Salt-tolerance in *Vicia faba* L. is mitigated by the capacity of salicylic acid to improve photosynthesis and antioxidant response

Kada Souana^a, Khaled Taïbi^{a,b,*}, Leila Ait Abderrahim^a, Mokhtar Amirat^a, Mohamed Achir^a, Mohamed Boussaid^a, José M. Mulet^b

^a Faculty of Life and Natural Sciences, University of Tiaret, 14000, Algeria

^b Instituto de Biología Molecular y Celular de Plantas (IBMCP), Universitat Politècnica de València-Consejo Superior de Investigaciones Científicas, Avd. de los Naranjos s/n, Valencia, 46022, Spain

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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 present study is to evaluate the role of salicylic acid (SA) application in mitigating the adverse effects of salinity on faba bean (*Vicia faba* L.) at the physiological and molecular levels. For this purpose, two faba bean genotypes were subjected to various concentrations of NaCl and salicylic acid in a full factorial design. After that, growth, water status, gas exchanges, photosynthesis parameters, ions homeostasis and antioxidant enzymes activities were evaluated. The obtained results demonstrated that salinity induced several limitations in plants growth and physiological attributes. In response, salt stressed faba bean plants improved water status and enhanced antioxidant enzymatic activities. Remarkably, salt-tolerance of both 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. Overall, the difference between genotypes is rather quantitative than qualitative even if the genotype Aguadulce displayed better growth, physiological and molecular response under salt stress than the genotype Histal. Besides, the beneficial effects of salicylic acid vary according to its concentration, the tested genotype and the studied parameter; 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. Therefore, salicylic acid can be considered as potential growth regulator to improve the salt response of faba bean. 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.

1. Introduction

Salinity is one of the most major threats to plants and crops yields in the XXI century worldwide and its adverse effects are expected to increase under the burden of climate change mainly in arid and semiarid regions. Recent statistics have revealed more than 45 million hectares of irrigated land damaged by salt stress around the world and this number is in perpetual increase (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, further causing reduced uptake of nutrients and decline of photosynthetic performance (Taïbi et al., 2016).

In addition, salt stress produces plenty of reactive oxygen species which damage the biomolecules (lipids, proteins, and nucleic acids) and other enzymatic activities and even cause the degradation of cell membrane system (Demiral and Türkan, 2005). To deal with this constraint, plants activate several pathways to control salt damage and modulate cellular homeostasis and growth. Salt tolerance is often decrypted by the activation of cell signaling pathways including synthesis of antioxidant enzymes, stress hormones and osmoprotectant metabolites such as amino acids and sugars (Isayenkov and Maathuis, 2019).

Salicylic acid, an endogenous phenolic plant growth regulator, has been found to generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development

* Corresponding author.

E-mail address: khaledtaibi@hotmail.com (K. Taïbi).

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(Khan et al., 2015). The positive effects of salicylic acid in mitigating stress are attributed to nutrient uptake improve, membrane protection, photosynthesis repair, as it can interact also with reactive oxygen species signal pathways and reduce oxidative stress (Herrera-Vásquez et al., 2015; Xu and Brosché, 2014). 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 susceptible to salt regions and attempts are being made to enhance its production and yields mainly 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) the recovery photosynthetic performance and antioxidant enzymatic activity enhancement under salinity are correlated with the applied concentration of the hormone. Therefore, the main objective of the work is to evaluate the role of salicylic acid application at various concentrations in mitigating the adverse effects of salinity on two faba bean genotypes at the physiological and molecular levels. Examining the magnitude of intra-specific and genotypic variation for salinity tolerance along with a comprehensive description of the physiological and molecular response could provide vital information to faba bean breeding programs for the selection of the most suited genotypes for specific environmental conditions for their introduction in salt affected areas.

2. Material and methods

2.1. Plant material

Two genotypes of *Vicia faba* L., Aguadulce and Histal, widely cultivated in developing countries of the Mediterranean region were tested throughout the present study.

2.2. Growth conditions

Healthy and homogeneous seeds were surface sterilized using sodium hypochlorite (0.5 %, v/v) during 5 minutes followed by several washes with distilled water. After that, seeds were allowed to germinate between wet filter paper in Petri dishes in a growth chamber at controlled conditions (18–25 °C temperature, 70–90 % relative humidity) in the dark.

Seedlings cultivation were performed as described in Taïbi et al. (2016). Uniform healthy seedlings were selected and individually transplanted in culture pots containing a mixture of commercial peat and vermiculite (V: V). Culture was conducted under controlled conditions in a greenhouse (600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ of light intensity, 25/18 °C day/night temperature, 16 h photoperiod, and 70% relative humidity). Seedlings were irrigated by a Hoagland full nutrient solution twice to three times a week.

2.3. Stress application and monitoring

Experiments were performed on seedlings of four-weeks-old. Salt stress was set-up with NaCl at two levels of 100 or 200 mM. Moreover, the hormone application consisted of treating plants with 0.5 mM or 1.0 mM salicylic acid ($\text{C}_7\text{H}_6\text{O}_3$; 2-hydroxybenzoic acid; Sigma-Aldrich, C 99.0%) 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.

Control plants were irrigated by Hoagland full nutrient solution without NaCl and SA. Untreated plants with salicylic acid received the same volume of Tween-20 added to the nutrient solution for homogeneity. It should be noted that no visible damages on the stressed plants were observed during the experiment.

Overall, plants were arranged in a full factorial design with two faba bean genotypes, three salt treatments (i.e. 0, 100 and 200 mM NaCl) and three salicylic acid treatments (i.e. 0, 0.5 and 1 mM SA). For each treatment, eight replicates were set and processed.

Plants were harvested after ten days of treatment. The dry weights of the aerial parts and roots were determined and samples were conserved at $-80\text{ }^{\circ}\text{C}$ for further analyses.

2.4. Water status measurements

Leaf water potential (Ψ_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:

$$\text{RWC (\%)} = [(\text{fresh weight-dry weight})/(\text{turgid weight-dry weight})] \times 100$$

2.5. Gas exchange and photosynthesis measurements

Instantaneous assessments of stomatal conductance (g_s , $\text{mol m}^{-2}\text{s}^{-1}$), transpiration (E , $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), net CO_2 assimilation (P_n , $\mu\text{mol CO}_2\text{ m}^{-2}\text{s}^{-1}$), intercellular CO_2 concentration (C_i , $\mu\text{mol CO}_2\text{ mol}^{-1}$) and instantaneous water use efficiency (WUE_{inst} , $\mu\text{mol CO}_2\text{ mmol}^{-1}\text{H}_2\text{O}$) 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 (F_v/F_m) was assessed at predawn using a handy PEA portable fluorometer (Hansatech, United Kingdom).

2.6. Determination of electrolyte leakage

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

$$\text{Electrolyte leakage (\%)} = [(\text{EC}_1 - \text{EC}_0)/(\text{EC}_2 - \text{EC}_0)] \times 100,$$

where EC_0 is the electrical conductivity recorded at 0 time point; EC_1 is the electrical conductivity measured after contents heated at $60\text{ }^{\circ}\text{C}$; EC_2 is the electrical conductivity after contents heated at $100\text{ }^{\circ}\text{C}$.

2.7. Determination of ion content

Concentrations of sodium (Na^+) and potassium (K^+) were determined in oven dried leaf samples, after wet-digesting the samples in $\text{HNO}_3\text{-HClO}_4$ acid mixture (4:1 v/v), by atomic absorption spectrometry (Perkins Elmer, Norwalk, CT, USA) as described by Taïbi et al. (2016).

2.8. Antioxidant enzymes assay

Antioxidant enzymes were extracted at $4\text{ }^{\circ}\text{C}$ 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 $1\text{ }\mu\text{g/mL}$ aprotinin in a pre-chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 15 minutes at $4\text{ }^{\circ}\text{C}$ 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.

2.9. 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 Duncan test was used to estimate homogeneous groups. Correlations between the measured parameters were determined based on the coefficient of Pearson. All the statistical analyses were performed using the computing environment R.

3. Results

The effect of salt stress on faba bean seedlings growth was evaluated throughout the determination of shoot and root dry weight (Figs. 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 Hístal while root dry biomass decreased by around 70 % in both genotypes under high salinity. However, the exogenous 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 Hístal). However, better effect concerning root dry biomass was noted in the treatment with 1 mM SA for both genotypes.

Fig. 1.

As expected, salinity affects seedlings water status; leaf relative water content (RWC) diminished by more than 32 % for both genotypes and water potential (Ψ_w) decreased significantly by around 153 % in Aguadulce and 135 % in Hístal. 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 Hístal and therefore, the recovery of relative water content to 72 % and 74 % respectively in Aguadulce and Hístal. Nevertheless, under high salinity, better improving effect of water status was observed through treatment with 0.5 mM SA (Fig. 2A, B).

Fig. 2.

Likewise, stomatal conductance (g_s), transpiration (E), net photosynthesis (P_n) and intercellular CO_2 concentration (C_i) decreased significantly when seedlings were subjected to salt stress (Figs. 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 Hístal subjected to high salinity. Additionally, maximal efficiency of PSII (F_v/F_m) decreased as well under salinity for both genotypes by around 7 % (p -value<0.01**). Nevertheless, exogenous 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 ($F_v/F_m = 0.77$) 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 Hístal (Fig. 3 E).

Subsequent increase in water use efficiency (WUEinst) was induced by salinity and salicylic acid application which improved more the obtained values in both the two genotypes (Fig. 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.

Fig. 3.

Being the last sink, leaves constitute the most sensitive 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 subjected to 200 mM NaCl (Fig. 4 B). Consequently, the ratio K^+/Na^+ , which declined under salinity, was improved significantly with salicylic acid treatment (Fig. 4 C).

In addition, salinity affected significantly membrane stability throughout the induced increase of electrolyte leakage by more than four-folds in both genotypes (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 Hístal under high salinity in comparison to salt-stressed seedlings untreated with SA (Fig. 4 D).

Fig. 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 Hístal while GR activity increased by 198 % in Aguadulce and 192 % in Hístal. 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 Hístal. Nevertheless, APX activity increase was about 280 % for Aguadulce and 270 % for Hístal. By the same, CAT activity increased to 260 % for Aguadulce and 270 % for Hístal 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).

Fig. 5.

4. Discussion

Salinity is among the major challenging threats to horticulture by inducing several limitations in plants growth, development and overall productivity. Selection and improvement of salt-tolerant genotypes represents an effective strategy to improve plants productivity in areas

Fig. 1. Effects of salicylic application on shoot (a) and root (b) dry weight of two faba bean genotypes Aguadulce (grey bars) and Hístal (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 (Capital letters for Aguadulce and small letters for Hístal) 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 $n = 8$.

Fig. 2. Effects of salicylic application on leaf water potential (a) and relative water content (b) of two faba bean genotypes Aguadulce (grey bars) and Hystal (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 (Capital letters for Aguadulce and small letters for Hystal) 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 $n = 8$.

Fig. 3. Effects of salicylic application on gas exchanges and photosynthesis of two faba bean genotypes Aguadulce (grey bars) and Hystal (white bars) subjected to salinity. Stomatal conductance (g_s) (a), transpiration (E) (b), Net photosynthesis (P_n) (c), intercellular CO_2 concentration (C_i) (d), Maximal efficiency of PSII (e), and instantaneous water use efficiency (WUE_{inst}) (f). The letters above the bars marks the significant difference among the different treatments with salt and salicylic acid for each genotype (Capital letters for Aguadulce and small letters for Hystal) 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 $n = 8$.

Fig. 4. Effects of salicylic application on ions content and electrolyte leakage of two faba bean genotypes Aguadulce (grey bars) and Hystal (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 (Capital letters for Aguadulce and small letters for Hystal) 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 $n = 8$.

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

Superoxide dismutase activity (SOD) (a), Ascorbate peroxidase activity (APX) (b), Catalase activity (CAT) (c) and Glutathione reductase activity (GR) (d).

The letters above the bars marks the significant difference among the different treatments with salt and salicylic acid for each genotype (Capital letters for Aguadulce and small letters for Hista) 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 $n = 8$.

suffering from adverse environmental conditions (Hernández 2019). Therefore, comparison among genotypes for salt tolerance can be very useful to describe the relevant mechanisms that might help to recognize stress tolerant genotypes and reveal the limiting factors which could be used for molecular breeding (Hanin et al., 2016; Taïbi et al., 2017; Taïbi et al., 2018). 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 (Khan et al. 2015).

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

Severe growth limitations in terms of shoot and root dry biomass were observed under salinity for both genotypes. This finding, as one of the most visible effects of salt stress, 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 and Eroğlu 2011; Dawood and El-Awadi, 2015; Moussa and Hassan, 2016) and other leguminous species like common bean (Taïbi et al., 2012a, b; Taïbi et al., 2013a, b; Taïbi et al., 2016). 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. 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 (Li et al., 2014a; Li et al., 2014b; Khan et al., 2015). 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 (Ψ_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 (Taïbi and Chorfi, 2010; Abdul Qados, 2011; Chorfi and Taïbi, 2011a, b; 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 CO₂ 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 photo-inhibition, 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 photochemistry (F_v/F_m) 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., 2017; Taïbi et al., 2018). 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 (Bulut et al., 2011; Taïbi et al., 2016; Ahmad et al., 2018). 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 increases 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, 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 might 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 suggests 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 attack 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 the two genotypes throughout the maintenance of cell membrane and photosynthetic process and the diminution of sodium toxicity and oxidative damages. 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 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 effects of salicylic acid vary 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 to deal with salt-affected soils, mainly in arid regions, for the reclamation of marginal and wastelands under-cultivated.

CRedit authorship contribution statement

Kada Souana: Methodology, Validation, Formal analysis, Investigation, Writing-Original Draft, Visualization. **Khaled Taïbi:** Conceptualisation, Methodology, Validation, Formal analysis, Investigation, Writing-Original Draft, Writing-Review & Editing, Resources, Visualization, Supervision, Project administration. **Leila Ait Abderrahim:** Methodology, Investigation, Writing-Original Draft, Writing-Review & Editing, Visualization. **Mokhtar Amirat:** Investigation, Validation, Visualization. **Mohamed Achir:** Investigation, Validation, Visualization. **Mohamed Boussaid:** Resources, Visualization, Funding acquisition. **José M. Mulet:** Resources, Visualization, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <https://doi.org/10.1016/j.scienta.2020.109641>.

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