Physiological and Cytogenetical Effects of Glutamine Treatment in Onion (*Allium cep*a L.) Seeds Exposed to Salt Stress

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Citation
Çavuşoğlu, K., Duygu, T., & Çavuşoğlu, D. (2020). Physiological and Cytogenetical Effects of Glutamine Treatment in Onion (*Allium cep*a L.) Seeds Exposed to Salt Stress. *Rastenievadni nauki, 57*(1) 60-65

Abstract
The effects of glutamine on the seed germination, seedling growth (radicle lenght, radicle number and fresh weight), mitotic index and chromosome aberrations of *Allium cepa* L. germinated under saline conditions were examined in this study. Salt stress markedly inhibited the seed germination and seedling growth of *A. cepa*. Moreover, it reduced the mitotic index in the root-meristem cells of the seeds and fairly increased the number of chromosome aberrations. On the other, the inhibitive effect of salt stress on the seed germination, radicle lenght, fresh weight and chromosome aberrations was significantly decreased with glutamine application. However, this amino acid was ineffective in reducing of salt damage on the radicle number and mitotic index.

Keywords: cytogenetically parameters; glutamine; onion; physiological parameters; salt stress

INTRODUCTION

Salt stress generally is provided by Na salts especially NaCl. The highly soluble salt concentration in growing environments results in water shortage and salt stress due to physiological drought. Salty soils commonly are exist in arid and semi-arid climate regions of the world and cause salinity stress in plants. Saltness is an important abiotic stress factor that significantly affects survival and plant productivity. When a plant subjected to salt stress, its activity, salt concentration and chemical potential were higher than normal limits (Eker et al., 2006). Plants are stemless organisms, for this reason they need to cope with changing environmental conditions as adapted to to stress situations via various physiological and molecular processes. Plants need to get over water stress, exposed low external water potential and by ion toxicity (Demidchik et al., 2018).

Although glutamine has been classified as a non-essential amino acid (e.g sepsis, intense radiotherapy, chemotherapy), when its consumption exceeds its synthesis, it becomes a conditionally essential amino acid (especially in trauma patients or critically ill) (Buchman, 2001). The amino acid glutamine has recently been the focus of extensive scientific interest because of its many different physiologic role (including many aspects of nitrogen metabolism, as an important fuel source for the intestine and immune system, in acid–base balance in the kidney and as an anabolic precursor for muscle growth) and its importance in tissue and cell cultures. L-Glutamine (Gln,2,5-diamino-5-oxo-pentanoic acid) uses as an energy source and is a component of proteins. Gln has become increasingly popular as ingredients in functional foods, beverages dietary and supplements (Melis et al., 2004).

Allium cepa test, which is later simply called *Allium* test, is one of the most frequently used plant bio-assays. The *Allium* test has been used since it was introduced to evaluate mutagenic effects in the root tips of onions. Additionally, the importance of

the *Allium* test is supported by the very similar of the mutagenic activity of numerous compounds on mammalian cells and *Allium* test cells (Gajalakshmi & Ruban, 2014). The present study was designed to examine the influences of glutamine in the reducing of detrimental effects of salt stress on the seed germination, seedling growth, mitotic activity and chromosomal aberrations of *Allium cepa* L.

MATERIALS AND METHODS

In this study, *Allium cepa* L. seeds (2n=16 chromosome; *Amaryllidaceae*) were used. Salt (NaCl) concentration used was 0.125 M. L-glutamine concentration used in the experiments was 50 mg L^{-1}. L-glutamine and NaCl concentrations were determined in a preliminary investigation conducted by us. The present study has realized in Plant Physiology and Cytogenetic Laboratories of Biology Department in Süleyman Demirel University.

Germination of seeds of *Allium cepa* L. was carried out at a constant temperature (20°C), in the dark in an incubator. Healthy and approximately equal-sized *A. cepa* seeds were chosen. 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 0.125 M NaCl alone for 7 consecutive days.
- Group III was treated with a 50 mg L^{-1} dose of L-glutamine for 7 consecutive days.
- Group IV was treated with a 50 mg L^{-1} dose of L-glutamine+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. 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.

For cytogenetic analysis, root tips of germinated *A. cepa* were excised (1-1.5 cm segment) after several days. They were then 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°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) mounted 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 of all parameters was made by using SPSS program according to DMRT.

RESULTS AND DISCUSSION

As shown in Table 1, the germination percentage, radicle length and radicle number of the group III seeds treated with L-glutamine statistically showed

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

Groups	Growth parameters			
	Germination percentage (%)	Radicle length (mm)	Radicle number	Fresh weight (g/seedling)
Group I	*100 ± 0.0^{c}	58.7 ± 0.7^{c}	45.1 ± 0.7^{b}	10.5 ± 0.3^{c}
Group II	27 ± 2.8^{a}	13.5 ± 0.2^{a}	18.4 ± 1.4^{a}	7.1 ± 0.2^{a}
Group III	100 ± 0.0^{c}	58.6 ± 0.3^{c}	43.3 ± 1.2^{b}	12.7 ± 0.1^{d}
Group IV	75 ± 0.0^{b}	15.2 ± 0.4^{b}	18.3 ± 0.3^{a}	9.3 ± 0.4^{b}

*At the level 0.05 (±SD), the difference between values with the same letter in each column is not significant. Group I (control) treated distilled water, Group II treated 0.125 M NaCl alone, Group III treated 50 mg L^{-1} dose of L-glutamine, Group IV treated 50 mg L^{-1} dose of L-glutamine+0.125 M NaCl.

the same values as the group I (control) seeds germinated in distilled water medium while their fresh weights partly increased according to ones of the group I seeds.

Salt stress showed the restrictive effect on all examined growth parameters. For instance, the group I (control) seeds germinated in distilled water medium displayed germination 100% on the 7th day while this value became 27% in the group II seeds germinated in 0.125 M salinity. In other words, NaCl prevented 73% 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 (Flowers & 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. As expected the results from Table 1 clearly demonstrate that the seed germination and seedling growth of *A. cepa* seeds inhibited in salinity conditions. Results of these statements are are consistent with the present study in tems of displaying the diminish in water content and fresh weight of the seedlings in salinity conditions. The inhibitive 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-glutamine application markedly mitigated the inhibitive effect of salt stress on the seed germination. The group IV seeds treated with L-glutamine showed 75% germination (Fig. 1).

L-glutamine also continued its success on the radicle length and fresh weight. The radicle length and fresh weight of the group II seeds grown in 0.125 M salinity were 13.5 mm and 7.1 g, respectively while these values were 15.2 mm and 9.3 g in the group IV seedlings treated with L-glutamine (Tab. 1). But, the mentioned application was unsuccessful in alleviation of the inhibitive effect of salt stress on the radicle number of the seedlings. No study have been conducted about role of L-glutamine on the seed germination and seedling growth under both normal and saline conditions until now. That L-glutamine 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 0.125 M NaCl medium, L-glutamine application significantly 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).

So far, there is no reported data relating to effects of L-glutamine on the mitotic activity and chromosomal aberrations in non-stress and salt stress conditions. Therefore, in the present study

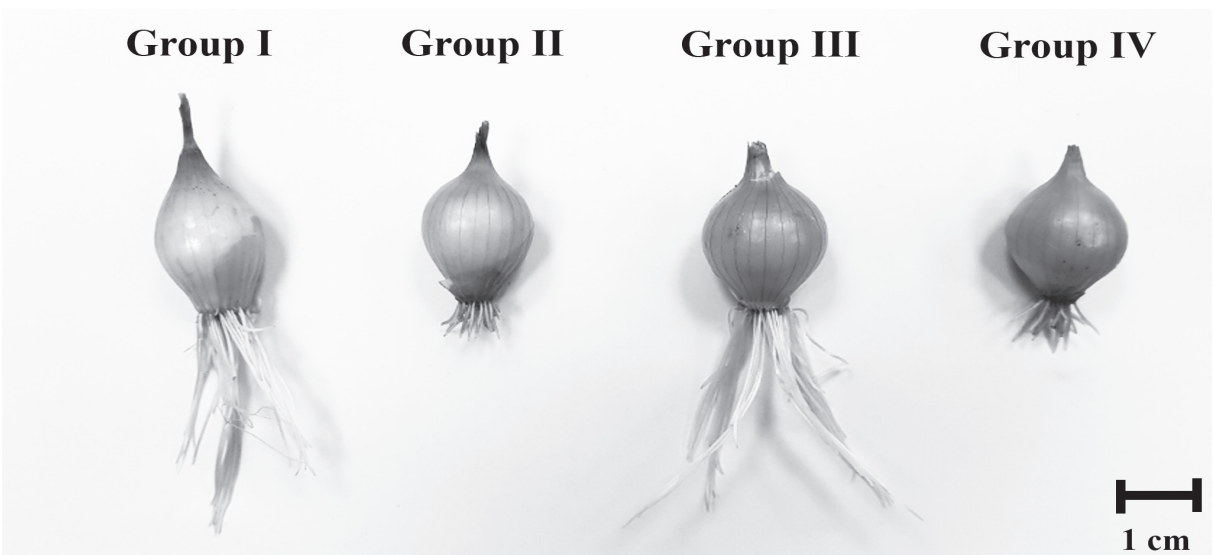

Figure 1. Root tip cells of *Allium cepa* showing germination situations at the end of 7 day. Group I (control): distilled water, Group II: 0.125 M NaCl alone, Group III: 50 mg L^{-1} L-glutamine and Group IV: 50 mg L^{-1} L-glutamine + 0.125 M NaCl. Scale bar = 1 cm

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

Groups	Mitotic index (%)	Chromosome aberration (%)
Group I	*7.6 ± 1.0[b]	1.5 ± 0.5[a]
Group II	3.1 ± 0.3[a]	10.2 ± 0.3[c]
Group III	3.1 ± 0.3[a]	13.6 ± 0.8[d]
Group IV	4.1 ± 0.2[a]	3.9 ± 0.7[b]

*Shows values with insignificant difference ($P < 0.05$) for each column shown with same letters. Group I (control) treated distilled water, Group II treated 0.125 M NaCl alone, Group III treated 50 mg L^{-1} dose of L-glutamine, Group IV treated 50 mg L^{-1} dose of L-glutamine+0.125 M NaCl.

was carried out to find whether L-glutamine is affecting these parameters in normal and saline conditions. The data obtained in this work indicated that mitotic index of the group III seeds germinated in the medium with L-glutamine alone partially decreased compared to the group I (control) seeds germinated in distilled water medium while their chromosomal aberrations excessively increased according to ones of the group I seeds. In this case, it may be said that some aberrations may result from this amino acid. Mitotic activity expressed as mitotic index decreased at 0.125 M salt concentration (group II) as compared to those of group I (control) samples germinated in distilled water. At the same time, the salt concentration caused a significant increase on the chromosomal aberrations in root tips of *A. cepa*. For instance, while mitotic index and chromosomal aberrations were 7.6 and 1.5 at control (group I), respectively, they were 3.1 and 10.2, respectively, at 0.125 M NaCl concentration. The inhibitory and cytotoxic effects of salt stress on the mitotic activity are known for a long time (Radic et al., 2005). According to some researchers, high salt concentration causes to total inhibition of the mitotic activity and chromosomal abnormalities in root-tip cells (Çavuşoğlu et al., 2017; 2019). On the other hand, L-glutamine+NaCl application (Group IV) showed a good performance in ameliorating the negative effects of salinity on the chromosomal aberrations (3.9). However, the mentioned amino acid application was ineffective in reducing of salt damage on the mitotic index (4.1). Statistically, all values mentioned here are substantially significant (Table 2).

The normal mitotic phases observed during the microscopic examination of root tip mitotic cells of *A. cepa* are shown in Figure 2 and abnormal mitosis phases are shown in Figure 3. The chromosomal aberrations majorly noticed were nuclear budding in this study. L-glutamine alone (Group III) and L-glutamine+NaCl treatments (Group IV) induced a number of nuclear budding when compared with other treatment groups. In this case, it may be said that glutamine might have caused the formation of nuclear buds. Other chromosomal damages observed in the cells are as follows: nuclear erosions, binucleolars, metaphase with ring chromosome, metaphase with chromosome loss, sticky metaphase, abnormal anaphase, anaphase with vagrant chromosomes, diagonal at anaphase, telophase with micronucleus, telophase with chromosome loop (Figure 3).

Chromosomal abnormalities (CAs) are a change in chromosomal structure from a break or exchange in the chromosome material resulting from breakage. As well as, CAs induction is considered as a result of genotoxic effects of various chemical and

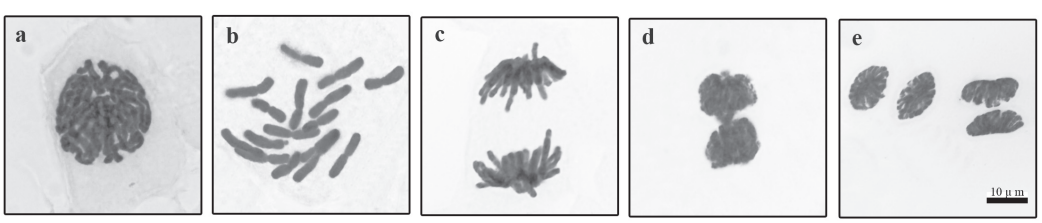

Figure 2. Normal stages mitosis phases in *A. cepa* root tip meristematic cells, Scale bar = 10 µm (a) prophase (b) 2n = 16 metaphase (c) anaphase (d) early telophase (e) telophases

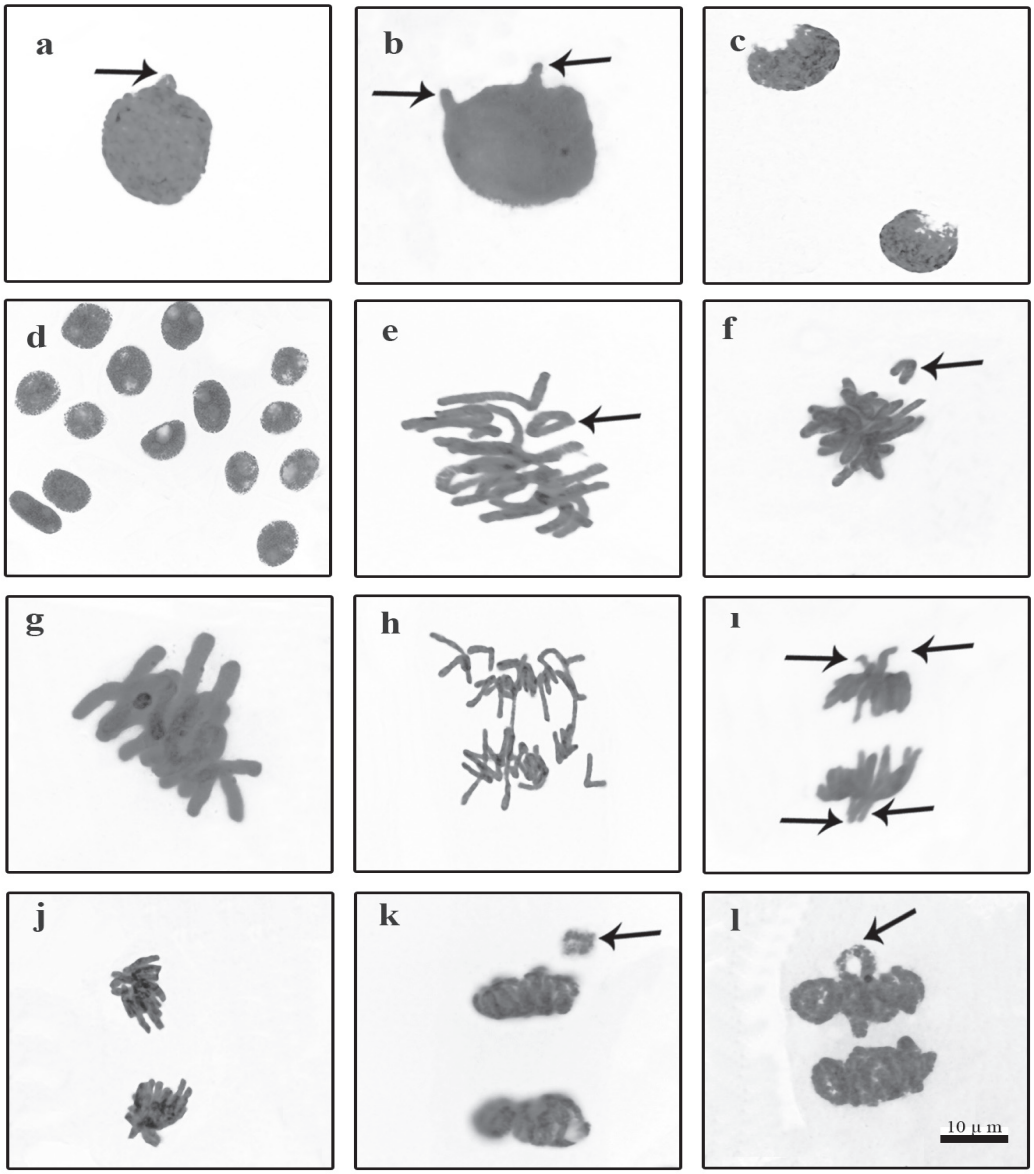

Figure 3. Different types of chromosomal aberrations; a: nuclear budding=arrow b: nucleus with nuclear buds=arrows c: nuclear erosions d: binucleolars e: metaphase with ring chromosome=arrow f: metaphase with chromosome loss=arrow g: sticky metaphase h: abnormal anaphase ı: anaphase with vagrant chromosomes=arrows j: diagonal at anaphase k: micronucleus in telophase=arrow l: telophase with chromosome loop=arrow (Scale bar = 10 µm)

physical agents and is also estimates of exposure of various organisms to different chemical and physical (Pohren et al., 2013). Nuclear erosion, which may result from the disintegration of chromatid proteins, represents irreversible toxicity (Karaismailoglu et al., 2013). Excess proteins and nucleic acids production induced by cytotoxicans result in nuclear buds (Fig. 3a) (Fenech et al., 2011). Ring chromosome (Fig. 3e) can spontaneously occur after breakage of the chromosomal ends and after the joining of the raw ends of the chromosomes (Khanna & Sharma, 2013). Chromosome loss (Fig. 3f) are typically associated with mitotic spindle malfunction. Abnormal anaphase (Fig. 3h) might be due to spindle apparatus disturbance which allows that the chromosomes to spread irregularly over the cell. As a result of spindle dysfunction, micronucleus (Fig. 3k) formation occurs as a result of chromosomal breaks and all chromosomes that do not migrate during the anaphase (Luzhna et al., 2013). Diagonal orientation

at anaphase (Fig. 3j) was caused by a slight tilt in the spindle apparatus (Renjana et al., 2013).

CONCLUSIONS

There is no present literature data related to the effects of L-glutamine application in both normal and saline conditions on the cytogenetically and physiological parameters studied here. Therefore, results of this study have been reported for the first time in non-stress and salt stress conditions. As a result, this study showed that L-glutamine can significantly increase the activations like the seed germination and seedling growth under normal or saline conditions. But the mechanisms by which salt inhibits growth are controversial and complex, also they might vary according to cultivar and species. An universal mechanism has still not been established. While the reasons of saltinity have been determined, it is still very poor to understand the mechanisms by which salty prevents plant growth. Therefore further work should be done to learn more about the effect of L-glutamine on cell division, cell cycle and germination molecular metabolism. For designing salinity tolerance hypotheses in plants, this literature study can serve to present new conceptual tools.

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