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EFFECTS OF SODIUM HYPOCHLORITE ON SOME PHYSIOLOGICAL AND CYTOGENETICAL PARAMETERS IN *ALLIUM CEPA* L. EXPOSED TO SALT STRESS

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Abstract

The effects of sodium hypochlorite (NaClO) on the seed germination, seedling growth (radicle length, radicle number and fresh weight), mitotic activity and chromosomal aberrations of *Allium cepa* L. germinated under salt stress were studied. Salt stress considerably inhibited the seed germination and seedling growth of *A. cepa.* Furthermore, it markedly reduced the mitotic index in root tip meristems of the seeds and increased the number of chromosomal aberrations. Whereas, the detrimental effects of salt on the seed germination, seedling growth, mitotic activitiy and chromosomal aberrations were dramatically alleviated in varying degrees by NaClO application.

Introduction

Accumulation of excess salts in the root zone resulting in a partial or complete loss of soil productivity is a worldwide phenomenon. Approximately 20% of the world's cultivated land, which account for over 6% of the world total area, is threatened by salinity (Fao 2015). The salt affected soils contain excess salts which affect plants by decreasing the osmotic potential of the soil solution (osmotic stress), interfering with normal nutrient uptake, inducing ionic toxicity and associating nutrient imbalances (An *et al.* 2003). Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of production (Sairam and Tyagi 2004).

Sodium hypochlorite is frequently used as a disinfectant or a bleaching agent, which releases oxygen gas as a by-product and is highly effective against all kinds of bacteria, fungi and viruses, by oxidizing biological molecules such as proteins and nucleic acids (Bewley and Black 1994). The effects of NaClO on the seed germination and seedling growth are conflicting. Thus, NaClO has been reported to either promote (Vujanovic *et al.* 2000) or inhibit (Ilahi and Hussain 1988) the germination and growth. Although there are many reports about the effects of NaClO on the seed germination and seedling growth under normal conditions (Nwangburuka *et al.* 2012, Varasteh *et al.* 2015), the protective mechanisms of NaClO on salt stress in plants is still unknown. The present study was designed to examine the influence of NaClO in reducing the detrimental effects of salt stress on the seed germination, seedling growth, mitotic activity and chromosomal aberrations of *Allium cepa* L.

Materials and Methods

Germination of seeds of *Allium cepa* L. was carried out using 0.225 M NaClO at a constant temperature (20°C), in the dark in an incubator. Healthy and approximately equal-sized *A. cepa* seeds were selected. The seeds were sterilized with 2.5% sodium hypochloride solution for 10 min

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and washed for 24 hrs in ultra-distilled water. Twenty seeds from each treatment group were placed into the plastic containers. The seeds were divided into four groups: Group I (control) was treated with distilled water for 7 consecutive days. Group II was treated with 0.225 M NaCl alone for 7 consecutive days. Group III was treated with a 0.1 % NaClO for 7 consecutive days. Group IV was treated with a 0.1% NaClO + 0.225 M NaCl for 7 consecutive days. Plastic containers were placed into an incubator for germination. It was assumed that the radicle should be 10 mm long for germination. At the end of the 7th day, after determination of the final germination percentages, radicle numbers were also recorded, and radicle lengths of the seedlings were measured in mm and in addition, the fresh weights in g/seedling were determined. All experiments were repeated 3 times.

After several days, root tips of germinated *A. cepa* were excised (1-1.5 cm segment) for cytogenetic analysis. Then, they were pretreated with saturated para-dichlorobenzene for 4 hrs, fixed in solution of ethanol: acetic acid (3 : 1) overnight at room temperature and stored at 4^{o}C in 70% ethanol until used. The root tips were hydrolysed in 1 N HCl at 60ºC for 15 min, stained with Feulgen for 1 - 1.5 hrs, smashed in a drop of 45% acetic acid and squashed. After 24 hrs, microscopic slides were made permanent by mounting in balsame. The mitotic phases and mitotic aberrations were photographed (100X) with a digital camera (Olympus C-5060) mounted on an Olympus CX41 microscope.

Mitotic index, i.e. percentage of dividing cells scored was evaluated by analysing at least 30.000 cells per treatment (approx. 10.000 per slide). Chromosomal abnormalities were calculated for each concentration as the percentage of 2000 dividing cells counted. Statistical evaluation of all parameters was made by using SPSS program according to DMRT.

Results and Discussion

As shown in Table 1, the radicle length, radicle number and fresh weight of the group III seeds germinated in the medium with NaClO alone reduced as compared to ones of the group I (control) seeds germinated in distilled water medium while their germination percentage was statistically the same as ones of the group I seeds (Table 1). There are enough studies about the effects of NaClO on the seed germination and seedling growth under normal conditions. However, from these studies, no conclusion could be reached. Thus, NaClO has been reported to promote (Vujanovic *et al.* 2000, Varasteh *et al.* 2015), inhibit (Ilahi and Hussain 1988) or be ineffective (Nwangburuka *et al.* 2012) the seed germination and seedling growth. These differences of observations may have resulted from differences in treatment times, concentrations used and plant species (Mendes de Jesus *et al.* 2016).

Salt exhibited an inhibitive effect on all examined growth parameters. For example, the group I (control) seeds germinated in distilled water medium displayed 100% germination on the 7th day while this value became 25% in the group II seeds germinated in 0.225 M salinity. In other words, salt prevented 75% the germination of *A. cepa* seeds. Salt stress can perform its preventive effect in many ways. It may interfere with seed germination by changing the water status of the seed so that water uptake is inhibited. The present results showing the decrease in the fresh weight and water content of the seedlings in saline medium may be explained by the failure of the roots to receive sufficient water due to the high osmotic pressure of the medium. The inhibitive effect of salt on the radicle length and radicle number may result from reducing cell division, nucleic acid and protein synthesis.

NaClO application markedly alleviated the inhibitive effect of salt stress on the seed germination. The group IV seeds treated with NaClO showed 98% germination. Finally, *A. cepa* seeds showed a performance such as germinated under normal conditions, are not in saline conditions (Fig. 1). NaClO also continued its success on the seedling growth parameters such as

the radicle length, radicle number and fresh weight. The radicle length, radicle number and fresh weight of the group II seedlings grown in 0.225 M salinity were 10.4 mm, 16.8 and 12.3 g, respectively while these values were 33.5 mm, 34.1 and 13.2 g in the group IV seedlings treated with NaClO (Table 1). There are a few studies which examined effects of NaClO on the seed

Table 1. Effect of NaClO on some growth of *Allium cepa*.

Groups	Growth parameters			
	Germination (%)	Radicle length (mm)	Radicle number	Fresh weight (g/seedling)
Group I	*100 ± 0.0^{b}	75.2 ± 1.0^{d}	48.5 ± 1.4^{d}	17.7 ± 0.7^{c}
Group II	25 ± 0.0^{a}	10.4 ± 0.2^{a}	16.8 ± 1.1^{a}	12.3 ± 1.2^{a}
Group III	100 ± 0.0^{b}	53.2 ± 0.2^{c}	42.8 ± 1.8^{c}	14.4 ± 1.1^{b}
Group IV	98 ± 2.8^{b}	33.5 ± 1.0^{b}	34.1 ± 1.9^{b}	13.2 ± 0.1^{ab}

*The difference between values with the same letter in each column is not significant at 0.05 (±SD). Group I (control) was treated with distilled water; group II was treated with 0.225 M NaCl alone; group III was treated with a 0.1 % dose of NaClO; group IV was treated with a 0.1 % dose of NaClO + 0.225 M NaCl.

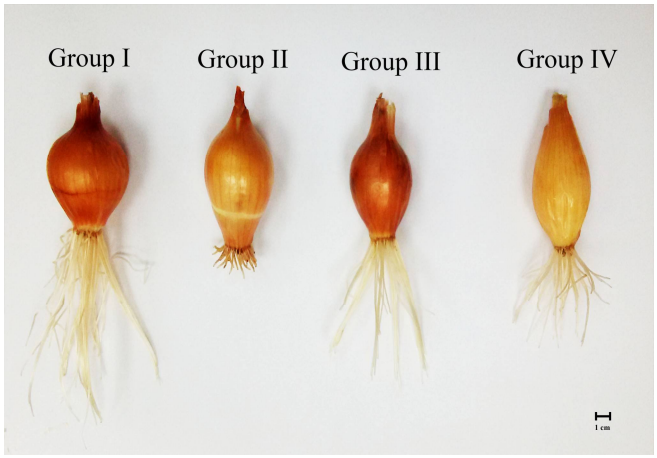

Fig. 1. The germination situations at the end of the 7th day of *Allium cepa* L. seeds. Group I (control) was treated with distilled water; Group II was treated with 0.225 M NaCl alone; Group III was treated with 0.1% NaClO; Group IV was treated with 0.1% NaClO + 0.225 M NaCl. Scale bar = 1 cm.

germination and seedling growth under saline conditions until now. Khan and Zia (2007) reported that 10% NaClO application significantly showed the negative effect of salt stress on the germination of *Limonium stocksii* (Boiss) Kuntze seeds. This result is in agreement with the present findings. That NaClO alleviates salt stress on the seed germination and seedling growth can be understood from the decrease in the salt's osmotic effects. For example, at 0.225 M NaCl medium, NaClO application partly increased the fresh weights of the seedlings compared to the control indicates this probability (Table 1). Moreover, it reduced the preventive effect of salt on the seed germination and seedling growth by stimulating mitotic activity of the embryo (Table 2). It could have made a counter-attack against the ABA being a germination inhibitor whose amount probably increases due to the salt existence.

Some growth regulators may particularly cause mitotic irregularities, cell distortions and chromosomal aberrations even without stress conditions (Tabur and Demir 2010b). So far, there is no reported data relating to effects of NaClO on the mitotic activity and chromosomal aberrations in non-stress conditions. Therefore, in the present study investigation was carried out to find whether NaClO is affecting these parameters in normal conditions or not. The data obtained in the present work indicated that the mitotic index in root meristems of *A. cepa* (group III) seeds exposed to NaClO application in normal conditions reduced 18% according to ones of the group I (control) seeds germinated in distilled water. 0.1% NaClO application showed a repressive effect on the mitotic activity by slowing down cell division. Moreover, frequency of chromosomal aberrations was increased 46-fold with this dose of NaClO application. In this case, it may be said that some aberrations may result from this chemical (Table 2).

Table 2. Effect of NaClO on mitotic index and frequency of chromosomal aberrations in *Allium cepa* L. root tip meristems.

Groups	Mitotic index (%)	Chromosome aberration (%)
Group I	*6.3 ± 0.2^{c}	0.0 ± 0.0^{a}
Group II	3.7 ± 0.4^{a}	50.1 ± 1.0^{d}
Group III	5.2 ± 1.0^{b}	46.3 ± 1.7^{c}
Group IV	9.4 ± 0.7^{d}	40.7 ± 0.4^{b}

*The difference between values with the same letter in each column is not significant at 0.05 (±SD). Group I (control) was treated with distilled water; Group II was treated with 0.225 M NaCl alone; Group III was treated with a 0.1% NaClO; Group IV was treated with a 0.1% NaClO + 0.225 M NaCl.

The cytotoxicity level of a test compound can be determined based on the increase or decrease in the mitotic index (MI), which can be used as a parameter of cytotoxicity in studies of environmental biomonitoring (Fernandes *et al.* 2007). The inhibitory and cytotoxic effects of salt stress on mitotic activity are known for a long time (Radic *et al.* 2005, Tabur and Demir 2009, 2010a, b). According to some researchers, high salt concentration causes to total inhibition of mitotic activity and chromosomal abnormalities in root-tip cells (Radic *et al.* 2005). In the present work, it is observed that salinity adversely affected the mitotic activity and chromosome behaviors in root meristem cells of *A. cepa*. The present data indicated that salinity according to the control decreased 41% the mitotic index and showed higher number of chromosomal abnormalities. The frequency of aberrations by salinity increased 50 times as compared to the control group. For example, the frequency of chromosomal aberrations in the root tip meristems of the seeds germinated in distilled water was 0.0 while it was 50.1 at 0.225 M salinity. Besides, simultaneous application of NaClO+NaCl could be successful in alleviating the negative effect of salinity on the mitotic activity. In addition, simultaneous application of NaClO+NaCl showed marked achievement in decreasing the detrimental effect of salinity on the chromosomal aberrations as compared to NaClO alone. That is, frequency of chromosomal aberrations was approximately decreased 20% by the simultaneous application (Table 2). These results indicated the repair role of NaClO against salt injuries during *A. cepa* mitosis.

Normal and abnormal mitotic phases observed during microscopic examination of *A. cepa* root tip meristem cells were indicated in Figs 2 and 3. The most striking aberrations observed in all applications were micronucleus, irregular prophase, uncoiling chromosome, irregular anaphase, bridge in anaphase, lagging chromosome in anaphase, bridge in telophase, vagrant chromosome in

telophase and fault polarization in telophase (Fig. 3a-l). The majority of chromosomal abnormalities in root tip cells treated with NaClO or salt were determined as disorderly prophase (Fig. 3b), uncoiling chromosome (Fig. 3c, d) and fault polarization in telophase (Fig. 3l).

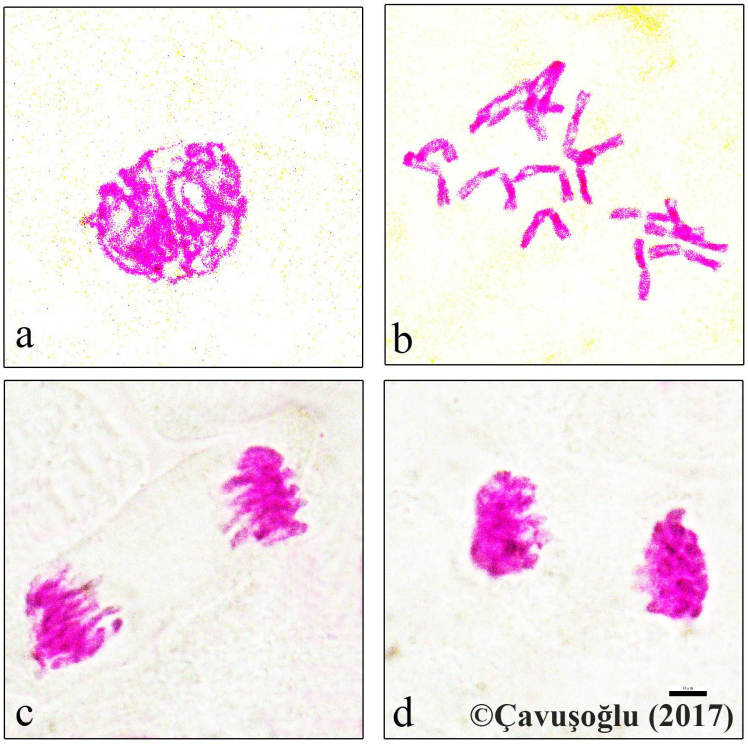

Fig. 2. Normal mitosis phases in root tips meristems of *Allium cepa* root tip cells. Prophase (a), metaphase (b), anaphase (c) and telophase (d). Scale bar = 10 μm.

In general, accurate chromosome segregation in mitosis requires that sister kinetochores attach to microtubules emanating from opposite spindle poles. Because kinetochore attachment is a stochastic process, it is error prone and can result in chromosome malorientation (Rieder and Salmon 1998). Mitotic irregularities such as disorderly prophase and anaphase, fault polarization, alignment anaphase, vagrant chromosomes and bridges may be mainly the result from mentioned reasons or spindle dysfunction and constitute a significant portion of chromosomal aberrations. The formations of micronucleus are likely the consequence of vagrant chromosomes and fragments (Briand and Kapoor 1989). The lagging chromosomes are presumably the result of a weak mitotic effect. NaCl may lead to the highest number of laggards. According to Fiskesjö (1997), NaCl caused c-mitotic effects including lagging chromosomes. Sticky chromosomes may result from improper folding of the chromatin fibres (Klasterska *et al.* 1976). It was previously reported that the stickiness reflects highly toxic effect on chromatin (Fiskesjo and Levan 1993). The prophase and metaphase cells with uncoiled chromosomes may be the result of disorderly chromosome contractions. Also, anaphase and telophase bridges could be the result of inversions (Tabur and Demir 2010b).

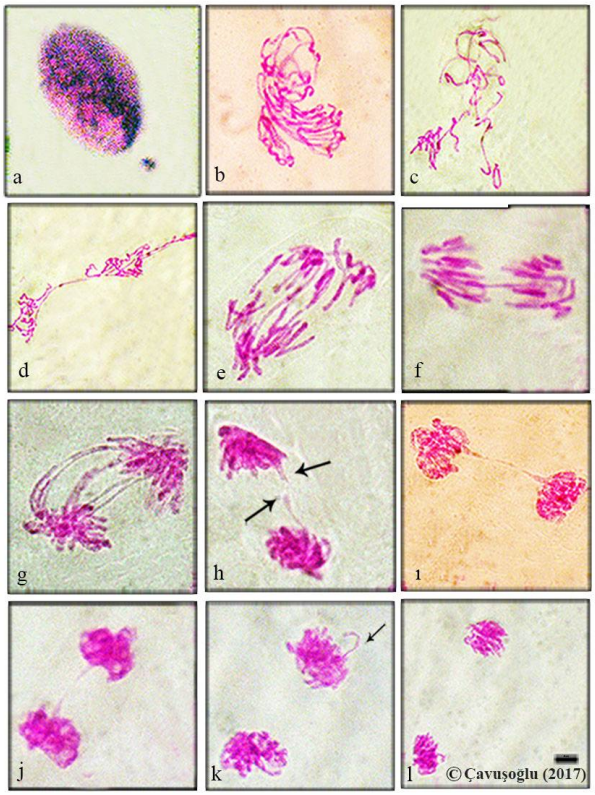

Fig. 3. Chromosomal aberrations examined in mitotic phases of *Allium cepa* root tip cells. Micronucleus (a), irregular prophase (b), uncoiling chromosome (c, d), irregular anaphase (e), bridge in anaphase (f, g), lagging chromosome in anaphase (h), bridge in telophase (i, j), vagrant chromosome in telophase (k) and fault polarization in telophase (l). Scale bar = 10 µm.

Reported data related to effects of NaClO application in saline conditions on the physiological and cytogenetical parameters are not available. Therefore, the present results in the present work are the first time report particularly in saline conditions. Consequently, this study indicates that NaClO may significantly improve the activations such as the seed germination, seedling growth and mitotic activity in saline conditions. However, the mechanisms by which salinity inhibits growth are complex and controversial. Moreover, they may vary according to species and cultivar. A universal mechanism has not been established yet. Although the causes of salinity have been characterized, understanding of the mechanisms by which salinity prevents plant growth is still rather poor. Therefore, further studies should be carried out in order to gain more knowledge about effect of NaClO on molecular metabolism of germination, cell division and cell cycle. This work may serve to provide new conceptual tools for designing hypotheses of salt tolerance in plants.

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