Taïbi, K.; Abderrahima, LA.; Boussaid, M.; Bissoli, G.; Taïbi, F.; Achir, M.; Souana, K.... (2021). Salt-tolerance ofPhaseolus vulgarisL. is a function of the potentiationextent of antioxidant enzymes and the expression profiles of polyamine encoding genes. South African Journal of Botany. 140:114-122. https://doi.org/10.1016/j.sajb.2021.03.045

Salt-tolerance of *Phaseolus vulgaris* L. is a function of the potentiation extent of polyamines and antioxidant enzymes

Abstract

Selection and improvement of crop salt tolerance constitute an urgent need for increasing agricultural and food production in order to feed the growing human population. Two bean (*Phaseolus vulgaris* L.) genotypes varying in their tolerance to NaCl were selected to investigate the determinant physiological and molecular mechanisms underlying salt tolerance. At the physiological level, salinity resulted in a marked decrease in growth, water status, stomatal conductance and photosynthesis for both genotypes. The potassium content was higher in the salt-tolerant genotype under normal and saline conditions. Regarding the molecular response, both genotypes revealed a significant increase in proline, glucose, fructose and sucrose concentrations, but glucose and fructose were differentially higher in the salt-tolerant genotype. Indeed, while putrescine and spermidine concentrations decreased, spermine concentration significantly enhanced under salt stress along with antioxidant enzymes activities namely catalase, glutathione reductase and ascorbate peroxidase, and again, the increase was higher in the salt-tolerant genotype. The expression levels of genes encoding enzymes involved in spermine biosynthesis has revealed an upregulation of spermine synthase (*SPMS*) along with a downregulation of polyamine oxidase (*PAO4*). Overall, the difference between genotypes is more quantitative than qualitative; the salt-tolerant genotype displays a better physiological and molecular response under salinity. We suggest that, by measuring these molecules in salt-stressed plants, several genotypes could be screened for breeding programmes leading to improve salt tolerance in beans.

Key words

Salt tolerance, *Phaseolus vulgaris*, physiological response, antioxidants enzymes, polyamines, genes expression.

1. Introduction

Abiotic stresses constitute major threats to crop yields in the XXI century and their adverse effects are likely going to be significantly increased under the pressure of climate change and the rapid population growth (Arunanondchai et al., 2018). Among the appropriate solutions to feed increasing populations, beans are crops that play an important role in food security and improving human nutrition in developing areas.

The common bean, *Phaseolus vulgaris* L., is an important legume for a large part of the human population, as it is a major source of protein, fiber, vitamins and minerals (Celmeli et al., 2018). In addition, common bean and other legumes are regarded as appropriate crops for the rehabilitation and improvement of soil as they are able to enrich the soil's nitrogen content due to its symbiotic association with rhizobium (Castro-Guerrero et al., 2016).

The increasing salinization of arable lands, mainly in arid and semi-arid regions is a major threat for plant productivity (Taïbi et al., 2016). Plants exposed to salinity are known to experience osmotic stress, water deficit, sodium toxicity and ion imbalance which in turn reduce membrane permeability, plant growth and yield. In response, plants trigger pathways to modulate mainly cellular homeostasis, salt damage control and growth regulation. Salt-tolerant plants often activate cell signaling pathways including those that lead to synthesis of some antioxidant enzymes, abscisic acid and osmoprotectant metabolites (amino acids, glycine betaine, polyamines and sugars) (Isayenkov and Maathuis, 2019).

Polyamines are ubiquitous polycations implicated in different stress response pathways including salinity. These nitrogenous molecules of low molecular weight are implicated in several regulatory processes such as DNA replication and transcription, cell division and

elongation, cell differentiation and morphogenesis (Chen et al., 2019). Polyamines are part of the stress signal transduction pathways and, at physiological pH, interact with macromolecules charged negatively such as DNA, RNA and proteins and also are able to modulate the activity of several enzymes. Polyamines act as a base and as antioxidant agents; they attenuate stress effects and stabilize both membranes and the cell wall (Sequeramutiozabal et al., 2016).

In general, putrescine (Put) is produced via the conversion of ornithine to Put by ornithine decarboxylase (ODC; EC 4.1.1.17), and from arginine (Arg) via agmatine by Arg decarboxylase (ADC; EC 4.1.1.19). The subsequent conversion of Put to spermidine (Spd) then to spermine (Spm) is realized throughout the symmetrical addition of an aminopropyl moiety from decarboxylated S-adenosylmethionine (SAM). Spd and Spm synthesis is catalyzed respectively by Spd synthase (SPD; EC 2.5.1.16) and Spm synthase (SPM; EC 2.5.1.22). However, polyamine catabolism is assured by diamine oxidases (DAO, EC 1.4.3.6), with strong preference for diamines (Put), and by polyamine oxidases (PAO, EC 1.5.3.3) which oxidize only higher polyamines such as Spd and Spm (Murray-Stewart et al., 2003). Although considerable supporting evidences on the role of polyamines under salinity are available, there are no specific information relevant to common bean.

In this study, we hypothesised that increased antioxidant enzymatic activities (catalase, ascorbate peroxidase, and glutathione reductase) and free polyamines (putrescine, spermidine and spermine) would contribute equally to the protection of bean plants against salinity. Our second hypothesis was to explore possible correlations between physiological response, polyamines contents, expression levels of genes encoding enzymes involved in polyamine metabolism and antioxidants enzymes in both genotypes subjected to salinity. Hence, the main objective is to evaluate the effects of salt stress on two bean genotypes widely used in developing countries, that differ in their salt tolerance and explore the defence mechanisms

associated with stress at the physiological and molecular levels. The goal is to identify also the differential markers in salt tolerance that can be used as tools to predict the performance of new been genotypes and varieties under salty environments.

2. Material and methods

Plant material and growth conditions

Two genotypes of *Phaseolus vulgaris*, Tema and Djadida, were selected on the basis of our previous studies (Taïbi et al., 2012, 2013a, b). Seeds preparation and conditions of cultivation were performed as described in Taïbi et al. (2006). Three-week-old plants were irrigated either with nutritive solution (control plants) or with nutritive solution containing 50 mM, 100 mM or 200 mM NaCl. Five plants per genotype were harvested after two weeks of treatment. The fresh and dry weights of the aerial parts and roots were determined. Samples were conserved at -80°C for further analyses.

Physiological measurements

Water status

Leaf water potential (Ψ_w, MPa) was measured in five plants selected randomly per each treatment using a Scholander-type pressure pump (PMS 1000, USA). Nevertheless, relative water content (RWC) was assessed following the formula described by Barrs and Weatherley (1962): RWC (%) = [(fresh weight-dry weight)/(turgid weight-dry weight)]x100

Gas exchange and photosynthesis

Instantaneous determinations of net CO_2 assimilation (P_n, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), and instantaneous water use efficiency (WUE_{inst}; µmol CO_2 $mmol^{-1}$ H_2O) calculated as assimilation divided by

transpiration P_n/E, were also determined in five plants per treatment using a portable photosynthesis open-system (Model LI-6400, LI-COR Biosciences Inc., Lincoln, USA).

Ion content

Concentrations of sodium (Na^+) and potassium (K^+) were determined in roots and shoots by atomic absorption spectrometry (Perkins Elmer, Norwalk, CT, USA) as described by Taïbi et al. (2006).

Molecular analysis

Proline and soluble sugars

The concentration of proline (Pro) was assessed as described in Bates et al. (1973). However, soluble sugars concentrations were measured following the method described by Fayos et al. (2006).

Antioxidant enzymes and free polyamines

Catalase activity (CAT), Ascorbate peroxidase activity (APX) and Glutathione reductase activity (GR) were assayed according to the method of Cakmak et al. (1993). Endogenous free polyamines (putrescine, spermidine and spermine) concentrations were assessed as described by Kotzabasis et al. (1993).

RNA Isolation, qRT-PCR assay and expression analysis of spermine metabolism genes

The qRT-PCR technology was used to analyze the expression profile of selected genes from leaves tissues in plants grown under salt and controlled conditions. Total RNA was isolated from 200 mg of frozen leaves tissues using E.N.Z.A Plant RNA Kit (Omega Bio-tek, USA) following the manufacturer's instructions. In addition, genomic DNA removal, cDNA synthesis, and quality verification for qRT-PCR were performed using SYBR Green PCR

Master Mix (Applied Biosystems, Foster city, CA, USA) following the manufacturer's instructions.

Primer sets of genes encoding enzymes involved in spermine metabolism, spermine synthase (SPMS) and polyamine oxidase (PAO4), were designed after an extensive search in the available public databases, the National Center for Biotechnology Information (NCBI) and Plaza v.4 from consensus sequence of three distinct fabaceous species *Medicago trunculata*, *Cajanus cajan* and *Cicer arientinum*. Gene annotation (identification of exons/introns or open reading frames) was done manually for all sequences, comparing the identified sequences against annotated cDNAs from the other species. SPMS primer, 5'-GTTGTTTCTGGGTGGTTCTCA-3' (forward) and 5'-GATTGAATGGGCTTCTCCTG-3' (reverse). PAO4, 5'-CYGCAATWTCAGATCTTGGTGT-3' (forward) and 5'-CCRAGAGAGTTTGGATCTGTTC-3' (reverse). The amount of target mRNA was normalized by using a housekeeping gene β-Tub8 encoding for Tubulin β-8 chain (Borges et al., 2012; Pereira et al., 2017) which was amplified with the forward primer of β-Tub8, 5'-CGATCGYATGATGTTGACGT-3' and the reverse primer 5'-AGACAACAAGTAACTCCACTCATG-3'.

To implement qRT-PCR analysis, assays were run in 96-well plates using the 7500 Real-Time PCR System and 7500 System Software (Applied Biosystems, Foster city, CA, USA) with settings of: 50 ºC for 2 min, 95 ºC for 5 min, and 43 cycles of 95 ºC for 15 s and 58 ºC for 60 s. Three biological replicates were carried out for the determination of the transcript level of each gene. Relative expression for each sample was calculated with the comparative Ct method. The Ct value obtained after each reaction was normalized with the Ct value of the housekeeping gene for expression levels of transcripts.

Statistical analysis

Prior to the analysis, the Levene test was applied to check the ANOVA requirements. The significance of differences between different treatments was assessed using one-way ANOVA, at 95% confidence level. The post-hoc Tukey test was used to estimate homogeneous groups when more than two samples were compared. Correlations between the different parameters were determined on the basis of the coefficient of Pearson.

3. Results

Physiological response to salt stress

The effect of salt stress on bean seedling growth was examined through determination of the biomass of shoot and root dry weight. As illustrated in Figures 1A and B, salinity induced a significant decrease in shoot and root dry biomass in comparison to control seedlings for both genotypes (p-value<0.01**). The high-yielding genotype Tema presented higher shoot biomass weight independently of salt concentration. The decrease was around 31% in the high-yielding genotype Tema while it was around 27% in the low-yielding genotype Djadida under the treatment 200 mM NaCl. In addition, root dry weight decreased by 60% in Tema and by 47% in Djadida when salinity concentration increased in the medium comparing to their respective control (Fig. 1 A, B).

Water status was also affected by salinity. As expected, water potential (Ψw) decreased significantly by three-fold in both genotypes leading to an approximate decrease in the relative water content (RWC) by around 22% which indicates that the plants were experiencing drought stress concomitant to salinity (Fig. 1C, D). By the same, net photosynthesis (Pn), stomatal conductance (gs) and transpiration (E) showed a significant decrease when plants were subjected to salt treatments (Figures 1 E, F, G). Significant differences in water status and gas exchanges appeared between genotypes when the NaCl concentration exceed 100 mM. The concomitant decrease was approximately 18 % and 21 %

in net photosynthesis, 45 % and 49 % in gas exchange and 26 % and 36 % for transpiration in the genotype Tema as compared to the genotype Djadida respectively. Water use efficiency (WUEinst) was higher in the genotype Tema under higher salinity (Figure 1H). This improvement was consequence of a greater capacity to maintain higher Pn rates in the genotype Tema for similar E-values in both genotypes. In general, significant differences among genotypes are observed mainly under high salt conditions.

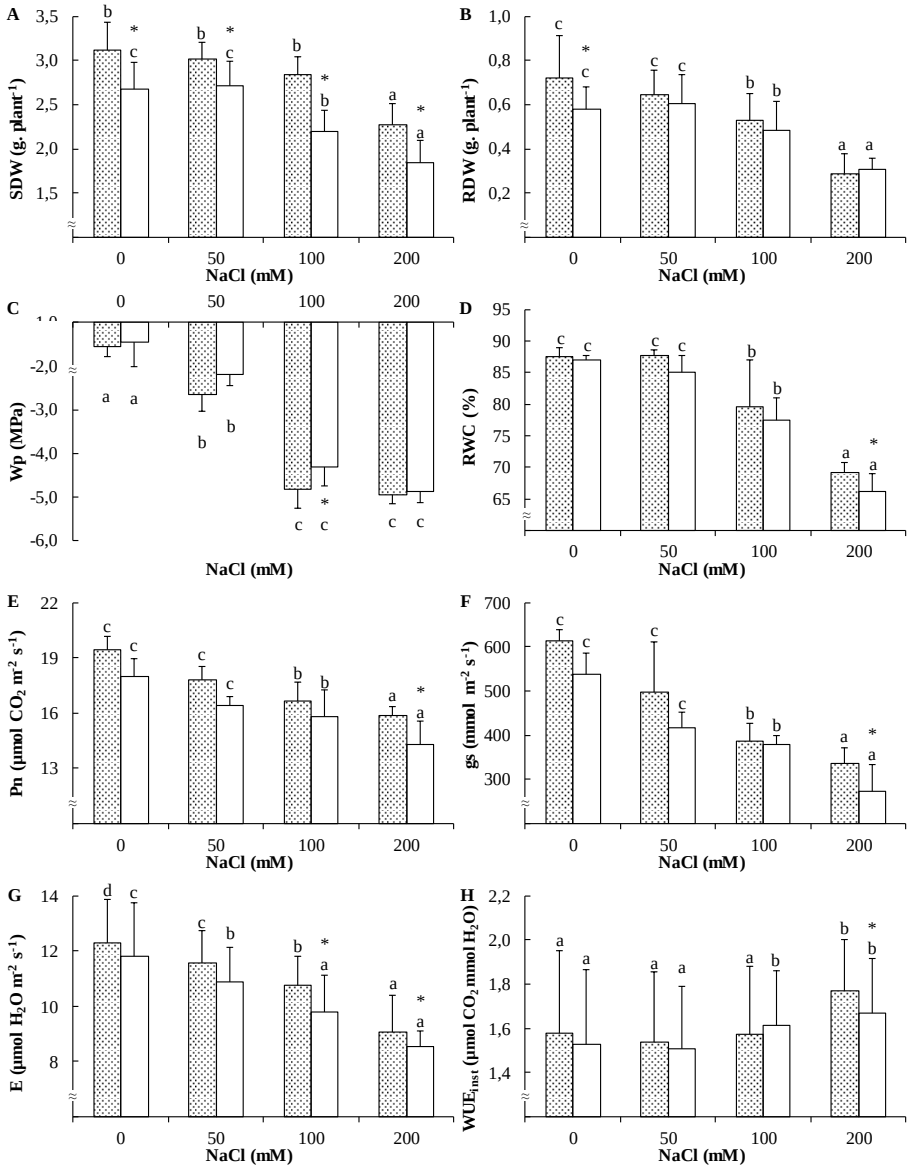

Figure 1. Measurements of physiological parameters of two bean genotypes under saline conditions.

Shoot dry weight (SDW) (A), Root dry weight (RDW) (B), Water potential (Ψw) (C), Relative water content (RWC) (D), Net photosynthesis (Pn) (E), stomatal conductance (gs) (F), transpiration (E) (G) and instantaneous water use efficiency (WUEinst) (H).

The high-yielding genotype Tema is represented by dotted bars while the low-yielding genotype Djadida is represented by white bars. The letters above the bars marks the significant difference among the different treatments of the same genotype following the post hoc Tukey's test. The asterisk marks the significant difference among the two genotypes under the same treatment. Bars represent the mean +SE, n = 5 for the variables analysed.

Leaves are the last sink so they constitute the most sensitive part of the plant where salt accumulates. As expected, for both genotypes, salt treatments affect significantly the content of Na^+ and K^+ in roots and shoots (p-value<0.001***). The increase of salinity concentrations induced significant increase in root and shoot [Na^+] against a significant decrease in [K^+]. Under high salinity, the concentration of Na^+ was significantly higher in the genotype Djadida. However, the concentrations of K^+ were significantly higher in roots and shoots of the genotype Tema independently to salinity (Fig. 2).

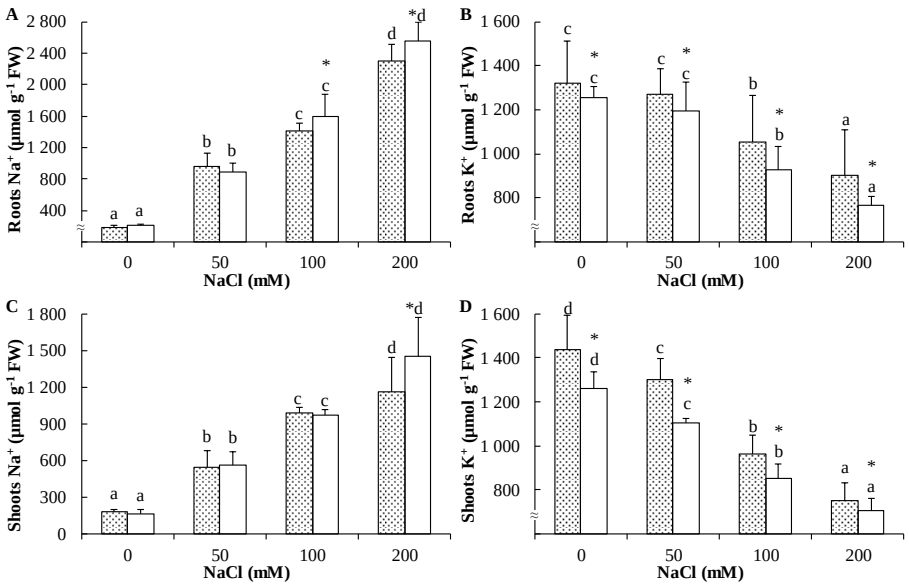

Figure 2. Sodium (Na^+) and potassium (K^+) content in roots and shoots of two bean genotypes under saline conditions.

The high-yielding genotype Tema is represented by dotted bars while the low-yielding genotype Djadida is represented by white bars. The letters above the bars marks the significant difference among the different treatments of the same genotype following the post hoc Tukey's test. The asterisk marks the significant difference

among the two genotypes under the same treatment. Scale bars are mean +SE, being the number of samples n = 5 for the analysed variables.

Molecular response to salt stress

It is known that under salinity there is a concomitant osmotic stress due to the high ionic potential of the external medium which increases the osmotic pressure and affects plant water uptake. Concentrations of several molecules known to act as osmolytes were measured. The proline (Pro) content increased significantly by around thirty-fold (p-value<0.001***) when the concentration of NaCl exceeded 100 mM in the medium for both genotypes (p-value>0.05, Fig. 3A). Furthermore, soluble sugar content increases when salinity concentration was increased in the medium for both genotypes (p-value<0.001***). Glucose (Glu) concentrations were higher in the high-yielding genotype Tema under salinity below 100 mM NaCl, while this situation was inverted under 200 mM NaCl (Fig. 3B). Nevertheless, the fructose (Fru) concentration was significantly higher in the high-yielding Tema genotype (p-value<0.05*). The increase of Fru concentrations under high salinity was around 37 % for the Tema genotype and approximately 46 % for the Djadida genotype (p-value<0.001***, Fig. 3C). In addition, the Sucrose (Suc) concentration was significantly higher in the Djadida genotype under low salinity. However, the opposite is observed when the salinity concentration exceeds 100 mM NaCl; the rate of increase is estimated at 50 % for the high-yielding genotype Tema and 15 % for the low-yielding genotype Djadida (Fig. 3D).

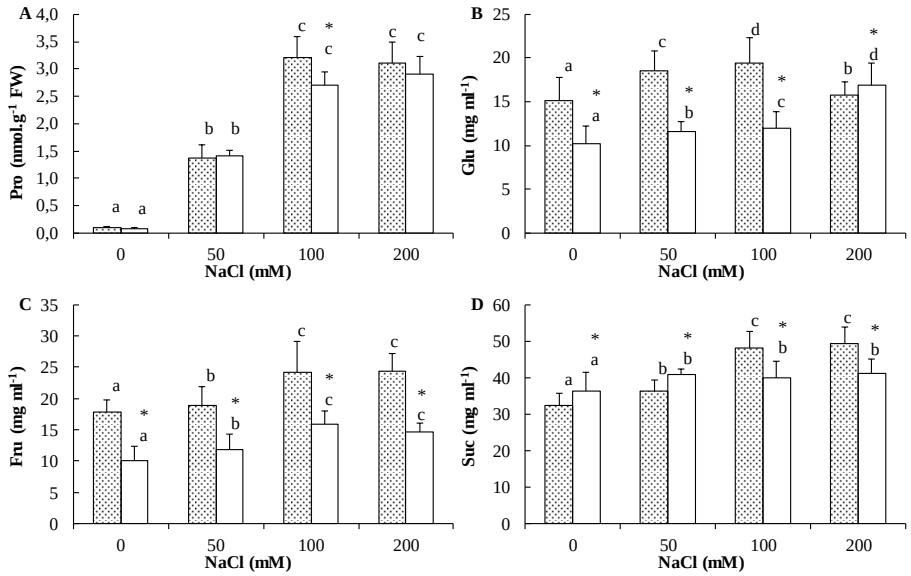

Figure 3. Proline and soluble sugars content of two bean genotypes under saline conditions.

Proline (Pro) (A), Glucose (Glu) (B), Fructose (Fru) (C) and Succrose (Suc) (D).

The high-yielding genotype Tema is represented by dotted bars while the low-yielding genotype Djadida is represented by white bars. The letters above the bars marks the significant difference among the different treatments of the same genotype following the post hoc Tukey's test. The asterisk marks the significant difference among the two genotypes under the same treatment. Scale bars are mean +SE, being the number of samples n = 5 for the analysed variables.

Spermine (Spm) was the most determinant polyamine, as its concentration was higher in the high-yielding Tema genotype under 200 mM NaCl (Fig. 4C), while the putrescine (put) (Fig. 4A) and spermidine (Spd) (Fig. 4B) concentrations did not differ significantly among genotypes (p-value>0.05). It is also interesting to note that Spm concentrations increased significantly under salinity for both genotypes, reaching the highest level when treated with 100 mM NaCl (p-value<0.001***). This is in contrast to the results observed for both Put and Spd under salt stress, which decrease significantly by three-fold and four-fold respectively (p-value<0.001***).

Concerning antioxidant enzymes activity, a significant increase in ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) activities was observed for both genotypes subjected to the increase of salinity concentrations (p-value<0.001***). Nevertheless, it should be noted that the enzymes activities were higher in the genotype Tema under all treatments.

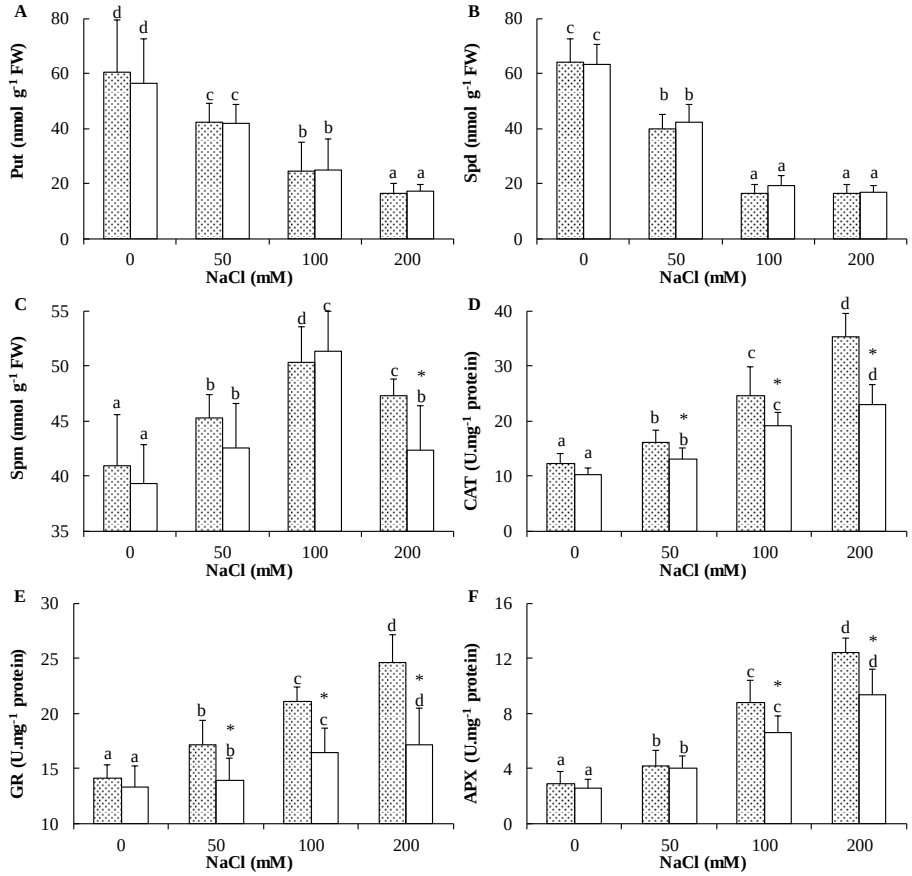

Figure 4. Polyamine content and antioxidant enzymatic activities of two bean genotypes under saline conditions.

Putrescine (Put) (A), Spermidine (Spd) (B), Spermine (Spm) (C), Catalase (CAT) (D), Glutathione reductase (GR) (E) and Ascorbate peroxidase (APX) (F).

The high-yielding genotype Tema is represented by dotted bars while the low-yielding genotype Djadida is represented by white bars. The letters above the bars marks the significant difference among the different treatments of the same genotype following the post hoc Tukey's test. The asterisk marks the significant difference

among the two genotypes under the same treatment. Scale bars are mean +SE, being the number of samples n = 5 for the analysed variables.

No significant difference observed between genotypes concerning the enzymatic activity under the control conditions. However, the rates of increase in CAT activity was estimated around three-fold in the genotype Tema and two-fold in the genotype Djadida under high salinity (Fig. 4D). By the same, GR activity increased by 70 % and 30 % in that order (Fig. 4E). In addition, APX activity increased by five-fold in Tema and four-fold in Djadida under the same conditions (Fig. 4F).

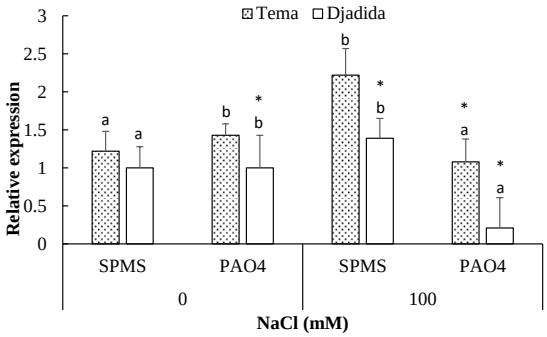

Figure 5. Expression levels of genes encoding enzymes involved in polyamine biosynthesis of two bean genotypes under saline conditions.

Spermine synthase (SPMS), Polyamine oxidase (PAO4). The high-yielding genotype Tema is represented by dotted bars while the low-yielding genotype Djadida is represented by white bars. The letters above the bars marks the significant difference among the different treatments of the same genotype following the post hoc Tukey's test. The asterisk marks the significant difference among the two genotypes under the same treatment. Scale bars are mean +SE, being the number of samples n = 5 for the analysed variables.

The change in gene expression between control and salt stressed plants was calculated with reference the housekeeping gene. Interestingly, differences in genes expression could be observed for both genes; *SPMS* expression was induced in both genotypes with the highest

induction more obvious in the tolerant genotype Tema under salinity, while *PAO4* was highly reduced under stress (Fig. 5).

Results indicate also several significant correlations between the measured parameters under salt stress. Interestingly, at the opposite of free polyamines putrescine and spermidine, spermine concentration was positively correlated with the antioxidant enzymes under salinity (r-value=0.76**).

4. Discussion

Salinity is a major environmental threat preventing crops from attaining their full genetic potential, as it induces several growth and developmental limitations. Therefore, selection and improvement of salt-tolerant genotypes constitutes an effective approach to increase crop productivity. In this context, comparison among bean genotypes differing in their salt tolerance can be extremely useful to describe deeply the most relevant physiological and molecular mechanisms underlying such a response (Gil et al., 2013), and to provide useful information regarding the distinctive features that could help identify new stress tolerant genotypes (Taïbi et al., 2016; 2017; 2018; Souana et al., 2020).

Our results indicate that salinity concentrations over 50 mM NaCl induce significant growth limitations for both genotypes (p-value<0.05*). One of the most visible effects of salt stress is the growth reduction (r-value=-0.62**), due to the inhibition of the developmental programme and the activation of stress response mechanisms (Isayenkov and Maathuis, 2019). Similar results reporting growth reduction under salinity have been also reported in other legumes including *Phaseolus vulgaris* (Delgado et al., 1994; Tejera et al., 2006; Taïbi et al., 2012, 2013a, b, 2016; Souana et al., 2020). Accordingly, measuring biomass accumulation under controlled conditions may allow for the determination of the relative degree of salt tolerance among bean genotypes.

From the physiological point of view, the reported growth inhibition could be attributed directly to the significant decrease of leaf water potential (Ψw) and relative water content (RWC) under salt stress for both genotypes (r-value=-0.66**). There is a substantial evidence that plants respond to salinity by adjusting their osmotic potential through accumulation of organic and inorganic compounds to maintain cell turgor and subsequently to sustain growth (Chorfi and Taïbi, 2011 a, b; Gil et al., 2013). Similarly, stomatal conductance *gs* and photosynthetic rate Pn were lower in plants of both genotypes subjected to salinity (r-value=-0.54*). The reduction in Pn was attributed mainly to *gs* decreases. Similar observations were made by Bayuelo-Jiménez et al. (2003) in several Phaseolus species. Omami and Hammes (2006) also reported that the first effect of a reduction in *gs* is an increase in WUE_{inter}. Regulation of ion transport and homeostasis is one of the main determinants of salt tolerance in plants (Isayenkov and Maathuis, 2019). Glycophytes, such as beans, cope with salinity generally through limiting the transport of toxic ions, mainly Na^+, to plant leaves (Tejera et al., 2006). The results obtained throughout this study indicate that the higher tolerance of Tema versus Djadida may be explained by the higher K^+/Na^+ ratio. The presence of mechanisms restricting the transportation of Na^+ from roots to the aerial of both genotypes. This mechanism, which is more efficient in the high-yielding genotype, allows to minimize the deleterious effects of Na^+ accumulation in leaves throughout reducing Na^+ transport from roots (Bayuelo-Jiménez et al., 2012; Assaha et al., 2017). The significant increases of Na^+ perceived in leaves and roots of both genotypes under salinity were accompanied by important decreases of K^+ concentrations which demonstrate that Na^+ was accumulated rather than being extruded. These results are in agreement with our previous findings reporting that bean plants subjected to salinity take up high amounts of Na^+ but loss significant amounts of K^+ (Taïbi et al., 2012, 2016). Flowers and Colmer (2008) reported that Na^+ uptake produces a depolarization of the plasma membrane triggering the activation of outward rectifying

Comentado [JM1]: Incluye esta referencia: https://www.researchgate.net/publication/31437652_A_Glimpse_of_the_Mechanisms_of_Ion_Homeostasis_during_Salt_Stress

K^+ channels and, therefore, the loss of cellular K^+. Nevertheless, the higher accumulations of Na^+ and K^+ were characteristics of the high-yielding genotype Tema which is in accordance with previous finding of Tejera et al. (2006) and Taïbi et al. (2016). Indeed, our findings confirm that the ability to tolerate salinity is a function of ions accumulation and the ability to take up potassium through the roots and transport it to the aerial part, as the differences in potassium content are significant in all cases.

Sodium concentrations differ among genotypes only under high salt concentrations (Fig. 2). This suggests that the sodium extrusion systems must perform in a similar manner in both genotypes under salt stress. Thus, it is the ability to uptake or retain potassium that determines the yield under salt stress. Potassium has several essential functions in plants and under salt stress it counteracts the toxicity of sodium (Wang and Wu, 2017). Interestingly, potassium transport is also a main determinant for regulating stomatal aperture, so the observed differences in figure 2 may be the underlying cause for the differences observed in WUE and *Pn* (Fig. 1).

Potassium homeostasis is important for salt tolerance, but there is substantial evidence that plants respond to salinity by adjusting their osmotic potential through the accumulation of organic and inorganic compounds to maintain cell turgor and subsequently to sustain growth (Gil et al., 2013).

Regarding the molecular response, plant cells are known to accumulate various osmolytes in order to adjust their osmotic potential and avoid cell injuries under salt stress. Here, proline and soluble sugars content increases in both genotypes to different extents when treated with salt. The accumulation of these metabolites plays an important role in osmoregulation, including controlling water status in plant cells (Nounjan et al., 2018), and in detoxification, throughout chelating Na^+ (Kanai et al., 2007), carbon storage and scavenging of reactive oxygen species (Gupta and Huang, 2014). Proline, one of the most important plant osmolytes,

Comentado [JM2]: Incluye esta referencia aquí https://pubmed.ncbi.nlm.nih.gov/31451552/

Comentado [JM3]:

was accumulated upon salt stress for both genotypes but significant difference was observed under 100 mM NaCl (r-value=0.71**). Interestingly, the salt-tolerant Tema genotype exhibited increased content of glucose and fructose under all circumstances indicating that this may constitute a differential trait and that the osmolyte potential of soluble sugars is determinant. The higher accumulation of glucose and fructose was also detected in the absence of stress. It could be thought that the accumulation of soluble sugars in the salt tolerant genotype is just a symptom, since it is performing better under salinity and therefore it is accumulating more sugars, but the fact that the salt tolerant genotype accumulates also higher concentrations of Glu and Fru in the absence of stress indicates that this attribute is a potential indicator of salt tolerance.

Antioxidant enzymes like catalase (CAT) and those involved in the ascorbate-glutathione cycle, ascorbate peroxidase (APX) and glutathione reductase (GR), are recognized to substantially decrease the oxidative damages in plants subjected to salinity (Kaymakanova and Stoeva, 2008). The activity of the antioxidant enzymes APX, CAT and GR was found to increase in response to the increase of salinity concentration in the medium. Nonetheless, the genotype Tema manifested higher enzymatic activities than the genotype Djadida. The increase of antioxidant enzymes activities is reported to alleviate lipid peroxidation under salt stress in *Lycopersicon pennellii* (Shalata and Tal, 1998), *Pisum sativum* (Hernandez et al., 2000), *P. vulgaris* (Taïbi et al., 2016) and *Vicia faba* (Souana et al., 2020). Azevedo Neto et al. (2006) have specified that APX and CAT are most important enzymes in regulating intracellular levels of H_2O_2. Our findings are in agreement with many reports suggesting that APX activity, coordinated with CAT activity, plays a central protective role in salt tolerance (Demirel and Turkan, 2005; Taïbi et al., 2016). In addition, the increase in GR activity is known to be implicated in maize (Zacchini et al., 2003) and rice (Maribel and Tobita, 2003) salt tolerance.

Polyamines (PAs) are known to be key molecules improving the plant's response to abiotic stresses, such as salinity (Duan et al., 2008). In the present study, salinity induced a significant decrease of putrescine and spermidine, but without any significant difference among genotypes. However, the spermine content increased upon stress, and the increase was about a 20 % higher in the genotype Tema under high saline concentrations, indicating that the accumulation of spermine upon salt stress could constitute a relevant trait for salt tolerance. Talaat (2014, 2015) and Li et al. (2017) have attributed salt tolerance to the concentrations of Pas in plant tissues; changes in free PAs and their catabolism have been shown to occur in plants under salt stress.

PA accumulation protects salt-stressed plants by scavenging free radicals, stabilizing the membranes and cellular structures and maintaining the ionic balance among others mechanisms (Liu et al., 2017). Spermine has a longer chain and more positive charges and thereby could provide more effective stabilizing actions of the membrane (Yamaguchi et al., 2006) and this is probably the main reason why it could constitute a limiting factor in salt tolerance.

The expression levels of genes encoding enzymes involved in polyamine biosynthesis mainly those responsible of spermine metabolism were analyzed in order to elucidate the molecular basis for such accumulation in response to salinity conditions. In general, the tolerant genotype Tema was able to increase higher Spm levels under salt stress through the increase of spermine synthase gene expression *SPMS* along with reduction of polyamine oxidase *PAO4* gene expression. However, the sensitive genotype Djadida showed the same pattern but it tried to compensate the lower synthesis of *SPMS* by the higher decrease in *PAO4* synthesis (Fig. 5). The expression of these genes was investigated in Arabidopsis (Urano et al., 2003) and maize under salt stress (Rodríguez-Kessler et al., 2006). In addition, polyamine oxidase (PAO) is thought to play a major role in the production of H_2O_2 via catabolism of Put and Spd

in plant tissues (Li et al., 2017). Enzyme activity measurements seem to be necessary to confidently link gene expression data to polyamine pool sizes. Our results suggest that the differential accumulation of Put, Spd, and Spm under salinity led to the differential antioxidant defense capacity among genotypes. Results suggest also that higher levels of spermine and antioxidant enzymes are significatively correlated with salt tolerance. Previous researches pointed out that polyamines' oxidation induced the expression of antioxidant enzyme encoding genes in *Nicotiana tabacum* (Guo et al., 2014), *Setaria italica* L. (Sudhakar et al., 2015) and *Zoysia japonica* Steud (Li et al., 2017). In this sense, we could report here that antioxidant system might be regulated by free polyamines in common bean. Shi et al. (2013) reported that exogenous polyamines supply increased drought and salt tolerance in *Cynodon dactylon* throughout the significant increase of antioxidant enzymes abundance along with several other stress-related proteins. Nevertheless, the decrease of antioxidant enzyme activities has led to the down-regulation of polyamine synthesis in transgenic rice (Chen et al., 2014).

5. Conclusion

The comparative analysis among genotypes differing in their tolerance to salt stress may help to identify the relevant mechanisms correlating their relative degree of tolerance to salinity and the associated changes in the physiological and molecular markers.

This strategy was applied on a major crop in this report, common bean, and the obtained results have demonstrated a higher accumulation of potassium, glucose, fructose, proline, spermine and antioxidant enzymes indicating that the ability to synthetize and/or accumulate these ions and molecules could be the main limiting factor for salt tolerance and therefore, may constitute potential targets for breeding programmes or biotechnological improvement for this species. It has been noted also that salinity tolerance in *P. vulgaris* is credited by the metabolism of

polyamines, through the conversion of putrescine and spermidine to spermine, which may enhance antioxidant enzymes activities.

We suggest that, following this strategy, a large number of genotypes could be easily and rapidly screened to select promising candidates to be used in beans breeding programmes to improvs salt tolerance.

6. References

Gupta, B., Huang, B., 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *International Journal of Genomics*. 2014, 18.

Li, S., Jin, H., Zhang, Q., 2016. The Effect of Exogenous Spermidine Concentration on Polyamine Metabolism and Salt Tolerance in Zoysiagrass (*Zoysia japonica* Steud) Subjected to Short-Term Salinity Stress. *Front. Plant Sci.* 7, 1221.

Barrs, H.D., Weatherley, P.E., 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci*. 15, 413–428.

Assaha, D.V.M., Ueda, A., Saneoka, H., Al-Yahyai, R., Yaish, M.W., 2017. The Role of Na^+ and K^+ Transporters in Salt Stress Adaptation in Glycophytes. Front. Physiol. 8, 509.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water stress studies. *Plant Soil*. 39, 200–207.

Castro-Guerrero, N.A., Isidra-Arellano, M.C., Mendoza-Cozatl, D.G., Valdés-López, O., 2016. Common Bean: A Legume Model on the Rise for Unraveling Responses and Adaptations to Iron, Zinc, and Phosphate Deficiencies. Front. Plant Sci. 7, 600.

Bayuelo-Jiménez, J.S., Debouck, D.G., Lynch, J.P., 2003. Growth, gas exchange, water relations, and ion composition of Phaseolus species grown under saline conditions. *Field Crop Res*. 80 (3), 207–222.

Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., Eker, T., Toker, C., 2018. The Nutritional Content of Common Bean (*Phaseolus vulgaris* L.) Landraces in Comparison to Modern Varieties. Agronomy, 8, 166.

Gil, R., Boscaiu, M., Lull, C., Bautista, I., Lidón, A., Vicente, O., 2013. Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes? *Funct. Plant Biol*. 40, 805–818.

Chen, M., Chen, J., Fang, J., Guo, Z., Lu, S., 2014. Down-regulation of S-adenosylmethionine decarboxylase genes results in reduced plant length, pollen viability, and abiotic stress tolerance. *Plant Cell Tissue Organ Cult.* 116, 311–322.

Edreva, A., 1996. Polyamines in plants. *Bulg. J. Plant Physiol*. 22, 73–101.

Isayenkov, S.V., Maathuis, F.J.M., 2019. Plant Salinity Stress: Many Unanswered Questions Remain. Front. Plant Sci. 10, 80.

Guo, Z., Tan, J., Zhuo, C., Wang, C., Xiang, B., Wang, Z., 2014. Abscisic acid, H_2O_2 and nitric oxide interactions mediated cold-induced *S*-adenosylmethionine synthetase in *Medicago sativa* subsp. *falcata* that confers cold tolerance through up-regulating polyamine oxidation. *Plant Biotechnol. J.* 12, 601–612.

Kanai, M., Higuchi, K., Hagihara, T., Konishi, T., Ishii, T., Fujita, N., Nakamura, Y., Maeda, Y., Yoshiba, M., Tadano, T., 2007. Common reed produces starch granules at the shoot base in response to salt stress. *New Phytol* 176, 572-580.

Omami, E.N., Hammes, P.S., 2006. Interactive effects of salinity and water stress on growth, leaf water relations, and gas exchange in amaranth (*Amaranthus* spp.), *New Zealand Journal of Crop and Horticultural Science*, 34(1), 33-44.

Rodríguez-Kessler, M., Alpuche-Solís, A., Ruiz, O., Jiménez-Bremont, J., 2006. Effect of salt stress on the regulation of maize (*Zea mays* L.) genes involved in polyamine biosynthesis. Plant Growth Regul. 48, 175–185.

Taïbi, K., Del Campo, A.D., Vilagrosa, A., Bellés, J.M., López-Gresa, M.P., López-Nicolás, J.M., Mulet, J.M., 2018. Distinctive physiological and molecular responses to cold stress among cold-tolerant and cold-sensitive *Pinus halepensis* seed sources. *BMC plant biology*, 18(1), 236.

Taïbi, K., Taïbi, F., Ait Abderrahim, L., Ennajah, A., Belkhodja, M., Mulet, J.M., 2016. Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. *South African journal of botany*, *105*, 306-312.

Wang, Y., Wu, W.H., 2017. Regulation of potassium transport and signaling in plants. *Curr Opin Plant Biol*. 39, 123-128.

Taïbi, K., del Campo, A.D., Vilagrosa, A., Bellés, J.M., López-Gresa, M.P., Pla, D., Calvete, J.J., López-Nicolás, J.M., Mulet, J.M., 2017. Drought Tolerance in *Pinus halepensis* Seed Sources As Identified by Distinctive Physiological and Molecular Markers. Fr*ont. Plant Sci.* 8, 1202.

Urano, K., Yoshiba, Y., Nanjo, T., Igarashi, Y., Seki, M., Sekiguchi, F., et al., 2003. Characterization of Arabidopsis genes involved in biosynthesis of polyamines in abiotic stress responses and developmental stages. Plant Cell Environ. 26, 1917–1926.

Sudhakarbabu, O., Sivakumar, M., Pandurangaiah, M., Kiranmai, K., Lokesh, U., 2015. Polyamine metabolism influences antioxidant defense mechanism in foxtail millet (*Setaria italica* L.) cultivars with different salinity tolerance. Plant Cell Rep, 34, 141.

Tejera, N.A., Soussi, M., Lluch, C., 2006. Physiological and nutritional indicators of tolerance to salinity in chickpea plants growing under symbiotic conditions. *Eviron Exp Bot* 58, 17-24.