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ROLE OF L-ORNITHINE IN MITIGATION OF SALT STRESS IN *ALLIUM CEPA* L.

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Abstract

Effects of L-ornithine (150 mg/l) on the germination, seedling growth, mitotic index, chromosome aberrations and micronucleus frequency of *Allium cepa* L. bulbs germinated at 0.125 M salinity were studied. The radicle number of the group III bulbs germinated in the medium with ornithine alone as compared to ones of the group I (control) bulbs which germinated in distilled water medium. But, their germination percentage, radicle length and fresh weight were statistically the same as ones of the group I bulbs. Besides, the micronucleus frequency and chromosomal abnormalities in the root-tip meristematic cells of the group III bulbs showed increased germination compared to ones of the group I bulbs. However, their mitotic index statistically showed the same value as the group I bulbs. Salt stress significantly inhibited the germination and seedling growth of *A. cepa* bulbs. Moreover, it reduced the mitotic index in the root-meristem cells of the bulbs and fairly increased the number of chromosome aberrations and micronucleus frequency. On the other hand, the inhibitive effect of salt on the germination, seedling growth, mitotic index and micronucleus frequency was dramatically alleviated in varying degrees by ornithine application. But, it was ineffective in reducing the detrimental effect of salinity on the chromosome aberrations. The germination percentage, radicle lenght, radicle number, fresh weight, mitotic index, micronucleus frequency and chromosomal aberrations of the group II seedlings grown in 0.125 M salinity were 27%, 13.5 mm, 18.4, 7.1 g, 5.5, 18.3 and 60.8%, respectively while these values became 68%, 16.4 mm, 16.4, 10.5 g, 15.6, 7.6 and 74.8% in the group IV seedlings treated with L-ornithine.

Introduction

Salinity is an important bordering environmental factor in crop production. Under salt stress, plants need to get over water stress, exposed by the low external water potential, and with ion toxicity, due to accumulation inside the plant. The plants must also cope with ion toxicity accumulated when exposed to salinity stress. As plants are sessile they must cope with changing environmental conditions by adapting to stress situations via various physiological and molecular processes. High salt concentration in soil may lead to three main types of stress, namely osmotic, oxidative and ionic stress (Shrivastava and Kumar 2015). Salt stress has various effects on plant physiological processes such as increased respiration rate and ion toxicity, mineral distribution, changes in plant growth, membrane permeability, membrane instability due to calcium displacement by sodium and reduced photosynthesis (Demidchik *et al.* 2018).

Ornithine, a non-essential amino acid, serves as the precursor for biosynthesis of polyamines, with the first and rate-limiting being catalyzed by ornithine decarboxylase. It is not a constituent of proteins, but is important in the regulation of nutritional state as a precursor of aliphatic polyamines. It can act in many biological processes including fruit ripening and plant protection from osmotic stress. In other words, it is used as the precursor of many metabolic pathways associated with stress resistance. L-ornithine is an important chemical used in medicine (wound healing, liver disease treatment), microorganism fermatiation, pharmaceutical and food industry (Mattoo and Handa 2008, Schneider *et al.* 2012).

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Allium cepa test one of the most frequently used plant bioassays has been used to evaluate the mutagenic effects in the root tips of onions. Its substantial value is supported by the fact that effects of mutagenic action of many compounds on the Allium test cells and mammalian cells are very similar. This test has been validated in internetional collaborative studies under the United Nations Environmental Program (UNEP), World Healt Organization (WHO) and US Environmental Protestion Agency (USEPA) as an effective test for environmental and genetic monitoring (Gajalakshmi and Ruban 2014). The present study was designed to examine the influences of L-ornithine in reducing the detrimental effects of salt stress on the germination, seedling growth, mitotic activity, micronucleus frequency and chromosomal aberrations of *Allium cepa* L.

Materials and Methods

Allium cepa L. bulbs were used as experimental material where ornithine doses (1, 5, 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000 mg/L^{-1}) and salt concentrations (0.05, 0.10, 0.125, 0.15, 0.175, 0.20, 0.25, 0.30, 0.40, 0.50 M) were applied in a preliminary investigation. The salt concentration preventing the germination of onion bulbs was determined as 0.125 M. The best promoting germination and seedling growth against the inhibitory effect of 0.125 M salinity was established as 150 mg/L^{-1} the ornithine concentration. Thus, the mentioned concentrations were used in this work. The present study was carried out in Plant Physiology and Cytogenetic Laboratories of Biology Department in Faculty of Art and Science, Süleyman Demirel University

Germination experiments were carried out at a constant temperature (20°C), in the dark in an incubator. Healthy and approximately equal-sized *A. cepa* bulbs were selected. Twenty bulbs from each treatment group were placed into the plastic containers. The bulbs were divided into four groups: Group I (control) was treated with distilled water for 7 consecutive days. Group II was treated with 0.125 M NaCl alone, for 7 consecutive days. Group III was treated with a 150 mg/L^{-1} dose of L-ornithine, for 7 consecutive days. Group IV was treated with a 150 mg/L^{-1} dose of L-ornithine + 0.125 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 to take place. At the end of the 7 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 5 N HCl for 20 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 (500X) with a digital camera (Olympus C-5060) after mounting on an Olympus CX41 microscope. Mitotic index, i.e. percentage of dividing cells scored was evaluated by analysing at least 9.000 cells per treatment (approx. 3.000 per slide). Statistical evaluation concerning all parameters was made by using SPSS program according to DMRT.

Results and Discussion

The germination percentage, radicle length and fresh weight of the group III bulbs germinated in the medium with L-ornithine were statistically the same as ones of the group I bulbs germinated in distilled water medium. But, their radicle numbers partly decreased according to ones of the

group I seedlings (Table 1). So far no study has been conducted regarding the effects of L-ornithine on the germination and seedling growth under normal conditions.

Table 1. Effect of L-ornithine on some growth parameters of *Allium cepa* L.

Groups	Growth parameters			
	Germination (%)	Radicle length (mm)	Radicle number	Fresh weight (g/seedling)
Group I	*100 ± 0.0^{c}	58.7 ± 0.7^{c}	45.1 ± 0.7^{d}	10.5 ± 0.3^{b}
Group II	27 ± 2.8^{a}	13.5 ± 1.2^{a}	18.4 ± 1.4^{b}	7.1 ± 0.2^{a}
Group III	100 ± 0.0^{c}	57.7 ± 1.1^{c}	38.9 ± 0.7^{c}	9.8 ± 0.9^{b}
Group IV	68 ± 2.8^{b}	16.4 ± 0.6^{b}	16.4 ± 0.6^{a}	10.5 ± 0.3^{b}

*The difference between values with the same letter in each column is not significant at the level 0.05 (±SD). Group I (control) was treated with distilled water, Group II treated with 0.125 M NaCl alone, Group III treated with 150 mg/L^{-1} dose of L-ornithine, Group IV treated with 150 mg/L^{-1} dose of L-ornithine+0.125 M NaCl.

Salt stress showed a restrictive effect on all examined growth parameters. For instance, the group I (control) bulbs displayed 100% germination on the 7 day while this value became 27 % in the group II bulbs germinated in 0.125 M salinity. In other words, salt prevented 73% the germination of *A. cepa* bulbs (Table 1). 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 (Flowers and Colmer 2015). 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 inhibitory effect of salt on the radicle length and radicle number may result from reducing cell division, nucleic acid and protein synthesis (Roy *et al*. 2014).

L-ornithine application markedly mitigated the inhibitive effect of salt stress on the germination. The group IV bulbs treated with L-ornithine demonstrated 68% germination in the mentioned salt level (Fig. 1). L-ornithine also continued its success on the seedling growth parameters such as the radicle length and fresh weight. The radicle length and fresh weight of the group II seedlings grown in 0.125 M salinity were 13.5 mm and 7.1 g, respectively while these values were 16.4 mm and 10.5 g in the group IV seedlings treated with L-ornithine. But, this application was unsuccessful in alleviation of the inhibitive effect of salt stress on the radicle number of the seedlings (Table 1). That L-ornithine alleviates salt stress on the germination and seedling growth can be estimated from the decrease in the salt's osmotic effects. For example, at 0.125 M NaCl medium, L-ornithine application partly increased the fresh weights of the seedlings compared to the control indicating this probability (Table 1). Moreover, it reduced the preventive effect of salt on the germination and seedling growth by stimulating mitotic activity of the embryo (Table 2).

Data relating to effects of L-ornithine on the mitotic activity, micronucleus frequency and chromosomal aberrations in non-stress and salt stress conditions are not still available. Thus the present study was carried out to find whether L-ornithine is affecting these parameters in normal conditions or not. The data obtained in the present work indicated that the mitotic index in root tip meristems of the group III bulbs germinated in the medium with L-ornithine alone was statistically the same as ones of the group I bulbs germinated in distilled water medium while their micronucleus frequency and chromosomal aberrations excessively increased according to ones of

the group I (Table 2). In this case, it may be said that some aberrations may result from this amino acid.

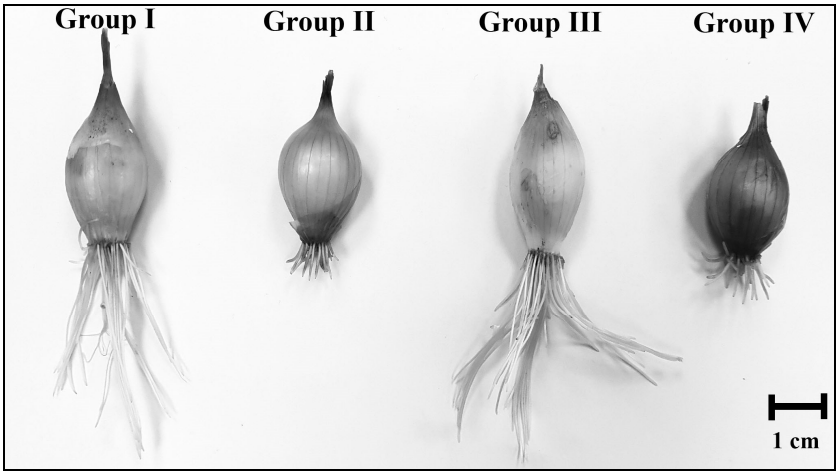

Fig. 1. The germination situations at the end of seventh day of *Allium cepa* bulbs. Group I was treated with distilled water, Group II was treated with 0.175 m NaCl alone, Group III was treated with 150 mg/l dose of L-ornithine and Group IV was treated with 150 mg/l dose of L-ornithine + 0.125 M NaCl. Scale bar = 1 cm.

Table 2. Effect of L-ornithine on some cytogenetical parameters of *Allium cepa* L.

Groups	Mitotic index (%)	Micronucleus frequency (%)	Chromosome aberration (%)
Group I	*15.9 ± 0.4[b]	0.3 ± 0.5[a]	0.8 ± 0.0[a]
Group II	5.5 ± 0.4[a]	18.3 ± 0.5[c]	60.8 ± 0.4[c]
Group III	15.9 ± 0.5[b]	7.3 ± 0.5[b]	52.6 ± 1.3[b]
Group IV	15.6 ± 0.9[b]	7.6 ± 0.5[b]	74.8 ± 1.4[d]

*The difference between values with the same letter in each column is not significant at the level 0.05 (±SD). Group I (control) treated with distilled water, Group II treated with 0.125 M NaCl alone, Group III treated with 150 mg/l dose of L-ornithine, Group IV treated with 150 mg/l dose of L-ornithine+0.125 M NaCl.

The inhibitory and cytotoxic effects of salt stress on mitotic activity are known for a long time (Radic *et al.* 2005). High salt concentration causes total inhibition of mitotic activity, micronucleus frequency and chromosomal abnormalities in root-tip cells (Çavuşoğlu *et al.* 2017, 2018). In the present work, 0.125 M salinity was found to decrease mitotic activity expressed as mitotic index according to ones of the control group and caused a significant increase on the micronucleus frequency and chromosomal aberrations. For instance, the mitotic index, micronucleus frequency and chromosomal aberrations in the root tip meristems of the bulbs germinated in normal conditions were 15.9, 0.3 and 0.8, respectively while they were 5.5, 18.3 and 60.8 at 0.125 M NaCl medium. Besides, L-ornithine + NaCl application (Group IV) showed a perfectly good performance in ameliorating the negative effects of salt on the mitotic index (15.6) and micronucleus frequency (7.6). However, this application was ineffective in reducing the salt

damage on the chromosome aberrations (74.8). All this values mentioned are substantially significant (Table 2).

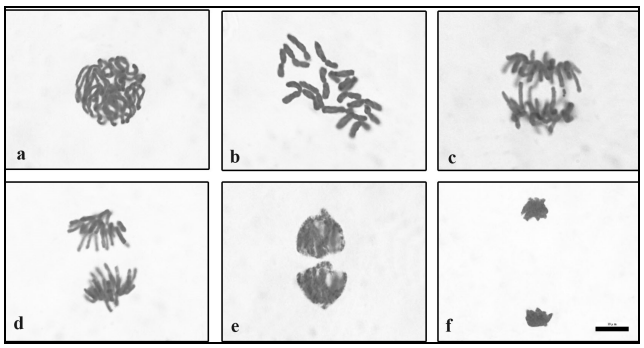

Fig. 2. Normal mitosis phases in root tip meristem cells of *Allium cepa* L. Prophase (a), metaphase (b), delayed anaphase (c), anaphase (d), early telophase (e), telophase (f). Scale bar = 10 µm

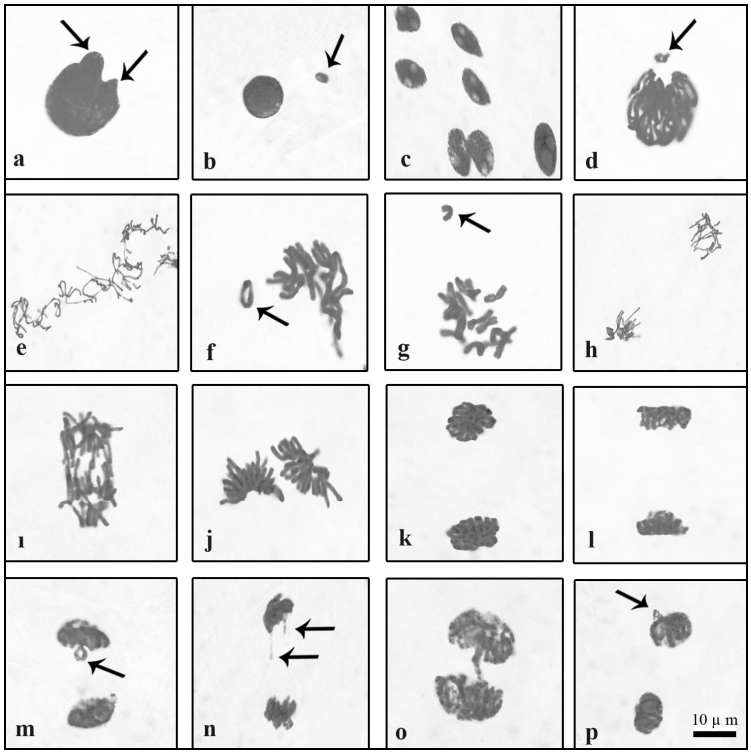

Fig. 3. Types of chromosomal aberration. Nucleus with nuclear buds = arrows (a), micronucleus = arrow (b), binucleolars (c), prophase with chromosome loss = arrow (d), irregular prophase (e), metaphase with ring chromosome = arrow (f), metaphase with chromosome loss = arrow (g), abnormal anaphase (h), multiple bridge formation in anaphase (ı), diagonal at anaphase (j), chained telophase (k), alignment telophase (l), telophase with chromosome loop = arrow (m), lagging chromosomes = arrows (n), bridge formation in telophase (o), diagonal at telophase with vagrant chromosome = arrow (p). Scale bar = 10 µm.

Normal and abnormal mitotic phases observed during the microscopic examination of *A. cepa* root tip mitotic cells were indicated in Fig. 2 and Fig. 3. The most striking aberrations observed in all applications were micronucleus, irregular prophase, abnormal anaphase, nuclear buds, chromosome losses, bridge formation in anaphase/telophase, diagonal at anaphase/telophase with vagrant chromosome, metaphase with ring chromosome, chained telophase, lagging chromosomes, alignment telophase, telophase with chromosome loop. The majority of chromosomal aberrations in root tip cells treated with L-ornithine or salt were determined as binucleolars (Fig. 3).

Chromosomal abnormalities (CAs) are changes in chromosome structure resulting from a break or exchange of chromosomal material. Induction of CAs can affect the fertility, vigour, competitive or yield ability of the exposed plants (Kara *et al.* 1994). Nuclear buds arise as a result of excessive production of proteins and nucleic acids, induced by cytotoxicans (Fig. 3a) (Fenech *et al.* 2011). Micronucleus (Fig. 3b) is composed either of small chromatin fragments, which arise as a result of chromosomal breakage or of whole chromosomes that do not migrate anaphase as a result of spindle dysfunction. Chromosome losses (Fig. 3d, g) are alterations typically associated with the malfunction of the mitotic spindle. Abnormal anaphase (Fig. 3h) might be due to disturbance of spindle apparatus which allows that the chromosomes spread irregularly over the cell (Luzhna *et al.* 2013). Bridge formation (Fig. 3ı, o) resulted from chromosome and/or chromatid breaks, indicating the mutagenic event in the cell (Leme and Marin-Morales 2009). Lagging chromosomes (Fig. 3n) are the direct results of breaks and fragmentation, which lead to the loss of centromere and the stopping of their movement (Paul *et al.* 2013). İrregular prophase failure (Fig. 3e) can induce chromosome loss once they cannot bind to the spindle and therefore not segregate (Gisselsson *et al.* 2004). Ring chromosome (Fig. 3f) might arise due to the spontaneous breakage of chromosomal ends, followed by the joining of the raw ends of the chromosomes (Khanna and Sharma 2013). Diagonal orientation at anaphase/telophase (Fig. 3j, p) was due to a slight tilt in the spindle apparatus (Renjana *et al.* 2013).

There is no present literature data related to the effects of L-ornithine application in both normal and saline conditions on the physiological and cytogenetic parameters. Therefore, results of the present study have been reported for the first time in non-stress and salt stress conditions. Results showed that L-ornithine may significantly improve the activations such as the germination, seedling growth and mitotic activity in saline conditions. However, the mechanisms by which salinity inhibits growth are complex and controversial. Moreover, they might vary according to cultivar and species. An universal mechanism has not been established yet. Although the reasons of salinity have been characterized, understanding of the mechanisms by which salinity prevents plant growth is still very poor. Therefore, further works should be carried out in order to gain more knowledge about the effect of L-ornithine on cell division, cell cycle and molecular metabolism of germination. This literature study may serve to provide new conceptual tools for designing hypotheses of salt tolerance in plants.

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