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EFFECTS OF L-LYSINE ON CYTOGENETICAL AND PHYSIOLOGICAL PARAMETERS IN *ALLIUM CEPA* L. UNDER SALT STRESS

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Abstract

The role of L-lysine (Lys) on some cytogenetic and physiological parameters in *Allium cepa* L. seeds exposed to salt was evaluated. NaCl stress on the other hand showed a significantly inhibitory effect on the seedling growth and seed germination of *Allium cepa.* Besides, it significantly reduced the mitotic index in the root tip meristems of seeds and increased micronuclei which are the most effective and simplest indicator of cytological damage and chromosomal abnormalities. However, the effects of salinity on chromosomal aberrations, seedling growth, seed germination and mitotic activity have decreased significantly with Lys application.

Introduction

The salinity in soil is one of the major abiotic factors affecting crop yield in irrigated, arid and semi-arid regions. Nearly 3/4 of the earth surface is covered with saline water, thus it is not surprising that salt affects the earth surface's a significant part. The salt affected soils contain sufficient amount of soluble salt, which causes toxicity in common crop plants. Salinity stress affects significantly the economic yield and the growth of a very important plants in agriculture (Maas and Hoffman 1977). Although most of the plants are salt tolerant during germination, this stress retards this process, although there is no difference in proportion of germinated seeds from one process to another (Maas and Poss 1989). This stage of development is categorized as "salt tolerant" for most products (Carter *et al.* 2005). Although the salt retards germination, the percentage of germinating seeds at the end of higher salt concentrations was found to decrease (Mauromicale and Licandro 2002).

Lysine an essential amino acid is synthesized in plants and strongly regulated by the rate of synthesis in the seeds of many crop plants. So, it is considered to be of great nutritional importance in human foods and animal feeds (Stepansky *et al.* 2006). Lysine (Lys), one of the key essential amino acids, is relatively rare in the plant kingdom and is very close to the standards that FAO determines for human nutritional needs (Schlick and Bubenheim 1993). It can act as regulator of interaction with the environment and as a plant growth regulator (Stepansky *et al.* 2006). Although there are a few reports on role of Lys on the seedling growth, seed germination under both saline and normal conditions (Falco *et al*. 1995, Galili 1995, Tian *et al*. 2010) unfortunately, there are no studies on the effects of said amino acid on micronucleus frequency, mitotic activity and chromosomal aberrations in salted and normal conditions. Thus, this work was designed to investigate the effects of Lys in reducing of the harmful effects of saltinity stress on the mitotic activity, seed germination, chromosomal aberrations, micronucleus frequency and seedling growth of *A. cepa* L.

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Materials and Methods

Seeds of *Allium cepa* L. were used in the experiment conducted in Plant Physiology Laboratory, Department of Biology, Faculty of Arts and Science, Süleyman Demirel University, L-lysine was obtained from Solgar ®. In a preliminary investigation of this study, Lys and salt concentrations were determined.

Seed germination was made in a (fixed temperature) incubator set to 20°C in the dark. *A. cepa* L. seeds of equal size were used as test material. The seeds were sterilized with the aid in 2.5% NaClO for ten min and washed in ultra-distilled water for 24 hrs. The seeds were placed into the plastic containers and divided in 4 groups for seven sequential days:

- Control (Group I) was processed with only distilled water.
- Group II was processed with 0.175 M NaCl alone.
- Group III was processed with 500 mg/l of Lys.
- Group IV was processed with 500 mg/l of Lys + 0.175 M NaCl.

It is assumed that the seeds in plastic containers placed in the incubator for germination should have a length of ten mm. After seven days, the final germination percentage was recorded, the number of radicle, the radicle lengths of onions were measured in mm, the fresh weights were also determined in g/seed. All experiments were repeated three times.

After a few days, 1 - 2 cm root tips of germinated onion were cut for use in cytogenetic analysis. They were pretreated with saturated para-dichlorobenzene for 4 hrs, fixed for overnight in (3 : ethanol 1 : acetic acid) in the room heat and kept in ethanol (70%) at 4°C due to experimental procedures. For the preparation, these were hydrolyzed at 1N HCl for 15 min at 60°C and then stained in Feulgen about one hour, crushed at 45% acetic acid, counted in the research microscope (Olympus CX41) and photographed (Olympus C-5060) at X500 magnification (Sharma and Gupta 1982). For the frequency of micronucleus (MN), a total of 1.000 cells were counted in each application group. MN, mitotic aberrations and mitotic phases were observed under a microscope and photographed at X500 magnification.

The mitotic index was calculated by means of the formula: Mitotic index (%) = Number of cells in mitosis × 100/total number of cells. 10.000 cells per group were counted for MI. Chromosomal abnormality (CA) was calculated by means of the percentage of 2000 dividing cells counted per group. The statistical analysis was determined by SPSS program following the Duncan's multiple range test. The MN determination is based on the criteria of Fenech *et al.* (2003). According to this: (i) MN should be 1/3 of the cell nucleus or smaller (ii) MN should be round or oval and (iii) MN membrane should be clearly distinguishable from cell nucleus.

Results and Discussion

The radicle length and number of germinated group III seeds in the medium with lysine decreased as compared with ones of the group I (control) seeds while their fresh weight and germination percentage were statistically the same as ones of the group I seeds (Table 1). Conversely, there are only a few reports about the effects of Lys on seedling growth and seed germination in normal conditions. But, it could not reach a consensus in these studies. Thus, Lys has been reported to increase (Tsai *et al.* 1975, Tian *et al*. 2010) and inhibit (Green and Philipps 1974, Jacobs *et al.* 1987, Falco *et al*. 1995, Galili 1995) the seedling growth and seed germination. These differences of observations may have resulted from concentrations used, plant species and differences in treatment times.

Inhibitory effect of salinity on all examined growth parameters was re-emphasized with this study. For instance, group I seeds germinated in distilled water illustrated 100% germination after

7 days but in group II seeds germinated at 0.175 M salinity, this value was 23%. That is, salinity prevented 77% germination of *A. cepa* seeds. It is possible to observe the negative effect of salt in many ways, for example, changing in the case water of seed may prevent the seed germination thus preventing water uptake. It can be explained by the reduction of the fresh weight and water content of the seedlings in saline condition and by the inability of the roots to get enough water due to the high osmotic pressure of the condition. Inhibitory effect of salinity on the radicle number and length might be due to the reduction of protein synthesis, cell division and nucleic acid. The inhibiting effect of salt stress on seed germination was markedly mitigated by Lys application. In the mentioned salt level, group IV seeds treated with Lys demonstrated 70% germination. *A. cepa* seeds showed a performance such as germinated under normal conditions, are not in salt conditions as may be seen in Fig. 1. Also, Lys continued its success on the seedling growth parameters such as the fresh weight and radicle number (out of the radicle length). The fresh weight and radicle number of the group II were determined as 12.7 and 7.0 g, respectively while in Lys + NaCl treated groups, were determined as 17.2 and 10.5 g (Table 1). There are no reports on the effects of lysine on seedling growth and seed germination to date.

Table 1. Effect of Lys on some growth parameters of *Allium cepa*.

Groups	Growth parameters			
	Germination (%)	Radicle length (mm)	Radicle number	Fresh weight (g/seedling)
I	*100 ± 0.0^{c}	63.5 ± 0.5^{c}	63.2 ± 0.6^{d}	14.2 ± 0.8^{c}
II	23 ± 2.8^{a}	10.3 ± 0.3^{a}	12.7 ± 0.5^{a}	7.0 ± 0.5^{a}
III	100 ± 0.0^{c}	56.3 ± 0.4^{b}	51.2 ± 0.5^{c}	13.0 ± 0.6^{c}
IV	70 ± 5.0^{b}	10.3 ± 0.4^{a}	17.2 ± 1.3^{b}	10.5 ± 0.5^{b}

*The difference between the values in each column and the same letters is not significant at $p < 0.05$ level (±Sd). Group I: (control) distilled water. Group II: 0.175 M NaCl. Group III: 500 mg/l L-lysine. Group IV: 500 mg/l L-lysine + 0.175 M NaCl.

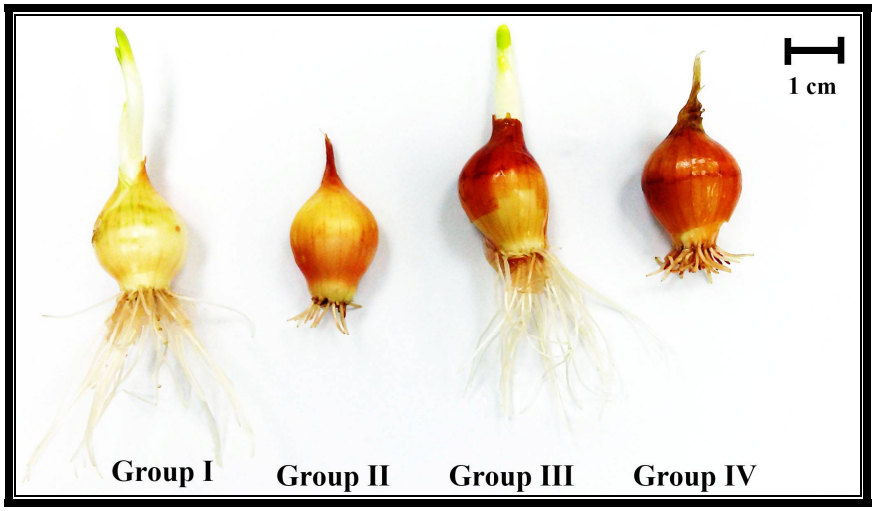

Fig. 1. Showing germination at the end of seven days of *A. cepa* L. seeds (Scale bar = 1 cm). Group I: distilled water, group II: 0.175 M NaCl, group III: 500 mg/l L-lysine and group IV: 500 mg/l L-lysine + 0.175 M NaCl.

Lysine on seedling growth and seed germination may be understood by the decrease in the osmotic effects of salt and by the reduction of salt stress. For example, in 0.175 M salt medium, Lys application increased significantly the fresh weight of the seedlings compared to posibility of the control group (Table 1). Moreover, it reduced the preventive effect of salt on the seedling growth and seed germination 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.

Table 2. Effect of L-lysine on some cytogenetic parameters of *Allium cepa*.

Groups	Mitotic index (%)	Micronucleus frequency (%)	Chromosome aberration (%)
I	*11.6 ± 1.0^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
II	1.2 ± 0.2^{a}	13.0 ± 1.0^{b}	17.0 ± 0.4^{d}
III	17.8 ± 0.8^{d}	0.3 ± 0.0^{a}	2.3 ± 0.5^{b}
IV	7.1 ± 0.2^{b}	12.3 ± 1.5^{b}	6.3 ± 0.7^{c}

*The difference between the values in each column and the same letters is not significant at $p < 0.05$ level (±Sd). Group I: distilled water, group II: 0.175 M NaCl, group III: 500 mg/l L-lysine and group IV: 500 mg/l L-lysine + 0.175 M NaCl.

If stress conditions are present in the environment, any plant growth regulator should be added in the germination process (Çavuşoğlu *et al.* 2017, 2018). There is no reports relating to effects of Lys on the mitotic activity and CAs in non-stress conditions until now. It is for this reason firstly whether lysine is affecting these parameters in salt and normal conditions have been investigated. According to results obtained from this work, MI in *A. cepa* meristem cells (group III) seeds exposed to Lys application in normal conditions ascended 53% according to ones of the group I seeds. In particular, 500 mg/l of Lys showed a perfectly successful effect on the mitotic activity by accelerating cell division. However, CAs frequency increased with this Lys application. In this case, some aberrations seem to result from this amino acid which can be deduced from the results (Table 2).

Mitotic Index (MI) is a parameter used to estimate the frequency of cell division in *A. cepa* root (Türkoğlu 2008). Chromosomal abnormalities (CA) are characterized by the chromosomal structure changes or in either total number of chromosomes which can occur spontaneously or as a result of the exposure to chemical or physical agents induced stress (Russel 2002). To evaluate the various chromosomal abnormalities, the prophase, metaphase, anaphase and telophase stages of the cell cycle were considered. The analysis of CA allows for the estimation of both genotoxic effects as well as for the evaluation of the clastogenic and aneugenic actions (Rank *et al.* 2002). It has been reported that high salt concentration causes chromosomal abnormalities and total mitotic activity inhibition in root-tip cells (Radic *et al.* 2005). This study re-indicate that salt negatively affects the mitotic activity and chromosome behaviors in *A. cepa* root meristem cells. These data indicated that salinity according to the control decreased 89% MI, showing higher number of chromosomal abnormalities and micronuclei. For example, the micronucleus frequency and CAs in the root tip meristems of the seeds germinated in distilled water were 0.0 and 0.0, respectively while it was 13.0 and 17.0 at 0.175 M salinity. In addition the simultaneous application of Lys + NaCl could be successful in alleviating the negative effect of salt on the mitotic activity. However, Lys application exhibited a perfect performance in alleviating the inhibitive effect of salt on MI.

Statistically, this performance would not be successful in decreasing the detrimental effects of salinity on the frequency of micronucleus. Besides simultaneous Lys + NaCl application has not showed remarkable achievement in reducing detrimental effect of salinity on the CAs as compared to Lys alone. The cause of these high abnormalities and micronuclei might be due to salt as mentioned above. The frequency of micronucleus and chromosomal aberration in root tip meristems of seeds germinated at Lys alone were less than those which were treated with 0.175 M NaCl (Table 2). These results showed that lysine had a protective role against salt injuries during *Allium* mitosis.

Figs 2, 3 showed normal and abnormal mitotic phases observed during microscopic examination of the root tip meristem cells of *A. cepa*. CAs are changes in chromosome structure resulting from a break or exchange of chromosomal material. In the present study different kinds of chromosomal aberrations were observed in cells with the frequency of occurrence as: micronucleus > irregular movement of chromosomes > hyperchromasia > lobed nuclei > chromosomal stickiness > telophase bridge formation > anaphase with bridges > ring chromosome at metaphase > metaphase with isolate chromosome > ball metaphase > giant cell (Fig. 3).

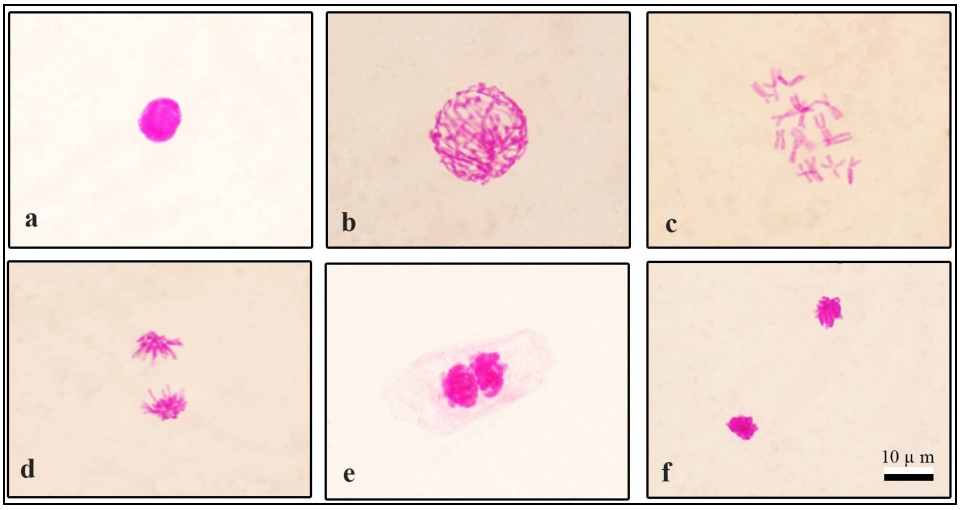

Fig. 2. Normal mitosis phases in root meristems tip cells of *A. cepa*. (Scale bar = 10 µm) a: İnterphase, b: Prophase, c: Metaphase, d: Anaphase, e: Early telophase and f: Late telophase.

Generally, misorientation of chromosomes may be due to the disturbed functioning of spindle apparatus, a change in the direction of movement of daughter chromosomes during anaphase or a tilt in the equatorial organization of metaphase chromosomes. Tripathy and Rao (2015) stated that disturbance in metaphase can be the result of disturbances in the spindle apparatus or due to inhibition of spindle formation. Kaymak (2005) attributed that inhibition of spindle treads and chromosome splits occur from various chemicals which affect the basic proteins required for spindle fibers. The cytotoxic effects of some chemicals in occurrence of fragments on DNA doubled spindles were observed (Çavuşoğlu 2008). Khora *et al.* (1997) declared that sister chromatid exchanges involve DNA breakage and reunion are believed widely to represent the interchanges of DNA replication products at apparently homologous loci. Kaymak (2005) claimed that the stickiness and bridges which are scored to be clastogenicity indicators in chromosomes are induced by chemicals considered to be clastogenic agents. The most effective and simplest

endpoint for analyzing mutagenic effects is the micronucleus, which may form a small individual nucleus as a result of not integrating the whole chromosome or the acentric fragmant into the new nucleus (Terradas *et al*. 2010).

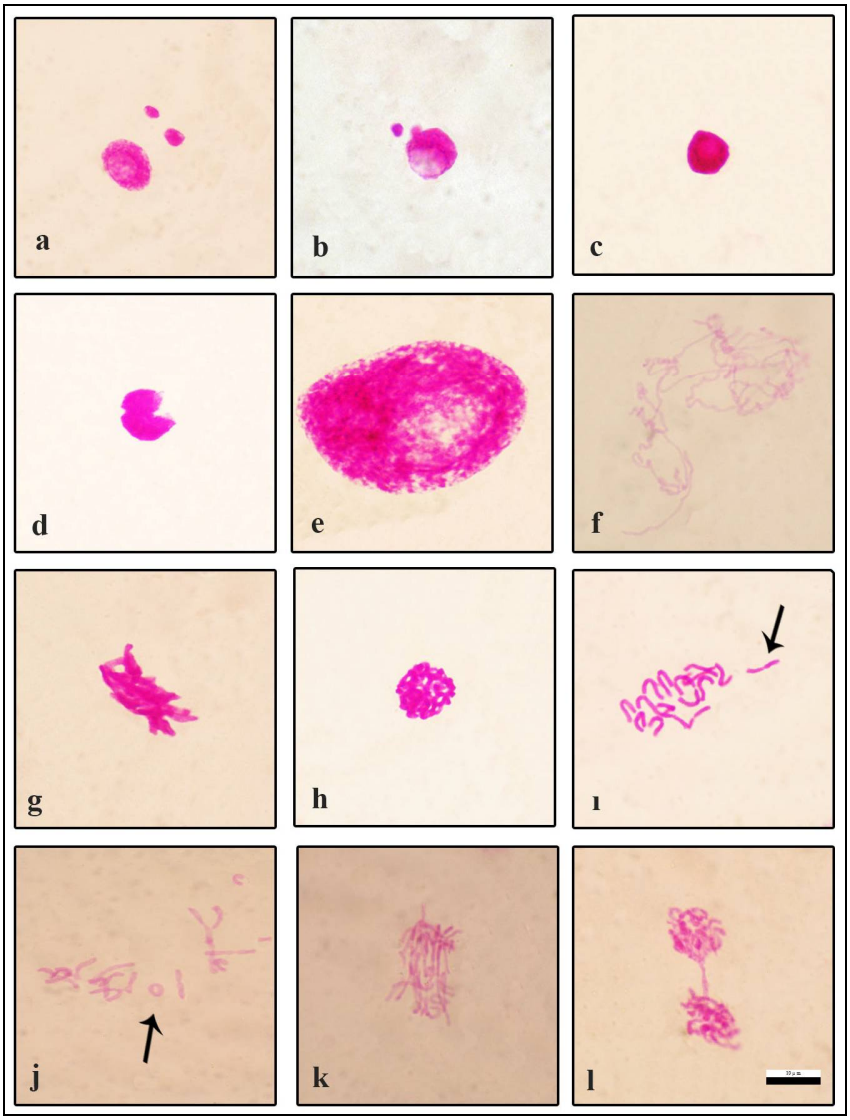

Fig. 3. Chromosomal damages in mitotic phases of *A. cepa* L. root tip cells (Scale bar = 10 µm); a: cell with micronuclei, b: micronucleus with nuclear bud, c: hyperchromasia, d: lobed nuclei, e: giant cell, f: irregular movement of chromosomes in prophase, g: chromosomal stickiness, h: ball metaphase, ı: metaphase with isolate chromosome = arrow, j: ring chromosome at metaphase = arrow, k: anaphase with bridges and l: telophase bridge formation.

There are no reports on effects of Lys application on physiological and cytogenetical parameters examined in saline conditions. Therefore, the results of this study have been

particularly reported for the first time in saline conditions and show that Lys can significantly increase activations such as seedling growth and seed germination under saline or alone conditions. However, mechanisms in which salt inhibits growth are controversial and complex. They can also vary by cultivar and species. An universal mechanism has not yet been established. In spite of characterized salinity causes, the understanding of the salt prevent mechanisms in plant growth remains weak. It is for this reason further work is needed to learn more about the effect of Lys on the cell cycle, cell division and molecular metabolism of germination. In summary, this study can serve to design salt tolerance hypotheses in plants for providing new conceptual tools.

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