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# Salt-tolerance in *Vicia faba* is enhanced by the effect of salicylic acid on photosynthesis and the antioxidant response

## Abstract

Selection and improvement of crops subjected to salinity constitutes an urgent need for increasing agricultural and food production in order to feed the growing human population. The aim of the study is to evaluate the role of salicylic acid application at various concentrations in mitigating the adverse effects of salinity of two faba bean genotypes at the physiological and molecular levels. For this purpose we have selected two genotypes of faba bean (Vicia faba L.) and we have applied to them various concentrations of NaCl and salicylic acid in a full factorial design. Growth, water status, gas exchanges, photosynthesis parameters, ions homeostasis and antioxidant enzymes activities were evaluated. One of the genotypes (Aguadulce) performed better under salt stress, but the difference among them was rather quantitative than qualitative. In general, salinity limited growth and several physiological processes. However, faba bean plants have triggered some mechanisms to deal with this constraint throughout the maintenance of water status and the enhancement of antioxidant enzymatic activities. The salt-tolerance of both genotypes at the growth and physiological level was improved by the application of salicylic acid. The beneficial effect of salicylic acid varies according to its concentration, the genotype and the studied parameter. Overall, the genotype Aguadulce performs better with 0.5 mM SA while the genotype Histal requires 1 mM SA. The genotype Aguadulce displayed better growth, physiological and molecular response under salt stress than the genotype Histal. Besides, salicylic acid can be considered as potential growth regulator to improve the salt response of both faba bean genotypes. The application of salicylic acid would provide a practical basis for wide cultivation of faba bean in marginal and wastelands under-cultivated and might propose an effective ecological and economical alternative solution to deal with salt-affected soils mainly in arid regions.

**Key words**

Salt tolerance, salicylic acid, *Vicia faba* L., water status, gas exchanges, photosynthesis, ions homeostasis, antioxidants enzymes.

## 1. Introduction

Salinity is one of the major threats to food production and agricultural yield in the XXI century and its adverse effects are expected to increase due to anthropogenic global warming and demographic increase. Arid and semiarid regions are more vulnerable to this threat. Recent statistics have revealed that more than 45 million hectares of irrigated land are affected by salt stress and this number is increasing (Isayenkov and Maathuis, 2019).

Plants subjected to salinity experience a combination of osmotic and ionic stresses that leads regrettably to various damages at the physiological, cellular and molecular levels (Serrano et al., 1999), further causing reduced uptake of nutrients and decline of photosynthetic performance (Taïbi et al., 2016). In addition, salt stress induces the production of reactive oxygen species which damage the biomolecules (lipids, proteins, and nucleic acids) and this causes a decrease of essential enzymatic activities and even causes the degradation of cell membrane systems (Mahajan and Tuteja 2005)

Salicylic acid is a phenolic phytohormone with roles in plant growth and development, photosynthesis, transpiration, ion uptake and transport. Salicylic acid is involved in endogenous signalling, mediating in plant response against pathogens.an a determinant of plant growth regulation, thereby generating a wide range of metabolic and physiological responses in plants (Khan et al., 2015). Salicylic acid is able to mitigate the effects of stress as induces nutrient uptake, membrane protection, photosynthesis repair, and also can reduce oxidative stress (Khan et al., 2015). There are several reports are available highlighting the role of salicylic acid in improving salt stress tolerance in plants (Ahmad et al., 2018; Jayakannan et al., 2013; Khan et al., 2014; Li et al., 2014a; Li et al., 2014b; Nazar et al., 2015).

Fabaceous plants constitute a suitable solution to feed growing populations, improve human and animal nutrition and enhance soil fertility since they are able to enrich the soil's nitrogen content throughout its symbiotic association with rhizobium (Castro-Guerrero et al., 2016). The faba bean or broad bean, *Vicia faba* L., is an important legume for human nutrition as it is a major source of protein, fiber, vitamins and minerals. This species is very sensitive to salinity and many strategies are being developed to enhance its production and yields in marginal arid lands.

In the present study, we hypothesised that (i) the extent of the beneficial effects of salicylic acid application varies among genotypes belonging to the same species and that (ii) evaluate the dose-response of the hormone and the physiological and biochemical outcomes under salt stress. To attain these objectives we have evaluated the effect of different concentrations of salicylic acid on two faba bean genotypes at the physiological and molecular levels. Examining the magnitude of intra-specific and genotypic variation for salinity tolerance, and a detailed description of the physiological and molecular response, as well as the effect of salicylic acid could provide vital information to faba bean breeding programs for the selection of the most suited genotypes for introduction in salt affected areas.

## 2. Material and methods

*Plant material and growth conditions*

Two genotypes of *Vicia faba* L., Aguadulce and Histal, widely cultivated in developing countries of the Mediterranean region were surface sterilized using sodium hypochlorite (0.5 %, v/v) during 5 minutes followed by several washes with distilled water. Seeds preparation and conditions of cultivation were performed as described in Taïbi et al. (2016). Four-week-old plants were arranged in factorial block design with eight replicates per treatment, two faba bean genotypes, three salt treatments (i.e. 0, 100 and 200 mM of NaCl) and three salicylic acid ($C_7H_6O_3$, Sigma-Aldrich, C 99.0%) treatments (i.e. 0, 0.5 and 1 mM). Salicylic acid (SA; 2-hydroxybenzoic acid) was dissolved in 0.02 % Tween 20 (Polyoxyethylenesorbitan monolaurate, Sigma Chemicals, UK). Either NaCl or salicylic acid mixed with Tween-20 were both dissolved in the nutrient solution of Hoagland which was applied directly to the pots for irrigation every alternate day. The same volume of Tween-20 was added to the nutrient solution untreated with salicylic acid for homogeneity. Plants were harvested after ten days of treatment. The dry weights of the aerial parts and roots were determined and samples were conserved at -80 °C for further analyses.

*Water status measurements*

Leaf water potential ($\Psi_w$, MPa) was measured in eight plants selected randomly per treatment using a Scholander-type pressure pump (PMS 1000, USA). Nevertheless, relative water content (RWC) was assessed on leaves following the formula:

RWC (%) = [(fresh weight-dry weight)/(turgid weight-dry weight)]x100

*Gas exchange and photosynthesis measurements*

Instantaneous assessments of stomatal conductance ($g_s$, mol $m^{-2}$ $s^{-1}$), transpiration ($E$, mmol $H_2O$ $m^{-2}$ $s^{-1}$), net $CO_2$ assimilation ($P_n$, μmol $CO_2$ $m^{-2}$ $s^{-1}$), and instantaneous water use efficiency ($WUE_{inst}$; μmol $CO_2$ $mmol^{-1}$ $H_2O$) were also measured on recently expanded leaves of eight plants per treatment by means of a portable photosynthesis (Model LI-6400, LI-COR Biosciences Inc., Lincoln, USA). Besides, maximal photochemical efficiency of PSII (Fv/Fm) was assessed at predawn using a handy PEA portable fluorometer (Hansatech, United Kingdom).

*Determination of electrolyte leakage*

Electrolyte leakage in fresh leaves was determined following Li et al. (2014b) method:

Electrolyte leakage (%) = [(EC1 – EC0)/(EC2 – EC0)] × 100,

where $EC_0$ is the electrical conductivity recorded at 0 time point; $EC_1$ is the electrical conductivity measured after contents heated at 60 ºC; $EC_2$ is the electrical conductivity after contents heated at 100 ºC.

*Determination of ion content*

Concentrations of sodium ($Na^+$) and potassium ($K^+$) were determined in oven dried leaf samples, after wet-digesting the samples in $HNO_3$-$HClO_4$ acid mixture (4:1 v/v), by atomic absorption spectrometry (Perkins Elmer, Norwalk, CT, USA) as described by Taïbi et al. (2016).

*Antioxidant enzymes assay*

Antioxidant enzymes were extracted at 4 ºC from 500 mg fresh leaf sample in Tris-HCl (100 mM, pH 7.5) containing dithiotrol (5 mM), $MgCl_2$ (10 mM), EDTA (1.0 mM), magnesium acetate (5 mM), PVP (1.5%), PMSF (1.0 mM) and 1 µg/mL aproptinin in a pre-chilled pestle and mortar. The homogenate was centrifuged at 10.000 rpm for 15 minutes at 4°C and the supernatant was used as enzyme source then Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX) and Glutathione reductase (GR) activity were assayed according to the method described by Cakmak et al. (1993).

*Statistical analysis*

Prior to the analysis, the Levene test was applied to check the analysis of variance requirements. The significance of differences between different treatments was assessed using analysis of variance, at 95% confidence level. The post-hoc Tukey test was used to estimate homogeneous groups. Correlations between the measured parameters were determined based on the coefficient of Pearson.

## 3. Results

The effect of salt stress on faba bean seedling growth was evaluated throughout the determination of shoot and root dry weight (Figures 1A and B). In general, dry biomass decreased significantly in comparison to control seedlings for both genotypes in response to the increase of NaCl concentration (p-value<0.01**). Shoot dry biomass decreased by 60 % in Aguadulce and 65 % in Histal while root dry biomass decreased by around 70 % in both genotypes under high salinity. However, the exogeneous application of salicylic acid enhances both shoot and root biomass over control and salt stressed seedlings; under high salinity, less decrease in shoot dry biomass was observed in the treatment with 0.5 mM SA (50 % for Aguadulce and 60 % for Histal). However, better effect concerning root dry biomass was noted in the treatment with 1 mM SA for both genotypes.

**Figure 1.**

As expected, salinity affects seedlings water status; leaf relative water content (RWC) diminished by more than 32 % for both genotypes and water potential (Ψw) decreased significantly by around 153 % in Aguadulce and 135 % in Histal. This finding indicates that seedlings were experiencing drought stress concomitant to salinity. However, salicylic acid application improves significantly seedlings water status through the decrease of water potential to 165 % for Aguadulce and 150 % for Histal and therefore, the recovery of relative water content to 72 % and 74 % respectively in Aguadulce and Histal. Nevertheless, under high salinity, better improving effect of water status was observed through treatment with 0.5 mM SA (Fig. 2A, B).

**Figure 2.**

Likewise, stomatal conductance (gs), transpiration (E), net photosynthesis (Pn) and intercellular $CO_2$ concentration (Ci) decreased significantly when seedlings were subjected to salt stress (Figures 3 A, B, C, D). The concomitant diminution under high salinity was around 48 % in gas exchange, 52 % in transpiration and intercellular $CO_2$ concentration, and 34 % in net photosynthesis for both genotypes. Exogenous application of salicylic acid improved significantly these parameters; less decreases were estimated around 34 % and 32 % in gas exchange, 48 % and 53 % for transpiration, 30 % and 31 % in net photosynthesis and 33 % and 35 % in intercellular concentration of $CO_2$ respectively in Aguadulce and Histal subjected to high salinity. Additionally, maximal efficiency of PSII (Fv/Fm) decreased as well under salinity for both genotypes by around 7 % ($p$-value<0.01**). Nevertheless, exogeneous salicylic acid enhanced the maximal efficiency of PSII under controlled and salt stressed conditions by around 2 to 3 %; the highest value obtained under 200 mM NaCl (Fv/Fm=0.77) was observed in the treatment with 0.5 mM SA for the genotype Aguadulce and in the treatment with 1 mM SA for the genotype Histal (Figure 3 E).

Subsequent increase in water use efficiency (WUEinst) was induced by salinity and salicylic acid application improves more the obtained values in both the two genotypes (Figure 3 F). Nevertheless, water use efficiency was higher in the genotype Aguadulce under controlled and salt-stressed conditions. This improvement translates a higher capacity to maintain elevated photosynthetic levels in the genotype Aguadulce for comparable transpiration values in both genotypes.

**Figure 3.**

Being the last sink, leaves constitute the plant part where salt ions accumulate. Salt stress induced a significant increase of sodium content by around 156 % against a significant decrease of potassium content by around 50 % for both genotypes (p-value<0.001***). However, exogenous salicylic acid supply decreased remarkably sodium content in leaves of both genotypes by around 10 % for the concentration 0.5 mM SA and 15 % for the concentration 1 mM SA under high salinity (Fig. 4 A). Concomitantly, potassium content increased for both genotypes by around 30 % under 0.5 mM SA and 50 % under 1 mM SA when plants were irrigated with 200 mM NaCl (Fig. 4 B). Consequently, the ratio $K^+/Na^+$ was improved significantly with salicylic acid treatment (Fig. 4 C).

In addition, salinity increased the electrolyte leakage by more than four-folds in both genotypes, indicating membrane damage (p-value<0.001***). Treatment of seedlings with salicylic acid diminished widely the electrolyte leakage either in controlled or salt-stressed seedlings. This effect was estimated around 17 % of decrease in Aguadulce and 22 % in Histal under high salinity in comparison to salt-stressed seedlings untreated with SA (Fig. 4 D).

**Figure 4.**

Regarding antioxidant enzymes activity, a significant increase was observed for both genotypes subjected to salt stress in terms of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) activities (p-value<0.001***). The rates of increase were estimated around to 260 % for SOD and APX activity however, CAT activity increased by 210 % in Aguadulce and 224 % in Histal while GR activity increased by 198 % in Aguadulce and 192 % in Histal. The application of salicylic acid induced further increase of the enzymatic activity mainly under high salinity. SOD activity increase was estimated around 300 % for Aguadulce and 320 % for Histal. Nevertheless, APX activity increase was about 280 % for Aguadulce and 270 % for Histal. By the same, CAT activity increased to 260 % for Aguadulce and 270 % for Histal while GR activity raised to 240 % for both genotypes. Better effects were observed in the treatment supplied with 0.5 mM SA for APX, CAT and GR. However, SOD activity was higher in the treatment with 1 mM SA (Fig. 5 A, B, C, D).

**Figure 5.**

## 4. Discussion

Salinity is among the major threats to horticulture by inducing several limitations in plant growth, development and overall productivity. Selection and improvement of salt-tolerant genotypes represents an effective strategy to improve yield in areas suffering from adverse environmental conditions. Therefore, comparison among several genotypes for salt tolerance can be very useful to describe the relevant mechanisms that might help to recognize stress tolerant genotypes and unveiling the limiting factors which can constitute objectives for breeding or molecular breeding (Taïbi et al., 2018; Taïbi et al., 2017; Taïbi et al., 2016). Besides, phytohormones constitute a strong tool to alleviate the adverse effects produced by abiotic stress. Recently, an increasing interest has been focused on the effect of salicylic acid in salinity tolerance.

In general, salinity induced severe restrictions of physiological and molecular responses of faba bean which affected significantly plant growth. However, salicylic acid restored most of these parameters and improve the plant stress response.

Severe growth limitations in terms of shoot and root dry biomass were observed under salinity for both genotypes. This finding is one of the most visible effects of salt stress and it is generally attributed to the inhibition of the developmental program and the stimulation of stress response mechanisms (Isayenkov and Maathuis, 2019). Growth arrest under salinity has been already reported in faba bean (Bulut et al., 2011; Dawood and El-Awadi, 2015; Moussa and Hassan, 2016) and other leguminous species like common bean (Taïbi et al., 2016; Taïbi et al., 2012; Taïbi et al., 2013a, b). Therefore, biomass accumulation measure may help in the evaluation of the relative degree of salt tolerance among faba bean genotypes. However, the application of salicylic acid enhanced both shoot and root dry biomass in salt-stressed plants. Here we show that the ameliorating effects of salicylic acid could be attributed to its role in membrane protection from salt damage (r-value=0.73**), and enhancement of chlorophyll content and antioxidant enzymes activity (r-value=0.82**) which in turn supports the photosynthesis process, alleviate oxidative stress and regulate ion homeostasis (Khan et al., 2015; Li et al., 2014a; Li et al., 2014b). On the other side, growth limitations could be due to the significant deterioration of relative water content (RWC, r-value=-0.68**) and water potential ($\Psi w$, r-value=-0.71**) under salinity. It is well admitted that plants including faba bean respond to salt stress by regulating their osmotic potential and ionic balance throughout the accumulation of organic and inorganic compounds in order to sustain cell turgor and subsequently to maintain growth (Abdul Qados, 2011; Nazar et al., 2015). Maintenance of water status through adjustment of water potential is considered as an important adaptation strategy under salinity (Ashraf and Harris, 2013).
Application of salicylic acid enhanced leaves diffusive resistance (r-value=0.58**) and control transpiration level (r-value=-0.54**) which might improve water status of the plants (Ahmad et al., 2018).

Likewise, gas exchanges and net photosynthesis decreased in plants subjected to salinity (r-value=-0.44*). The stomatal closure, as an earliest response of salt-stressed plants, was significantly marked in the present study leading to the decrease of stomatal conductance and transpiration. In general, higher stomatal conductance enhances $CO_2$ diffusion into the leaves and improves photosynthetic rate which is an important determinant of growth and yield (Li et al., 2014b). Therefore, the decrease in photosynthesis rates under saline conditions is attributed mostly to the limitation of stomatal conductance (r-value=0.61**). Besides, diminution in photosynthesis might be attributed to perturbations in pigments content and chlorophyll fluorescence, PSII photoinhibition, conformational modifications in membrane-bound ATPase enzyme complex, and the diminution in Rubisco activity (Lawlor and Cornic, 2002). Nevertheless, salicylic acid was found to enhance photosynthetic rates and gas exchanges. Previous studies of Khan et al. (2014) and Ahmad et al. (2018) have reported that salicylic acid increases pigments content, improves the rates of transpiration and photosynthetic electron transport, sustains higher Rubisco and antioxidant enzymatic activities, and boosts the PSII efficiency under salt stress. The maximal quantum yield of PSII phytochemistry (Fv/ Fm) decreased under saline conditions but restored after the addition of salicylic acid. This finding indicates reduced leaves capacity for absorption of excitation energy and diminution in photosynthetic assimilation under salinity (Taïbi et al., 2018; Taïbi et al., 2017). The protective effects of salicylic acid could be associated to the diminution of oxidative damage and the restriction of massive sodium accumulation in leaves (Khan et al., 2015).

By the same, regulation of ion homeostasis is one of the main determinants of plants salt tolerance (Isayenkov and Maathuis, 2019). In the present study, salinity increased the content of sodium and decreased the content of potassium. These findings are in agreement with (Ahmad et al., 2018; Bulut et al., 2011; Taïbi et al., 2016). Competition exists for the uptake of sodium and potassium since they share similar physicochemical structure. Under salt conditions, the passive entrance of sodium ions under salinity throughout the plasma membrane could induce membrane depolarization that prevents considerably potassium uptake and increase its leakage through depolarization-activated potassium outward-rectifying channels (Jayakannan et al., 2013). The suppression of potassium uptake induces various cell damages and reduces plant growth since it constitutes an important activator of several cytosolic enzymes and contributes as well for osmotic adjustment (Almeida et al., 2017). Besides, potassium plays a determinant role in regulating stomatal aperture, and this is subjected to a complex regulation so the observed differences may be the underlying cause for the differences observed in water use efficiency and photosynthesis rates. Exogenous salicylic acid supply was found to be very effective in dropping sodium excess and enhancing potassium content in salt-stressed faba bean plants. Salicylic acid reduces the xylemic translocation of sodium to the aerial parts from a part, and the extent of membrane polarization through the stimulation of H+-ATPase activity under salt conditions from another part which could, consequently, restore potassium retention and decrease its efflux (Khan et al., 2015). Nevertheless, the higher accumulations of sodium and potassium were characteristics of the genotype Aguadulce. This suggest that it is the ability to uptake and retain potassium that determines faba bean genotypes growth and yield under salt stress.

In addition, an increase of electrolyte leakage was associated to salt stress reflecting consequently the occurrence of membrane injuries. Membrane disorganization is due mainly to the decrease of phospholipids and sterols in response to salinity (Wu et al., 1998). Reactive oxygen species attacks linolenic and linoleic polyunsaturated fatty acids and produce complex of lipid hydroxy-peroxides which reduce membrane permeability (Ahmad et al., 2018). It should be noted that membrane protection from salt injuries constitutes a determinant criterion for salinity tolerance mechanisms and salicylic acid demonstrated its benefits to decrease electrolyte leakage. Salicylic acid effects are attributed to the stimulation of the antioxidative system and improvement of calcium uptake (El-Tayeb, 2005).

Salt stress affects various physiological and molecular mechanisms associated with plant growth and development. Plants with potent antioxidant system, either constitutive or induced, acquire significant salt-tolerance (Isayenkov and Maathuis, 2019). The activity of the antioxidant enzymes SOD, APX, CAT and GR increased in response to salt stress. The genotype Aguadulce revealed higher SOD and CAT activities while the genotype Histal showed higher APX and GR activities.

The role of antioxidant enzymes is to scavenge reactive oxygen species produced by oxidative stress and therefore protect cell membrane integrity (Taïbi et al., 2016). SOD converts superoxide radicals into which will be reduced by CAT to $H_2O$ and oxygen (Demiral and Türkan, 2005). Besides, the increase in GR activity under salt conditions has been also reported (Maribel and Tobita, 1998; Taïbi et al., 2016). The antioxidant enzymatic activities increased in controlled and salt-stressed conditions after salicylic acid treatments. Exogenous application of salicylic acid mitigated salt stress in *S. lycopersicum* throughout the stimulation of GST gene family (Csiszár et al., 2014). The boosted expression of antioxidants genes such as GST1-2, GPX1-2 and ASA-GSH pathway in response to salicylic acid supply restores cell membrane integrity and enhances the content of photosynthetic pigments (Li et al., 2014b). It should be noted that the effect of salicylic acid varied according to the applied concentration; CAT, APX and GR activities were stimulated the maximum at 0.5 mM SA under high salinity while SOD activity was stimulated at 1 mM SA. Taken all together, even if the genotype Aguadulce performed better than the genotype Histal in terms of growth, physiological and molecular response under salt stress, the difference between genotypes remains more quantitative rather than qualitative. Salicylic acid application improved significantly the salt-tolerance of both genotypes throughout the maintenance of cell membrane and photosynthetic process and the diminution of sodium toxicity and oxidative damage. Nevertheless, the action of salicylic acid varies in function of the applied concentration of the hormone, the tested genotype and the studied parameter. Overall, the genotype Aguadulce performs better with the treatment 0.5 mM SA while the genotype Histal manifests greater behaviour with the treatment 1 mM SA.

## 5. Conclusion

The intraspecific comparative studies among genotypes for salt-tolerance might help to identify the relevant mechanisms correlating their relative degree of tolerance to salinity and to select the most suited genotypes for very specific environmental conditions.

The present study revealed that the difference between genotypes is more quantitative rather than qualitative even if the if the genotype Aguadulce performed better in terms of growth, physiological and molecular response under salt stress. In general, the salt stress promoted several limitations in growth, gas exchanges, photosynthesis, ion homeostasis and other physiological attributes. Nevertheless, faba bean plants have triggered some mechanisms to deal with this constraint throughout the maintenance of water status and the enhancement of antioxidant enzymatic activities. However, the salt-tolerance of both the two genotypes was significantly improved by salicylic acid application which allowed the maintenance of cell membrane and photosynthetic process, restoring of ion homeostasis and the diminution of oxidative damages. The beneficial effect of salicylic acid varies according to its concentration, the tested genotype and the studied parameter. Overall, the genotype Aguadulce performs better under the treatment with 0.5 mM SA while the genotype Histal manifests greater behaviour under the treatment with 1 mM SA. Based on our results, salicylic acid can be considered as potential growth regulator to improve the salt response of faba bean. Plants treatment with salicylic acid might constitute a sustainable approach and effective alternative to restore the adverse effects of salinity. The application of salicylic acid would provide a practical basis for wide cultivation of faba bean and might propose an ecological and economical solution for horticulture in salt-affected soils, mainly in arid regions, for the reclamation of marginal and wastelands under-cultivated.

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# Figures

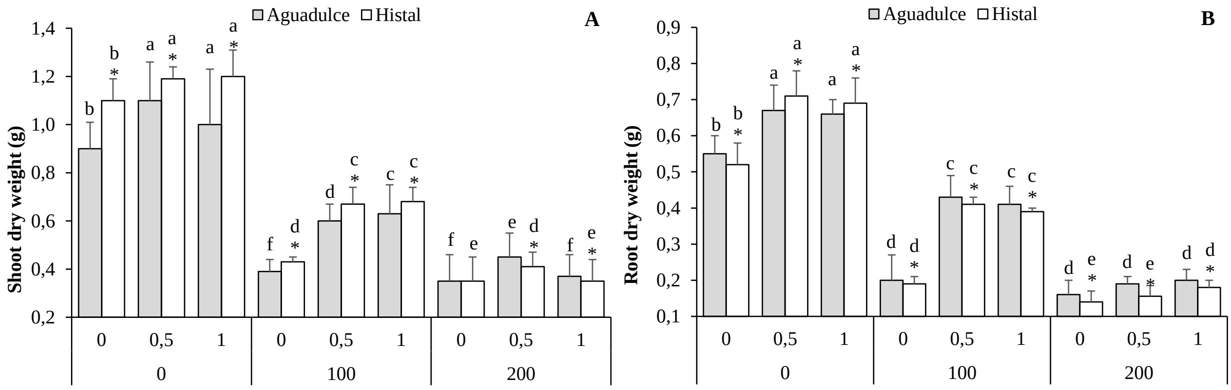


**Figure 1.** Effects of salicylic application on shoot (**a**) and root (**b**) dry weight of two faba bean genotypes Aguadulce (grey bars) and Histal (white bars) subjected to salinity.

The letters above the bars marks the significant difference among the different treatments with salt and salicylic acid for each genotype following the post hoc Duncan's test. The asterisk shows the significant difference between the two genotypes within the same treatment. Scale bars are mean + SE, being the number of samples $n = 8$.

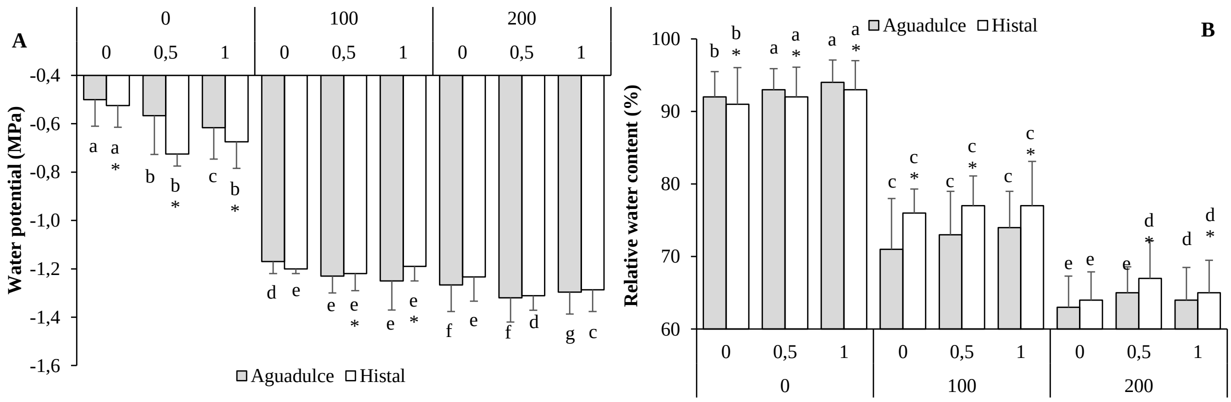


**Figure 2.** Effects of salicylic application on leaf water potential (**a**) and relative water content (**b**) of two faba bean genotypes Aguadulce (grey bars) and Histal (white bars) subjected to salinity.

The letters above the bars marks the significant difference among the different treatments with salt and salicylic acid for each genotype following the post hoc Duncan's test. The asterisk shows the significant difference between the two genotypes within the same treatment. Scale bars are mean + SE, being the number of samples $n = 8$.

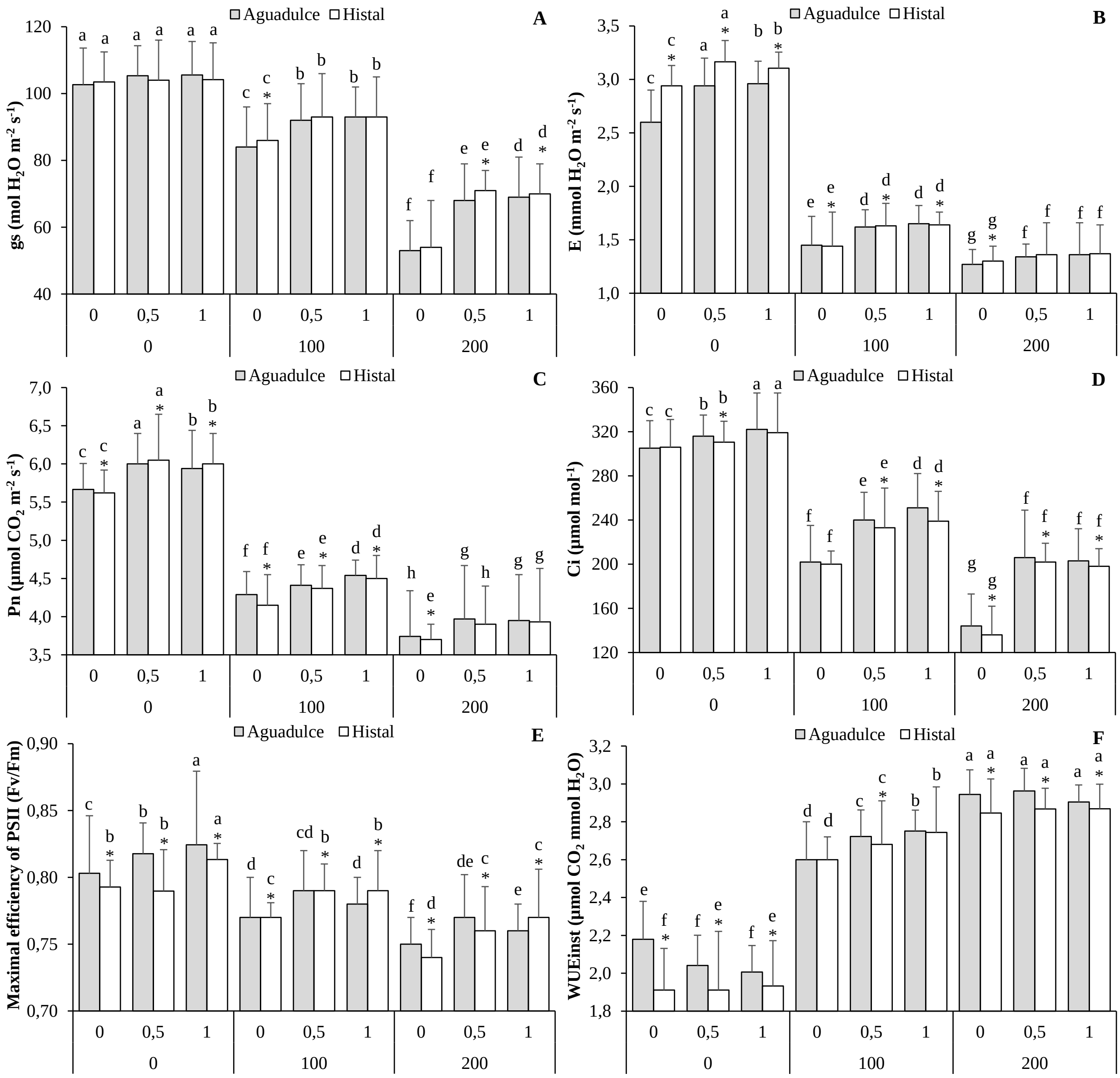


**Figure 3.** Effects of salicylic application on gas exchanges and photosynthesis of two faba bean genotypes Aguadulce (grey bars) and Histal (white bars) subjected to salinity.

Stomatal conductance (gs) (**a**), transpiration (E) (**b**), Net photosynthesis (Pn) (**c**), intercellular $CO_2$ concentration (Ci) (**d**), Maximal efficiency of PSII (**e**), and instantaneous water use efficiency (WUEinst) (**f**).

The letters above the bars marks the significant difference among the different treatments with salt and salicylic acid for each genotype following the post hoc Duncan's test. The asterisk shows the significant difference between the two genotypes within the same treatment. Scale bars are mean + SE, being the number of samples $n = 8$.

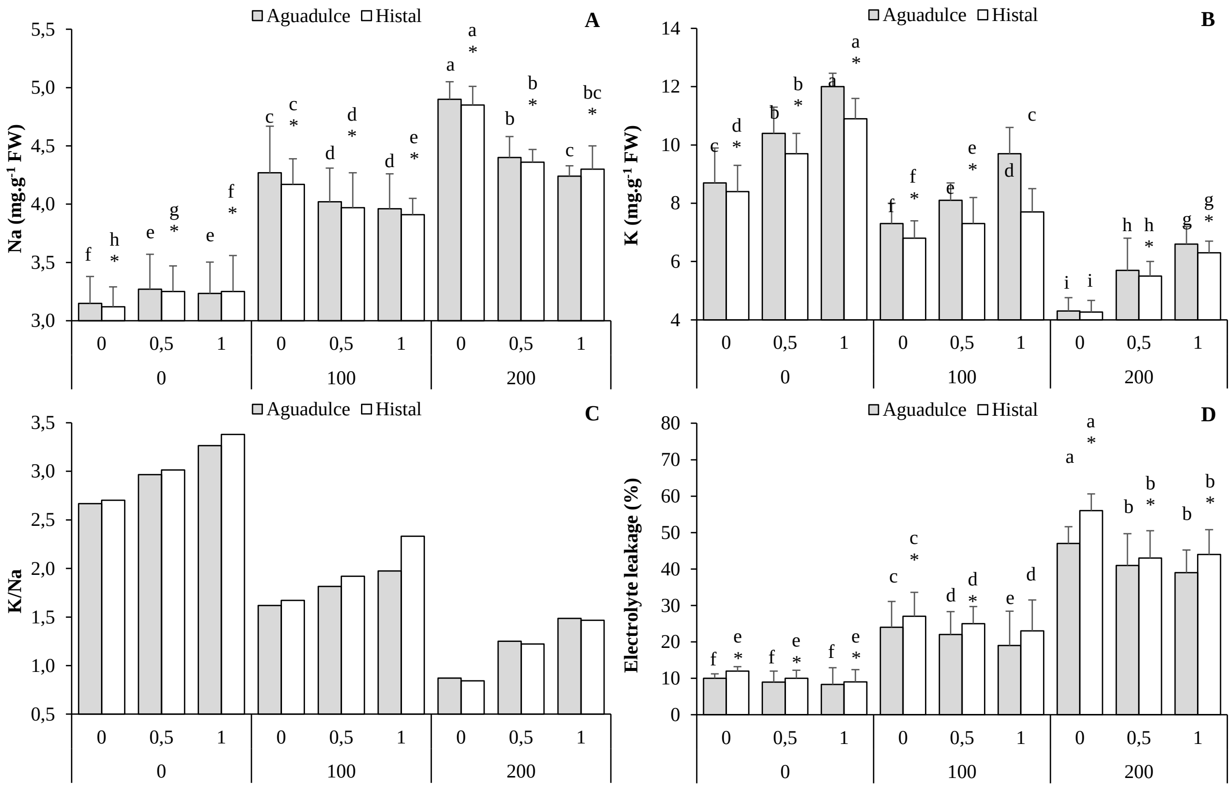


**Figure 4.** Effects of salicylic application on ions content and electrolyte leakage of two faba bean genotypes Aguadulce (grey bars) and Histal (white bars) subjected to salinity.

Sodium content ($Na^+$) (**a**), potassium content ($K^+$) (**b**), ratio $K^+/Na^+$ (**c**) and electrolyte leakage (**d**) The letters above the bars marks the significant difference among the different treatments with salt and salicylic acid for each genotype following the post hoc Duncan's test. The asterisk shows the significant difference between the two genotypes within the same treatment. Scale bars are mean + SE, being the number of samples $n = 8$.

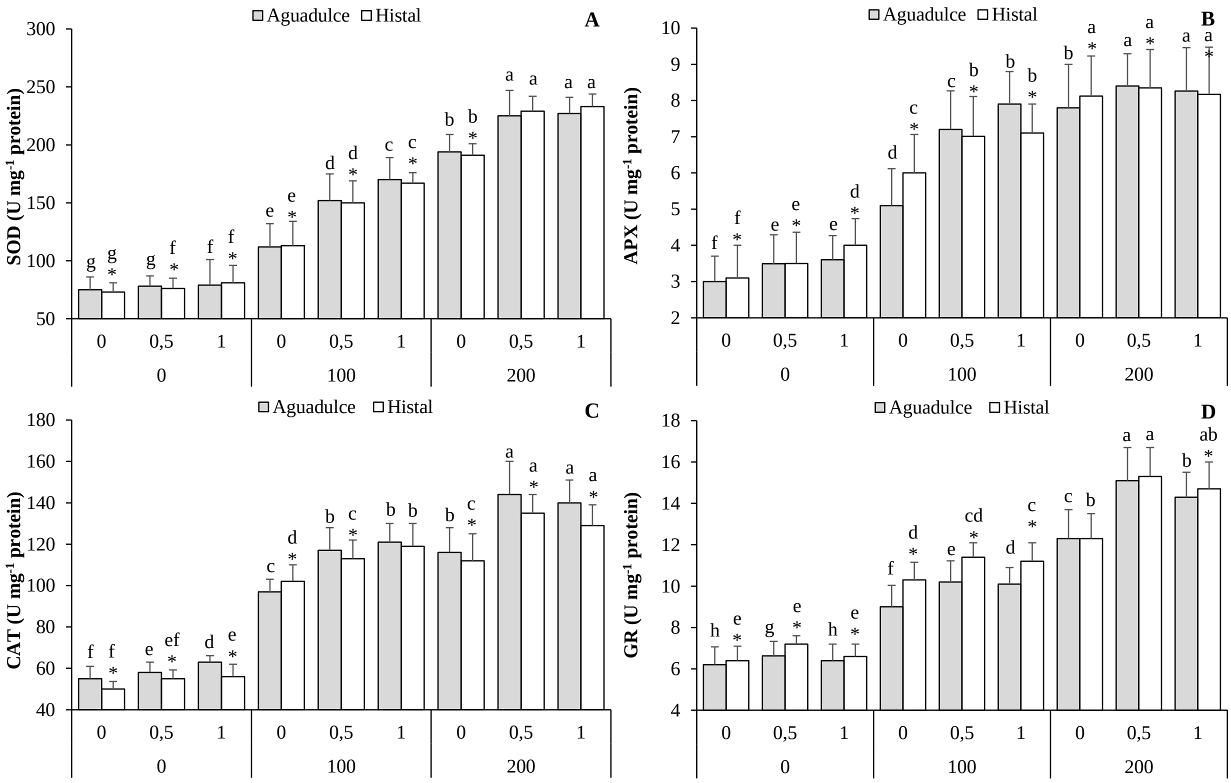


**Figure 5.** Effects of salicylic application on antioxidant enzymes activities of two faba bean genotypes Aguadulce (grey bars) and Histal (white bars) subjected to salinity.

Superoxide dismutase (SOD) (**a**), Ascorbate peroxidase (APX) (**b**), Catalase (CAT) (**c**) and Glutathione reductase (GR) (**d**).
The letters above the bars marks the significant difference among the different treatments with salt and salicylic acid for each genotype following the post hoc Duncan's test. The asterisk shows the significant difference between the two genotypes within the same treatment. Scale bars are mean + SE, being the number of samples $n = 8$.