physiological and biochemical parameters of *V. faba* L. shoot and root were investigated. Results show that increased salinity reduces Leaf water potential and stomatal conductance. However, this inhibitory effect was alleviated due to SA treatment. Salinity induces oxidative stress in shoots and roots, and increases the concentration of total phenolic contents, soluble sugar, protein, proline, MDA and some antioxidant enzymes such as SOD and PPO, while seeds soaked in SA reduce their concentration/activity. In addition, salinity decreased chlorophyll 'a', total Chlorophyll, carotenoids content and the activity of POD only in control plants. Therefore, Pre-soaking the seeds in SA improved plant tolerance to salinity compared to the control plants. These findings indicate that SA might have an important protective effect in plants under salt stress and may help to alleviate the adverse effect of salinity on the growth of *V.faba* L.

1. Introduction

Agriculture plays a pioneering role in economical development in many countries, such as Morocco. There has been a renewed interest in faba bean [1] throughout the world, which may not be unconnected with its high nutritional value, characterized by its important proteins components (20–41% of seed dry matter) and carbohydrates (51–68% of seed dry matter). Most of these proteins are globulins (79%), albumins (7%), and glutelins (6%) [1]. Therefore, there is need to increase its production by expansion through newly reclaimed areas. However, reports about its physiological characteristics were limited and sporadic. Consequently, improvement in faba bean relies on better understanding of the bean itself, including its genome, physiology and behavior in growth and development under biotic and abiotic stresses.

Salinity is one of the major environmental constraints to plants growth and productivity. Increasing salinity leads to a reduction and/or delay in germination of plants and death of seeds before germination [2]. Salt stress causes a number of changes in plant metabolism, ion toxicity, osmotic stress, and production of reactive oxygen species (ROS) are most prominent [3].

Tolerance to high salinity is not a simple attribute, but it is an outcome of various features that depend on different physiological interactions, which are difficult to predict. The morphological appearance presented by the plant in response to salinity, may not be enough to determine its effects, so it is important to recognize other physiological and biochemical factors [4]. In order to survive in salt stress conditions, plants develop the network responses of physiological and biochemical defense mechanisms to protect themselves against stress [5]. A high salinity induces serious metabolic perturbations in plants, as it generates ROS, which disturb the cellular redox system [6]. The generation of ROS is limited or scavenged by an antioxidant system including antioxidant compounds (ascorbate, salicylate, glutathione, tochopherols, etc.) and antioxidant enzymes like superoxide dismutase, ascorbate peroxidase, and polyphenoloxydase [7, 8].

Salicylic acid (SA) is one of plant growth regulator involved in various physiological processes in plants, such as growth regulation, photosynthesis, stomatal conductance, nutrient uptake, plant water relations and mechanisms of plant resistance and tolerance to biotic and abiotic stresses [9, 10]. Therefore, exogenous application of SA to the stressed plants can potentially alleviate the toxic effects, generated by salinity. Many studies support that SA enhanced tolerance against abiotic stress and increase the resistance of maize [11, 12], and wheat [13] to salinity. Findings were similar to wheat [14, 15] grown under osmotic stress. However, the plant adaptation to salinity may depend on plant species, concentration, method and time of SA application [16].

The present study aimed to investigate the effects of salt stress on the physiological traits (leaf water potential and stomatal conductance) and biochemical parameters (antioxidant activity, MDA, soluble sugar, total phenolic contents and Photosynthetic pigments) of *V. faba* L shoots and roots and to determine the most effective SA concentration to alleviate salt stress effect.

2. **Materials and methods**

2.1 Plant Material and Growth Conditions

This study was carried out in a growth chamber with one faba bean variety "Reina Mora". Intact seeds, which were homogeneous and identical in size and color, and free from wrinkles, were chosen and then disinfected for 1 min in 70% (v/v) ethanol and then soaked in 20% (v/v) commercial bleach for 10 min. Seeds were rinsed several times in sterile distilled water. *Vicia faba L.* seeds were soaked with the following solutions for 12 hours in the ambient conditions: 0 mM SA, 0.5 mM SA and 1 mM SA. After soaking, the seeds were sowed directly in pots 20 cm in diameter and 30 cm high filled with sterile sand and peat at 2:1 ratio respectively. The number of plants was adjusted to 1 per pot. Taking into account the soaking treatment, pots were arranged in a completely randomized design and each one was considered as one replicate with ten pots per treatment. Irrigation was with a one-half strength Hoagland solution once a week. Every three days, the pots were watered, the plants irrigated with distilled water were taken as the experimental control. After 21 days of salt treatment, plants were subjected to different physiological and biochemical analysis.

2.2 Plant water status

The plant water status was characterized by the leaf water potential and Stomatal conductance.

2.2.1 Leaf water potential (Ψ)

Leaf water potential (Ψ) was measured using a Scholander pressure chamber (SKPD 1400, Skye Instruments, Powys, UK). A branch with newly expanded leaves per plant (four plants per treatment) was detached immediately severed at the petiole, and scaled into the humidified chamber for determination of balancing pressure.

2.2.2 Stomatal conductance (gs)

The stomatal conductance was measured on sunny days at 10 am to 12 am with a steady-state diffusion porometer (Leaf Porometer, Decagon Device, Inc., Washington, USA). On two upper leaves in each treatment (6 leaves per treatment). The system was calibrated each use with the supplied calibration plate. The terminal part of the main leaf lobe was placed into the cup on the head unit which was positioned normal to the sun.

2.3 Enzyme assays

Leaf segments (0.1 g) were crushed into fine powder in a mortar placed in an ice-bath. 1ml of 0.05 mol l-l pH 6 phosphate buffer with 1% polyvinylpyrrolidone (PVP) was used as an extraction buffer. The homogenate was centrifuged at 15000xg for 15 min at 4°C, and the supernatant was used for soluble protein content and enzyme analysis of superoxide dismutase (SOD), polyphenoloxydase (PPO) and peroxidase (POD). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetra-zolium (NBT) according to Beauchamp and Fridovich [17]. One unit of SOD activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of NBT. The enzyme activities were expressed as unit mg-1 protein

The PPO activity was determined according to the method designed by Hori, et al. [18]. The reaction mixture contained 20 mM catechol in 0.1M phosphate buffer pH 6. The assay was initiated by the addition of 100µl of enzymatic extract. PPO activity was expressed in enzyme unit mg-1 protein. One unit of PPO activity was defined as the amount of enzyme, which caused an increase in absorbance of 0.001/ min at 420 nm.

For measurement of POD activity, assay solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H_2O_2 and 0.1 ml enzymatic extract. The reaction was initiated by adding the enzyme extract. Increase in absorbance of the reaction solution at 470 nm was recorded after every 30 s. One unit POD activity was defined as an absorbance change of 0.01 unit min-1. Total soluble proteins content determined by the method of Bradford (1976) and a standard curve was drowned out with the serum bovine albumin.

2.4 Lipid peroxidation

The level of lipid peroxidation was determined in terms of malondialdehyde content (MDA) according to the method of Rao and Sresty [19]. MDA concentration was calculated from the absorbance at 532 nm and measurements were corrected for non specific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM−1 cm−1.

MDA = DO532-DO600/∑ MDA*V

Whereas ∑ MDA= 155 mM/cm and V (ml) is the volume of crushing medium.

2.5 Determination of soluble sugar

The soluble sugar in faba bean was estimated following Dubois et *al.* [20]. After the test tube was cooled, absorbance was recorded at 485 nm. All determinations were carried out in triplicate. The concentration of soluble sugar was determined against a standard curve prepared by using a glucose solution (concentration range from 50 to 1000µg/ml). Results were expressed as µg of glucose equivalents for g of fresh weight of *Vicia faba* L.

2.6 Total phenolic contents

The total phenolic content of aqueous methanol extracts was determined by using the Folin Ciocalteu Method. Briefly, 50µl of sample was added into a 5 ml thick test tube followed by the addition of 250 µl Folin-Ciocalteu reagent (diluted 3 times). The mixture was shaken slowly and left to react at room temperature for 3 min. After 3 min, 500µl of sodium bicarbonate (20 % w/v) was added to the mixture. The test tube was filled with 1.745 ml distilled water; the mixture was heated at 40°C for 30 min. Distilled water was used as blank. Sample absorbances were recorded at 760 nm against the blank. The total phenolic content was expressed as µg cafeic acid equivalents g-1 fresh weight though the calibration curves with cafeic acid.

2.7 Photosynthetic pigments

For each sample 50 mg of fresh leaves were cut and ground in 3 ml of cold 80% acetone. The extracts were centrifuged at 1000g for 10 minutes. The supernatants were then collected in test tubes and incubated in the dark for two hours before the assay. The optical density (O.D.) of the extract was measured at wavelengths 663, 645, and 440.5 nm [21] to estimate chlorophyll 'a' and 'b' and carotenoids respectively. Three replicates were used for each treatment, and die amount of pigment present in each sample was calculated according to the following equations:

mg (chlorophyll a)/g (FW) = 12.7 (O.D) 663 - 2.69 (O.D) 645 × v/(w×1000)

mg (chlorophyll b)/g (FW) = 22.9 (O.D) 645 - 4.68 (O.D)663 × v/(w×1000)

mg (carotenoids)/g (FW) = 46.95 {O.D. 440.5 -0.268 x chlorophyll 'a* + 'b')

mg (chlorophyll total)/ / g (FW) = 20.2 (O.D) 645 + 8.02 (O.D) 663 x v/(w×1000)

Whereas W, the fresh weight by grams for extracting tissue; V, the final size of the extract in 80% acetone; O.D., optical density at specific wavelength.

2.8 *Statistical analyses*

The experiments were carried out with a completely randomized design. Values are means of four replicates, the means were separated with least significant difference test using CoStat version 6.3. However, the principal component analysis (PCA) was preferment using SPSS version 10.

3. Results

3.1 *Effect of Salt stress and Salicylic acid on Plant water status of Vicia faba L.*

3.1.1 *Leaf water potential (Ψ)*

The Figure 1.a shows a comparison of the leaf water potential values observed in the *Vicia faba L.* in tow salinity levels. Statistical analysis (Table 1) provides a higher significant effect ($p<0.001$) of different treatments on the Leaf water potential. Salt treatment decreased water potential by 118%, 95% and 64% under 0 mM, 0.5mM and 1mM of SA respectively. Supplementation of SA to salt treated plants proved ameliorative effect on the water potential. In contrast, the mean comparison shows a significant difference between the three doses of SA under salinity (Figure 1.a). Under non-saline condition, application of 0.5 mM and 1 mM SA increased slightly the leaf water potential, but the difference was not significantly to the control. This result shows that the exogenous salicylic acid (SA) significantly improved abiotic tolerance in higher plants.

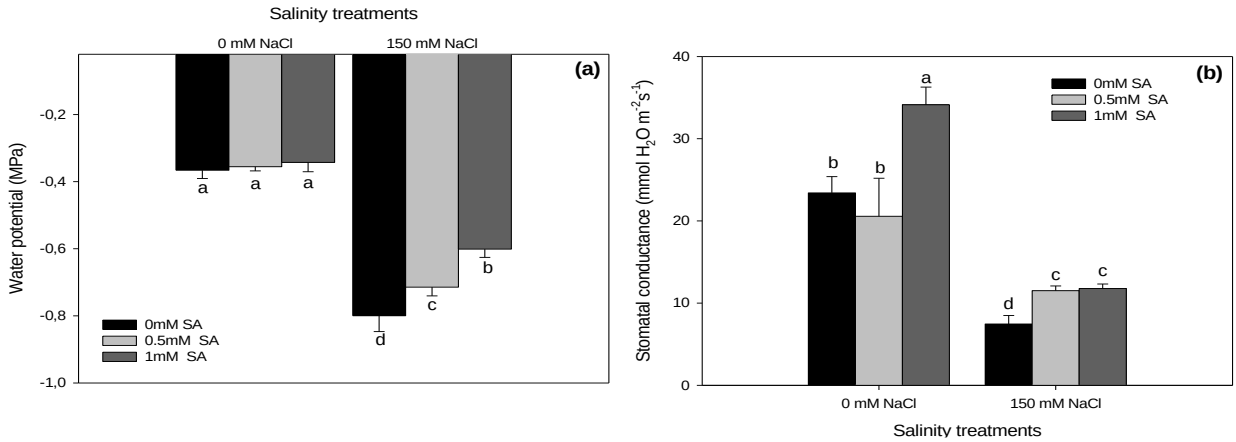

Figure 1: Effect of SA and/or NaCl application on leaf water potential (a) and Stomatal conductance (gs) (b) of faba bean (cv. Reina Mora) submitted to 150mM NaCl for 21days. Data are means of 10 replicates ± SE. Means with similar letters are not different at $P \leq 0.05$ according to LSD test.

3.1.2 *Stomatal Conductance (gs)*

The stomatal conductance values observed in the faba bean plants under tow salinity levels are shown in figure 1.b. Statistical analysis showed that the gs was significantly ($p <0.001$) affected by salinity, SA treatments and their combinations. SA treatment increased gs of non stressed plants increased by 46% in comparison to the control. Elsewhere, stomatal conductance values between control and 0.5mM SA/0 mM NaCl treatment were not significantly differed. However, under salinity treatments gs readings showed a decreasing trend compared to control. Nevertheless, it was significantly increased with both SA doses (0.5 mM and 1mM SA/150 mM NaCl) in comparison to salinity treatment without SA application, whereas mean comparison demonstrates non significance difference between both SA doses.

3.2 *Effect of Salt stress and salicylic acid on protein of Vicia faba L.*

The Figure 2 shows the effect of different levels of salinity and SA treatments on the protein contents in shoots and roots of *Vicia faba L.* In the absence of salinity, SA had no significant effect on the shoots protein (Figure 2.a). However, the protein contents in shoots was significantly increased ($p<0.05$) under salinity treatments relative to the control. The application of SA reduced significantly ($p<0.05$) the protein contents under 150 mM NaCl. Moreover, at the concentration of 150 mM of NaCl, the protein of *Vicia faba L.* shoots decreased significantly ($p<0.05$) with SA application. The protein contents were reduced by 23% and 12% in the presence of 0.5 mM and 1mM of SA. The roots protein contents increased significantly ($p<0.05$) with SA application both under saline conditions (figure 2.b).

Table 1: ANOVA table summarizing two-way completely randomized effects of salinity treatment, salicylic acid and their interactions on different parameter of *Vicia faba L.*

	Salinity Treatment	Salicylic acid treatment	Salicylic acid treatment * Salinity treatment
DF	1	2	2
Stomatal conductance (gs)	257.87545***	24.061338***	15.282054***
Leaf water potential (Ψ)	793.89314 ***	26.794048 ***	16.615802 ***
Protein Shoots	390.51236 ***	8.0333341 **	7.8787619 **
Protein Roots	1280.07 ***	116.12863 ***	6.8593758 **
Superoxide dismutase (SOD) Shoots	126.70124 ***	12.832348 ***	8.6527108 **
Superoxide dismutase (SOD) Roots	333.92101 ***	39.757271***	10.113568**
Peroxidase (POD) Shoots	0.2145488 ns	10.967046 **	17.39655 ***
Peroxidase (POD) Roots	557.69739 ***	30.223464 ***	11.446725 ***
Polyphenoloxydase (PPO) Shoots	510.94525 ***	0.7528572 ns	30.989514 ***
Polyphenoloxydase (PPO) Roots	339.90206 ***	7.0010059 **	2.747886 ns
Lipid peroxidation (MDA) Shoots	272.31801 ***	12.669558 ***	6.7298593 **
Lipid peroxidation (MDA) Roots	121.82703 ***	98.601791***	134.72631***
Total phenolic contents Shoots	228.84139 ***	8.9816334**	281.1898***
Total phenolic contents Roots	953.43249 ***	413.46606***	455.49407***
Soluble sugar Shoots	154.62835***	41.86079***	39.828943***
Soluble sugar Roots	275.36071***	150.15414***	69.335827***
PROLINE Shoots	219.61602***	17.722631***	78.288664***
PROLINE Roots	1573.9043***	2.9856672ns	1.5296215ns
Chlorophyll a	43.85***	104.15599***	6.9545649**
chlorophyll b	1679270.8***	35934283***	38739173***
Carotenoids	1457987.3***	525524.33***	400895.62***
Total Chlorophyll	47954075***	64093045***	2171617.3***

ns = not significant; * difference statistically significant at $p < 0.05$; ** difference statistically significant at $p < 0.01$; *** difference statistically significant at $p < 0.001$.

3.3 *Effect of Salt stress and salicylic acid on antioxidant system of Vicia faba L.*

3.3.1 Superoxide Dismutase (SOD)

Adaptation to salt stress may depend on different mechanisms, including the capacity to maintain high levels of antioxidants and through the induction of antioxidant enzymes. Compared with the control, all salinity stress and SA increased SOD activities in the shoots and roots. Table 1 illustrates that SOD in both shoots and roots was significantly ($p<0.001$) affected by salinity and SA. The mean comparisons in shoots (Figure 3.a) show a significant increase by 64% of SOD activity under non saline condition with application of 1 mM in comparison to the control. Under salinity treatment the increasing was 262, 654 and 784% respectively for 0, 0.5 mM and 1mM of SA in comparison to the control. In roots, the SOD activity had a similar tendency, but with higher activities compared to shoots. As presented in Figure 3.b the activity was significantly increased under non saline condition with application of 0.5 mM of SA to reach an increasing of 84% of the control. Though, stressed plants present a higher increasing of SOD activity which reached 127, 251 and 234% respectively for 0, 0.5 and 1mM of SA in comparison to the control.

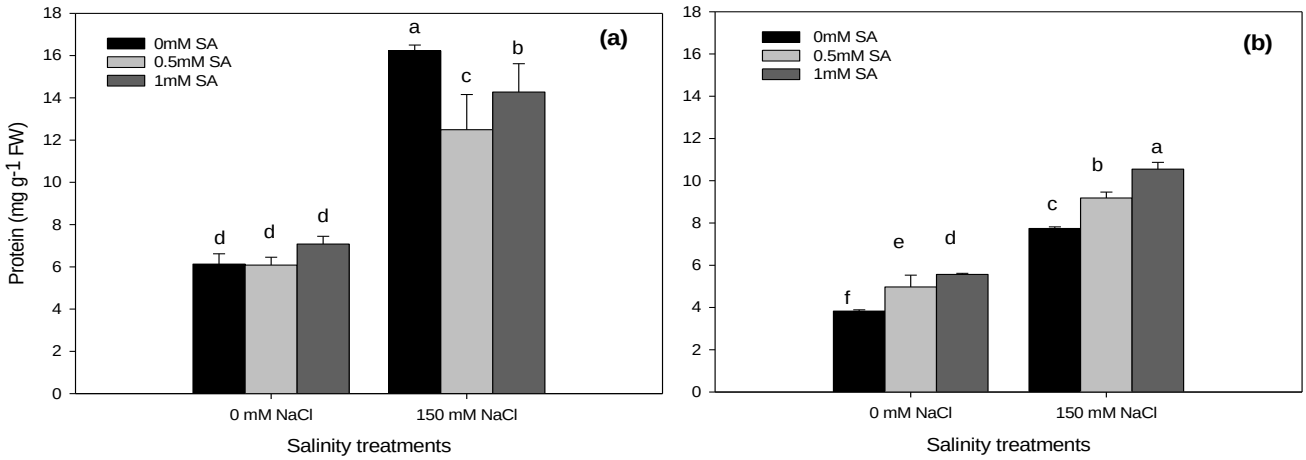

Figure 2: Effect of SA and/or NaCl application on Protein content in shoots (a) and roots (b), of faba bean (cv. Reina Mora) submitted to 150mM NaCl for 21days. Data are means of 10 replicates ± SE. Means with similar letters are not different at $P \leq 0.05$ according to LSD test.

3.3.2 *Peroxidase POD*

The Figure 3 (c and d) shows the peroxidase activity (POD) in shoots and roots parts under different treatment of salinity and SA. However, in shoots statistical analysis (Table 1) show that salinity had no significant effect on the POD activity, nevertheless SA and combination between both factors were highly significant ($p<0.001$), whereas mean comparison show no significant effect between the control and 0.5mM of SA/0mM NaCl, 0.5 mM SA/150mM NaCl and 1mM SA/150 NaCl (figure 3.c). Elsewhere, the POD activity in the roots was higher than shoots under no saline condition. Thought, there was no significant difference ($p<0.05$) between 0 and 0.5 mM of SA under no saline condition, and between all SA doses under saline condition (figure 3.d).

3.3.3 Polyphenoloxydase PPO

The Polyphenoloxydase activities (PPO) were significantly affected by salinity (Table 1) in both compartments (shoots and roots). However, the activities were more pronounced in the shoots part than in the roots. As presented in the Figure 3.f SA had no effect on the PPO activity in roots inside both salinity treatments. However, the PPO activity in shoots part (figure 3.e) was more affected by the saline stress. There we can note an increasing by 277, 330 and 200% respectively in 0, 0.5 mM and 1mM of SA under saline conditions compared to the control. With the exception of control plants and 0 mM NaCl /0.5mM SA, mean comparison (Figure 3.e) shows a significant difference between PPO activities in response to salinity and SA treatments.

3.4 *Lipid peroxidation MDA*

The Lipid peroxidation (MDA) of the *Vicia faba* L. shoots and roots (Figure 4) was significantly ($P<0.001$) affected by salinity and SA (Table 1). The MDA content in shoots part (figure 4.a) was significantly increased with the increasing of SA doses by 36% and 127% respectively for 0.5 mM and 1mM SA under non saline conditions. Salt treatment induces an increasing of 631 % relative to the control and the combination of Salt and SA increased MDA contents by 367 and 525% respectively for 0.5 mM and 1mM SA/150 NaCl compared to control. At the roots level, there was also an increase in MDA contents with SA application and with NaCl stress. However, MDA content was not affected by the application of SA under saline conditions (Figure 4.b).

3.5 *Total phenolic contents (TPC)*

The total phenolic contents variation in shoots and roots under salinity and SA regimes are shown in Figure 5. In shoots (figure 5.a), the phenolic content decreased with the salinity, while its contents increased under 0.5mM SA by 16% compared to the control under non saline conditions. Whereas, the total phenolic contents decreased by 35% under 1mM SA in comparison to the control. However, under saline conditions the phenol contents decreased by 28% in comparison to the control.

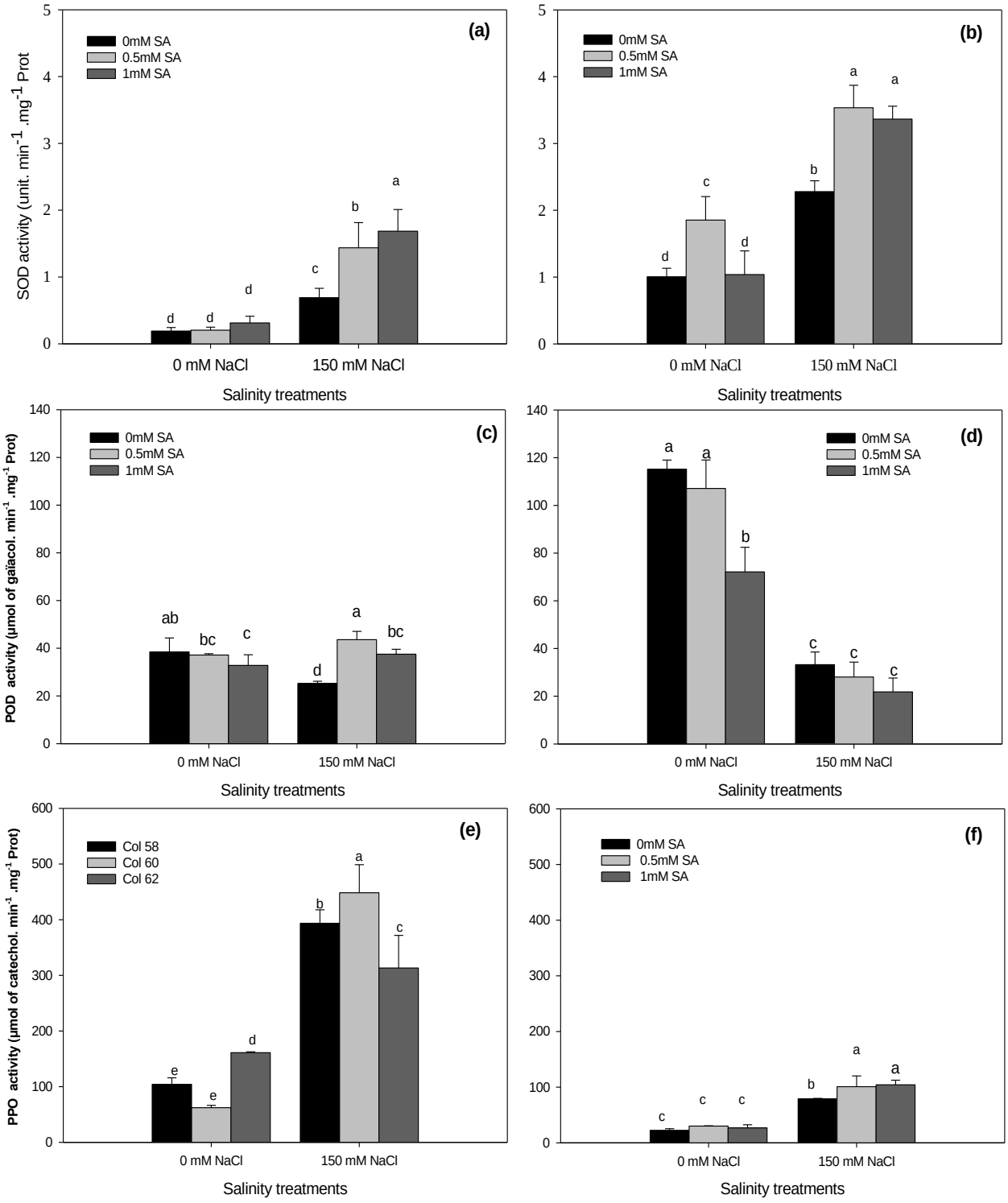

Figure 3: Effect of SA and/or NaCl application on antioxidant activity (in shoots/roots) superoxidase activity (SOD) (a/b), peroxidase activity (POD) (c/d) and Polyphenoloxidase activity (PPO) (e/f), of faba bean (cv. Reina Mora) submitted to 150mM NaCl for 21days. Data are means of 10 replicates ± SE. Means with similar letters are not different at $P \leq 0.05$ according to LSD test.

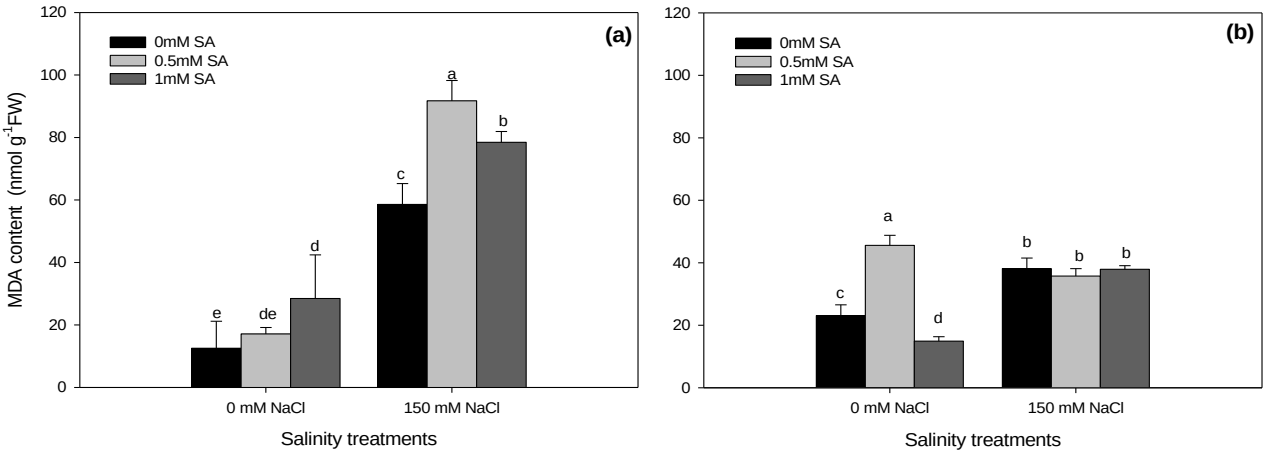

Figure 4: Effect of SA and/or NaCl application on MDA in shoots (a) and roots (b), of faba bean (cv. Reina Mora) submitted to 150mM NaCl for 21days. Data are means of 10 replicates ± SE. Means with similar letters are not different at $P \leq 0.05$ according to LSD test at 95%.

Elsewhere, under 0.5 mM SA the total phenolic contents decreased by 39% and by 56% respectively in comparison to the 150mM NaCl and to the control. Nevertheless, under 1mM the phenolic contents was increased significantly ($p<0.05$) by 25% in comparison to 150mM NaCl. At roots compartment (figure 5.b), mean comparison show a significance difference ($p<0.05$) between all treatment except 0.5 mM and 1mM SA under salinity. Therefore, we note an increasing of the total phenolic content by 137 and 73%, respectively under 0.5 mM and 1mM SA application in the absence of salinity in comparison to the control.

3.6 *Total soluble sugars*

The Figure 5 (c and d) presents the total soluble sugars in response to SA treatments under two levels of salinity in shoots and roots compartments. Statistical analysis shows that total soluble sugars in both compartments was significantly ($p<0.001$) affected by salinity, Salicylic acid and the combination of the two factors. Thereafter, we not a significant ($p<0.05$) increasing under the effect of salt stress in the shoots by 33% and 39 % under 1mM and 0.5 mM SA/0mM NaCl respectively, and by 100%, 150%, respectively under 0mM and 0.5mM SA under salt treatment of 150mM in comparison to the control(figure 5.c). At roots, mean comparison shows significant difference ($p<0.05$) between all treatments. The salinity treatment had a significant effect on the total soluble sugars. Thereafter the total soluble sugars increased with increasing salinity. However the SA had an increasing effect of the total soluble sugars under booth salinity treatment with increasing SA dose (Figure 5.d). Elsewhere, under the 0.5mM SA we note a decreasing effect by 41% in comparison to the control.

3.7 *Proline content*

The proline responses of *Vicia faba* L. plant to salinity and SA is summarized in the Figure 5 (e and f). The Table 1, point out the significant ($p<0.001$) effect of salinity, SA and the interaction between the booth factors on the proline contents in the shoots. However, in the roots, statistical analysis (Table 1) shows no significant effect of SA and the interaction between salinity and SA on the proline content. Therefore, mean comparison (as present Figure 5.f) reveled two statistical groups difference depended only to the salinity levels. Nonetheless the proline content was in increased by 73% with increasing salinity to reach a maximum of 47µg. mg-1FW under 150 mM NaCl/0.5 mM SA. In the other hand, the proline content in shoots was increased significantly ($p<0.05$) with salinity to reach a maximum of 57.5 µg. mg-1FW under combination with 1 mM SA (figure 5.e). However, under absence of salinity the proline content was decreased by 22% with increasing SA dose to reach a minimum of 28 µg. mg-1FW.

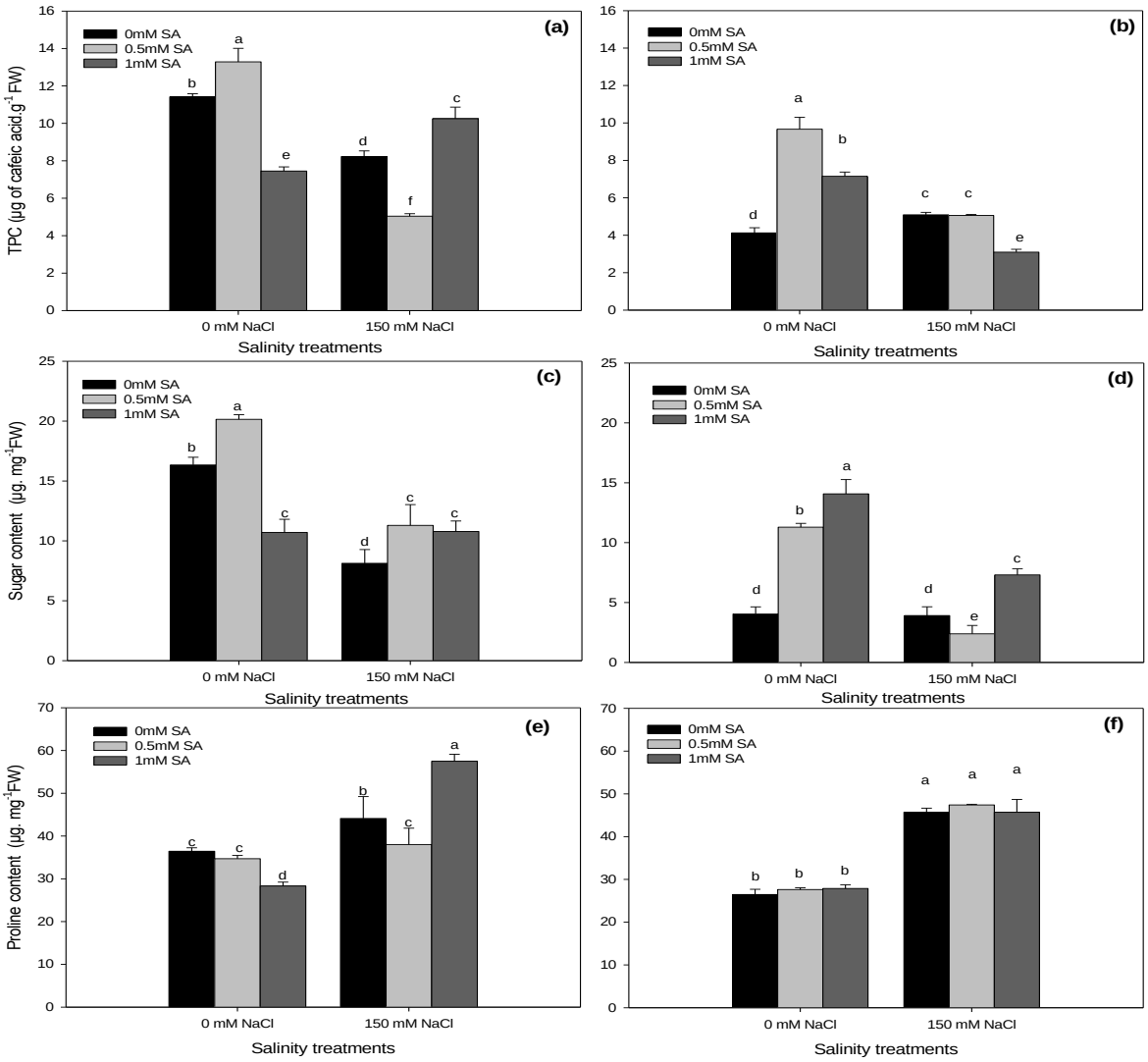

Figure 5: Effect of SA and/or NaCl application on total phenol content (a/b), sugars concentrations (c/d) and Proline content (e/f) respectively in shoots/roots, of faba bean (cv. Reina Mora) submitted to 150mM NaCl for 21days. Data are means of 10 replicates ± SE. Means with similar letters are not different at $P \leq 0.05$ according to LSD test at 95%.

3.8 *Photosynthetic pigments*

The Figure 6 illustrates the effect of salt stress, on the chlorophyll content of faba bean. Chlorophyll contents was significantly ($p<0.001$) affected by salinity stress, SA and the combination of two factors (Table 1). The results show an inverse relationship between salt stress and chlorophyll. "a" content. Whenever, the concentration increased, chlorophyll "a" content decreased. However, the application of 0.5 mM SA led to an increasing of the chlorophyll. "a" content by 30% and 15% respectively for 0 and 150mM of NaCl compared to the control plant (figure 6.a). The chlorophyll "b" content was decreased with increasing salinity in combination with SA. However, under salinity and in absence of SA the chlorophyll "b" increased significantly ($p<0.05$) by 45% in comparison to the control (figure 6.b). Carotenoids content in leaves of *Vicia faba L.* seedlings is shown in Figure 6.c. The Carotenoids content in both SA treated and non SA treated seedlings significantly decreased with the increasing of NaCl concentrations. The Cartenoid contents of SA treated seedlings (with SA) under non salinity treatment reduced by 9% and 14% respectively to 0.5 and 1mM of SA compared to control. Under saline conditions combined to 1mM SA treatment led to about 50% compared to the control.

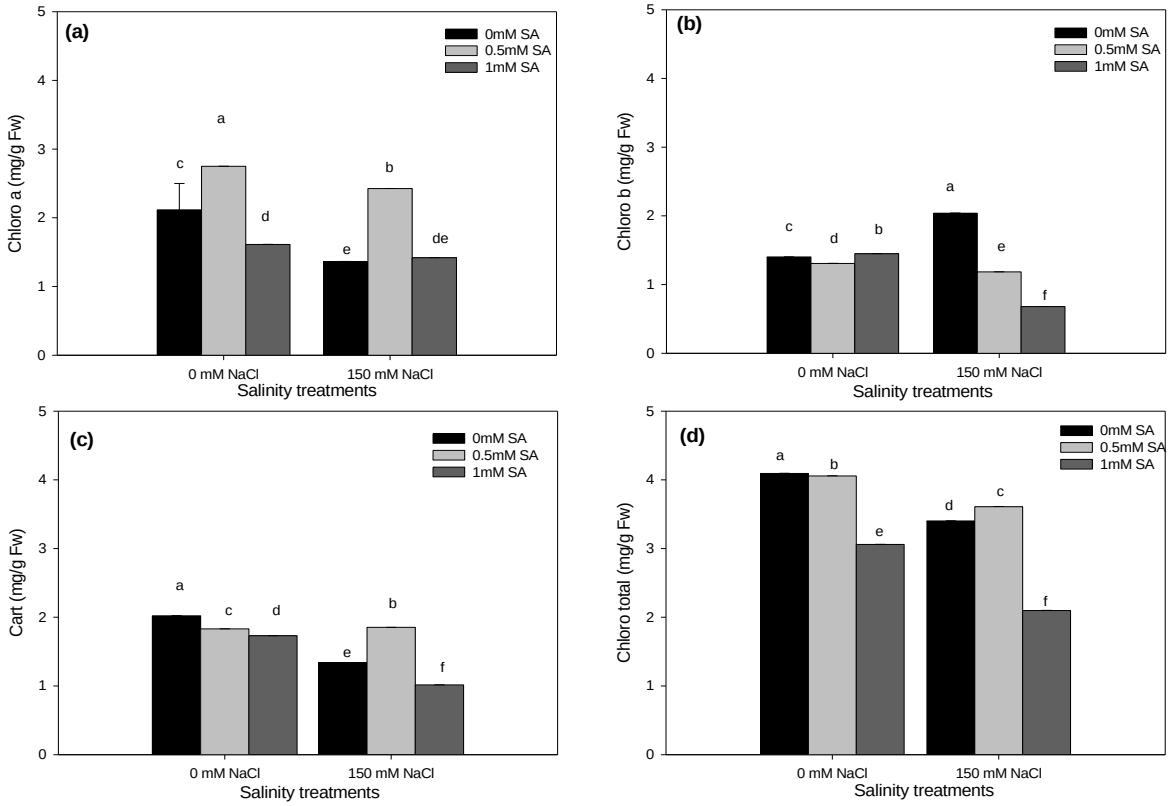

Figure 6: Effect of SA and/or NaCl application on chlorophyll a (a), b (b), carotenoid (c) and chlorophyll total (d) concentrations in shoots of faba bean (cv. Reina Mora) submitted to 150mM NaCl for 21days. Data are means of 10 replicates ± SE.Means with similar letters are not different at $P \leq 0.05$ according to LSD test.

3.9 *Correlations between parameters*

The Table 2 presents different correlations between parameters studied in shoots. A significant correlation was detected inside parameters studied, so, two major groups were highlighted the first includes water potential, stomatal conductance, sugar, total phenol contents and cartenoids, and the second group formed by protein, SOD, PPO, proline and MDA. All parameters inside both groups were positively and negatively correlated with the parameters of another group. However, the POX chlorophyll a and chlorophyll b presented no significant correlation with the majority of the studied parameters, however the POX present a significant correlation with chlorophyll a (positive) and chlorophyll b.

However, the correlation between parameters studied at the roots levels presented in the Tables 3. The correlation reveille on a positive and significance ($p<0.001$) correlation between protein, SOD, PPO and proline, though these parameters were negatively correlated to POX, total phenol content and sugar. The MDA at the roots levels present no significance with all parameters studied, except the SOD witch present a positive and a significant ($p<0.05$) correlation.

The principal component analysis (PCA) is the statistical tool used to explain differences between samples and to obtain more information on the variables that mainly influence the sample similarities and differences. This procedure extracts the dominant patterns in the data matrix in terms of a complementary set of scores and loading plots. PCA allows us to achieve a reduction in dimensionality, data exploration for finding relationships between objects, an estimation of the correlation structure of the variables and an investigation of how many components (a linear combination of original features) are necessary to explain the greater part of variance with a minimum loss of information (Rodrıguez et al., 2002).

Table 2: Pearson's linear correlations among Correlation analysis between evaluated parameters in shoots

		LWP	Protein	SOD	POX	PPO	MDA	Total phenolic content	sugar	Proline	Ch a	Ch b	carotioide
gs	**r**	**0,84**	**-0,80**	**-0,61**	**0,084**	**-0,72**	**-0,67**	**0,17**	**0,32**	**-0,67**	**0,12**	**-0,01**	**0,51**
	p	***	***	**	**ns**	***	***	**ns**	**ns**	***	**ns**	**ns**	*
LWP	**r**		**-0,93**	**-0,64**	**0,18**	**-0,91**	**-0,80**	**0,56**	**0,68**	**-0,54**	**0,36**	**-0,19**	**0,52**
	p		***	***	**ns**	***	***	**	***	**	**ns**	**ns**	**
Protein	**r**			**0,68**	**-0,38**	**0,83**	**0,801**	**-0,469**	**-0,756**	**0,713**	**-0,565**	**0,0702**	**-0,747**
	p			***	**ns**	***	***	*	***	***	**	**ns**	***
SOD	**r**				**0,17**	**0,73**	**0,88**	**-0,43**	**-0,50**	**0,70**	**-0,25**	**-0,55**	**-0,59**
	p				**ns**	***	***	*	*	***	**ns**	**	**
POX	**r**					**0,04**	**0,08**	**-0,09**	**0,38**	**-0,23**	**0,67**	**-0,46**	**0,48**
	p					**ns**	**ns**	**ns**	**ns**	**Ns**	***	*	*
PPO	**r**						**0,88**	**-0,74**	**-0,74**	**0,41**	**-0,34**	**0,043**	**-0,45**
	p						***	***	***	*	**ns**	**ns**	*
MDA	**r**							**-0,64**	**-0,65**	**0,59**	**-0,27**	**-0,30**	**-0,53**
	p							***	***	**	**ns**	**ns**	**
Total phenolic content	**r**								**0,72**	**0,081**	**0,26**	**-0,14**	**0,04**
	p								***	**Ns**	**ns**	**ns**	**ns**
Sugar	**r**									-0,37	0,75	-0,19	0,56
	p									ns	***	ns	**
Proline	**r**										-0,43	-0,42	-0,82
	p										*	*	***
Ch a	**r**											-0,14	0,71
	p											ns	***
Ch b	**r**												0,247
	p												ns

r = Pearson r correlation coefficient; p; significance level of the test; ns = not significant; * difference statistically significant at $p < 0.05$; ** difference statistically significant at $p < 0.01$; *** difference statistically significant at $p < 0.001$.

Table 3: Pearson's linear correlations among Correlation analysis between evaluated parameters in roots

		SOD	POX	PPO	MDA	Total Phenol content	Sugar	Proline
Protein	r	0,83	-0,93	0,91	0,30	-0,37	-0,21	0,89
	p	***	***	***	ns	ns	ns	***
SOD	r		-0,72	0,89	0,48	-0,30	-0,48	0,83
	p		***	***	*	ns	*	***
POX	r			-0,85	-0,16	0,47	0,28	-0,84
	p			***	ns	*	ns	***
PPO	r				0,39	-0,34	-0,30	0,85
	p				ns	ns	ns	***
MDA	r					0,09	-0,11	0,17
	p					ns	ns	ns
Total Phenol content	r						0,462	-0,23
	p						*	ns
Sugar	r							-0,41
	P							*

r = Pearson r correlation coefficient; p; significance level of the test; ns = not significant; * difference statistically significant at $p < 0.05$; ** difference statistically significant at $p < 0.01$; *** difference statistically significant at $p< 0.001$.

The principal analysis (PCA) reveals two principal components the first explains 49.47% and the seconds explains 33.88% of the information variation, so a total of 83.36%. Figure 7 present tow principal groups the first is correlated with the PC1 and formed by the antioxidant components and shows a negative correlation with the second PC2. The second group composed by the POX, total phenol content and the sugar, correlated negatively with the PC1 and positively with the second PC2.

The analysis of the treatments in the cartography presents an assortment of all treatments of 150mM NaCl with a positive correlation with PC1 and a negative correlation with PC2. On the other hand, a significant difference was observed between treatments under 0 mM NaCl based on the SA dose applications.

The PCA reveals two principal components the first explains 49.47% and the seconds explain 33.88% of the information variation, so a total of 83.36%. Figure 7 present two principal groups the first is correlated with the PC1 and formed by the antioxidant components and shows a negative correlation with the second PC2. The second group composed by the POX, total phenol content and the sugar, correlated negatively with the PC1 and positively with the second PC2. The cartographic analysis reveals a grouping of 150 mM NaCl treatments with a positive and negative correlation respectively with the PC1 and PC2. However, under treatment of 0mM NaCl we note a distinctions between treatments based on the SA dose applications. Where, we note that treatment of 0mM SA was negatively correlated to PC1 and PC2 , the 0.5mM SA was positively correlated PC2, and 1mM SA was negatively correlated to PC2.

4. **Discussion**

Salinity is one of the most important limiting of plant production in the world. This research was undertaken with the objective of understand and improve our knowledge on the physiological response of plant to salinity stress and how SA application can alleviate negative effect of this stress on the physiological and biochemical parameters. The different salinity and SA treatments resulted in a different plant water status. It is noteworthy that plant water status, as evaluated by leaf water potential Ψ [22], was not affected by SA seed soaking under non saline condition. But under salinity the leaf Ψ decreased significantly. Many studies have reported negative effects of salinity on the Ψ in the citrus and olive seedlings [23]. Suárez et al. [24] showed that high-salinity had more negative values of leaf water potential and the relationship between Ψ and RWC was modified in the Avicennia genninans seedlings growing under water salinity compared to the control, where the RWC was in decreasing with the Ψ decreasing. Jamali and Eshghi [25] demonstrated that SA had a positive effect on the RWC of the Strawberry plants grown in hydroponically conditions in comparison to treatments with salinity alone. The decreasing of Ψ, increased root-sourced signal, transported upwards in the transpiration stream, which is considered as a potential cause of the observed stomatal closure as previously shown in several studies [26-28].

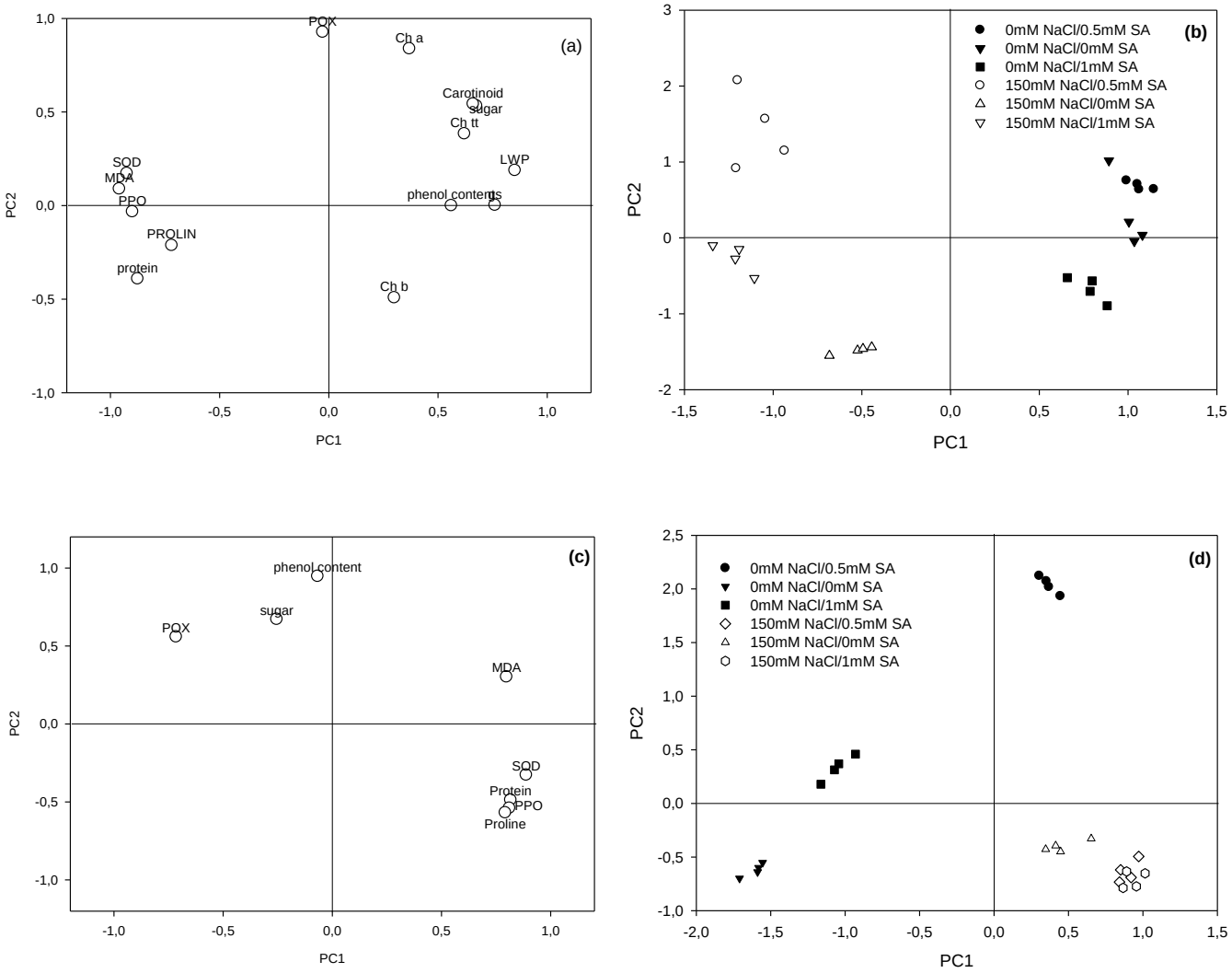

Figure 7: The principal component analysis (PCA) showing: (a and c) correlation of biochemical and physiological parameters with PC1 and PC2 respectively in shoot and root, and (b and d) relationship of treatments respectively in shoots and roots. PCA was performed on the correlation matrix of standardized values of physico-chemical attributes.

The gs was significantly decreased under salinity stress. The addition of SA improved the gs under both saline and non saline conditions. These results are in accordance with this presented by Barba-Espín, et al. [29] in pea plant under salt stress, where the gs has decreased by 75% (plants treated with 100 µm SA) to 87% (plants not treated with SA). Yusuf, et al. [30] indicate that stomatal conductance was significantly reduced by the salt treatment in Brassica juncea. However, when the plants received SA treatment, it overcomes the inhibition generated by NaCl treatment.

The generation of ROS is an inevitable process under normal condition in plant cells. However, under abiotic stress the ROS levels is enhanced [7]. Fortunately plants have an efficient defense system (antioxidant) may regulate the ROS level [8]. Based on this antioxidant system we can get a good idea on the degree of tolerance in plants. In this study, investigated antioxidants showed different reactions to salt stress and SA treatment; where the SOD, POD and PPO activities were stimulated under combination of SA and Salinity treatment, in both physiological compartments (shoots and roots). This result is corroborated by previous reports, in which the protective mechanism of SA in plants against abiotic stress focused on the oxidative status of the plants, and the activities of antioxidative enzymes were discussed. For example, SA was found to enhance the activities of peroxidase (POD) and superoxide dismutase (SOD) when sprayed onto drought-stressed Lycopersicon esculentum plants [31] or onto salinity-stressed B. juncea plants [30]. Ananieva, et al. [32] reports that SA treatment alone resulted in an increase in the activity of SOD and POD by 17% and 25% compared to the control plants of barley, respectively. Eraslan, et al. [33] found that treatment with SA caused induction of a new

isoperoxidase which can hardly be seen in the case of control. As it is known, cellular peroxidases are heterogeneous groups that not only participates in different physiological processes but also consume H202, thus minimizing its accumulation in the plant cell [32]. Therefore, it could be suggested that SA may influence the ability of cell to metabolize H202 or change the rate of oxidation of some substrates of POD, thus exerting certain effects on cell metabolism like changing hormonal balance and cell wall lignification, as has been shown by Kawano, et al. [34]. Taken in consideration these results, we conclude that antioxidative reaction is one of the major mechanisms of SA-induced resistance.

Under salt stress the ROS production increases, and leads to an increasing of lipide peroxidation of membrane. This decomposition product of polyunsaturated fatty acids has been utilized as a suitable biomarker for lipid peroxidation [3]. Based on the occurrence of this indicator to evaluate the damage caused by salt stress in this study, we note that MDA content was accumulated significantly under salt stress treatment and in the seedlings without SA pretreatment, than thus pretreated with SA. This conclusion is in accordance with the result presented by [35] in cucumber seedlings. Gunes, et al. [36] indicate that membrane permeability and lipid peroxidation (MDA concentration) increased significantly with salinity stress, and this oxidative damage was alleviated by increasing levels of exogenously applied SA in maize plants. Taking into consideration these results we can affirm that pretreatment of SA could increase the tolerance to salinity stress in *V. faba* seedlings.

Phenolic content was induced in pretreated plants with SA under non salin condition. Higher induction was observed in plants pretreated with SA at 0.5 mM than the other treatments. However, under salt stress we not a reduction of Phenolic content of the plants pretreated with SA. Elsewhere, higher concentration of SA may have led to low phenolic content [37]. Phenolic compounds defend plants against a number of biotic and abiotic stresses [38, 39]. Oxidation of phenols produces many defensive compounds that alter the plant physiology and metabolism, which in turn enable it to withstand various stress either directly or by mediating different plant signaling pathways [39]. Furthermore, ROS such as superoxide anion, hydroxide radicals, H202, and singlet oxygen produced by oxidation of phenols activate plant defense enzymes [40, 41].

Under stress conditions, plants besides producing antioxidants also accumulate compatible solutes in the cytosol, such as proline, that originally were thought to function as osmotic agents. In the present study, booth salinity and SA interaction caused significant increases in proline content. Similarly, an increase was found in proline content under salinity in maize [42]. In the same way, exogenous SA increased significantly the accumulation of proline in wheat [43], carrot [33] and strawberry [44] under both normal and salt stress. Since proline is one of the important components of the adaptation of plants to salinity, SA would contribute to the accumulation of this amino acid under stress.

Soluble sugars have been considered as osmotic regulators, and of which non-reducing sugars, such as disaccharides and oligosaccharides, are the carbohydrates most directly involved in membrane stability, while high levels of reducing sugars are associated with high metabolism and a loss of desiccation tolerance [45]. Salinity significantly increases the concentration of total soluble sugar in seedlings of V. faba, combination with SA also increases the concentration, these results are in accordance with that presented in wheat plant [46]. Dong, et al. [35] suggested that after SA treatment, the percentage of soluble sugars, especially the percentage of non-reducing sugars in roots, could be significantly up-regulated. Moussa and Khodary [47] noted a progressive increase in soluble sugars with increasing salinity in maize, and Balibrea, et al. [48] observed increase in soluble sugars in tomato plants in relation to salt stress.

Chlorophyll parameters were significantly affected by salinity and SA pre-treatments while salinity and SA pre-treatments significantly decreased chlorophyll a, b, carotenoid and total chlorophyll. Whereas, 0.5 mM SA treatment significantly increased chlorophyll a carotenoid and total chlorophyll under salinity compared with 0 SA mM treatment. Similarly Tohma and Esitken [44] report that Salinity negatively affected chlorophyll contents in strawberry plants. However, SA treatment significantly increased chlorophyll under saline conditions. In previous studies, it was determined that chlorophyll contents decreased by salt treatment [47, 49, 50] in spite of this SA treatment increased chlorophyll content in different plants [44, 47, 50].

Conclusion

The data obtained from this study suggests that seeds soaked in SA can mitigate the deleterious effects of salt stress by increasing chlorophyll content, the activity of the antioxidant enzyme; proteins; sugars; total phenol and proline content; thus increase salt tolerance in faba bean plants. The results suggest that seeds soaked in 0.5 mM SA improve plant tolerance to salinity compared to control plants. Therefore, SA treatments might help mitigate the negative effect of salinity on growth of *V. faba*. However, salt stress effects on plant growth may change with respect to the developmental stage of the plant. Thus, the evaluation of the effects of SA must be investigated in the other stages of plant development such as the reproduction stage.

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