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ORIGINAL PAPER

# Investigation of the response to salinity and to oxidative stress of interspecific potato somatic hybrids grown in a greenhouse

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**Abstract** Salinity is one of the major stresses threatening potato plants (*Solanum tuberosum* L.) by affecting their growth and yield. It leads to oxidative stress by the production of reactive oxygen species responsible for alteration of macromolecules. To improve the tolerance of potato to salt stress, we have used somatic hybridization to produce interspecific potato hybrids by protoplast fusion between the BF15 variety and the wild *Solanum berthaultii* species. These hybrids showed an improved tolerance to salt stress when cultivated in vitro. The present work aims to analyze the response of the hybrids to salt stress in greenhouse conditions. Thus, the development of plants and their antioxidant capacity in response to salt stress were followed. All hybrids showed better growth and stable chlorophyll content compared to those of the BF15 parent plant. Membrane lipid peroxidation, evaluated by measuring the malondialdehyde accumulation (MDA) in plant organs, showed low levels in the hybrids. Higher antioxidant enzyme activities were measured in the roots of the hybrids when compared to those of the BF15 parent. These hybrids also showed an improved control of $Na^+$ accumulation and a stable $K^+/Na^+$ ratio. These results therefore confirm the better tolerance of these hybrids to salt stress when compared to their BF15 parent.

**Keywords** Antioxidant enzymes · Potato · Somatic hybrids · Salinity · Oxidative stress

**Abbreviations**

| | |
|---|---|
| APX | Ascorbate peroxidase |
| CAT | Catalase |
| GPX | Glutathione peroxidase |
| GR | Glutathione reductase |
| GSH | Glutathione |
| MDA | Malondialdehyde |
| ROS | Reactive oxygen species |
| SOD | Superoxide dismutase |

## Introduction

An option to cope with the increase of population densities in developing countries consists in increasing productivity of cultivated land. Such extension can be difficult in arid and semi-arid areas because of water shortage and poor water quality. This makes the understanding of environmental stress phenomena and related tolerance mechanisms in plants in such countries a necessary step.

For decades, agriculture in arid and semi-arid environments has faced an increase in soil salinity that has become one of the major problems worldwide (Joseph et al. 2010). Indeed, salinity is one of the most important abiotic stresses limiting plant growth and productivity (Khan and Panda 2008; Khan et al. 2012; Iqbal et al. 2014; Rodziewicz et al. 2014).

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In Tunisia, plants are subjected to extreme climatic factors such as high temperatures and drought, in addition to the frequent use of saline water for irrigation. The dissolved salts may then accumulate in soils because of the insufficient leaching of ions. An accumulation of salt in upper soil layers may be also due to an unsuitable irrigation management. As a result, 1.8 million hectares corresponding to 25 % of irrigated areas are salinized in Tunisia (Hachicha 2007). Such an abiotic stress negatively influences survival, biomass production and crop yield (Beck et al. 2007).

Salt stress on plants can result in a hyper-osmotic stress, an ionic imbalance and toxicity (Nazar et al. 2011). The first evident symptom of salt toxicity is the inhibition of growth in the roots and shoots (Beck et al. 2007). Exposure to salinity is found to enhance oxidative stress (Iqbal et al. 2014). The overproduction of reactive oxygen species (ROS) is one of the important causes of the damages due to salinity. Chloroplasts are the main sites of ROS formation, and photosynthesis electron transport provides the main means of ROS formation. ROS such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), and singlet oxygen ($^1O_2$) are highly reactive and can alter normal cell metabolism through oxidative damage of membranes by lipid peroxidation. They can also lead to protein denaturation and DNA mutation (Baby and Jini 2011). However, the oxidative stress observed in plant tissues submitted to high salinity can be counteracted by the plant via the activation of a complex antioxidant system (Gill and Tuteja 2010).

To scavenge free radicals and to counter the hazardous effects of ROS, plants have developed a number of antioxidative mechanisms (Ghassemi-Golezani et al. 2009; Noctor et al. 2012) based on both enzymatic and non enzymatic pathways. Indeed antioxidant compounds such as glutathione (GSH), ascorbate (AA) and carotenoids (CAR) are employed by plants to eliminate ROS (Blokhina et al. 2003). The enzymatic pathway involves superoxide dismutase (SOD), a family of enzymes catalysing the dismutation of superoxide anion radicals to hydrogen peroxide in organelles and in the cytosol. Catalases (CATs; in peroxisomes) remove the bulk of hydrogen peroxide generated in photorespiration. Peroxidases possess broad specificities located in vacuoles, cell walls and the cytosol (Simova-Stoilova et al. 2008).

Although, salinity is regarded as one of the most important stress factors in agriculture (Caldiz 1994; Jithesh et al. 2006), breeding for salinity tolerant crop plants can be difficult and slow (Donnelly et al. 2007). The tissue culture techniques can be used as convenient aids for selection procedures of salt tolerant plants. However, screening at the cell level cannot 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 by NaCl and mannitol. Ochatt et al. (1999) reported an in vitro recurrent selection procedure that allowed isolation of stable salt tolerant cell lines and the subsequent regeneration and characterization of complete plants from these cultures.

Potato (*Solanum tuberosum* L.) is an economically important crop species worldwide (Kashyap and Panda 2003). It is a salt sensitive species with a threshold tolerance that does not exceed 50 mM (Bouaziz 1980).

To our knowledge there are to date no salt tolerant commercial potato varieties available. Breeding of new potato variety tolerant to biotic and abiotic stresses can be envisaged by using somatic hybridization. The wild *Solanum* species can be regarded as a source of different resistance traits in potato breeding, mainly against aphids, leafhoppers, thrips as well as against *Phytophthora infestans* (Thieme et al. 2010).

Somatic hybridization by protoplast fusion can overcome sexual incompatibilities between wild and cultivated *Solanum* species. Moreover, the simultaneous transfer of both nuclear and cytoplasmic genes can allow the exchange of part or all the nuclear and cytoplasmic genome allowing the transfer of multigenic traits such as those responsible for the tolerance to abiotic stress (Thieme et al. 2008). Somatic hybridization permitted introgression of mono- and polygenic traits from wild *Solanum* species into the *S. tuberosum* gene pool (Smyda et al. 2013). PVY resistance is a monogenic trait (Solomon-Blackburn and Barker 2001), while salinity stress tolerance is a polygenic one (Shahzad et al. 2013; Smyda et al. 2013).

In this context, interspecific somatic hybrids were produced by protoplast fusion between the dihaploid BF15 potato line and the wild *Solanum berthaultii* species (Serraf et al. 1991).

Three tetraploid symmetric somatic hybrids (STBa, STBc and STBd) were characterized and exhibited a recombinant chloroplast genome. Their tolerance to saline conditions was investigated under in vitro culture conditions (Bidani et al. 2007).

Our study, we focused on the physiological evaluation and the characterization of the antioxidant capacity of these hybrid lines cultivated in vivo and submitted to salt stress.

The salt stress tolerance of hybrid lines and the parental BF15 potato variety was evaluated by the analysis of growth parameters and by considering some antioxidant activities (SOD, CAT and GPX) together with physiological analyses.

## Materials and methods

### Plant material

The study was conducted on a commercial potato variety (BF15) and three interspecific somatic hybrid lines "STBa,

STBc and STBd" resulting from protoplast fusion between the wild species *S. berthaultii* and *S. tuberosum* variety BF15 (Serraf et al. 1991; Bidani et al. 2007).

*Solanum berthaultii* wild species is especially potent source of resistance to biotic stress (Simko et al. 2007). It has been also reported as a source of resistance to cold stress (Liu et al. 2010; Chen et al. 2012). In our knowledge no resistance trait has been reported to salinity in *S. berthaultii.* The cultivated potato parent (BF15 variety) is sensitive to salt (Bidani et al. 2007; Akossiwoa Quashie et al. 2004).

Plant multiplication was performed by in vitro as described by node culture (Bidani et al. 2007) on MS medium (Murashige and Skoog 1962) supplemented with vitamins (Morel and Wetmore 1951) and 30 g $L^{-1}$ sucrose. They were placed in a growth chamber at 21°C with a photoperiod of 12 h light (62 µE $m^{-2}$ $s^{-1}$) per day.

## Greenhouse planting

For weeks-old plantlets, grown in vitro and well developed (about 8 cm in height) on MS medium (Murashige and Skoog 1962), were transferred to similar standard pots with 16 cm in diameter containing a mixture of sand (1/3) and sheep manure (2/3) and covered with plastic to increase the surrounding humidity. The culture was carried out in a greenhouse at a temperature ranging from 20 to 30°C in the presence of sunlight. The plants were watered with tap water every 2 days.

Two weeks after the transfer in the greenhouse, sixty plants were watered with 15 mL of tap water supplemented with 100 mM NaCl for the stress conditions. Sixty other plants watered with the same volume of tap water (~20 mM NaCl) were used as controls.

The salt effect on plant growth was estimated for 25 days by monitoring the growth of stem and roots of both hybrid and BF15 lines. Leaves and roots samples were taken from six harvested plants at 2, 4, 6, 8, 10, 12 and 15 days to analyse chlorophyll content as well as the lipid peroxidation products and to assess the CAT, glutathione peroxidase (GPX) and SOD activities. The $Na^+$ and $K^+$ contents were estimated after 25 days of culture.

The data were recorded from three separate experiments.

## Measurement of MDA content

Lipid peroxidation was estimated by measuring the level of MDA production using the thiobarbituric acid (TBA) method as described by Hodges et al. (1999) but modified here to take into account the specific turbidity of the solution tested by measuring the OD at 600 nm.

The analytical method involves homogenizing the fresh material (150 mg) in 1.5 mL of 0.1% TCA followed by centrifugation at 13,000 g for 30 min at 4°C. The supernatant was mixed with 2 volumes of TBA/TCA solution (0.8% TBA and 15% TCA dissolved in 0.25 N HCl). The mixture was then heated at 95°C for 15 min. After centrifugation at 13.000*g* for 10 min, the absorbance of the supernatant was determined at 532 and 600 nm. The MDA concentration was calculated based on a standard curve of MDA (0.25–0.5–2.5–5–7.5 µM).

## Extraction and determination of protein content

Proteins were extracted from the roots and leaves by grinding tissues in a mortar in the presence of 3 volumes of EPSO buffer (10 mM Tris–HCl pH 8; 10 mM EDTA; 50 mM KCl; 20 mM $MgCl_2$; 1 mM DTT; 0.1 % Triton X100; 10 % PVP (polyvinylpyrrolidone); 0.5 mM PMSF (phenylmethylsulfonyl fluoride). A 30 min centrifugation at 4°C and 14,000*g* was then performed to retrieve the proteins from the supernatant.

The soluble protein concentration was determined as described by Bradford (1976). 5 µL of supernatant were diluted in 200 µL of Bradford reagent, and 795 µL of distilled water were added. After 10 min of incubation, the optical density was measured at 595 nm.

The soluble protein content was determined by reference to a standard curve corresponding to: 0, 2, 4, 6, 8, 12 and 16 mg $mL^{-1}$ BSA (bovine serum albumin).

## Determination of CAT activity

CAT activity was measured as described by Aebi (1984). This method is based on the measurement of the decrease of the absorbance at 240 nm because of the dismutation of $H_2O_2$. The amount of $H_2O_2$ converted into $H_2O$ and $O_2$ per min under standard conditions is accepted as the enzyme reaction velocity.

Assays were carried out at room temperature in a final volume of 3 mL containing 100 µL of extract added to 0.1 M phosphate buffer (pH 7) supplemented with 10 mM $H_2O_2$. The CAT activity was determined as follows: CAT U/mL: [(3.45 × slope)/0.05] × (1,000 µL/50 µL)].

## Determination of SOD activity

The SOD activity was determined spectrophotometrically using the pyrogallol assay (Ben Mansour et al. 2008). This technique is based on the competition between the dismutation reaction and the oxidation of pyrogallol. The rate of inhibition of pyrogallol oxidation is proportional to the

SOD activity. A sample of protein extract (25 µL), was mixed with 935 µL of Tris-cacodylic acid diethylene triamine penta-acetic acid buffer (pH 8-8.2). Pyrogallol (40 µL) was added to this mixture at time zero. The decline in OD at 420 nm (ODE) was measured after 1 min of reaction.

The percentage of inhibition of pyrogallol oxidation was determined using the following formula: Inhibition (%) = [(OD max − ODE/OD max] × 100; where OD max (0.030–0.031) is the change in absorbance at 420 nm of the control test after 1 min of reaction. It corresponds to 0 % inhibition of pyrogallol oxidation.

### Determination of GPX activity

GPX allows the oxidation of glutathione (GSH) by $H_2O_2$ in the presence of 5-5′-dithio-bis 2-nitrobenzoic acid (DTNB).

GPX activity was measured by determining the level of reduced glutathione (GSH) at 412 nm based on the published method Floh and Gunzler (1984). It involved mixing 200 µL of enzyme extract and 400 µL of a 0.1 mM GSH solution in 200 µl of phosphate buffer (67 mM; pH 7.8). The mixture was incubated at 25°C for 5 min, and 200 µL of 1.3 mM $H_2O_2$ were then added and incubation pursued for 10 min. The reaction mixture was supplemented with 1 mL 1 % TCA. After centrifugation at 3,000 rpm for 10 min, the supernatant (480 µL) was removed and 2.2 mL of 0.32 M $Na_2HPO_4$ and 320 µL of 1 mM DTNB were added. The oxidized glutathione (GSSG) produced was measured at 412 nm. The GPX activity was calculated as follows:

$$\mu M\ GSH_{\text{Reduced disappeared/min/mg of protein}} = \left[\left(\frac{OD_{essai} - OD_{blanc}}{OD_{blanc}}\right) \times \left(\frac{0.04 \times 5}{X \times 10}\right)\right]$$

X: protein concentration (mg $mL^{-1}$); 0.04: initial amount of reduced GSH; 5: to move from activity in 200 µL to activity in 1 mL; 10: reaction time.

### Determination of the chlorophyll content

Leaf discs (0.01 g) were ground in a mortar in the presence of 500 µL of pure acetone supplemented with 1 mL of 80 % acetone. The mixture was then centrifuged for 15 min at 12,000 rpm and the supernatant volume was adjusted to 2 mL with 80 % acetone. Absorbance measurements in the supernatant at 645 and 663 nm were performed. The results represent the average of three individual experiments. The rates of chlorophylls a and b were determined according to the formulae of Arnon (1949):

$$\text{Chlorophyll a } \left(\mu g\, mL^{-1}\right) = (12.7 \times OD_{663nm}) - (2.69 \times OD_{645nm})$$

$$\text{Chlorophyll b } \left(\mu g\, mL^{-1}\right) = (22.9 \times OD_{645nm}) - (4.68 \times OD_{663nm})$$

### Determination of the mineral content

The determination of the major ion contents ($Na^+$ and $K^+$) was performed for different organs of the plants grown in a greenhouse for 25 days in the absence or presence of 100 mM NaCl. The weighed fresh material was dried for 48 h in an oven at 80°C (Väänänen et al. 2005). The dry matter obtained was calcinated at 550°C for 2 h in a muffle furnace. The ashes obtained were treated with 5 ml of hot concentrated nitric acid. The extracts were then filtered and the final volume adjusted to 25 mL with distilled water. The mineral contents were determined by using a ZenithP700 atomic absorption spectrophotometer (Analytik Jena).

### Statistical analyses

Values are expressed as mean ± standard deviation. The statistical studies were carried out with the ANOVA test using the statistical software GraphPad Prism Version 5, taking $p < 0.05$ as significant.

## Results

### Effect of salinity on plant growth and development

Plants from hybrid lines and parental lines were transferred to a greenhouse and sprinkled with tap water supplemented with 100 mM NaCl. Plants watered only with tap water were used as controls.

Salt effect on plant growth was estimated for 25 days by monitoring the growth of organs and by determining the chlorophyll content in the leaves.

The elongation of stems and the determination of root weight of the different lines were followed to estimate plant growth in the presence or absence of salt stress (Fig. 1). The growth rate of the STBc and STBd hybrid plants was almost similar in the presence or absence of salt. However, significant growth inhibition was observed for plants from the BF15 parent and STBa hybrid subjected to salt stress. The inhibition rate was much more important for the BF15 parent than for the STBa hybrid. This inhibition was associated with symptoms of yellowing and necrosis of some of leaves (Fig. S1) and a high mortality rate in the BF15 variety (30 %) after 25 days of culture.

Although the STBa hybrid showed inhibition of stem elongation, it maintained significant development of its

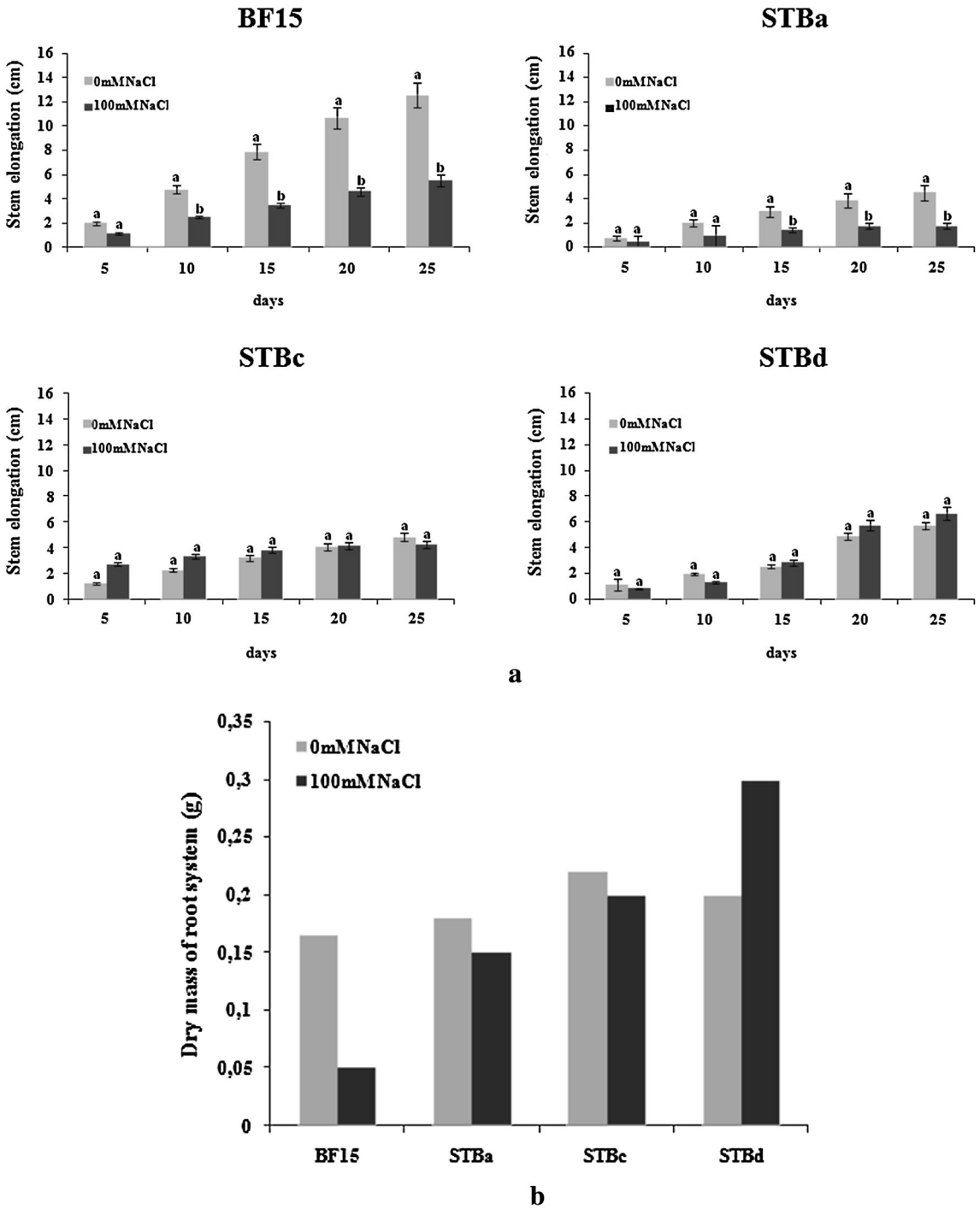


**Fig. 1** Stem elongation for 25 days (**a**) and dry mass of root system at the end of the treatment (**b**) of the plant cultivated under standard or salt stress conditions. Each value was represented by the mean ± SD. Means followed by the *same letter* indicated a highly significant difference between the stressed plant and the control plant ($p < 0.05$)

roots in the presence of salt and also showed a mortality rate (18 %) similar to that of the STBc line (20 %). Nevertheless the STBd line seems to be the most tolerant since no mortality was noticed on plants cultivated for 25 days in the presence of 100 mM NaCl.

## Impact of salt stress on the chlorophyll levels

The chlorophyll a/chlorophyll b ratio was determined in stressed and control plants during the first 15 days of salt treatment (Fig. 2). This ratio remained constant in the

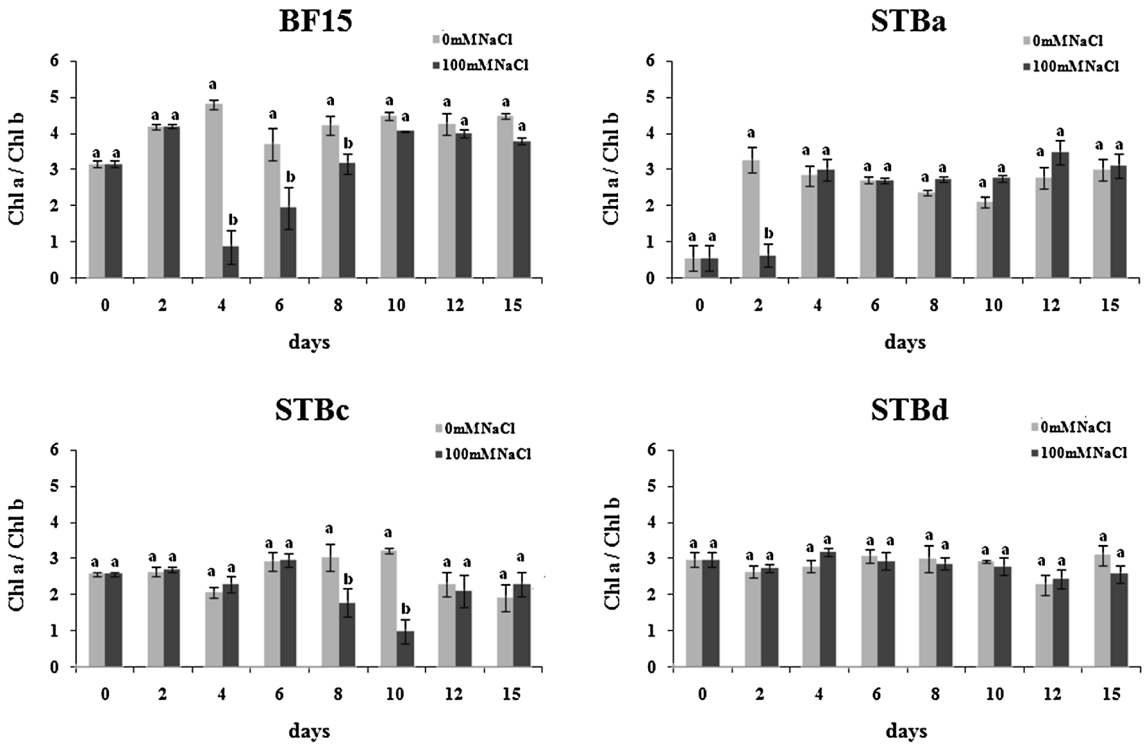


**Fig. 2** Variation of the chlorophyll a/chlorophyll b ratio (Chl.a/Chl.b) for 15 days of stress in different lines cultivated under standard or salt stress conditions. Each value was represented by the mean ± SD. Means followed by the *different letter* indicated a highly significant difference between the stressed plant and the control plant ($p < 0.05$)

presence of stress for the STBd hybrid during the first 2 weeks of treatment. This could reflect a better control of photosynthetic activity in this line compared to that of the parental line. A decrease in relative ratio of chlorophyll was observed in the BF15 parent after 4 days of stress. This decrease was also observed in plants of the STBa hybrid after 2 days of stress. However, a rapid recovery (as of day 4) was observed regarding this line suggesting some adaptation to stress conditions. The plants of the STBc line showed a decrease in chlorophyll a/chlorophyll b ratio from days 8–12 followed by a recovery later on. This behaviour suggests salinity tolerance of this line.

## Analysis of some oxidative stress parameters

### *Measurement of MDA*

The degree of lipid peroxidation established in the organs was estimated by the MDA assay in leaves and roots of plants.

The MDA contents in the organs were determined every 2 days in plants of the different lines cultivated for 15 days in the absence or presence of salt (Fig. 3). The presence of NaCl in the medium led to an increase of the MDA levels in the leaves of the BF15 parental line. However, in all plant lines the MDA levels in the leaves were higher than those in roots. Plants from the STBd line showed the lowest levels of MDA content (Fig. S2). The STBa line showed a significant increase of MDA content on day 6 both in roots and leaves, but these MDA levels stabilized after 8–10 days of culture both in the presence and absence of salt. These data suggest adaptation and salt stress tolerance of the plants. These results also suggest some membrane stability in the presence of salt stress as regards the hybrid lines. This stability has been associated with salt tolerance in plants. Lipid peroxidation was more important in the BF15 parents cultivated in saline conditions. It reached its maximal level in the roots after 10 days of culture and after 8 days in the leaves. The STBa line showed a similar behavior to BF15 but displayed a better tolerance to salt stress compared to that of BF15.

## Measurement of antioxidant enzyme activities

### *SOD activity*

The SOD activity was estimated by measuring the percent inhibition of the pyrogallol oxidation using the protein extract from leaves or roots of the stressed and control plants. When measured, SOD activities were found to be higher in roots than in leaves for all plant lines (Fig. 4). The SOD activity was higher in the hybrids compared to

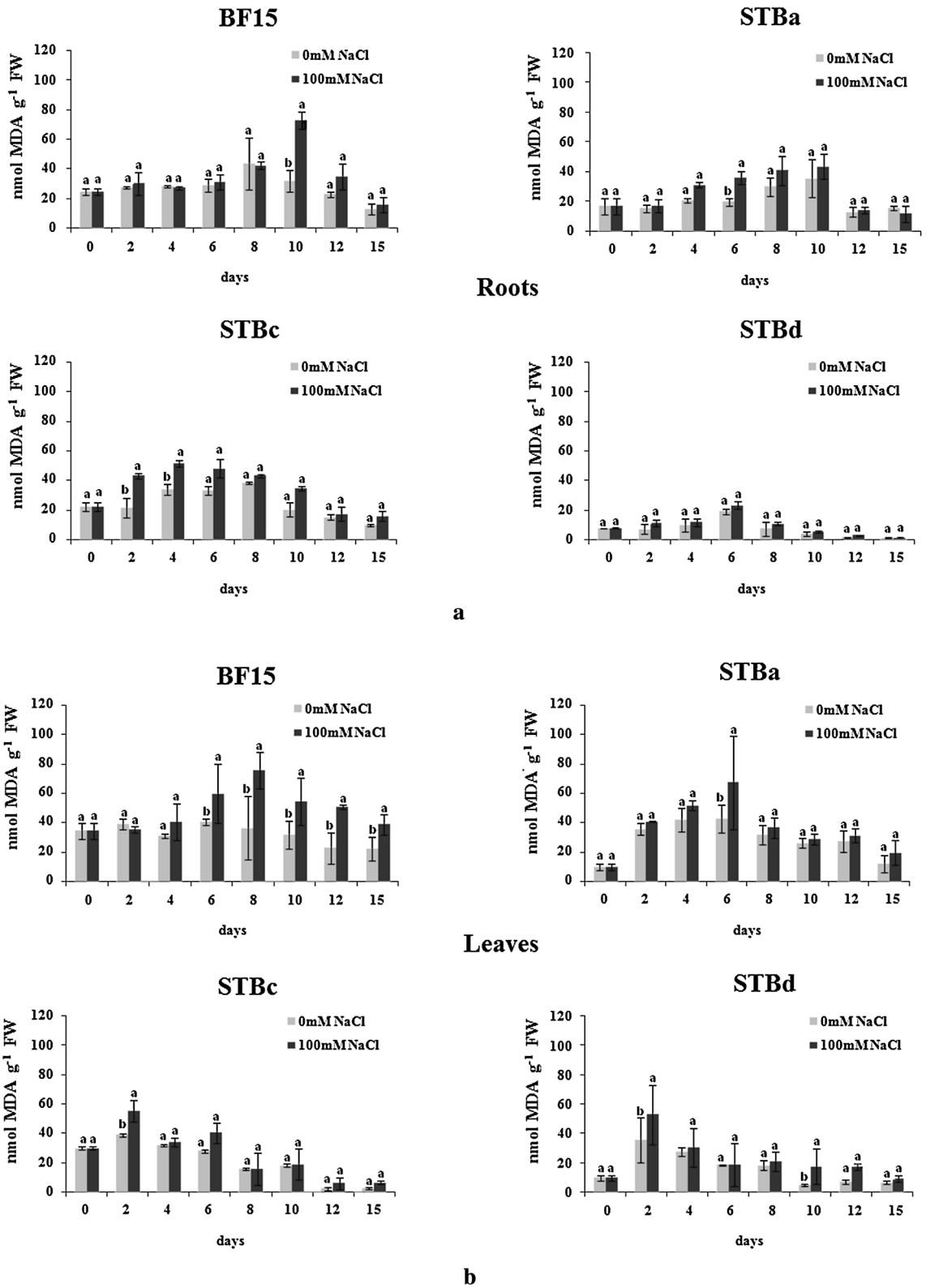


**Fig. 3** Measurement of MDA contents in the roots (**a**) and leaves (**b**) of different hybrid lines (STBa, STBc, STBd) and their BF15 parent cultivated under standard or salt stress conditions. Each value was represented by the mean ± SD. Means followed by the *same letter* indicated a highly significant difference between the stressed plant and the control plant ($p < 0.05$)

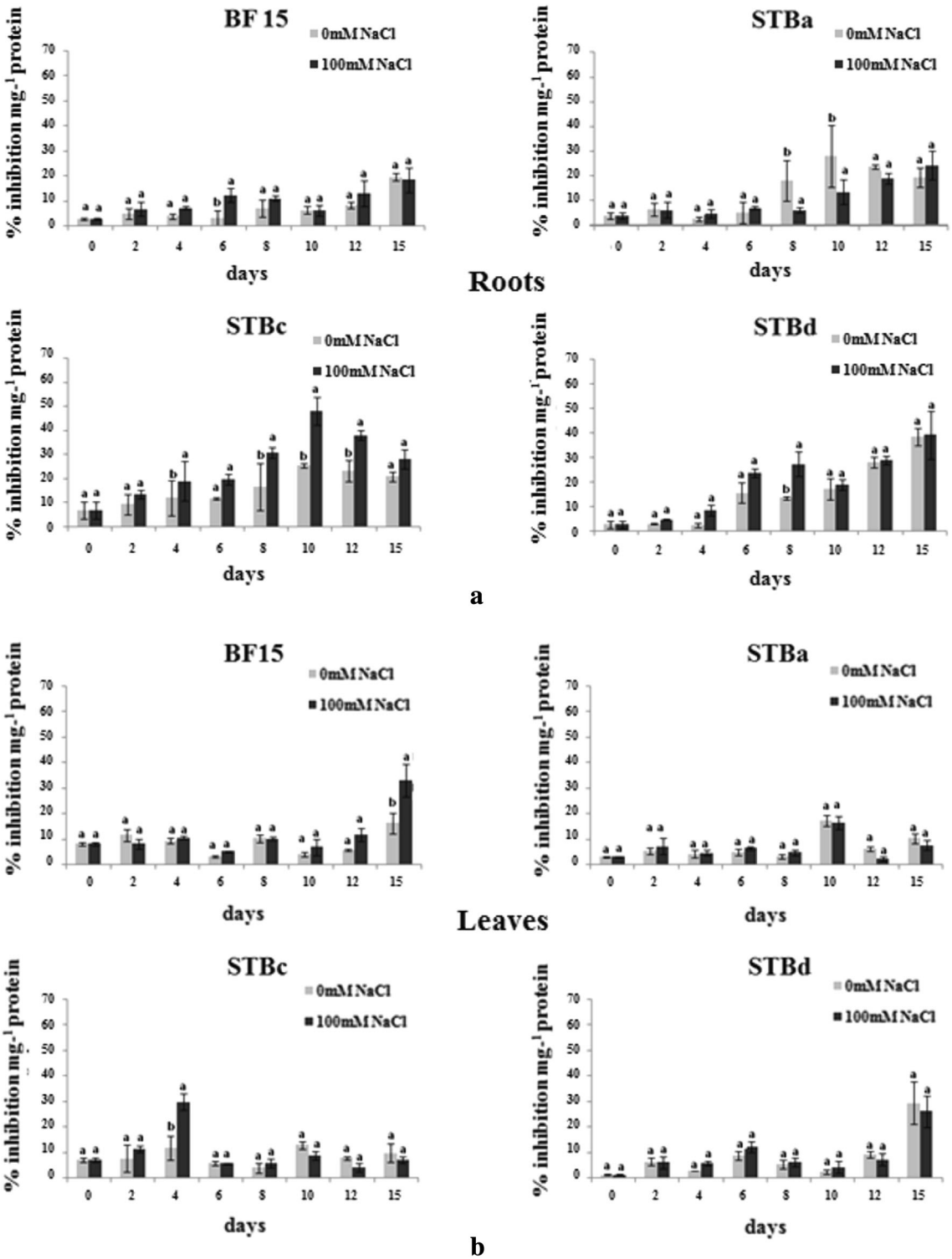


**Fig. 4** Estimation of SOD activity in the roots (**a**) and leaves (**b**) of different hybrid lines (STBa, STBc, STBd) and their BF15 parent cultivated under standard or salt stress conditions. Each value was represented by the mean ± SD. Means followed by the *same letter* indicated a highly significant difference between the stressed plant and the control plant ($p < 0.05$)

the BF15 parental variety (Fig. S3). It increased significantly in the roots of stressed STBc plants starting on the fourth day and seemed to reach its maximum on day 10. An increase of SOD activity was also observed in the roots of stressed STBd plants on days 6–8. Beyond this period, the SOD activity was stable and similar to that measured in

control plants. These results support the low level of MDA content observed in the roots of these plants. They suggest a better stress tolerance of these lines compared to the BF15 parent line. The STBa line showed a similar evolution of the SOD activity when compared to the BF15 parent. However, higher enzyme levels than in BF15 were observed in this line. Very low SOD activities were measured in leaves of all the plant lines.

*POX and CAT activities*

The effectiveness of the $H_2O_2$ removal mechanism generated by SOD between the different lines was evaluated by measuring the CAT and peroxidase activity systems in the roots and leaves of plants grown in the presence of salt.

*POX activity*

Hydrogen peroxide formed by SOD dismutation of superoxide anions, may be removed by the peroxidase system. Thus, the measurement of the glutathione peroxidase activity in roots and leaves of plants cultured in the absence or presence of salt was used to evaluate the effectiveness of $H_2O_2$ removal in the different lines (Fig. 5). This assay made it possible to assess the effectiveness of not only the elimination of $H_2O_2$ but also of toxic lipid peroxides resulting from the peroxidation of fatty acids in different cellular compartments (Noctor et al. 2002).

The most important GPX activity was observed in the roots of the STBc hybrid which showed a significant increase in the presence of salt after 6 days of salt treatment (Fig. S4A). Similarly a moderate increase of GPX was also observed in the roots of the STBa hybrid after 6 days of salt stress. These results would support the salt tolerance of such hybrids that exhibited an increased ability to destroy ROS and thus a better control of membrane oxidative damage generated by salt stress conditions. No significant increase in GPX activity was noticed in leaves of all plant lines cultivated either under salt stress or control conditions (Fig. S4B).

*CAT activity*

The CAT activities measured in the different lines showed the highest levels in the roots of the STBc and STBd hybrids (Fig. 6a). They became important from the 10th day of treatment. The highest increase was in the STBd line submitted to salt stress. In contrast, significantly low levels of CAT were registered in the BF15 parent and STBa hybrid (Fig. S5).

The CAT activity remained low in the leaves of the BF15, STBa and STBc lines while it increased slightly in STBd (Fig. 6b). These results confirm the best response to salt stress in this hybrid line.

These data therefore suggest that the plant response to salinity is most effective in the roots leading to less oxidative damage in the leaves. The STBd hybrid displays the best antioxidant activity.

Analysis of the content of some minerals

The levels of $Na^+$ and $K^+$ were determined in roots, stems and leaves of plants cultivated for 25 days in the absence or in the presence of salt (100 mM NaCl). An influx of $Na^+$ in the various organs was observed in plants cultivated under salt stress conditions (Table 1). The highest level of $Na^+$ accumulation was observed in roots and leaves of the BF15 parent line. All the hybrid lines displayed significantly lower $Na^+$ accumulation especially in the leaves. The STBa line showed the best control of $Na^+$ influx in the organs. It was followed by STBc and then by STBd. These results suggest a better regulation of ionic homeostasis in hybrids compared to the parent BF15. They also allowed to better understand the response of the different lines to oxidative stress. The presence of NaCl in the medium could affect the plant uptake of cations such as $K^+$ which could cause nutritional stress. The hybrids showed a better readjustment of the $K^+$ level to maintain the selectivity $K^+/Na^+$ in the leaves than the BF15 parental line (Table 1).

## Discussion

The development of salt-tolerant crops is arguably the most important strategy to mitigate the salinity adverse effects. Success, however, has been limited, largely due to the genetic and physiological complexity of the salt tolerance trait. Understanding the physiological processes involved in salt tolerance is essential to foster the improvement of salt tolerance in crops and to identify markers that might help develop agronomic strategies to improve crop yields in soils affected by salinity. As many features associated with salt tolerance are expressed at the cellular level, in vitro selection of salt stress tolerant material provides useful model systems to study the salt tolerance mechanisms (Tester and Davenport 2003).

In a previous report we showed that the STB potato interspecific hybrid lines displayed tolerance to salt stress under in vitro culture conditions. The results presented here and those obtained previously (Bidani et al. 2007) suggest that different salt tolerance mechanisms are undertaken by these hybrid lines. The variation in salt tolerance level observed in these somatic hybrids may be due to different

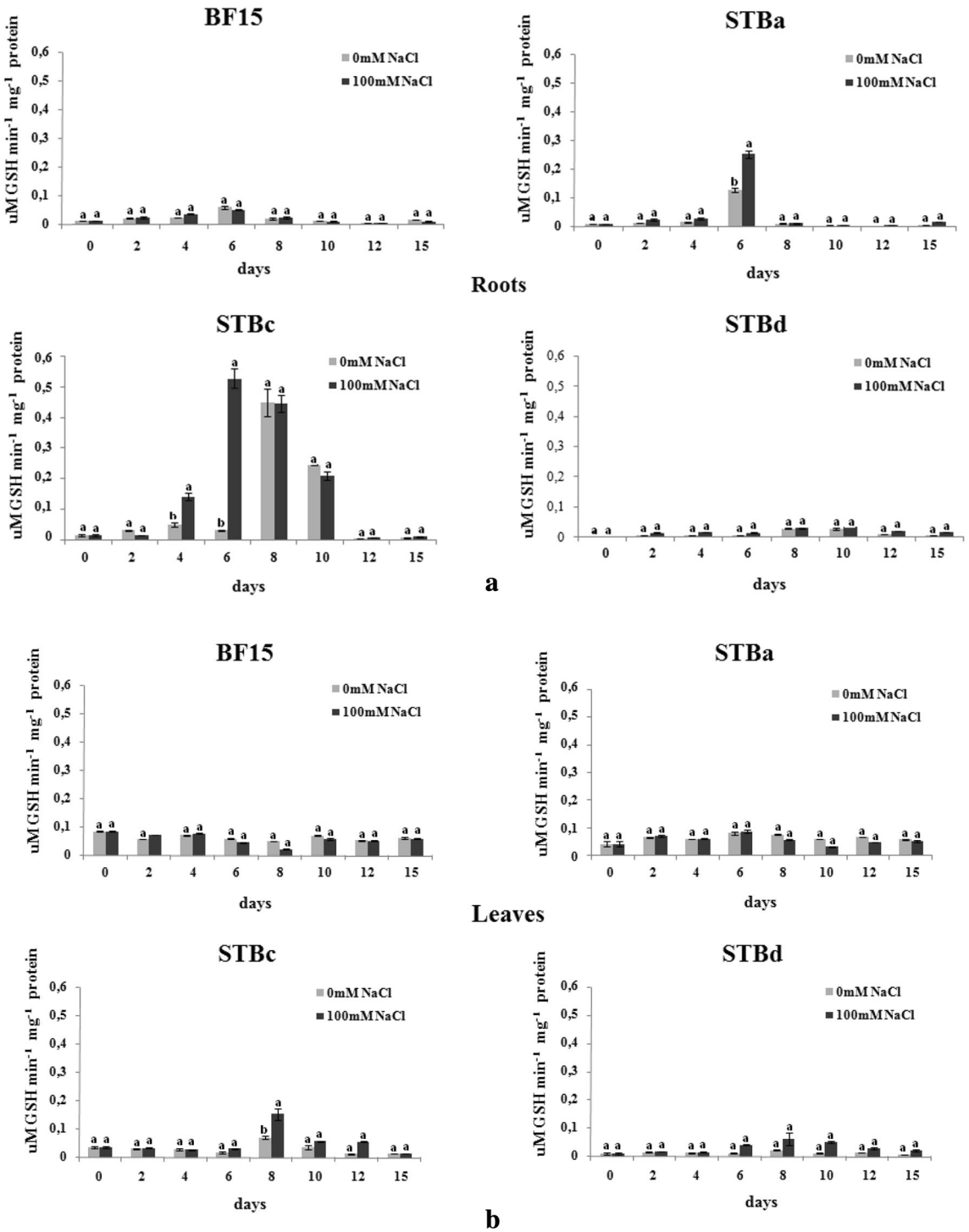


**Fig. 5** Measurement of GPX activity in the roots (**a**) and leaves (**b**) of different hybrid lines (STBa, STBc, STBd) and their BF15 parent cultivated under standard or salt stress conditions. Each value was represented by the mean ± SD. Means followed by the *same letter* indicated a highly significant difference between the stressed plant and the control plant ($p < 0.05$)

recombination events between parental genomes (Xu et al. 2014).

Excess $Na^+$ disturbs cellular ion homeostasis, and can lead to oxidative stress. To prevent these problems, plants developed different strategies to suppress the influx and accumulation of $Na^+$ especially in roots. Such inhibition of $Na^+$ influx in plant tissues was observed in the potato hybrid lines, but not in their BF15 parent that presented

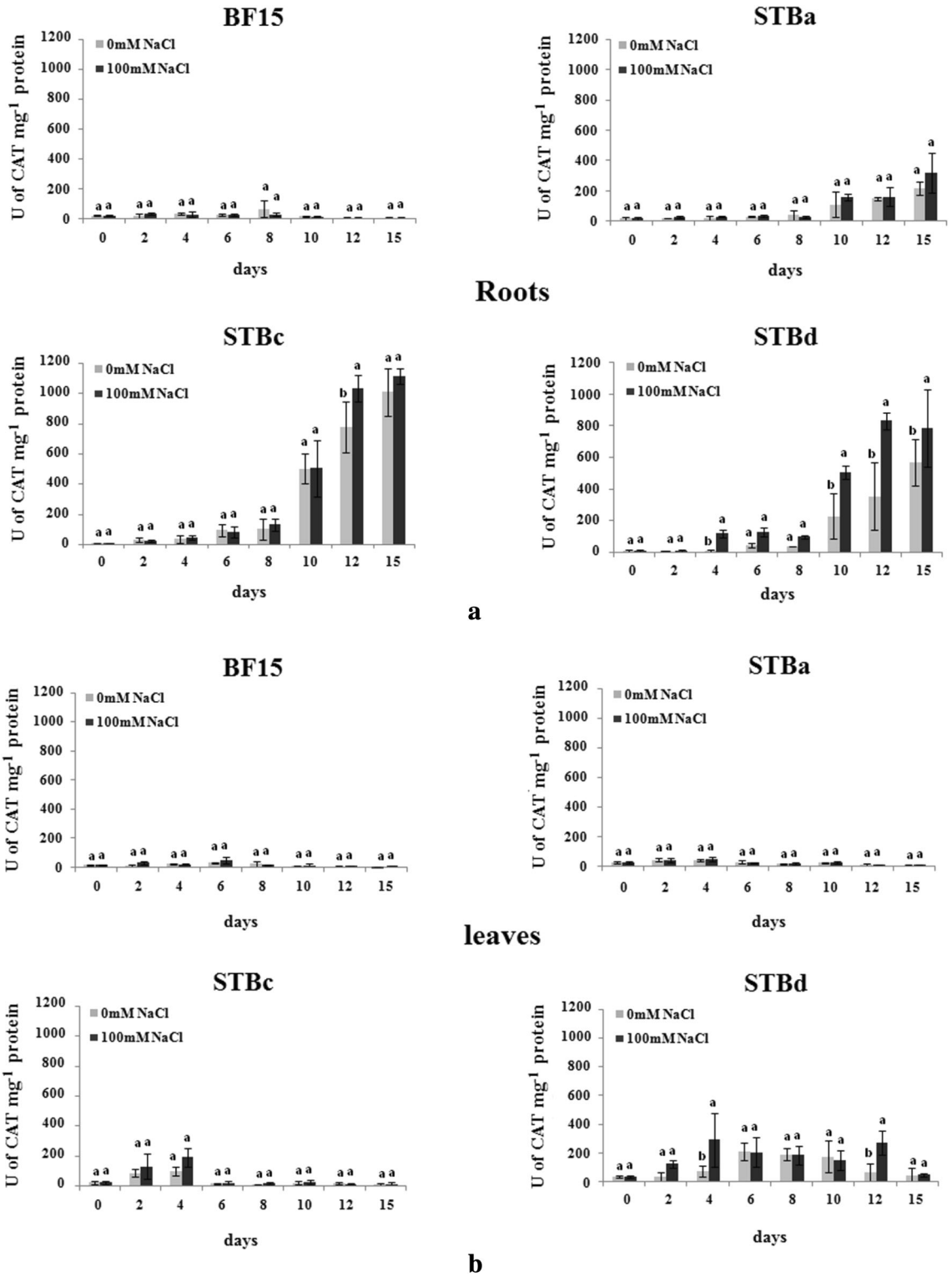


**Fig. 6** Measurement of CAT activity in the roots (**a**) and leaves (**b**) of different hybrid lines (STBa, STBc, STBd) and their BF15 parent cultivated under standard or salt stress conditions. Each value was represented by the mean ± SD. Means followed by the *same letter* indicated a highly significant difference between the stressed plant and the control plant ($p < 0.05$)

more important $Na^+$ accumulation than the hybrids. The best control of $Na^+$ influx in roots and leaves was observed in the STBa plant followed by the STBc line. These data corroborate those described by Takahashi et al. (2007) who reported that salt-sensitive plants accumulated more $Na^+$ than salt-tolerant plants that upset the ion balance.

**Table 1** Analysis of the accumulation of $Na^+$ and $K^+/Na^+$ ratio in organs

| | BF15 | | STBa | | STBc | | STBd | |
|---|---|---|---|---|---|---|---|---|
| | 0 mM NaCl | 100 mM NaCl | 0 mM NaCl | 100 mM NaCl | 0 mM NaCl | 100 mM NaCl | 0 mM NaCl | 100 mM NaCl |
| Accumulation of $Na^+$ (%) | | | | | | | | |
| Roots | | 84 | | 20 | | 42 | | 58 |
| Stem | | 23 | | 34 | | 16 | | 02 |
| Leaves | | 43 | | 12 | | 11 | | 20 |
| $K^+/Na^+$ | | | | | | | | |
| Roots | 0.50 | 0.35 | 0.80 | 0.25 | 0.80 | 1.11 | 0.65 | 0.71 |
| Leaves | 7.25 | 1.00 | 0.75 | 0.72 | 1.91 | 3.20 | 2.10 | 3.65 |

$$\text{Percentage of } Na^+ \text{ accumulation in stressed organ} = \frac{Na^+ \text{ accumulation in stressed organ} - Na^+ \text{ accumulation in control organ} \times 100}{Na^+ \text{ concentration in control organ}}$$

To prevent excessive accumulation of $Na^+$ in the cytoplasm, plants can develop selective ion uptake through high-affinity potassium transporters. The intracellular $K^+/Na^+$ balance is an important factor in salt tolerance, reflecting the selectivity and maintaining a high level of $K^+$ by restricting the entry of $Na^+$ (Mäser et al. 2002; Abideen et al. 2014; Tang et al. 2014). Such $K^+/Na^+$ control was clearly observed in all the hybrid lines. These results further support the tolerance of these hybrids to salt stress (Kahlaoui et al. 2011).

In this study, the increase in NaCl and stress duration had a negative influence on the growth of the parental BF15 variety. This line also showed leaf yellowing suggesting a disturbance of photosynthetic efficiency that might be due to salinity.

The salinity did not cause significant inhibition of the growth parameters such as root dry weight of the potato hybrid lines. The STBd line exhibited the highest salt tolerance as a function of roots dry weight. Our results are similar to those described by Sobhanian et al. (2011) who reported that generally, salt-tolerant *Poaceae* species exhibited an increase in fresh and dry mass when grown in NaCl-supplemented media. The negative effect of salinity on plant growth was due mainly to the increase in $Na^+$ content in the leaves observed in the sensitive parental cultivars (Marvin et al. 2011).

Stability of biological membranes has been taken as a screening tool to assess the effects of salinity stress (Kukreja et al. 2005). Salinity has a pronounced effect on the peroxidation of membrane lipids. It enhances their permeability and modulates the patterns of ion leakage (Kukreja et al. 2005).

MDA, a secondary breakdown product of lipid peroxidation, has been widely used as an indicator of oxidative damage (Baby and Jini 2011; Costa et al. 2005). Our data show that MDA accumulation in salt-stressed STB hybrid lines was lower than that of the BF15 potato parent. The highest MDA accumulation was observed after 10 days of stress in the 100 mM NaCl-treated BF15 parental line. STBa also showed a significant increase in the MDA levels in the leaves that coincided with the maintenance of chlorophyll levels and a low $Na^+$ uptake. These data suggest that treatment with 100 mM NaCl may result in a shock that leads to photo-oxidative stress, resulting in MDA accumulation in leaves. Similarly, it was indicated (Tounekti et al. 2011) that the highest MDA accumulation observed in stressed Rosemary plants also coincided with maintenance of chlorophyll levels. It is worth noting that chlorophyll loss is an efficient photoprotection mechanism that finely modulates the amount of light intercepted by leaves.

However, as stress progressed, hybrid plants were able to limit the extent of MDA accumulation in leaves. These data are in agreement with other studies (Ruiz et al. 2005) that concluded that lipid peroxidation in salt-sensitive lines increased more than in those of salt-tolerant lines. The MDA increase observed in roots can be related mainly to mitochondrial production of ROS (Gill and Tuteja 2010).

There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids. It can also have a negative effect on protein synthesis, energy production, and lipid metabolism. However, tolerant species have an excellent capacity to protect themselves from salt-induced oxidative stress through mechanisms of photoprotection and antioxidant protection (Azevedo Neto et al. 2006).

Salt treatment has a negative effect on the chlorophyll content in sensitive plants (Mostan et al. 1988; Parida and Das 2005) while an increase in chlorophyll content can be observed in the presence of salt in tolerant lines (García-Valenzuela et al. 2005; Surekha et al. 2014). Such increase of chlorophyll content was observed in all hybrid lines cultivated for 15 days under salt stress conditions. However, the BF15 chlorophyll content decreased during the same treatment period (Table. S1). STBd and STBa hybrid lines displayed a constant Chlorophyll a/chlorophyll b ratio during 15 days of salt treatment while the STBc line seems to behave similarly to BF15 parent. Indeed, the latter line

showed an important decrease ratio at day 4 followed by a recovery whereas the Chlorophyll a/chlorophyll b ratio decreased less than BF15 at day 8 and 10 in STBc. A recovery was observed later on. All these data confirm the salt tolerance of hybrid lines.

Antioxidant enzymes are important components in preventing oxidative stress in plants by scavenging free radicals and peroxides via the increase of their activities when exposed to stressful conditions. Almost all the antioxidant enzymes are greatly activated in tolerant species under stress conditions (Thounaojam et al. 2012). Such increases in antioxidant enzyme activities were correlated with significantly enhanced salt tolerance (Thounaojam et al. 2012; Long et al. 2014; Shen et al. 2013).

SOD is a key antioxidant enzyme that converts $O_2^{\cdot-}$ into $H_2O_2$ and can be activated by different stress factors. The important increase in SOD activity observed in the STBc and STBd lines may explain the low MDA levels reported in these plants. These data are similar to those of Scandalios (1993) who reported that the efficient protection observed against oxidative damage, caused by salt treatment, resulted from high SOD activities. A strong correlation between the antioxidant defense system and salt tolerance has also been reported in many plants. A constitutively high antioxidant capacity under stress conditions can prevent damages due to ROS formation.

In addition to being an important agent of cellular toxicity, $H_2O_2$, the product of SOD activity, is also an important signal molecule between environmental stress and adaptive response (Seckin et al. 2009). In plants, CAT and GPX are considered to be the most important enzymes regulating intracellular levels of $H_2O_2$. When the activities of the $H_2O_2$ scavenging enzymes were investigated in this report, a significant increase in CAT activity was observed in leaves and roots of STBd and STBc lines. High GPX activity was also observed in roots of salt-stressed STBc plants. Our data suggest that CAT is the most important $H_2O_2$ scavenging enzyme acting in the STBd potato hybrid line under salt stress especially in the roots. Similarly, Marvin et al. (2011) reported that in plant leaves, CAT activity increased and GPX decreased with salinity. The highest GPX activity observed in the roots of the STBc line compared with the other lines indicates that GPX plays a major role in $H_2O_2$ detoxification in this hybrid line. Peroxidase activities, alternative modes of $H_2O_2$ destruction, are found throughout the cell and have much greater affinity for $H_2O_2$ than CAT (Jiménez et al. 1997).

In this study, GPX appeared to be an important enzyme in overcoming NaCl-imposed oxidative stress since there was a considerable increase in GPX activities in the STBc and STBa lines, especially in the roots, as reported regarding other plant species (Azevedo Neto et al. 2006). The STBc hybrid line exhibited the highest CAT and GPX activities in the roots and leaves suggesting that CAT and GPX activities coordinated with SOD activity play a central protective role in the $O_2^{\cdot-}$ and $H_2O_2$ scavenging process (Liang et al. 2003). The active involvement of these enzymes is related, at least in part, to salt-induced oxidative stress tolerance in hybrid potato lines. Similarly, the activity of the $H_2O_2$ scavenging enzymes has been shown to be closely related and enhanced by NaCl stress in other species such as maize (Neta-Sharir et al. 2005).

Our data also show that CAT and GPX have a much higher $H_2O_2$ scavenging activity in roots than in leaves of both control and salt-stressed hybrid lines. These results are in agreement with those of others (Shalata et al. 2001) who suggested that the reduction of MDA content in *Lycopersicon pennellii* was due to increased antioxidative enzyme activities that reduced $H_2O_2$ levels and membrane damage. Therefore, it could be hypothesized that under salt stress, CAT is the most important among the $H_2O_2$ scavenging enzymes in leaves and roots of the STBc and STBd lines, while GPX seems to play a key role in STBc and STBa leaves and especially in STBc roots. The same data were reported for other species such as mulberry, cotton and barley (Liang et al. 2003).

## Conclusion

The assessment of the salinity effect on the growth parameters in potato somatic hybrid lines made us conclude that all parameters were affected by salinity in the BF15 salt sensitive parent. However, STBc and STBd can be considered as tolerant while STBa exhibited an intermediate behavior. Moreover, the use of some enzymatic antioxidants as potential selection criteria to improve plant salt tolerance was considered here as reported by others (Ashraf 2010).

A close relationship between the antioxidant capacity and NaCl tolerance has been demonstrated in numerous crops. Indeed, under salt stress, the antioxidant system of hybrid lines revealed that CAT, GPX activities in conjunction with SOD might have an essential protective role in scavenging of ROS. Further studies, especially dealing with gene expression profiles of CAT, GPX and SOD, need to be conducted to better understand the complex response of hybrid lines to salt stress.

The field performance of these salt tolerant STB somatic hybrids needs to be evaluated in the future to support the results obtained in the greenhouse study.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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