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Effect of Salt Stress on Polyamine Metabolism in Two Bean Cultivars

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ABSTRACT

In agricultural areas, salinity affects plant growth, development and productivity, causing loss of economically important crops. Nitrogen-derived compounds such as polyamines (PAs) are differentially accumulated in diverse plants species in response to salinity. PA and chlorophyll (Chl) contents, as well as water potential (Ψw), were assessed in leaves of two common bean cultivars subjected to salt stress for one and seven days; these bean cultivars, 'Pinto Villa' and 'Canario 60', differ in their drought tolerance phenotype. Salt stress induced a phenotypic behaviour similar to that of drought in which the sensitive 'Canario 60' showed a pronounced decrease in Ψw, in comparison to the tolerant 'Pinto Villa'. Regarding PAs, after the first day of treatment, the levels of all of them (putrescine (Put), spermidine (Spd) and spermine (Spm)) increased in tolerant cultivar ('Pinto Villa') while in the sensitive one ('Canario 60') the levels of Spd and Spm only increased at 400 mM NaCl. At the seventh day, the tolerant cultivar showed an accumulation of Spm at the higher concentrations of NaCl used (150 and 400 mM), whereas a decrease in PA content occurred in the sensitive cultivar at all concentrations assayed. Furthermore, the effect of salt stress on the expression of the main genes involved in PA biosynthesis, including a new *S*-adenosylmethionine decarboxylase (*SAMDC*) gene identified in this work, was analysed. It is suggested that Spm accumulation in 'Pinto Villa' might be part of the mechanism conferring salt tolerance.

Keywords: *Phaseolus vulgaris*, sensitive and tolerant cultivars, water potential
Abbreviations: ***ADC***, arginine decarboxylase; **Chl**, chlorophyll; **PA**, polyamine; **Put**, putrescine; ***SAMDC***, *S*-adenosylmethionine decarboxylase; **Spd**, spermidine; ***SPDS***, spermidine synthase; **Spm**, spermine; ***Ψw***, water potential

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is the most cultivated legume in the world and is one of the main nutritional elements in Mexico. Its production reached 8.3% of the worldwide harvest; however, it has decreased as a result of environmental problems in the last few years (SAGAR 2000). In agricultural areas, salinity affects plant growth, development and productivity causing loss of whole cultures; most of the commercial crops are sensitive to relatively low salt concentrations. Around 20% of the cultivated and irrigated areas in the world are affected by salinity (Zhu 2001). Plants vary in their stress tolerance because they differ in their capacities of stress perception, signalling and response.

Some nitrogen compounds are accumulated in plants as a response to stress, including amino acids like proline, amides, ammonium, quaternary ammonium and polyamines (PAs) (Rabe 1990). PAs, putrescine (Put), spermidine (Spd) and spermine (Spm) in plants are frequently considered as growth regulators, involved in cell division and differentiation (Galston and Sawhney 1990). Put biosynthesis in plants can be addressed by two decarboxylases: ornithine decarboxylase (ODC; EC 4.1.1.17) and arginine decarboxylase (ADC; EC 4.1.1.19). The higher PAs, Spd and Spm are synthesized by Spd and Spm synthase (SPDS; EC 2.5.1.16 and SPMS; EC 2.5.1.22) respectively by the successive addition of aminopropyl groups to Put. The aminopropyl moiety is derived from methionine, which is first converted into *S*-adenosylmethionine (SAM) and then decarboxylated via *S*-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50).

PA biosynthesis increases greatly in response to stresses and its function is presumed to be protective with a role in scavenging free radicals (Mansour 2000). NaCl-salinity causes both ionic imbalance and water stress creating membrane damage (Tiburcio *et al*. 1994). PAs prevent uptake of Na^+, loss of K^+ and leakage of amino acids and electrolytes from plant tissues (Chattopadhayay 2002). Furthermore, it has been suggested that PAs contribute to the maintenance of the cellular cation-anion balance as well as in the stabilization of the membrane integrity through their low molecular mass and polycationic nature (Smith 1985). Salt tolerant rice and tomato cultivars have shown increased Spd and Spm levels which did not occur in the sensitive genotypes (Krishnamurthy and Bhagwat 1989; Santa-Cruz *et al*. 1997). Although a correlation between stress and PA levels have been established in diverse plants, the role of PAs in relation to plant stress tolerance is still not well understood. In view of the fact that environmental stress problems are increasing progressively, the understanding of the mechanism leading to salinity tolerance in commercial crops such as bean is of growing interest.

In order to study the effects of salinity and establish the possible bases of salinity tolerance in bean, a comparative analysis was carried out between 'Pinto Villa', tolerant to drought, and 'Canario 60' considered as drought sensitive (Acosta-Gallegos *et al*. 1995; Terán and Singh 2002; Rosales-Serna *et al*. 2004; Acosta-Gallegos pers. comm.). We analyzed salt-induced changes in PAs and chlorophyll (Chl) contents, and water potentials (Ψw). Effects of salt stress on the expression of the main genes involved in PA biosynthesis, including a new SAMDC (*PvSamdc1*) isolated in this work, were also assessed.

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MATERIALS AND METHODS

Plant material and growth conditions

Phaseolus vulgaris L., cvs. 'Pinto Villa' and 'Canario 60' seeds, sterilized with 50% commercial sodium hypochlorite during 10 min and rinsed three times with distilled water, were germinated in Petri dishes and sown in pots with a mix of sand and perlite (2:1), sub-irrigated with the nutrient solution Hoagland (Hoagland and Arnon 1950), and grown for 10 d in a bioclimatic camera (RIELSA, Mexico, Mexico) with a 16/8 h photoperiod at 25/21°C and 55/75 + 5% RH (day/night). Light intensity (80 µmol $m^{-2}.s^{-1}$) was provided by day-light and grolux® fluorescent lamps (F 40 W). Afterwards, plants were subjected to salt stress for 1 and 7 d by adding NaCl to the nutrient solution to final concentrations of 0, 25, 150 and 400 mM. Leaves were harvested and used for Chl and PAs determinations, as well as for the RT-PCR assays. After treatment, all samples were frozen in liquid nitrogen. All treatments were performed in triplicate.

Water potential determination

Water potentials (Ψw) were determined in leaf discs excised from the younger expanded leaves for each plant. Discs (6 mm in diameter) were sealed immediately after excision and placed in insulated psychrometer chambers (C-52-SF, Wescor). Ψw were determined after 1 h equilibration periods, using a dew point microvoltmeter (HT-33T, Wescor Inc. USA).

Chlorophyll determination

Chl was extracted from leaves of the NaCl treated plants described previously based on the Arnon (1949) method. Fresh prepared cold 80% acetone (0.75 mL) was added to 0.1 g of vegetal material and ground; tubes were protected from light entirely to avoid Chl degradation. The samples were centrifuged 5 min at 2000 rpm. Carefully, the supernatant was removed, and Chl *a* and *b* were determined spectrophotometrically at 645 and 663 nm, respectively, and then total Chl was calculated. The 80% acetone was used as a standard.

Free polyamines determination

An extract from the frozen and ground 10 d old plant material (0.3 g), obtained with 1 mL of 5% perchloric acid and incubated overnight at 4°C, was centrifuged and 0.04 mL of 0.1 mM 1-7 diamino heptane (Sigma-Aldrich Munich, Germany) was added as an internal standard to 0.2 mL of extract. Then, 0.2 mL of saturated Na_2CO_3 and 0.4 mL of dansylchloride (10 mg mL^{-1} acetone, (Sigma-Aldrich Munich, Germany) were added and the mixture was incubated overnight in the dark at room temperature; 0.1 mL proline (100 mg mL^{-1}) was added to stop the reaction. Dansylated PAs were extracted in 0.4 mL toluene and the organic phase was vacuum evaporated. PAs were dissolved in 0.1 mL acetonitrile and analyzed by reverse phase HPLC as described by Marcé *et al.* (1995). Two-way-ANOVA analysis was performed to assess statistical significance between treatments, by the GraphPad Prism 3.0 software. Experiments were repeated twice with similar results.

Cloning of *S*-adenosylmethionine decarboxylase gene (*PvSamdc1*)

A cDNA library was constructed from the leaves of common bean plants, cultivar Pinto Villa, by Suppression Subtractive Hybridization (SSH) under saline conditions (unpublished data). Two identical expressed sequence tags (ESTs) of 853 bp were identified from such library, as part of the 5′-UTR region of the *SAMDC* gene, which contained the characteristic upstream open reading frame (uORF) reported in many plant SAMDC's. When analyzed the new ESTs found, these turned out to be different from the *PvSamdc2* gene isolated previously by our group (Jiménez-Bremont *et al.* 2006). Hence, in order to obtain the cDNA sequence of this new *SAMDC* gene, we designed the UTR5SSH sense primer located at the end of the uORF in the 5′-UTR region (**Fig. 5**) of the new gene fragment, and it was used in combination with the antisense 2299 primer (designed to the end of the ORF of the previously isolated *PvSamdc2* gene). The PCR amplifications were performed in a 0.05 mL reaction mixture containing 0.001 mL of the RT reaction product as template. Primers and PCR conditions are described in the next section. The new *SAMDC* amplified gene fragment of 1118 bp, referred in this work as *PvSamdc1*, was cloned in the pCR 4-TOPO vector (Invitrogen, Carlsbad, USA) and sequenced. The sequence obtained was registered in the GenBank under the accession no. EF580132.

RT-PCR analyses of *PvAdc*, *PvSamdc1*, *PvSamdc2* and *PvSpds* transcripts

Total RNA (0.005 mg) from the NaCl treated bean samples was extracted using the Plant RNA Purification Reagent (Invitrogen, Carlsbad, USA). First strand cDNA synthesis was performed with the SuperScript™ First Strand Synthesis System for reverse-transcriptase polymerase chain reaction (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The PAs biosynthetic genes were amplified using the next sets of primers: for the *PvAdc* (GenBank AY671973) the 38 sense 5′-CTCATTACCAAGGTGTTTATCCGGTGAA-3′ and the 42 antisense 5′-GGCTTCTCGTCCAAACGGTGAAT-3′, for the *PvSamdc1* (GenBank EF580132) the UTR5SSH sense 5′-CTGGTTGTGTGACATAGCCCCCGTCTTCA-3′ and the 2299 antisense 5′-CCACCCATTCCAAGC-3′, for the *PvSamdc2* (GenBank AY327898) the 2274 sense 5′-GAAAAGAGGTTGGAAATATCCT-3′ and the 2299, for the *PvSpds* (GenBank AY674166) the Spe-3-5 sense 5′-CCTGGATGGTTCTCTGAAATTAGCC-3′and the Spe-3-3 antisense 5′-CTCTTCGCAAAAGATGGCAAACAGAA-3′. The actin used as loading control was amplified by the 5-Act sense 5′-ATGGGGCAGAAGGATGCGTATG-3′ and 3-Act antisense 5′-AGCCTTCATAGATGGGGACCGT-3′. The PCR conditions were, in general, 5 min at 94°C for the first denaturalization, 50 s at 94°C, 45 s at each melting temperature (56°C for *PvAdc* and *PvSamdc1*, 50°C for *PvSamdc2*, 58°C for *PvSpds* and 55°C for actin), 80 s at 72°C, and finally 8 min at 72°C for the last polymerization. Amplification was performed in duplicate, with a different number of cycles to ensure a linear response in the PCR reaction, and we present in this work one of such assay at 25 cycles. Samples were analyzed by electrophoresis on 1% agarose gels, and were analyzed using Doc-It® LS Image Analysis Software. The gene fragments amplified in the RT-PCR were verified by sequencing for each cultivar.

RESULTS

Effect of salt stress on water potential and total chlorophyll content in bean leaves

The physiological status of the bean plants subjected to salt stress for 1 and 7 days was assessed considering two parameters, the water potential (Ψw) measurements as well as the total Chl content in stressed leaves. No visible damage was observed in either cultivar at the first day of salt treatment, however, at the seventh day, the sensitive plants ('Canario 60') showed much clearer dried and folded leaves than the tolerant plants ('Pinto Villa') at 150 and 400 mM NaCl (**Fig. 1**). During salinity treatments the leaves water potentials were reduced in both 'Pinto Villa' and 'Canario 60', but such decrease was more pronounced in latter than the former (**Fig. 2**). Since the first day of stress there was a difference among the Ψw of both cultivars, mainly at the higher NaCl concentrations tested (150 and 400 mM); for example at 400 mM NaCl, the sensitive cultivar decreased about -0.8 MPa, while the tolerant one just reached -0.7 MPa. Such difference in Ψw was even more noticeable at the seventh day of stress, at 25 and 150 mM NaCl, 'Pinto Villa' registered -0.6 and -0.85 MPa, in comparison with 'Canario 60' which descended to -1.0 and -1.45 MPa, respectively (**Fig. 2**). At 400 mM NaCl, 'Pinto Villa' showed a Ψw of -1.4 MPa; however the sensitive cultivar showed no registration of Ψw, since it was much damaged.

It is well established that the rate of photosynthesis diminishes with stress, so, in this sense, we determined the Chl content in the same samples described previously for

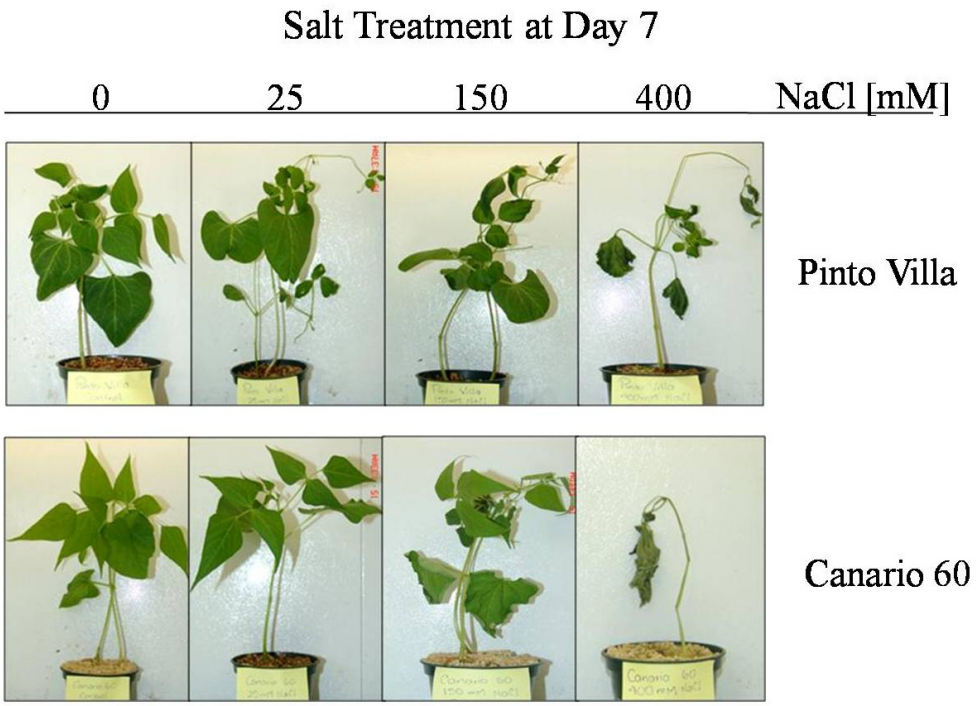

Fig. 1 Photographs of the two bean cultivars ('Pinto Villa' and 'Canario 60') at 7 days of treatment with 0, 25, 150 and 400 mM NaCl.

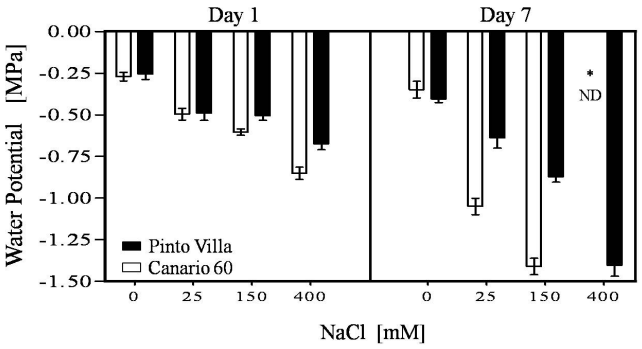

Fig. 2 Effect of NaCl on the leaf water potential of *Phaseolus vulgaris*. Leaves from two bean cultivars, 'Pinto Villa' (black bars) and 'Canario 60' (white bars), subjected to 0, 25, 150 and 400 mM NaCl for one day and seven days were used to determine the plant water potential (Ψw), and reported in MPa. ND, not determined. Data are mean ± SE (n=3).

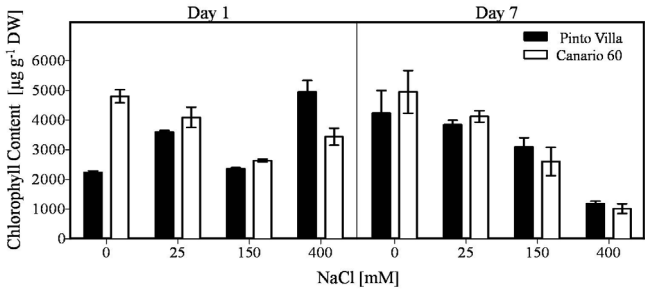

Fig. 3 Chlorophyll content in leaves of *Phaseolus vulgaris* under salt stress. Leaves from two bean cultivars, 'Pinto Villa' (black bars) and 'Canario 60' (white bars), subjected to 0, 25, 150 and 400 mM NaCl for one day and seven days were detached and subjected to chlorophyll (Chl) extraction by the Arnon method. Chl content is expressed in dry weight. Data are mean ± SE (n=3).

water potentials estimation. As shown in **Fig. 3**, 'Pinto Villa' presented an increased Chl content at 25 and 400 mM NaCl (1.6 and 2.2-fold, respectively) after the first day of stress, but at the seventh day the Chl level decreased 0.8-fold at 400 mM respective to the control. 'Canario 60' displayed a general decrease during both treatments periods.

Effect of salt stress on polyamine content

Free PAs (Put, Spd and Spm) were estimated as dansyl-derivatives by reversed phase HPLC (**Fig. 4**). After the first day of treatment, 'Pinto Villa' accumulated Put, Spd and Spm (4.7, 2.3 and 2.7-fold, respectively) at 400 mM. 'Canario 60' showed a decrease in the three PAs at 25 and 150 mM NaCl respect to control, while at 400 mM, an increase in Spd and Spm (1.3 and 3-fold, respectively) was observed. At the seventh day, the highest accumulation of PAs in 'Pinto Villa' was detected at 150 mM with Put (181.2 treated vs 94.9 control nmol g^{-1} DW) and Spm 2-fold each. Furthermore, an increase in Spm content was also observed at 400 mM (1.5-fold). Conversely, the Spd content in this cul-

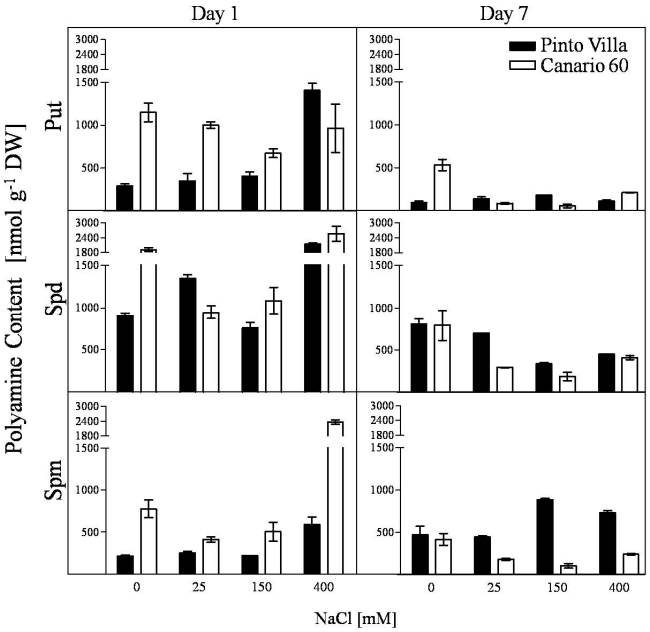

Fig. 4 Polyamine content in leaves of *Phaseolus vulgaris* under salt stress. Leaves from two bean cultivars, 'Pinto Villa' and 'Canario 60', subjected to 0, 25, 150 and 400 mM NaCl for 1 d and 7 d were harvested and subjected to Put, Spd and Spm determination by reverse phase HPLC. PAs content is reported in dry weight. A Two-way-ANOVA analysis of the data was performed comparing both, treatment and cultivars, and the differences found were statistically significant with a value of $P<0.05$.

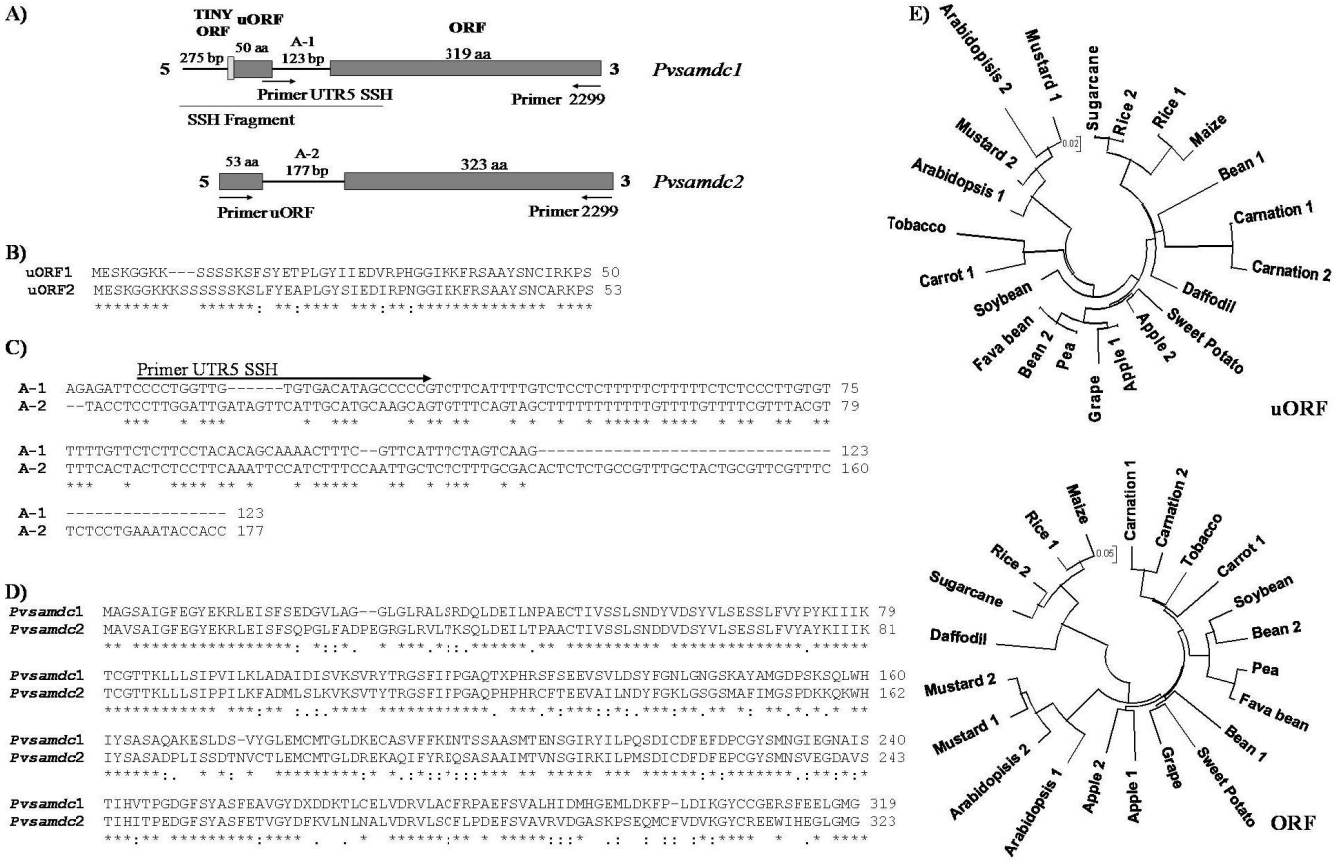

Fig. 5 Comparison among the two *Phaseolus vulgaris SAMDC*'s sequences studied. A new *SAMDC* sequence isolated in this work was compared to the *PvSamdc2* reported previously by our group. Panel (**A**), shows a scheme representing the differences between both *PvSamdc*'s sequences, as well as the amplification strategy (see text for details). Panel (**B**), shows a comparison of the deduced amino acid sequences of the both small uORF harbored in the *SAMDC* mRNA 5′ leader sequences. Panel (**C**) represents an alignment of the 5'UTR region corresponding between the uORF and the translation initial codon of the main ORF from both sequences represented as A-1 and A-2. In the panel (**D**) are compared the main ORFs of the sequences studied. All these comparisons were made by a ClustalW analyses; identical residues are marked by asterisks, and amino acid conserved substitutions are indicated as dots. The Panel (E) shows two phylogenetic trees generated from the two *PvSamdc* plus twenty SAMDC uORFs and ORFs from Arabidopsis (NM_001084624 and AJ251915), Mustard (X95729 and AY444341), Carrot (AY491510), Tobacco (AF033100), Carnation (U38526 and U38527), Rice (Y07766 and AJ251899), Maize (Y07767), Daffodil (AY232672), Apple (AB077441 and AB077442), Grape (AB240537), Fava bean (AJ250026), Sweet potato (AF188998), Soybean (AF488307), Pea (AB087841) and Sugarcane (EF520728).

tivar decreased 0.6-fold at 150 mM and 0.4-fold at 400 mM, respectively compared to the control. Such a decrease is inversely proportional to the increase observed in Spm at these NaCl concentrations, so it might be attributed to Spm biosynthesis. 'Canario 60' at seven days of stress showed an important decrease in all PA levels. A Two-way-ANOVA analysis of the data was performed comparing both, treatments and cultivars, and the differences found were statistically significant with a value of $P<0.05$.

Cloning and sequence analysis of a new *S*-adenosylmethionine decarboxylase gene (*PvSamdc1*)

A new *SAMDC* sequence from an EST corresponding to the 5′-UTR region including the uORF *SAMDC* was isolated from a cDNA library in 'Pinto Villa' by Suppressive Subtractive Hybridization under salt stress (unpublished data). Recently, our group reported the *PvSamdc2* cDNA sequence from bean including ORF and uORF (Jiménez-Bremont *et al*. 2006), but when comparing the newly isolated sequence versus the uORF reported previously, it turned out to be different (**Fig. 5A**). In order to isolate the main ORF *SAMDC* gene, we designed the primer UTR5SSH at the end of the new uORF sequence, specific for this sequence but not for the previous 5′-UTR region of *PvSamdc2* gene; the second primer 2299, used to obtain the *PvSamdc2* gene, was designed based on the conserved regions of SAMDC enzymes from several plants. Comparisons between the 5′-UTR region as well as the main coding region (ORF) of both bean *SAMDC* genes were performed as shown in **Fig. 5B-D**. The sequence of the new gene obtained was Registered in GenBank under the accession no. EF580132, and we refer to it as *PvSamdc1*, to differentiate it from *PvSamdc2*, as previously reported. The PvSAMDC1 protein shares 66% identity with the bean PvSAMDC2 (GenBank AAR00210) and the uORFSAMDC1 peptide shares 85% identity with the bean uORFSAMDC2, while the remaining 5′-UTR regions only share 19% identity. Phylogenetic trees of SAMDC proteins and uORF peptides from different plant species were constructed using MEGA 3.1 version (Kumar *et al*. 2004) (**Fig. 5E**). As observed in the SAMDC tree, the PvSAMDC1 is grouped with sweet potato and grape proteins, while the PvSAMDC2 protein is clustered in a separate group with the leguminous plants (soybean, pea and fava bean). Respect to the uORF2 (Bean2) is grouped with fava bean and pea uORFs, similar to PvSAMDC2 protein, while a different pattern was observed in the uORF1 (Bean1), which is grouped with the uORFs 1 and 2 from carnation.

Effect of salt stress on the expression of genes involved in polyamine biosynthesis

In order to assess effects of salt treatments on expression, PAs biosynthetic genes were evaluated. The same samples as those described above for PA assays were used for RT-PCR analyses (**Fig. 6**). In 'Pinto Villa', *PvAdc* was down regulated by NaCl conditions during the first day of stress, contrasting to one week when the transcript increased. 'Ca-

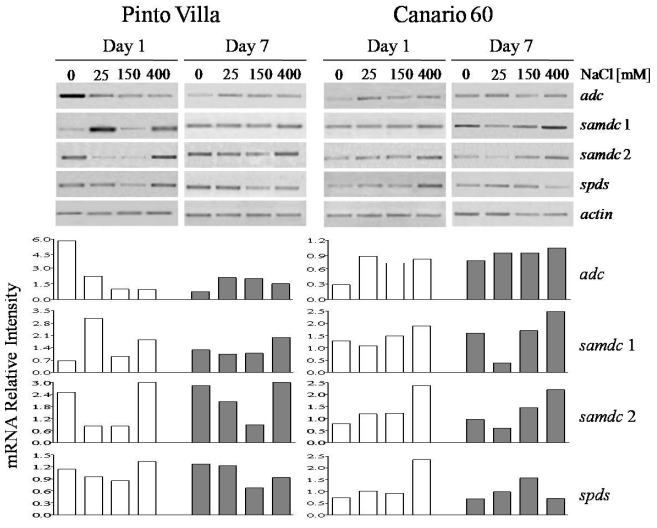

Fig. 6 Expression analyses of Arginine decarboxylase (*ADC*), *S*-adenosylmethionine decarboxylase (*SAMDC1* and *SAMDC2*) and Spermidine synthase (*SPDS*), in two bean cultivars 'Pinto Villa' and 'Canario 60' under salt stress. Ten day old bean plants were subjected to 0, 25, 150 and 400 mM NaCl and leaves were used for RT-PCR assays. Amplification was performed in duplicate, with a different number of cycles to ensure a linear response in the PCR reaction, and we present in this figure one of such assay at 25 cycles. The amplification products were loaded on each lane and separated by electrophoresis on 1% agarose gel. Actin was used as loading control.

nario 60' *PvAdc* gene showed an up regulation during both treatment periods, but was more remarkable at the first day in respect to the control condition. The new *PvSamdc1* gene showed a general up regulation in both cultivars as much to the first day as to seventh, mainly at 400 mM NaCl. In the case of *PvSamdc2* transcript, a down regulation in 'Pinto Villa' at 25 and 150 mM NaCl was observed, while 'Canario 60' maintained an expression pattern similar to *PvSamdc1* for both times. Finally, the *PvSpds* gene was repressed on the seventh day of treatment in 'Pinto Villa' under the highest salt concentrations, which correlated with the decrease observed in Spd content in this cultivar during this period. In 'Canario 60', the *PvSpds* was induced, presenting two peaks of the transcript at 400 and at 150 mM NaCl, during the first and seventh day, respectively.

DISCUSSION

Plant development and productivity are negatively affected by environmental stresses, and the cultivation of the common bean is not an exception, because more than 60% of the world production of this crop is obtained under abiotic stress conditions (Singh 1995). PAs are involved in a wide range of physiological processes in plants (Kaur-Sawhney *et al*. 2003), but the greater changes in their metabolism happen in response to several conditions of stress (Liu *et al*. 2007; Pang *et al.* 2007; Rodriguez-Kessler *et al*. 2008), including osmotic and saline (Aziz *et al.* 1997; Benavides *et al.* 1997; Maiale *et al.* 2004; Jiménez-Bremont *et al*. 2007) and have been considered like salt tolerance modulators and biochemical indicators of this stress (Sanchez *et al*. 2005). These universal multifunctional regulators of physiological processes manifest distinct anti-stress protective actions and their accumulation and protective function against various stresses are of special interest.

In this work, we studied the response to salt stress of two bean cultivars, at one day and seven days, and found a differential accumulation of PAs. These two periods were selected to investigate the effect since the beginning of the salt stress (one day) being mainly by osmotic stress, and salt (ionic) effects even more at long term at seven days. Although 'Canario 60' shows higher PA levels in the control (untreated plants) than the tolerant one, it presents an important decrease in all PA levels under salt stress. Contrary, in 'Pinto Villa', the PA levels were generally maintained and/or increased at both 1 and 7 days, suggesting that the cultivar tend to carry on the pool of these amines under stress conditions. After the first day of treatment, at 400 mM NaCl the levels of all PAs increased in the tolerant cultivar and, in sensitive cultivar the content of Spd and Spm also increased, in comparison to control. At the seventh day, Spm increased in leaf of bean plants ('Pinto Villa') at 150 and 400 mM NaCl, while the levels of other PAs decreased or no significant changes occurred. Opposite, a decrease in PA content occurred in the sensitive 'Canario 60' at all concentrations assayed. In *Lotus glaber*, under long-term salt stress, a similar trend concerning higher polyamines content was shown, as salt induced a decrease of Spd and an increase of Spm (Sanchez *et al.* 2005). Salt tolerant Pokkali rice plants accumulate higher PAs such as Spd and Spm in response to salinity stress, while the sensitive cultivar M-1-48 is unable to maintain high levels of these PAs under similar conditions (Chattopadhayay *et al*. 2002). Yang *et al*. (2007) investigated the changes in contents of PAs in rice plants subject to water stress by using cultivars differing in drought resistance. They found that the contents of Spd and Spm in rice leaves correlated with drought resistance. Considerable controversy exists as far as which PA offers stress tolerance in plants, in which some authors affirm that Put is the crucial one for defence to stress since they have observed its accumulation. For example, in stress by potassium deficiency (Murty *et al.* 1971; Tachimoto *et al.* 1992) where apparently Put plays role replacing potassium like organic cations; however, this hypothesis cannot explain the role of Put in phosphate deprived plants, since phosphate is an inorganic anion. However, the majority of the reports agree that the higher PAs (Spd and Spm) are implied in response to diverse stresses. Again, for example, it has been reported that Spd and Spm represent an important role in stress tolerance of plants, through inhibiting the NADPH oxidation activity (Cuevas *et al*. 2004).

A sequence coding a new bean SAMDC was isolated, other than the previously reported by our group, *PvSamdc2* (Jiménez-Bremont *et al.* 2006). The bean SAMDC1 and SAMDC2 proteins shares 66% of identity between them, however, their own uORFs peptides show 86% identity. The presence of more than one *SAMDC* transcripts in plants is common; for example, it has been reported in carnation, Arabidopsis and mustard (Lee *et al.* 1997; Franceschetti *et al.* 2001; Hu *et al*. 2005). Several plant *SAMDC* mRNAs have been shown to possess a uORF of 50-54 amino acids in the 5′-UTR region (Franceschetti *et al.* 2001). These peptides have been shown to be highly conserved among different plant SAMDCs, with a variation towards the N-terminus, in serine residues, as we observed in both uORFs reported in the present work. Several reports have shown that the uORF plays important roles in translational regulation of the downstream cistron (Hanfrey *et al.* 2002). We reported an induction of *PvAdc* transcript in both cultivars studied, except for the first day of treatment in 'Pinto Villa' where a dramatic down regulation was observed. *PvSamdc1* and *PvSamdc2* were differentially regulated between the two bean cultivars, while 'Canario 60' showed a global induction in expression of both genes, apparently in a salt concentration dependent manner; 'Pinto Villa' manifested an induction peak of *PvSamdc1* at 25 and 400 mM NaCl at first and seventh days, respectively. Recently, a report in transgenic tobacco plants overexpressing a *SAMDC* gene from carnation showed an increased photosynthetic rate and attenuation of stress induced damage, like yellowing and chlorophyll degradation under salt, cold and acidic treatment (Wi *et al.* 2006). In tolerant rice (cv. 'Pokkali'), an up regulation of *Samdc2* at 3 h of salt stress was observed (Kawasaki *et al.* 2001). Some reports have shown a high tolerance to salinity in transgenic rice, tobacco, sweet potato, pear and Arabidopsis plants overexpressing *ADC*, *ODC*, *SAMDC* or *SPDS* under the control of cauliflower mosaic virus 35S or ABA-inducible promoters (Roy and Wu 2001;

Kumria and Rajam 2002; Waie and Rajam 2003; Kasukabe *et al.* 2004, 2006; Wen *et al.* 2008). In respect to *PvSpds*, the first day showed an induction in both cultivars at 400 mM NaCl and at the seventh day 'Canario 60' manifested an induction peak at 150 mM. *PvSpds* showed a down regulation in 'Pinto Villa' at the seventh day of stress which correlated with a decrease in free Spd observed to the seventh day of salinity in 'Pinto Villa'. Differential expression of *ZmSpds2* gene has been reported in maize cultivar under salt stress (Rodriguez-Kessler *et al*. 2006). Additionally, two *ZmSpds2* splicing variants (*ZmSpds2A* and *ZmSpds2B*) were up-regulated under these conditions. In this study, it seems that expression of these four bean genes involved in PA biosynthesis did not parallel the change with the free PA levels. Inconsistency in gene expression and PA contents has been reported in many studies (Alcázar *et al.* 2006; Hummel *et al.* 2004; Liu and Moriguchi 2007) which should be explained by the existence of other PA biosynthetic genes in the bean genome, the regulation of PAs at the post-transcriptional and/or translational levels, and PA conjugation and/or oxidation.

Salt stress decreases the availability of water to plant cells. This decreased availability of water is quantified as a decrease in water potential. Plants resist low water potential by modifying water uptake and loss to avoid low water potential, accumulating solutes and modifying the properties of cell walls to avoid the dehydration, and utilizing protective proteins and mechanisms to tolerate reduced water content by preventing or repairing cell damage (Verslues *et al.* 2006). The results showed a decrease in the leaves water potential in both cultivars under salt stress with a stronger decrease in 'Canario 60'. The lower decrease in the water potential of the leaves in 'Pinto Villa' showed that this cultivar contain mechanisms for counteracting the effect of stress. On the other hand, changes in leaf Chl content can be used as an indicator of maximum photosynthetic capacity, leaf developmental stage, productivity and stress. The growth decrease induced by salt stress is frequently associated to a diminution on its photosynthetic capacity and accompanied with the degradation of Chl and Chl protein complexes (Curran *et al*. 1990; Lichtenthaler *et al.* 1996; Zagolski *et al.* 1996). Accordingly, we observed a reduction in the total Chl content in both cultivars throughout the stress period; however, the tolerant cultivar at the beginning of stress (one day) raised its Chl content at 25 and 400 mM NaCl, compared to the control. Furthermore, in this cultivar the Chl content displayed a similar tendency to PA content, particularly Spd. In this sense, a possible correlation between the accumulation of PAs and the rise in Chl is suggested, with a protective role on the photosynthetic apparatus under various environmental stresses (Navakoudis *et al.* 2007). Tassoni *et al.* (2000) reported that exogenous application of Spd increased the amount of total Chl in *A. thaliana* seedlings at both the rosette and flowering plant stage.

Our results agree with the concept that PAs may be involved in salt stress protection. PA accumulation, specifically Spm, might be part of mechanism conferring salt tolerance to 'Pinto Villa' and not to 'Canario 60'. There are several ways in which PAs could confer salt tolerance; they could act by stabilizing proteins and membranes, by scavenging radicals, by affecting ion uptake or by inhibiting stomatal opening (Tiburcio *et al.* 1994; Drolet *et al.* 1986, Yamaguchi *et al.* 2007). In particular, Spm having a longer chain and more positive charges could provide more effective neutralizing and membrane stabilizing. A recent report in an Arabidopsis double knockout mutant plant which cannot produce Spm showed hypersensitivity to high salt, and this phenotype was abrogated by exogenously applied Spm. Furthermore, this mutant exhibited a symptom of Ca^{2+} deficiency, evidencing the protective role for Spm in the context of responses to ionic stress (Yamaguchi *et al.* 2006).

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