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Salt stress affects mitotic activity and modulates antioxidant systems in onion roots

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Abstract This study was aimed to explore the effect of short-term salt stress and recovery on cytology and the activity of antioxidant enzyme in roots of onion (*Allium cepa* L.) plant. Roots of *A. cepa* were treated with different concentrations of NaCl (0, 50, 100, 150, 200 mM), and cytological and physiological indicators in the root of plant were measured in different processing time. With the increase of NaCl concentrations and processing time, mitotic activity and relative division rate (RDR) were reduced. However, during recovery, mitotic activity and RDR were restored but their values were less than control values. Salt stress caused an increase while in recovery experiments there was a slight reduction in chromosomal aberrations. A significant increase in SOD and POX activities except in 200 mM occurred after 18 h of stress which was still higher than control in recovered plants. CAT activity showed 53.90 % decrease after 18 h of salt stress and also a significant decrease was observed after 24 h post stress. The results suggest that at high salt stress up to 150 mM, the roots of *A. cepa* are capable to rapidly activate antioxidant defence system to resist the salt-induced oxidative stress, but could not control the cytogenetical activities. The results also suggest that the recovery is possible at physiological and cytogenetical level by retaining chromosomal and DNA integrity.

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Keywords Abnormalities · Antioxidant enzyme · Post stress · Salt stress

Abbreviations

MI	Mitotic index
RDR	Relative division rate
ROS	Reactive oxygen species
S	Stickiness
C	Clumping
B	Bridge
Dist. Meta	Disturbed metaphase
Dist. Ana	Disturbed anaphase
Bc	Binucleated cells
DN	Disorganized nucleus
NB	Nuclear bud
FW	Fresh weight
CAT	Catalase
POX	Peroxidase
SOD	Superoxide dismutase

Introduction

Soil salinity is one of the most important abiotic stresses limiting the productivity of agricultural land. It has been estimated that about 8.6 mha land of India is affected by salinity, and this issue is increasing every year (Pathak 2000). The water used for irrigation has sometimes a high concentration of salts and there is no possibility of exporting this salt to a sink, so salts can accumulate in the soil. Plants can tolerate and cope up with the adverse effect of salt stress by generating various mechanisms subject to the salinity sensitive and tolerant of plants which varies with plant species like halophytes (*Limonium cossonianum*,

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Kandelia candel) (Luque et al. 2013; Wang et al. 2014) and glycophytes (*Solanum lycopersicum*, *Solanum chilense*) (Martinez et al. 2014). The prevention of salt-mediated DNA fragmentation and apoptosis was strived by overexpression of anti-apoptotic genes such as Bcl2 or SOS1 in *Arabidopsis* (DeWald et al. 2001). Being primary receptor of salt stress, roots act as the most reliable system to study the cellular and molecular mechanism (Munns and Tester 2008). Salt stress brings membrane blebbing, DNA fragmentation and deformation in cells and its organelle. Earlier many studies reported that salt can induce DNA fragmentation in barley roots and programmed cell death in root cells of rice (Lin et al. 2006; Li et al. 2007). The outcomes from high salinity generally resulted in slow root and shoot growth; yield loss and can often lead to death. Salinity deteriorates plant growth by increasing ionic toxicity, changes in water relations, impairment of mineral nutrition and oxidative stress (Bray et al. 2000; Munns and Tester 2008). The accumulation of Na^+ and Cl^- ions can limit the germination and development of several species that lead to morphological, biochemical, cellular and molecular alterations (Lima and Bull 2008; Barroso et al. 2010).

In addition to osmotic and ionic stress, salinity also generates secondary stress such as oxidative stress which imbalances the cellular redox causing overproduction of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$). The production of ROS and its scavenging under normal condition are adequately regulated by cell metabolism; however, under abiotic condition, plants induce for high ROS production to unsustainable levels (Liszkay et al. 2004). Excessive amounts of ROS in plants are immensely cytotoxic and can damage lipids, proteins and nucleic acids by oxidation.

Plants have complex antioxidant defence mechanisms that consist of enzymatic and non-enzymatic pathways to scavenge excessive ROS. The enzyme SOD, POX and CAT are the most important antioxidant enzyme of plants that play key role in elimination of $O_2^{\bullet -}$ and H_2O_2 (Zhang et al. 2014). It has been reported that genetically engineered plants (e.g. tobacco, rice, wheat) containing higher levels of ROS scavenging enzyme, such as SOD, APX, POX, etc. have improved tolerance to abiotic stresses (Roxas et al. 2000; Xue et al. 2004; Verma et al. 2007).

The short-term exposure to salt stress enhances free radicals formation in plants, which can be ascertained through cytological and biochemical alterations (Radić et al. 2005; Achenbach and Brix 2014). We have investigated the effect of salt stress in onion roots at different concentrations and time intervals by considering post stress/recovery. The effect of salt stress was measured by analysis of mitotic, chromosomal and antioxidant enzyme activities.

Materials and methods

Plant material and treatments

Healthy and equal-sized bulbs of *Allium cepa* L. ($2n = 16$) were chosen from local market. Prior to use, the loosened outer scales were removed and the dry bases were scraped to expose the root primordia. The bulbs were placed in a tank containing sand under a 12 h photoperiod (Mukherjee and Gichner 2009). After 4 days, bulbs with well developed roots of 2–3 cm were transferred in solution containing different concentrations of NaCl (0, 50, 100, 150 and 200 mM) and plant roots were sampled at 4, 8 and 18 h after treatment. Treatments were stopped by washing the intact roots in running tap water. Then plants from each treatment were transferred to distilled water and roots were sampled at 24 h of post stress (recovery) corresponding to 28, 32 and 42 h. Solution of 0 mM of NaCl was used as the control.

The excised roots were processed for cytological study and analysis of antioxidant enzyme level as follows

Cytological study

For estimation of cell divisions, the roots were treated with 0.05 % colchicines for 3 h, fixed in 3:1 ethanol:acetic acid (v/v), hydrolyzed in 1 N HCl at 60 °C for 5 min and stained in 2 % aceto-carmine. Segments of 1 mm starting from the tip were dissected and squashed (Mišík et al. 2014). For each treatment 3 squash preparations were made; approximately 1000 cells were scored for each slide under a binocular light microscope (Olympus BX 51) at ×40. The number of cells in mitosis, stickiness, clumping, c-mitosis, bridge, disturbed metaphase/anaphase, binucleated cells, disorganized nucleus and nuclear buds were scored.

Calculations

The mitotic index (MI) of 1000 dividing cells was described as percentage of dividing cells. The relative division rate (RDR) was calculated by the method of Hoda et al. (1991). The presence of aberrant cells in dividing cells at each concentration was represented as percentage. Phase indices were also expressed as percentage of the cells in the several phases of division.

Extraction and determination of antioxidant enzyme activities

For extraction of all enzymes, root materials were crushed to a fine powder using ice-cold mortar and pestle in specific

enzyme buffers. The homogenates were centrifuged at 4 °C for 20 min at 15,000×*g*. The supernatant was collected and used for estimation of enzyme activities.

SOD activity was estimated by slight modification of Fridovich (1974) method. The reaction mixture (3 mL) was made of 100 mM sodium phosphate buffer (pH 7.8), deionized water, 3 mM EDTA, 2.25 mM NBT, 0.2 M methionine, 1.5 M sodium carbonate, 2 µM riboflavin and 300 µL enzyme extract. The reaction was initiated at room temperature by placing tubes below 40 W fluorescent mercury tube for 10 min, and were stopped by keeping the tubes in dark. The absorbance of formazone so formed was recorded at 560 nm using a UV–Vis spectrophotometer (UV-2900). One unit of SOD activity was defined as the amount of the enzyme required to inhibit 50 % NBT photoreduction and expressed as units g^{-1} FW.

POX activity was estimated by the method of Britton and Mehley (1955) with slight modifications. The reaction mixture (5 mL) containing phosphate buffer (125 mM, pH 6.8), pyrogallol (50 mM), H_2O_2 (50 mM) and enzyme extract (1 mL), was incubated at 25 °C for 5 min. The reaction was terminated by adding 5 % H_2SO_4 (v/v). The absorbance of reaction mixture was measured at 420 nm and enzyme specific activity was determined and represented as µmol purpurogallin formed g^{-1} FW.

CAT activity was assayed according to the modified method of Aebi (1984). The reaction mixture (3 mL) contained 50 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2 (800 µL) and 200 µL enzyme extract. The CAT activity was measured by monitoring the decrease in absorbance at 240 nm for 1 min ($\varepsilon = 0.036\ mM^{-1}\ cm^{-1}$) and enzyme activity was expressed as mM of H_2O_2 utilized $min^{-1}\ g^{-1}$ FW.

Statistical analysis

Three replicates of each experiment were assessed for statistical validation. The software SPSS 16.0 was used for calculation of standard deviation and one-way analysis of variance (ANOVA) and determined significance at $P \leq 0.05$. Windows-Microsoft Excel 2007 software was employed for plotting graphs of data.

Results

Effect of salt stress on mitotic index, relative division rate and phase indices

The effect of salt stress on MI (%) was summarized in Table 1. According to the results, MI significantly decreased in different concentrations of salt at each exposure time in comparison to control. The highest MI values were obtained from 4 h applications of 50 mM with a score of 8.70 ± 0.82, whereas the minimum MI values were observed for 18 h treatment of 200 mM (1.91 ± 1.45).

There were great variations in MI percentage at different mitotic phases (Fig. 1). The result showed significant variation in the frequency of prophase indices in treated roots than that of control. It reached a maximum frequency of 50.42 % at 200 mM concentration after 18 h of treatment as compared with the control value of 42.71 % (Fig. 1a). On the other hand, the metaphase index decreased among different concentrations of NaCl. The minimum value of metaphase index was observed at 200 mM NaCl (22.29 %) after 18 h of treatment as compared to control (Fig. 1b). However, the frequency of anatelophase was not continuously decreasing, this irregularity was observed after 8 and 18 h of treatment (Fig. 1c).

The relative division rate (RDR) represented severity of mitotic inhibition. At different time periods (4, 8 and 18 h) the RDR values were negative corresponding to applied concentrations of NaCl. The highest RDR value (−2.02 %) was observed at 50 mM NaCl after 4 h of treatment. The percentage of RDR was low at 200 mM NaCl after 18 h of treatment as compared to other concentrations.

Effect of salt stress on chromosomal abnormalities

Table 1 shows different types of chromosomal abnormalities along with reduction in mitotic index. The observation showed that mitotic abnormalities increased with increased concentration and duration of salt treatment. All concentrations of NaCl were capable for inducing various types of chromosomal abnormalities. Various types of abnormalities such as stickiness, clumping, c-mitosis, bridge, disturbed metaphase/anaphase, binucleated cells, disorganized nucleus and nuclear bud were observed (Figs. 2–22). There were few abnormal cells in the non-treated (control) roots. However, the high frequency of various kinds of chromosomal abnormalities was noticed in treated roots. Stickiness in treated cells was predominant at 50 and 100 mM NaCl. Bridges also appeared at all concentration except 50 mM. Whereas binucleated cells were recorded at higher concentration (200 mM). Overall, the total percentage of abnormalities increased in regular order and reached a maximum value after 18 h treatment at 200 mM concentration.

Effect of salt stress on antioxidant enzyme activities

The activity of SOD was affected by generation of superoxide radical. SOD could catalyze the reaction of superoxide radical to generate oxygen and hydrogen peroxide, whereas other enzyme POX/CAT catalyzed the hydrogen peroxide. Effects of salt stress on SOD activities varied with different concentrations of NaCl and duration of treatment. The activities of SOD in the roots of *A. cepa*

Table 1 Chromosomal aberrations induced by salt stress in *Allium cepa*

Treatment	Total cell examined	MI (%) mean ± SD	RDR (%)	Different types of abnormalities (%)							
				S	C and c-mitosis	B	Dist. Meta.	Dist. Ana.	Bc	DN and NB	Total abnormalities
Treatment period: 4 h											
Control	3143	10.51 ± 0.87dc	–	–	–	0.21	–	–	–	–	0.21
50 mM	3122	8.70 ± 0.82c	−2.02	0.32	0.21	–	1.18	0.91	–	–	2.62
100 mM	3178	8.55 ± 0.93bc	−2.19	0.34	0.27	0.09	1.34	1.18	–	0.09	3.31
150 mM	3164	7.15 ± 0.82a	−3.75	0.43	0.39	0.13	2.87	2.53	–	0.13	6.48
200 mM	3252	2.32 ± 1.01a	−9.15	1.11	0.53	0.17	12.45	9.62	0.03	0.39	24.3
Treatment period: 8 h											
Control	3189	10.39 ± 1.34d	–	0.11	–	–	–	0.11	–	–	0.22
50 mM	3076	8.36 ± 0.62 cd	−2.27	0.34	0.29	–	1.61	1.27	–	–	3.51
100 mM	3094	7.91 ± 0.58bc	−2.77	0.36	0.36	0.11	1.97	1.31	–	0.11	4.22
150 mM	2993	6.45 ± 0.50a	−4.39	0.49	0.41	–	3.71	3.79	–	0.17	8.57
200 mM	3112	2.18 ± 0.83a	−9.16	1.23	0.53	0.21	16.53	15.35	0.11	0.49	34.45
Treatment period: 18 h											
Control	3077	10.17 ± 0.98cb	–	0.21	–	–	–	–	–	–	0.21
50 mM	3278	7.86 ± 0.85b	−2.57	0.37	0.37	–	2.34	1.49	–	–	4.57
100 mM	3258	7.55 ± 0.43ab	−2.92	0.41	0.41	0.13	2.91	1.93	–	0.18	5.97
150 mM	3187	6.16 ± 0.17ac	−4.46	0.62	0.49	0.17	4.63	4.31	–	0.17	10.39
200 mM	3161	1.91 ± 1.45a	−9.19	1.63	0.71	–	26.29	26.29	0.39	0.41	55.72
Treatment period: 28 h											
Control	3123	10.71 ± 0.47d	–	0.11	–	0.09	–	0.09	–	–	0.29
50 mM	2974	9.31 ± 1.29cb	−1.57	0.27	0.13	–	0.91	0.86	–	–	2.17
100 mM	2921	8.97 ± 0.42ad	−1.95	0.31	0.27	0.06	1.23	0.89	–	0.06	2.82
150 mM	2779	7.51 ± 1.04b	−3.58	0.43	0.35	0.12	2.57	2.39	–	0.09	5.95
200 mM	2828	2.52 ± 0.71a	−9.17	1.09	0.49	0.15	10.49	7.56	0.09	0.33	20.2
Treatment period: 32 h											
Control	3289	10.47 ± 1.43cb	–	0.19	–	–	–	–	–	–	0.19
50 mM	2780	8.74 ± 0.59b	−1.93	0.29	0.27	–	1.53	1.19	–	–	3.28
100 mM	2922	8.28 ± 1.02a	−2.45	0.33	0.31	0.09	1.89	1.21	–	0.09	3.92
150 mM	2961	6.69 ± 0.68ab	−4.22	0.49	0.43	–	3.53	3.17	–	0.11	7.73
200 mM	2936	2.31 ± 0.92a	−9.11	1.13	0.49	0.11	15.73	14.35	0.17	0.41	32.39
Treatment period: 42 h											
Control	3188	10.66 ± 0.97b	–	0.21	–	–	–	–	–	–	0.21
50 mM	2937	8.49 ± 0.65a	−2.43	0.33	0.29	–	2.17	1.39	–	–	4.18
100 mM	2966	8.11 ± 1.53ab	−2.85	0.41	0.39	0.09	2.63	1.49	–	0.13	5.14
150 mM	2906	6.60 ± 1.20ab	−4.54	0.59	0.43	0.15	4.29	4.43	–	0.15	10.04
200 mM	2988	2.47 ± 1.15a	−9.17	1.53	0.69	–	24.87	22.98	0.33	0.33	50.73

Means with the same letters do not significantly differ at 0.05 level (Dunnett test)

S stickiness, *C* clumping, *B* bridge, *Dist. Meta.* disturbed metaphase, *Dist. Ana*. disturbed anaphase, *Bc* binucleated cells, *DN* disorganized nucleus, *NB* nuclear bud

were increasing continuously and reached the maximum at 18 h (Fig. 23). At the early stage of treatment (4 h), the levels of SOD in roots were significantly higher than that of control and were not significantly different among each concentration. The activities of SOD in the roots treated with low concentration of NaCl had changed gradually (with increasing concentration) and optimized at 150 mM of NaCl and were declined at 200 mM NaCl.

Peroxidase could protect cells against the destructive influence of hydrogen peroxide by catalyzing its decomposition through oxidation of phenolic and endiolic co-substrates (Giannakoula et al. 2010). Figure 24 shows a

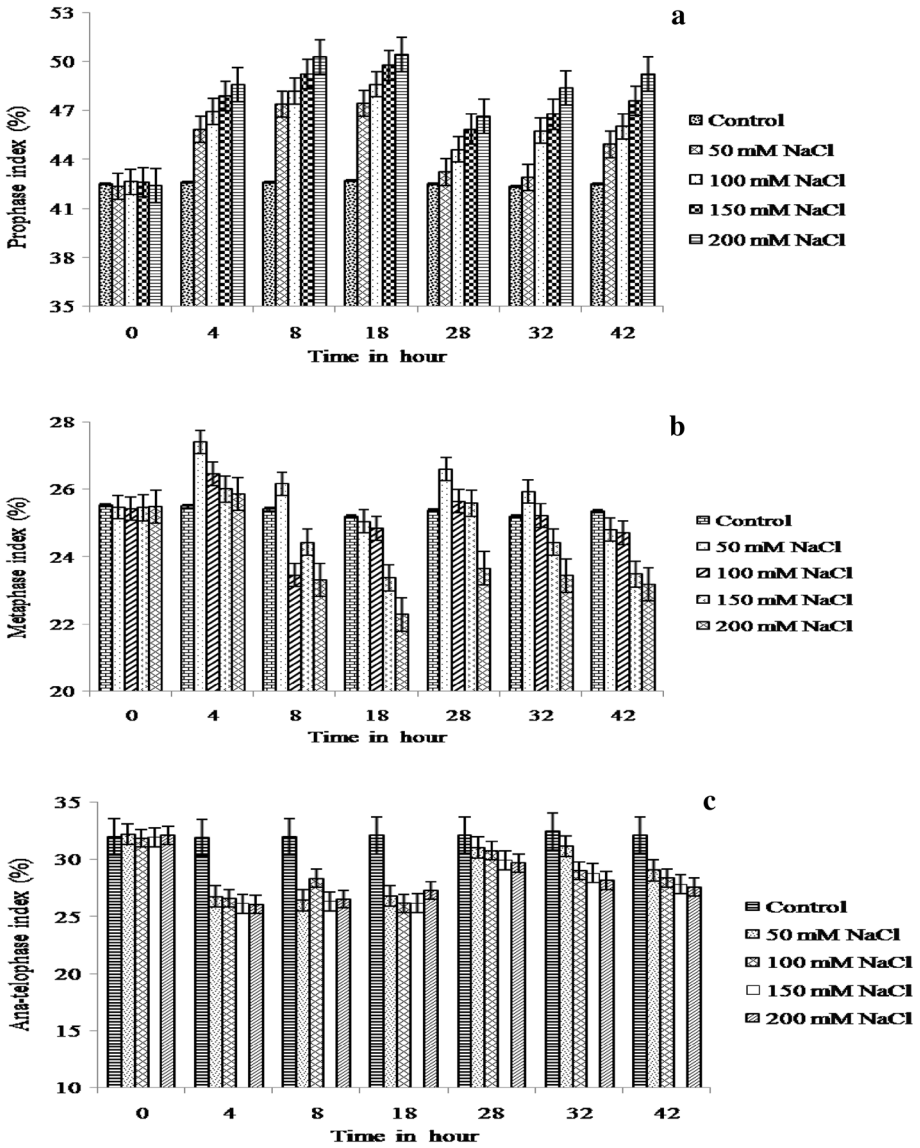

Fig. 1 Index rates of mitotic phases in root tip cells of *Allium cepa* under different concentrations and exposure time for NaCl **a** Prophase index; **b** Metaphase index; **c** Ana–telophase index

trend from ascent to descent, which had reached the maximum at 150 mM and were reduced at 200 mM. The results indicated that the activity of POX increased significantly up to 150 mM of NaCl for longer treatment periods. At the early stage of treatment (4 h), the POX activities of each concentration were not significantly different. The POX activity in treated roots with 150 mM concentration was significantly higher than that of other concentrations of NaCl during 4–18 h.

CAT could detoxify hydrogen peroxide into water and oxygen to remove peroxide from plant cells. The activities of CAT in roots under salt stress were determined, which showed that the activities of CAT were gradually decreased with increasing concentration of NaCl and duration of exposure (Fig. 25). The significant inhibition of CAT activity was accomplished. At 18 h treatment, the CAT activities of each treatment period had reached the minimum particularly in the roots treated with 200 mM of NaCl and decreased by 53.90 % as compared to control. The low concentrations of NaCl showed little effect on the CAT activities in the treated roots, while high concentration of NaCl (200 mM) could reduce 50 % CAT activity.

Effects of recovery period

Table 1 explained the effects of recovery on the mitotic index after NaCl treatment. A significant decrease in MI was noted at all concentrations and duration of treatments even after recovery for 24 h. In treated roots with 200 mM NaCl (18 h), the MI was increased from 1.91 to 2.47 after salt withdrawal. The results showed that recovery of MI was slow when treated roots were incubated in distilled

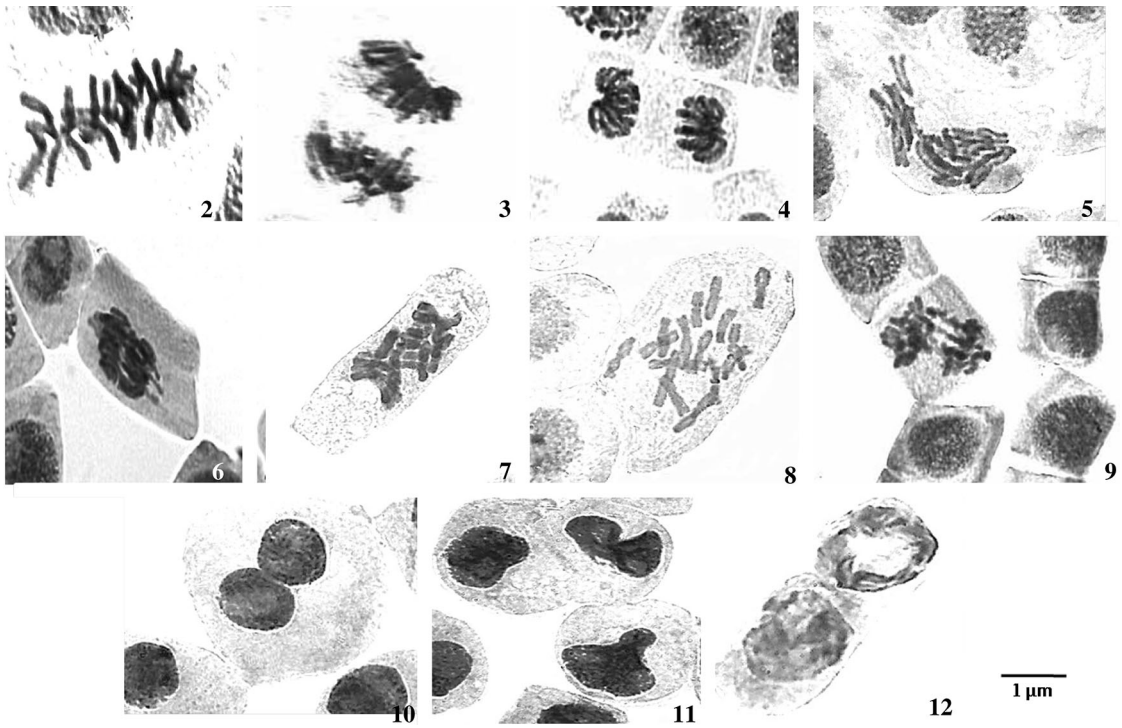

Fig. 2–12 Different chromosomal aberrations in *A. cepa* root tip cells, treated with no NaCl (**2** metaphase; **3**, **4** anaphase); 50 mM NaCl (**5** disturbed metaphase); 100 mM NaCl (**6** late separation in anaphase, **7** stickiness); 150 mM NaCl (**8** c-mitosis, **9** change in polarity) and 200 mM NaCl (**10** binucleated cell, **11** nuclear bud, **12** disorganized nucleus)

water for 24 h. The cause of improvement in the mitotic activity may be associated with salt withdrawal. The MI recovered by 2.09 and 1.34 % after salt withdrawal in 150 mM NaCl treatment of 4 and 18 h. In post-stress period, MI recovered by 4.15 and 1.45 % in 50 and 200 mM NaCl treatment of 4 h. The gradual reduction in the recovery of MI was observed in increasing concentration of NaCl. The phase indices were also recovered in post-stress period. The prophase and metaphase indices were moderately decreased, whereas ana-telophase index was increased in post-stress period.

It has been observed that post treatment with water could reduce chromosomal abnormalities from 2.6 to 2.2 % at 50 mM NaCl (4 h), but this water treatment after stress was less effective in checking abnormalities at higher concentrations, hence most dividing cells had noticeable abnormalities (Fig. 13–22).

In non-treated plants, the levels of SOD were around 4.75–5.28 unit g^{-1} fresh weight. However, in salt-treated plants a linear increase in SOD activity was observed (up to 2.6-fold of increase after 18 h of stress). A slight reduction in the SOD activity was observed in the recovered plants, although this value was still high (twofold higher than in control plants). POX activity increased significantly up to 150 mM concentration in salt-treated plants, showing a 47.40 % rise after 18 h of stress and a slight depletion was also observed in recovered plants which was still higher (42.20 %) than control plants. A strong decrease in CAT activity was observed after 18 h of stress (53.90 % decrease in relation to control plants at 200 mM NaCl). During the post-stress period, CAT activity progressively decreased, although after 24 h of recovery this value was still 51.97 % lower than in control.

Discussion

This study was undertaken to evaluate the possible changes in cytological and antioxidant enzyme activities in stress and post-stress conditions. The mitotic index is a reliable parameter for estimating the frequency of cellular division (Fernandes et al. 2007). Changes in mitotic activities and its inhibition are generally used for cytotoxicity measurement. In the present study, mitotic activity was reduced in both salt-stressed and recovered plants which indicated that NaCl resulted in decrease in cell number entering into mitotic division. The concentration and time-dependent inhibition of MI described the cytotoxic potential of NaCl in *A. cepa*. Significant reduction of MI may be a result of mitodepressive effect of salt, which interfere in the normal process of mitosis resulting in decrease in number of dividing cells. The response to salt stress involves

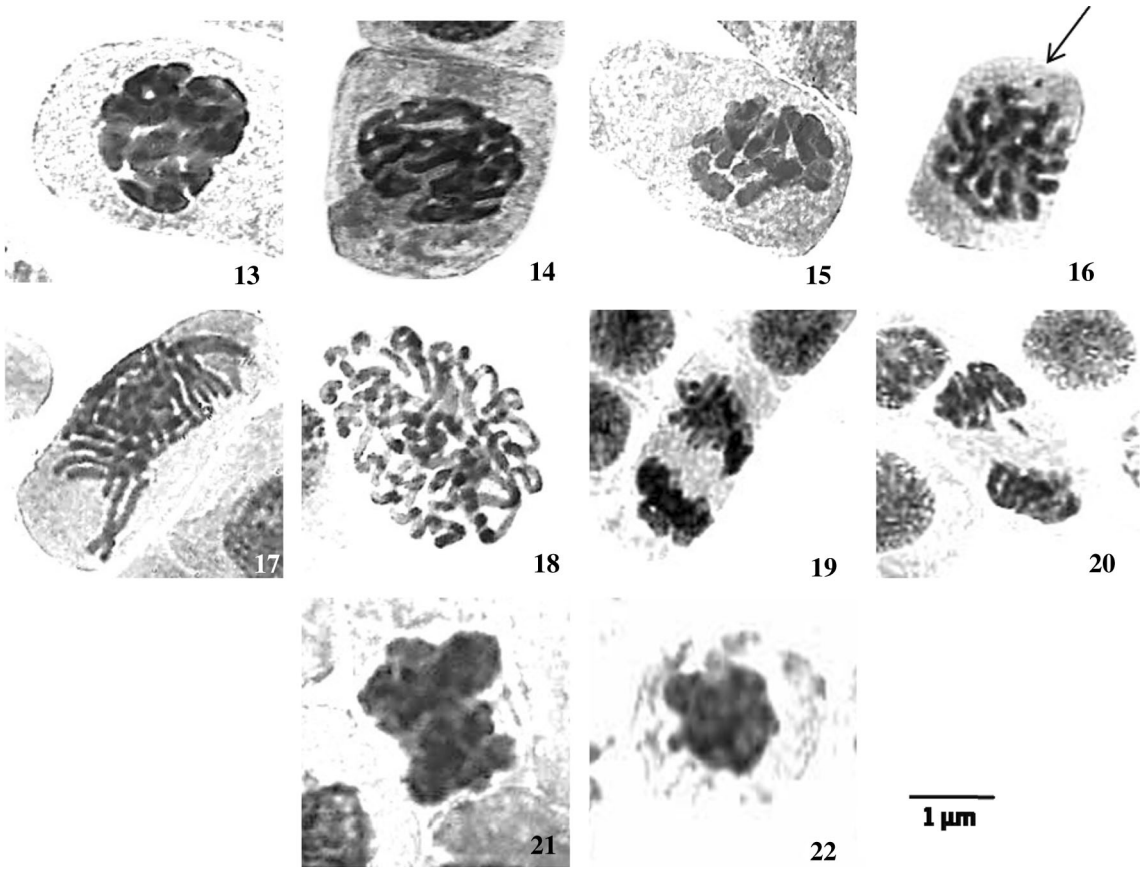

Fig. 13–22 Abnormalities found in different concentrations of NaCl during post treatment with water. **13**, **14** early condensation (50 mM NaCl); **15** sticky metaphase (100 mM NaCl); **16** star shaped metaphase with chromosome break, **17** stickiness, **18** fragments (150 mM NaCl); **19** abnormal anaphase, **20** laggard, **21** disorganized nucleus, **22** nuclear buds (200 mM NaCl)

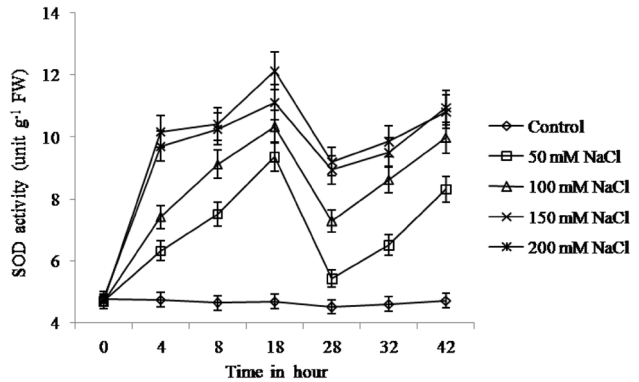

Fig. 23 Increasing superoxide dismutase activity under different concentration and exposure time for NaCl

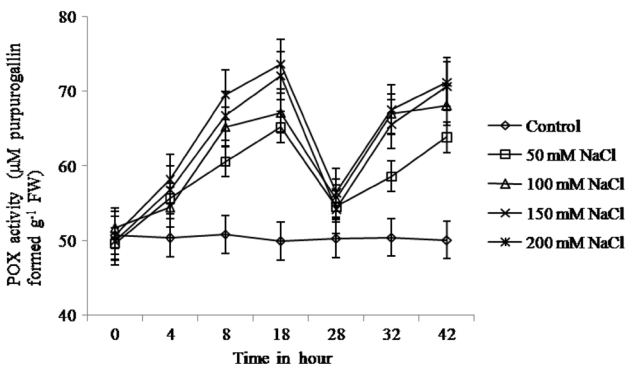

Fig. 24 Increasing peroxidase activity under different concentration and exposure time for NaCl

inhibition of cell production by inhibition of cell cycle and reduction of meristem size (West et al. 2004). In addition it may be due to inhibition of protein synthesis, DNA synthesis or effect on S or G2/M phase of cell cycle and reduction in cyclin-dependent kinase (CDK) activity (Zhao et al. 2014). Further, the value of RDR was found negative at each concentration and different periods of treatment. The finding of decrease in MI and increase in negative value of RDR was directly proportional to the severity of mitotic inhibition (Horemans et al. 2000).

In addition, application of NaCl also altered the frequencies of mitotic phases. These frequencies depended on the concentration and duration of treatment in salt-stressed and recovered plants. In comparison to control, NaCl

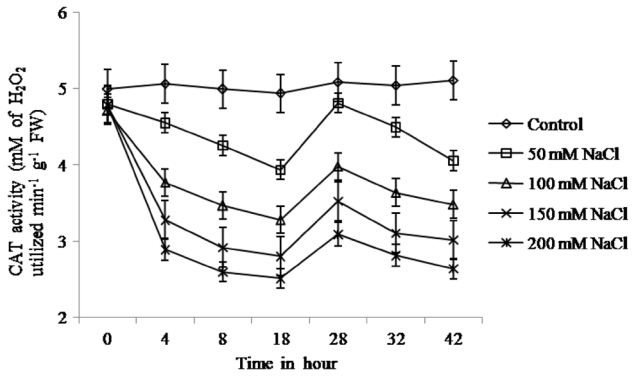

Fig. 25 Inhibition of catalase activity dependent on NaCl concentrations and period of exposure

increased the percentages of the prophase stages at all concentrations and exposure time that caused differentiation in the metaphase and ana–telophase. Our results showed that the phase indices of metaphase and ana–telophase were decreased with concentration of NaCl (Fig. 1). This decrease of the phase indices may be the result of prolonged prophase or blockage of dividing cells in prophase strongly delayed to further phases (Prasad and Das 1977). Accumulation of Na^+ and Cl^- may be highly toxic, which induced overproduction of ROS. This ROS imbalance causes significant delay in the transition from prophase to prometaphase that affect anaphase chromosome, chromosome movement and nuclear envelop dynamics (Foreman et al. 2003).

Different types of chromosomal anomalies were determined in this study. Among them four main types of chromosome aberrations were observed in different mitotic phases: disturbed metaphase, disturbed anaphase–telophase, stickiness and bridges. Other aberrations such as clumping, c-mitosis, binucleated cells, disorganized nucleus and nuclear bud were also evaluated in the experiment. The total percentage of chromosomal anomalies increased with an increase in the concentration of NaCl and exposure time in salt-stressed and recovered plants.

Chromosome stickiness is considered as chromatid type aberration (Badr 1986) which causes shortened and thickened chromosomes that are found in prophase and metaphase. This means that NaCl regarding organization of chromatin may be related to impact on physical and chemical properties of DNA, protein or both and leading to improper folding of chromatin (El-Ghamery et al. 2003). The bridges involving one or more chromosomes were the most important type of chromosomal anomalies in addition to sticky chromosomes. Bridges may arise due to stickiness, breakage and fusion of chromatids and chromosomes (El-Ghamery et al. 2000; Luo et al. 2004). The occurrence of nuclear bud may result in loss of genetic materials (Ruan et al. 1992). Nuclear buds are morphologically similar to micronuclei with the exception that they are joined to the nucleus (Fenech et al. 2011). Clumping of chromosomes at metaphase was frequently observed in root tip treated with NaCl. Clumping of chromosome might be result of the action of disulfide and sulfhydryl linkages which enter into spindle formation. The thickening and swelling of chromosome induced chromosome to stick together and form a compact clump (Banerjee 1992). Other chromosomal anomalies such as c-mitosis, binucleated cells, disturbed metaphase and disturbed anaphase-telophase were also observed in the experiment. Binucleated cells may originate from the inhibition of cytokinesis in any control point of the cell cycle (Ateeq et al. 2002). The c-mitosis, disturbed metaphase and disturbed anaphase-telophase might occur due to disturbed microtubules or might be result of disturbances in the spindle fibre formation (Haliem 1990; Fiskesjö 1993).

As a normal physiological response, plants generate oxidative stress immediately under salt stress. In the present study, enhancement in SOD activity was observed in salt-treated and recovered plants except at 200 mM NaCl, which was an agreement with the observations of Mittler and Zilinskas (1994). Our results suggested that the H_2O_2 produced by SOD activity was effectively consumed by POX/CAT. In contrast, the CAT activity was decreased and POX activity was increased in both salt-treated and recovered plants. The high activities of SOD and POX that were observed in the present study has been in a good agreement with several reports available on tomato, cotton and *Brassica napus* (Mittova et al. 2002; Meloni et al. 2003; Zare and Pakniyat 2012). The high activity of POX shows that it catalyzes the dismutation of H_2O_2 into water and oxygen. At low concentrations, H_2O_2 is mainly cleared by CAT. Possibly CAT is less efficient H_2O_2 scavenger than POX because of its low substrate affinity and seems to be more sensitive to high level of NaCl than SOD and POX. Declination in CAT activity suggested that either enzyme synthesis or assembly of enzyme subunits was inhibited under stress conditions (Jaleel et al. 2009).

Our findings suggest that the increased activities of SOD and POX are not sufficient to protect DNA against damages caused by ROS in salt-treated plants that results in increased chromosomal aberrations in cell cycle. However, during recovery, water involved in the maintenance of homeostasis (osmotic adjustment and ionic balance), counter action to damages and their repair such as scavenging of ROS and decrease oxidative stress (Horie et al. 2011). The increase in SOD and POX activities during recovery is probably the result of elimination of NaCl from treatment that is perceived by plants as a hypoosmotic stress situation (Hernández and Almansa 2002). Thus, the high activity of antioxidant enzyme (SOD and POX) in recovery period detoxifies ROS which probably helped plant cell to improve its mitotic activity.

Overall, it can be concluded that known components of osmotic stress and ion toxicity is manifested as an oxidative stress. During this short term of salt stress, recovery of antioxidant enzyme is possible after salt withdrawal.

In conclusion, it appears that induction of antioxidant defences can be at least one component of tolerance mechanism of crops to long-term salt stress. Therefore, further studies related to gene expression are needed to improve onion quality under saline conditions.

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