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Enhanced antioxidant enzyme activities and respective gene expressions in potato somatic hybrids under NaCl stress

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Abstract

Potato (*Solanum tuberosum* L.), a plant of great economic importance worldwide, is known to be highly sensitive to salinity. Improving the tolerance of this crop was envisaged using interspecific somatic hybridization. In this report, the impact of salinity on three hybrid lines (STBa, STBc, and STBd) produced by protoplast fusion between the cv. BF15 and the wild species *Solanum berthaultii* was investigated *in vitro*. An analysis of plant response to oxidative stress was considered when plantlets were submitted to 100 mM NaCl for 5 d. The peroxidation of membrane lipids was screened by measuring malondialdehyde accumulation in these lines. Moreover, gene expressions and activities of antioxidant enzymes, such as catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD), were assessed. The results show a lower degree of lipid peroxidation in the hybrid lines in comparison to the BF15 parent. These hybrids also showed higher activities of CAT, POX, and SOD than the BF15, especially in roots. The significant inductions of *FeSOD*, *(Cu-Zn)SOD*, *MnSOD*, and *CAT* genes in hybrid plants suggest their participation in salt tolerance. The differential expressions of the *SOD* and *CAT* genes between leaves and roots also indicate their tissue specificity.

Additional key words: catalase, malondialdehyde, oxidative stress, peroxidase, salinity, *Solanum tuberosum*, superoxide dismutase.

Introduction

The exposure of plants to abiotic stresses, such as high salinity, drought, extreme irradiance, and a high or low temperature, leads to a major loss in crop yields that can exceed 50 % (Hernandez *et al.* 2001, Tuteja 2007). Soil salinity is the biggest threat to modern agriculture, and it is a serious problem in agricultural systems that rely on irrigation (Jithesh *et al.* 2006). Salt detrimental effects on plant include ion toxicity, osmotic stress, nutrient deficiency, and oxidative stress (Tuteja 2007). Indeed, salt stress causes stomatal closure reducing the CO₂/O₂ ratio in leaves leading to subsequent inhibition of CO₂ fixation. These conditions enhance genesis of reactive oxygen species (ROS; Ben Amor *et al.* 2006). Accumulation of ROS is highly toxic and, in the absence of protective

mechanisms, can cause oxidative damage to the cell. The ROS are routinely generated during normal plant metabolic processes to maintain physiological functions (Mittler *et al.* 2004, Kim *et al.* 2005), whereas excessive ROS accumulation damages essential macromolecules such as proteins, DNA, and lipids (Gill and Tuteja 2010). To mitigate the oxidative damage, plants have developed a complex antioxidative system including low-molecular mass antioxidants as well as ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase, and glutathione peroxidase, differently distributed in cellular compartments (Apel and Hirt 2004). The SOD is the first defense enzyme as it catalyzes the dismutation of superoxide radical into O₂ and H₂O₂. This latter molecule can then be scavenged by CAT and peroxidase (POX) (Sairam *et al.* 2004). Peroxidases

Submitted 19 October 2018, last revision 11 March 2019, accepted 22 May 2019.

Abbreviations: CAT - catalase; MDA - malondialdehyde; MS - Murashige and Skoog; PAGE - polyacrylamide gel electrophoresis; POX - peroxidase; ROS - reactive oxygen species; RT - reverse transcription; SOD - superoxide dismutase; STB - interspecific somatic hybrid of potato; TBA - thiobarbituric acid; TCA - trichloroacetic acid.

Acknowledgments: This work was financed by the Tunisian Ministry of Higher Education and Scientific Research. Hybrid production was performed at the Laboratory of Experimental Plant Morphogenesis, the University of Southern Paris, Building 360, Orsay, France under the supervision of Dr. Darasinh Sihachakr. The authors are grateful to Dr. Anne-Lise Haenni from the Institute Jacques Monod (France) for reading the manuscript and improving the English language.

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display a higher affinity for H_2O_2 than catalases (Willekens *et al.* 1997) and consequently, these enzymes play an important role in salt tolerance in plants (Dionisio-Sese and Tobita 1998, Mittova *et al.* 2004).

Potato (*Solanum tuberosum* L.), an economically important crop species worldwide, is salt sensitive with a threshold in tolerance that does not exceed 50 mM (Kikuchi *et al.* 2015). Although salinity is regarded as one of the most important stress factors in agriculture, the number of *in vitro* studies that have actually resulted in the production of stably salt-tolerant plants is scant. Tissue culture techniques can be used as convenient aids for selection procedures of salt tolerant plants. However, screening at the cell level does not necessarily lead to the expression of salt tolerance in regenerated whole plants (Donnelly *et al.* 2007). Sabbah and Tal (1990) reported the efficient development of callus and suspension cell cultures of potato in media supplemented with NaCl and mannitol. Ochatt *et al.* (1999) reported an *in vitro* recurrent selection procedure that allows isolation of stable salt tolerant cell lines and the subsequent regeneration and characterization of complete plants from these cultures.

Somatic hybridization has the unique potential to produce efficient salt tolerant lines either by intraspecific or interspecific protoplast fusion (Trabelsi *et al.* 2005). Indeed, it allows simultaneous transfer of both nuclear and cytoplasmic genes. This exchange of a part or all of the nuclear and cytoplasmic genomes allows the transfer of multigenic traits such as those responsible for stress tolerance (Thieme *et al.* 2008). Many wild *Solanum* species are regarded as important sources of disease resistance and tolerance to a variety of abiotic stresses, but their use in potato breeding is limited due to a poor crossability and sterility of interspecific hybrids. Sexual incompatibility barriers can be overcome using somatic hybridization (Davey *et al.* 2005). The wild species *Solanum berthaultii* is considered a source of different resistance traits against aphids and leafhoppers, thrips, as well as against *Phytophthora infestans* (Hermanová *et al.* 2007). *S. berthaultii* was also used as a donor of tolerance trait to cold (Chen *et al.* 2012).

In this context, we have previously produced interspecific somatic hybrids (STBa, STBc, and STBd) by protoplast fusion between the dihaploid BF15 potato line and the *S. berthaultii* wild species (Serraf *et al.* 1991, Bidani *et al.* 2007).

In this report, we focused on the characterization of the salt stress tolerance of these hybrid lines *in vitro* by measuring their antioxidant capacity. The results obtained in the previous study revealed that the tolerance of these hybrids under salinity conditions implies better control of the oxidative stress generation (Jbir-Koubaa *et al.* 2015). In the present study, we investigate the regulation of activities of antioxidant enzyme and expressions of respective genes under salt stress during *in vitro* cultivation of STB hybrids and the BF15 parent cultivar.

Materials and methods

Plants and culture conditions: The study was conducted on a commercial potato (*Solanum tuberosum* L.) cultivar BF15 and three interspecific somatic hybrid lines STBa, STBc, and STBd resulted from protoplast fusion between the wild species *Solanum berthaultii* Hawkes and cv. BF15 (Serraf *et al.* 1991, Bidani *et al.* 2007). Plantlets were cultivated *in vitro* from single nodal stem explants on a Murashige and Skoog (MS) (1962) medium supplemented with the Morel and Wetmore (1951) vitamins and 30 g dm^{-3} sucrose. The vessels were placed in a growth chamber, and plantlets were grown at a temperature of 24 °C, a 12-h photoperiod, and an irradiance of 62 $\mu\text{mol m}^{-2} s^{-1}$. After 10 d of culture, six plantlets of each potato line were transferred to the MS medium supplemented with 0 and 100 mM NaCl. Stem elongation was measured during 40 d. Oxidative stress parameters were pursued in plants during the first 5 d of culture. Samples taken from leaves and roots were used to study lipid peroxidation products and to measure POX, CAT, and SOD activities and respective gene expressions. Data are recorded from three separate experiments.

Determination of Na^+ content was performed in the leaves and roots of plants cultivated *in vitro* in the absence or in the presence of 100 mM NaCl for 10 d. The fresh material was weighed and dried in a steam at 80 °C for 48 h. The resulting dry material was subjected to calcination in an oven at 550 °C for 2 h and, the ash was treated with 5 cm^3 of concentrated nitric acid to allow mineralization in a ramp until production of a colorless solution. The final volume was adjusted to 25 cm^3 with distilled water. Finally, Na^+ content was determined by atomic absorption spectrometry. Three independent assays were performed and the percentage of Na^+ accumulation in organs was determined as $[(Na^+ \text{ content in stressed plant} - Na^+ \text{ content in control plant}) / Na^+ \text{ content in control plants}] \cdot 100$.

Measurement of malondialdehyde content: Lipid peroxidation in tissues was estimated by measuring the production of malondialdehyde (MDA) using the thiobarbituric acid (TBA) method as described by Hodges *et al.* (1999) with a slight modification. It involved homogenizing fresh material (150 mg) in 1.5 cm^3 of 0.1 % (m/v) trichloroacetic acid (TCA) and then centrifugation at 13 000 g and 4 °C for 30 min. The supernatant was mixed with 2 volumes of TBA/TCA solution (0.8 % (m/v) TBA and 15 % (m/v) TCA dissolved in 0.25 M HCl). The mixture was heated at 95 °C for 15 min. After centrifugation at 13 000 g for 10 min, the absorbances of the supernatant was determined at 532 and 600 nm. The content of MDA was calculated based on a standard curve of MDA.

Proteins were extracted from roots or leaves by grinding them in a mortar and pestle in the presence of 3 volumes of EPSO buffer containing 10 mM Tris-HCl (pH 8), 10 mM Na₂EDTA, 50 mM KCl, 20 mM MgCl₂, 1 mM dithiothreitol, 0.1 % (v/v) Triton X100, 10 % (m/v) polyvinylpyrrolidone,

and 0.5 mM phenylmethylsulfonyl fluoride. Centrifugation at 4 °C and 14.000 g for 30 min was then performed to retrieve proteins from the supernatant. Soluble protein content was determined as described by Bradford (1976) using bovine serum albumin as a standard.

Determination of SOD activity: Superoxide dismutase activity was determined spectrophotometrically using the pyrogallol assay (Ben Mansour *et al.* 2008). This method is based on competition between the dismutation reaction and the oxidation of pyrogallol. The inhibition rate of pyrogallol oxidation is proportional to SOD activity. Protein extract (25 mm³) was mixed with 935 mm³ of Tris-cacodylic acid diethylene triamine penta-acetic acid buffer (pH 8.0 - 8.2). Then, pyrogallol (40 mm³) was added and the oxidation rate of pyrogallol was measured by a decline in absorbance at 420 nm after 1 min of reaction (AE). The percentage of inhibition of pyrogallol oxidation was determined using a formula: Inhibition [%] = [(A_{max} - AE) / A_{max}] · 100; where A_{max} is a change in absorbance at 420 nm of a control test conducted under the same conditions with the absence of protein extract.

Determination of CAT activity: Catalase activity was measured as described by Aebi (1984). This method is based on a principle that absorbance at 240 nm decreases following H₂O₂ dismutation. The amount of H₂O₂ converted into H₂O and O₂ in 1 min under standard conditions is accepted as an enzyme reaction velocity. Assays were carried out at room temperature in a final volume of 3 cm³ containing 100 mm³ of the extract added to 0.1 M phosphate buffer (pH 7); a final concentration of H₂O₂ was 10 mM. A decrease in absorbance of 0.05 corresponds to the decomposition of 3.45 μmol of H₂O₂. One unit of CAT activity corresponds to decomposition of 1 micromol of H₂O₂ per minute.

Determination of POX activity: Peroxidase activity was analyzed using native polyacrylamide gel electrophoresis (native-PAGE) as reported by Trabelsi *et al.* (2005). Total soluble proteins (25 μg) were separated on 10 % (m/v) acrylamide native PAGE by electrophoresis at 4 °C. The gels were washed in distilled water and then soaked in a reaction solution (2.4 mM 3-amino-9-ethyl carbazole, 50 cm³ dm⁻³ N,N dimethyl formamide, 10 mM guaiacol, and 2 mM CaCl₂ in 50 mM acetate buffer, pH 5). Five cubic centimetres of 30 % H₂O₂ was added and kept for few minutes at room temperature until stained bands appeared.

Preparation of total RNA and semi-quantitative reverse transcription PCR analyses : Total RNA was isolated from leaves and roots as described by Vaewoerd *et al.* (1989). The RNA extract was treated with DNase I at 37 °C for 30 min as reported by Bouaziz *et al.* (2012). The RNA concentration was determined by absorbance at 260 nm. Semi-quantitative reverse transcription (RT)-PCR was carried out as described by Degenhardt *et al.* (2005). Reverse transcription PCR analysis of genes encoding SOD and CAT was performed. Total RNA (2 μg) was used for

first strand cDNA synthesis using 200 units of *MoMLV* (Moloney murine leukemia virus) reverse transcriptase (*Invitrogen*, Carlsbad, USA) in a final volume of 20 mm³. The cDNA (1 mm³) was used as a template for RT-PCR with 2 units of *Taq* DNA polymerase (*Invitrogen*). The following PCR amplification conditions were used: heating at 95 °C for 5 min, then 25 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 5 min. A final extension at 72 °C for 10 min was performed. The *elongation factor 1 α* gene (GenBank ID: AB061263) was used to normalize the amount of template added. All primer sequences are presented in Table 1 Suppl. The RT-PCR amplified products were visualized on ethidium bromide stained 1.5 % agarose gels and quantified using the *Gel DocXR* documentation system (*Bio-Rad*, Hercules, USA).

Statistical analysis: Values are expressed as means ± standard deviations of three parallel measurements. The statistical studies and the comparison between the different values were carried out with *ANOVA* using the statistical software *GraphPad Prism Version 5*, taking *P* ≤ 0.05 as significant.

Results

The hybrid plants and their BF15 parent were grown *in vitro* on MS medium in the presence or absence of 100 mM NaCl for 40 d. Phenotypic differences between hybrids and the BF15 parent were observed (Fig. 1 Suppl.). Indeed, hybrid lines were more vigorous than the BF15. Their leaves remained green under salt stress conditions, whereas, the BF15 plants displayed an important reduction of leaf number and size and exhibited a loss of leaf greenness.

Stem elongation was measured regularly during the experiment (Fig. 2 Suppl.). On the medium without NaCl, growth of all lines was similar and no significant differences were observed (*ANOVA*, *P* ≤ 0.05). However, the addition of NaCl in the medium led to significant differences between the different plant lines. Distinct growth inhibition or even arrest was observed in the BF15 parent whereas the STB hybrids were capable of growing.

Assessment of Na⁺ content (Table 2 suppl.) shows an over-accumulation of Na⁺ in all plant lines submitted to salt stress. However, the uptake of Na⁺ into leaves and roots was more important in the BF15 suggesting that the STB hybrid lines may harbor a defense mechanism limiting Na⁺ entry into the cells. It may be also the result of an exclusion process responsible for an efflux of Na⁺ that has entered into the plant. These results are in favor of tolerance to salinity of these hybrid plants. The STB line showed the best control of Na⁺ accumulation.

The content of lipid peroxidation product (MDA) was determined in the roots and leaves of plants cultivated under control and salt stress conditions for 5 d (Fig. 3 Suppl.). In all lines, MDA content in leaves was higher than in roots. The presence of NaCl in the medium led to a significant increase in MDA content in the BF15 leaves. The line STB showed the lowest MDA accumulation. The STB

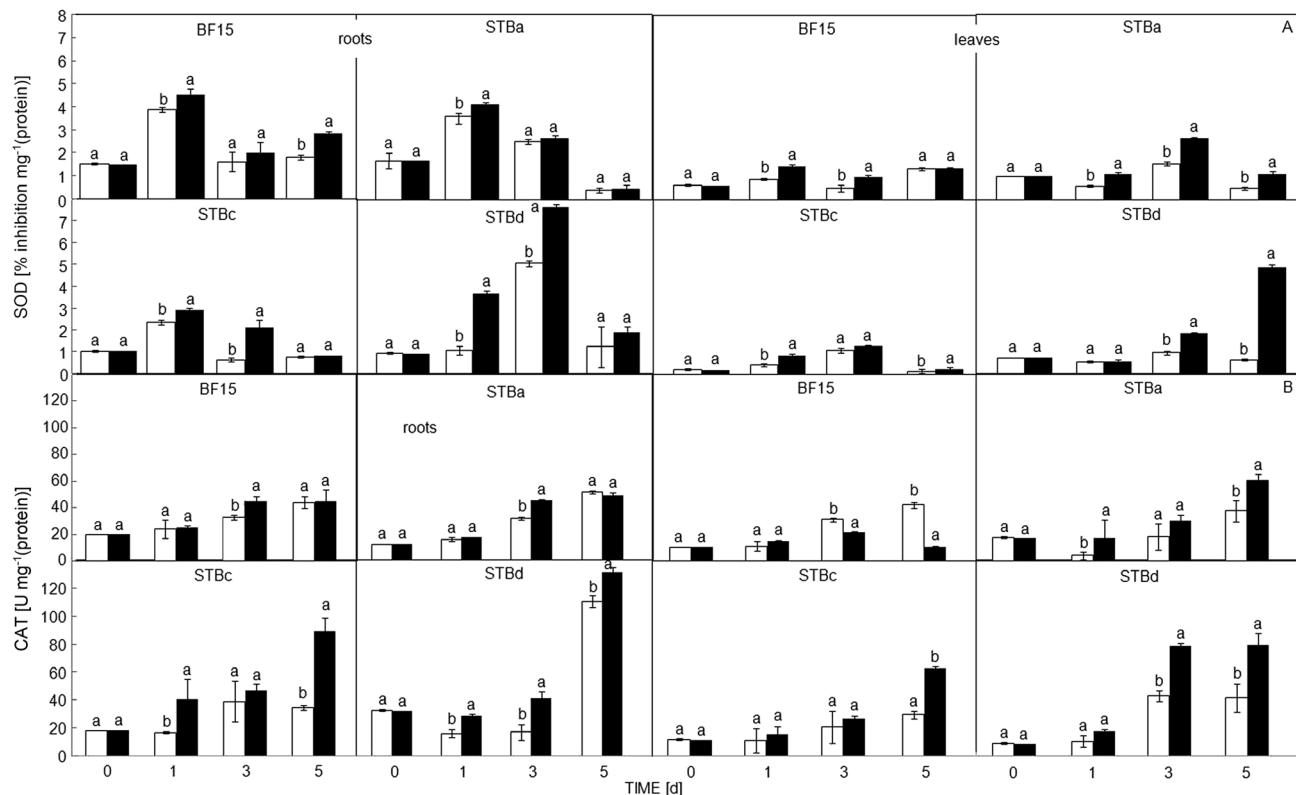


Fig. 1. Estimation of superoxide dismutase (SOD) (A) and catalase (CAT) (B) activities in roots and leaves of interspecific somatic potato hybrid lines STBa, STBc, and STBd and their parent BF15 cultivated on Murashige and Skoog medium without (white columns) or with (black columns) 100 mM NaCl. Means \pm SDs, $n = 10$; means followed by different letters are significantly different between the stressed and the control plants ($P \leq 0.05$).

line showed a significant increase of MDA content in stressed plants, but some recovery was observed on day 5. Salt stress induced a lower MDA increase in the leaves of the STBc line than in those of the BF15. These results support membrane stability and a better management of oxidative stress generated by salinity in the STBc and STBd hybrid lines than in the BF15.

The ability of plants to overcome oxidative stress relies in part on the induction of SOD activity (Farouk 2011). Superoxide dismutase activity was estimated by measuring the percentage of inhibition of pyrogallol oxidation in leaves and roots of plants cultivated under control and salt stress conditions for 5 d (Fig. 1). An increase in SOD activity was observed in all plants submitted to NaCl compared to control plants. Moreover, the results indicate that SOD activity was higher in roots than in leaves. The highest SOD activity was observed in the STBd hybrid. This result might explain a low MDA accumulation in the organs of this hybrid line. The slight increase of SOD activity in the BF15 and STBa lines correlates with an increase in MDA content in these plants submitted to salinity. Salt stress induced a significant increase in SOD activities in the roots of the STBc and STBd lines, which reached a maximum after 3 d of stress. These results suggest that the STBc and STBd lines exhibited salt stress tolerance that can be associated with a sufficient SOD activity.

Hydrogen peroxide produced by SOD-mediated dismutation of superoxide anions may be removed by CAT (Sairam *et al.* 2004). Activity of CAT measured in roots and leaves of hybrids and parental plants cultivated in the presence of NaCl was used to evaluate the effectiveness of H₂O₂ removal in the different lines (Fig. 1). Similarly to SOD activity, the STBa and BF15 lines showed lower CAT activities under salt stress when compared to the STBd and STBc hybrid lines. Nevertheless, CAT activity increased slightly in roots and leaves of the STBa line. More pronounced stimulations of this activity were observed both in the STBc line and in the STBd line. The highest increase of CAT activity was at day 5 in the STBc line and at day 3 and day 5 in the STBd line.

Salt treatment seemed to negatively affect CAT activity in leaves of the BF15 parent indicating a low antioxidant capacity in this line.

These results support the hypothesis that the tolerance of the hybrid lines to salinity was associated with a better control of oxidative stress through an effective activation of antioxidant enzymes such CAT.

The use of multiple isoforms of enzymes is one of the primary control mechanisms of cellular metabolism in plants. However, little data are available on the regulation of the expressions of antioxidant enzyme isoforms under salt stress (Kim *et al.* 2005). Peroxidase activities were investigated here by visualization of the various enzyme

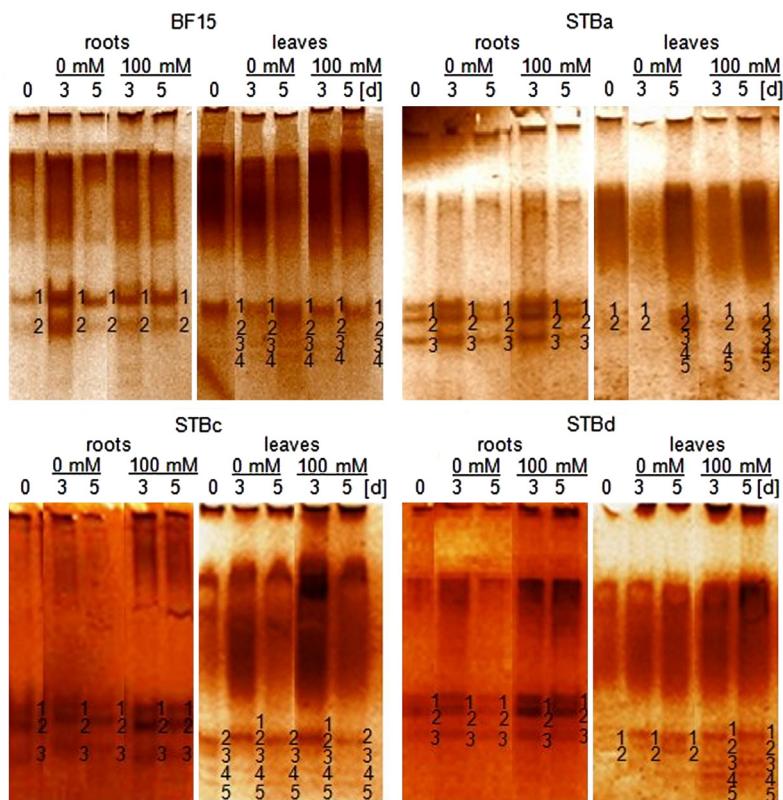


Fig. 2. Representative native gel electrophoresis of peroxidase isoforms (1, 2, 3, 4, and 5) in roots and leaves of interspecific somatic potato hybrid lines STBa, STBc, and STBd and their parent BF15 grown *in vitro* on Murashige and Skoog medium without or with 100 mM NaCl for 0, 3, and 5 d.

isoforms by native PAGE (Fig. 2). Peroxidase enzymes are frequently stable and long-lived enabling rapid and flexible responses (Pearse *et al.* 2005, Cheeseman 2007). Several POX isoforms were observed in all the lines tested. Differences between roots and leaves were clearly noticed among the low molecular mass isoforms. Indeed, leaves exhibited more POX isoforms than roots. Three isoforms were detected in roots whereas five isoforms seemed to be present in leaves. Induction of POX activity was more pronounced in both roots and leaves of the STBc and STBd lines. Activity of POX isoforms 1 and 2 were higher in the leaves of the BF15 and STBa lines cultivated under the control conditions than in plants submitted to NaCl. Enhancements of POX activities were observed in the STBc and STBd lines grown under salt stress. However, in all stressed plants, the activities of the isoforms 3, 4, and 5 were more pronounced (Fig. 2). It seems, therefore, that these isoforms may be related to salt stress tolerance of these potato lines. The NaCl treatment increased the activity of POX isoforms 1, 2, and 3 in roots (Fig. 2). Such an enhancement was not observed after 5 d of culture under the control conditions. This may be an adaptive response to salt stress (Yokoi *et al.* 2002). New POX isoform bands were detected in roots of the hybrid lines compared to the BF15. The STBc and STBd hybrid lines showed an increased POX activity after 3 d of salt treatment.

A total of six *SOD* genes designated *FeSOD1*, *FeSOD2*, *FeSOD3*, *MnSOD*, *(Cu-Zn)SOD*, and *(Cu-*

Zn)2SOD, and three *CAT* genes named *CAT1*, *CAT2*, and *CAT3* were identified by *in silico* analyses in the potato genome (data not shown). The results of the expressions of genes encoding SOD enzymes (Fig. 3 and 4) are generally in accord with those relating to the measurements of SOD activities in the hybrid lines and the BF15. Indeed, the transcripts of *FeSOD*, *(Cu-Zn)2SOD*, and *(Cu-Zn)SOD* remained constant in the BF15 under salt stress, and only a slight induction of *MnSOD* expression was observed subsequent to NaCl application. In the STBa line, a slight SOD activity enhancement observed under salt stress was consistent with a trend of the expression of the six SOD genes in roots. The expressions of *FeSOD1* and *(Cu-Zn)SOD* seemed not to be induced by NaCl in leaves of this line. These results indicate that the regulation of these genes is organ specific. The increases in SOD activities in the STBc and STBd lines were consistent with inductions of all the *SOD* genes. The *FeSOD2*, *MnSOD*, and *(Cu-Zn)2SOD* genes seem to be most induced in the different hybrid lines in response to salt stress. These expression patterns explain the high SOD activity after 3 and 5 d of salt stress in the STBd line. The upregulations of *FeSOD2*, *MnSOD*, and *(Cu-Zn)2SOD* expressions in leaves of the STBc line was significant under NaCl stress although no increase of activity on days 3 and 5 was recorded.

Investigation of *CAT* gene expression in the leaves and roots of the different lines (Fig. 5) was consistent with the analysis of enzyme activity. In the BF15 parent line, the

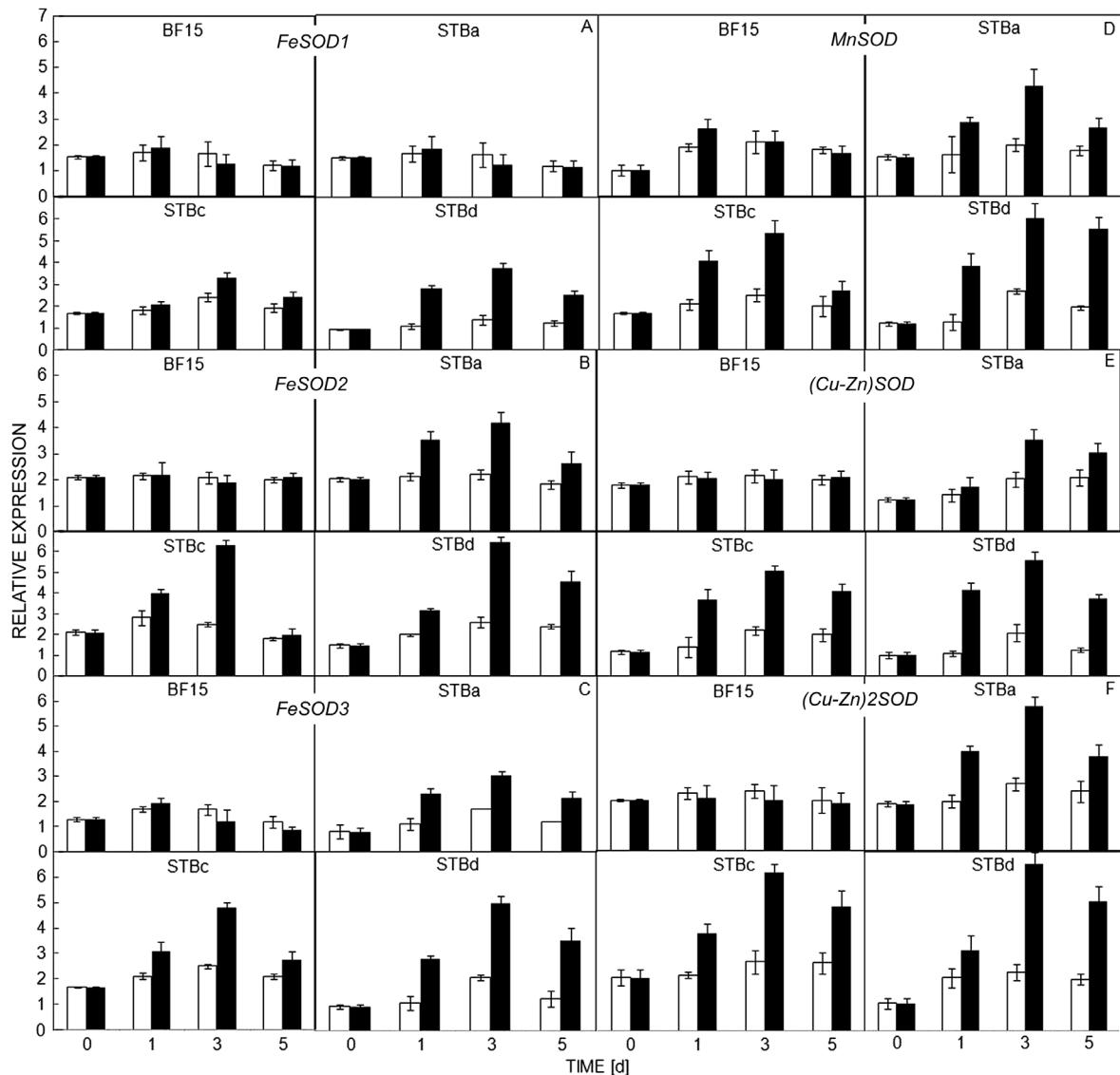


Fig. 3. Expressions of superoxide dismutase (SOD) genes in roots of interspecific somatic potato hybrid lines STBa, STBc, and STBd and their parent BF15. A: *FeSOD1*; B: *FeSOD2*; C: *FeSOD3*; D: *MnSOD*; E: *(Cu-Zn)SOD*; F: *(Cu-Zn)2SOD*.

expression of *CAT* genes seems to be reduced by salt stress. The expression of the *CAT2* gene was constant in the leaves of this plant grown in the presence of NaCl. These results might explain the decrease of CAT activity observed in the BF15 parent grown under salt stress. Expression of *CAT* genes increased in the hybrid plants subjected to salt stress. Transcriptions of them were higher in the roots than in the leaves of the hybrid lines. The correlations between expression profiles and CAT activities were observed. In the STBc and STBd lines, the expressions of *CAT* genes were activated by NaCl to a higher extent compared to the STBa. Only the expression of *CAT2* was activated in the leaves of the STBa line following NaCl treatment. The *CAT1* and *CAT2* genes seem to be most upregulated under salt stress conditions.

All these results show that the tolerance of the hybrid lines is related to their antioxidant capacity by the activation

of expressions of genes encoding SOD and CAT.

Discussion

In the present study, we investigated growth and antioxidant enzyme activities and their respective gene expressions in interspecific potato somatic hybrid plants submitted to salt stress during *in vitro* cultivation. The data show that these hybrid lines display an improved tolerance to salinity in comparison with their parent BF15. Tolerance seems to be related to a good control of Na⁺ influx in hybrid plant roots and leaves. Indeed, the BF15 potato parent showed the highest Na⁺ influx from medium to plant tissues. This Na⁺ entry generated an oxidative stress leading to the peroxidation of membrane lipids. This may be also related to low antioxidant enzyme activities. Indeed, low

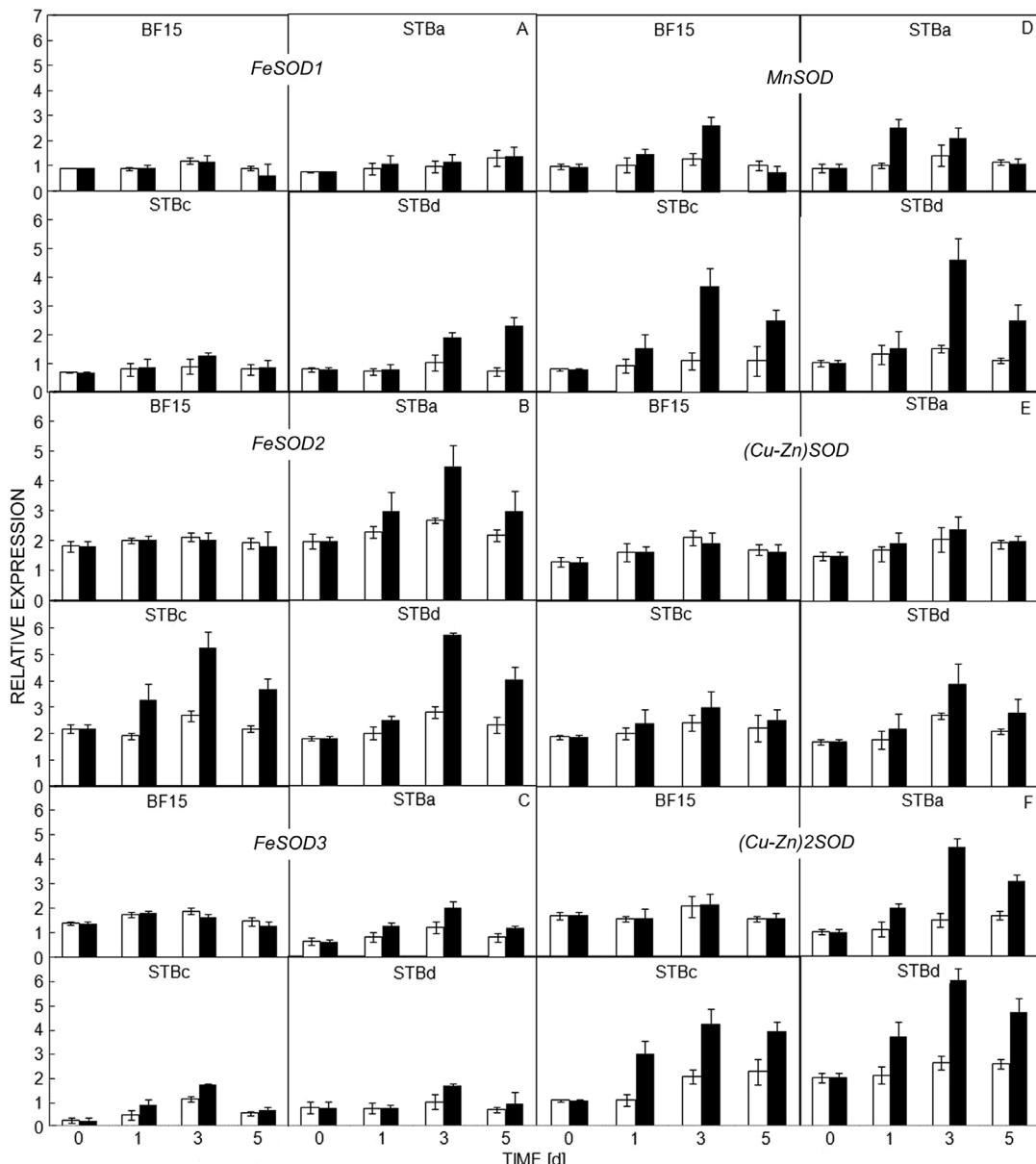


Fig. 4. Expressions of superoxide dismutase (SOD) genes in leaves of interspecific somatic potato hybrid lines STBa, STBc, and STBd and their parent BF15t. A: *FeSOD1*; B: *FeSOD2*; C: *FeSOD3*; D: *MnSOD*; E: *(Cu-Zn)SOD*; F: *(Cu-Zn)2SOD*.

activations of SOD, CAT and POX under salt stress may not be sufficient to limit the deleterious effects of stress. These results are similar to those described previously for barley plants submitted to salt stress (Costa *et al.* 2005). They also confirm the positive relationship between the amount of lipid peroxidation products and the degree of membrane damages due to the injurious effect of salt stress (Kattab 2007).

The efficient control of Na^+ influx in the STBa hybrid may have reduced its toxic effects. Consequently, low activities of antioxidant enzymes, such as SOD and CAT, are needed to overcome the low oxidative stress generated by salinity. The STBc and STBd hybrids accumulated intermediate Na^+ content in comparison to the BF15 and

STBa. However, this Na^+ uptake was counterbalanced by improved antioxidant activities. Such an improved oxidative stress response may contribute to the salt tolerance observed in these lines as previously suggested (Hernandez *et al.* 2001). The low lipid peroxidation observed in the STBc and STBd lines suggests membrane stability and efficient protection against salt stress in these lines. Such a phenomenon is related to tolerance to abiotic stresses in potato (Backhausen *et al.* 2005) and tomato (Shalata *et al.* 2001).

The STBd line accumulated the lowest amount of MDA probably due to the rapid expression of the SOD, CAT, and POX antioxidant enzymes. Such antioxidant enzymes are considered as reliable indicators of environmental

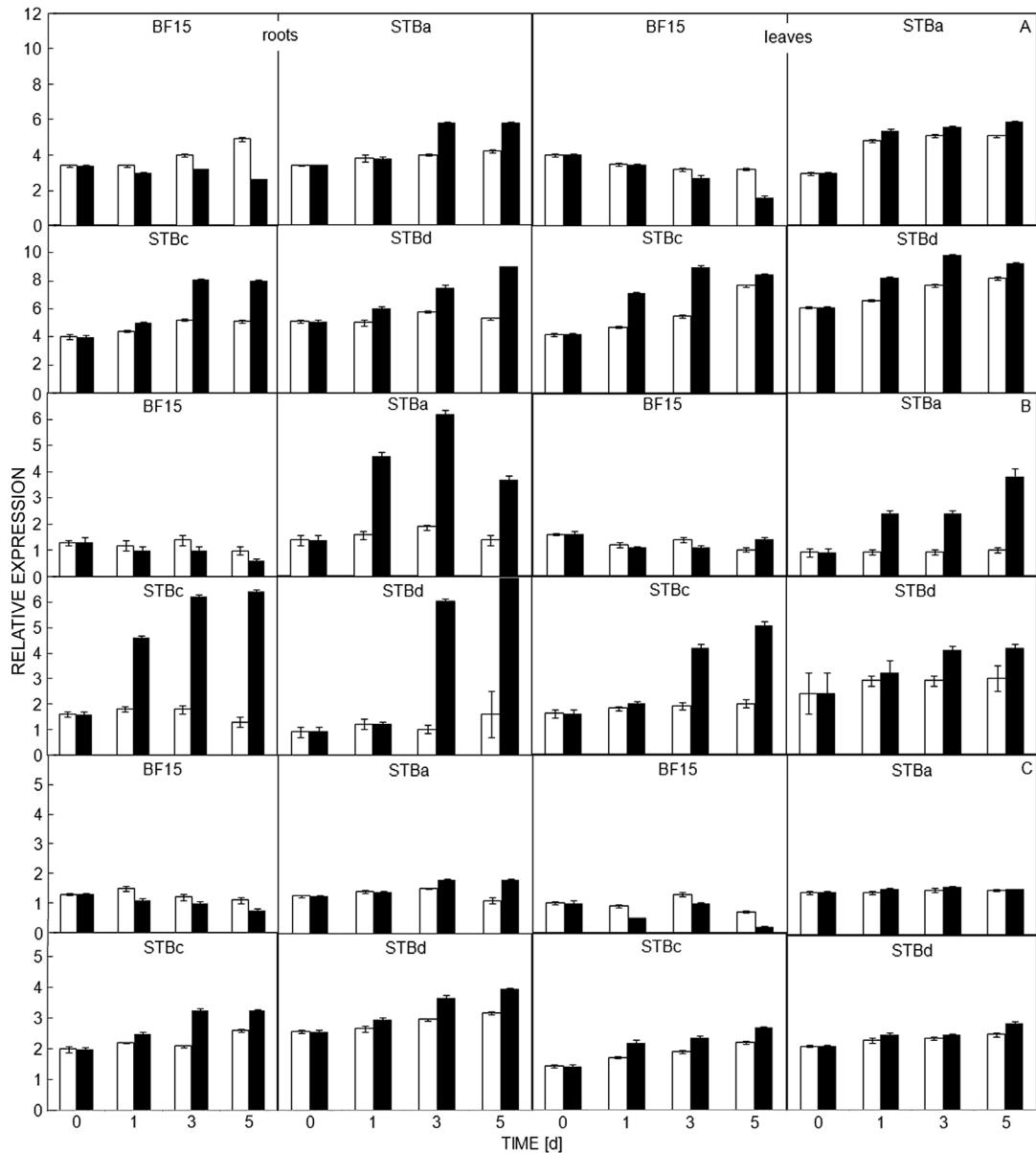


Fig. 5. Expressions of catalase (CAT) genes determined in roots and leaves of interspecific somatic potato hybrid lines STBa, STBc, and STBd and their parent BF15. A: *CAT1*; B: *CAT2*, and C: *CAT3*.

stress tolerance in plants (Harb *et al.* 2015). These data correlate with those of Scandalios (1993), who reported that the efficient protection against oxidative damage caused by salt stress results from high SOD and CAT activities. Such increases were also observed in other plant species (Bor *et al.* 2003, Koca *et al.* 2007, Dai *et al.* 2009). High activities of antioxidants under salt stress were also reported in salt-tolerant plant species (Gossett *et al.* 1994, Shalata and Tal 1998). Moreover, plants harboring a low CAT activity were shown to be sensitive to a high salinity (Joseph and Jini 2011). Other studies showed that the increase of antioxidant enzyme activities during oxidative stress does not exceed two times in susceptible plants (Benavides *et al.* 2000), whereas it may be more than four times in salt-tolerant plants (Kennedy and De

Filippis 1999). The increase of CAT activity and POX isoform patterns is associated with a decrease in content of hydrogen peroxide under abiotic stresses. Higher POX activities were observed in the hybrid plant roots and leaves when the separation of the isoforms of this enzyme was conducted. Upregulation of the constitutive isoforms and induction of new ones may be related to the salt tolerance of the hybrid lines (Kim *et al.* 2005, Rahnama and Ebrahimzadeh 2005, Carassay *et al.* 2012). The differential enzyme isoform patterns between roots and leaves also indicates their tissue specificity.

These enhanced antioxidant capacities prompted us to focus on the regulation of expressions of genes encoding antioxidant enzymes in these hybrid lines. Moreover, the data suggest that the behavior of each hybrid line may

rely on particular signaling pathways used in response to salinity. The expressions of antioxidant enzyme genes will help in the differentiation of the response mechanism to control salt stress in these hybrid lines. In the present study, the expression profiles were specific to each genotype but generally showed a correlation with the corresponding enzyme activities. The expressions of the *SOD* genes did not increase in the parent BF15 during 5 d of salt treatment. Only a slight upregulation of expression of *MnSOD* was observed, which is consistent with the slight increase of global SOD activity in this line. The highest transcriptions of all *SOD* genes in STBd leaves and roots on days 3 and 5 may explain the highest SOD activity. The increases of *FeSOD2*, *MnSOD*, and (*Cu-Zn*)₂*SOD* expressions, which seem not to be directly correlated with enzyme activity in leaves of the STBc line, can be explained by the complex regulation mechanisms of gene expression (Mazzucotelli *et al.* 2008, Harb *et al.* 2015). The STBc and STBd lines exhibited the highest *SOD* and *CAT* expressions as well as enzyme activities. However, each hybrid line had its unique pattern of gene expression. The low increase in antioxidant gene expression and enzyme activity in the STBa seems to be related to the best control of Na⁺ influx. The increases in *SOD* and *CAT* expressions in the STBc and STBd were correlated with a significantly enhanced salt tolerance. These data confirm that the STB hybrid lines exhibited a specific capacity to protect themselves from salt stress through different mechanisms.

Antioxidant enzymes such SOD and CAT may act synergistically. This direct connection suggests a correlation between activation of gene expressions of these enzymes. Both *CAT* and *SOD* gene expressions were higher in the hybrid lines. The low expressions of *CAT* genes in the BF15 can explain the low CAT activity.

This is the first report that describes simultaneously the expression regulation of the six *SOD* genes together with the three *CAT* genes in the potato plant hybrids as a response to salt stress. All these genes were activated in the hybrid plants and enabled them to tolerate salt stress. These results greatly increase our understanding of how potato responds to salt stress and provide evidence for the effectiveness of the different STB hybrid detoxification mechanisms.

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