The Effects of Aspartic Acid on Some Physiological and Cytogenetical Parameters in *Allium cepa* L. Seeds Germinated under Salt Stress

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Abstract

The effects of aspartic acid 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, seedling growth and mitotic index was significantly decreased with aspartic acid application. However, this amino acid was ineffective in reducing of salt damage on the mitotic index.

Keywords: aspartic acid; cytogenetical parameters; onion; physiological parameters; salt stress

INTRODUCTION

Agricultural productivity is very low in semi-arid and arid regions of the world. Crops in these regions are naturally exposed to multitude abiotic stresses which limits their growth and productivity. The soil salinity from these factors largely limits crop production in these regions of the world (Munns, 2002). Salt stress is cytotoxic due to excessive uptake of ions such as chloride (Cl^-), sodium (Na^+) and nutritional imbalance (Isayenkov, 2012). The response of the plants to salinity is divided into two main stages. An ion-independent growth reduction that occurs within a few minutes to days, predominantly leads to stomatal closure in attraction and inhibition of cell expansion in mainly the shoot (Rajendran et al., 2009). The second phase, which occurs for days or even weeks, is related to the build up of cytotoxic ions levels that slow down metabolic processes. These phase causes premature senescence and eventually lead to cell death (Roy et al., 2014).

Aspartic acid (ASP, AspA, AsA), a non-essential amino acid, is one of the 20 essential amino acids and plays an important role in the Krebs cycle and neuroendocrine system (D'Aniello, 2007). Aspartic acid is found in L-, D- and LD-form in nature (Alam & Ahmad, 2012). It has a special importance among the amino acids due to its property to racemize from L- to D-form, which can be used to determine age of living and non living systems (Waite et al., 1999). L-Aspartic acid ($C_4H_7NO_4$) is an input material of aspartame that a low calorie sweetener in the food industry, so it has a high commercial value in the world market (Kirk & Othmer, 1992). L-Aspartic acid is also widely used in chemistry,

detergents, pharmacy, cosmetic, medicines and agriculture industry. It is good for fatigue because it gives vitality, strength and force (Pamuk, 2000). It is also sometimes used to treat chronic fatigue and depression (Iadarola et al., 2015). This amino acid is an environmentally friendly and biodegradable product that can be widely used in fields (Nita et al., 2006).

Allium cepa, which is used as a plant model, has been widely used to both in toxicology and in vivo studies of the toxicity and genotoxicity of samples. Additionally, this test has several advantages given that it is cheap, it is sensitive to rapid response bioassays, it is easy to manipulate and the most importantly, it has a good correlation with models that use mammalian cells for this study types (Gajalakshmi & Ruban, 2014). So - the present study was aimed at description the influences of aspartic acid 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

Test Material and Application Doses

Salt (NaCl) concentration used was 0.125 M. Aspartic acid concentration used in the experiments was 50 mg L^{-1}. Aspartic acid and NaCl concentrations were determined in a preliminary investigation conducted by us. *Allium cepa* L. (*Amaryllidaceae*) seeds of equal-sized (25–30 mm in diameter) were used as a test material. Germination of *Allium cepa* L. seeds was carried out at a constant temperature (20°C) in the dark in an incubator. Healthy *A. cepa* seeds were selected. Twenty seeds from each treatment group were placed into the plastic containers. The seeds were divided into 4 groups with 1 control and 3 applications. The seeds in the control (group I) were germinated in distilled water for 7 consecutive days. Group II, Group III and Group IV as applications groups were treated with alone 0.125 M NaCl, a 50 mg L^{-1} dose of aspartic acid, a 50 mg L^{-1} dose of aspartic acid+0.125 M NaCl, respectively for 7 consecutive days.

Measurement of Physiological Parameters

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 with the milimetric ruler. In addition, the fresh weights in g/seedling were measured with the precision scale. Percentage of germination (%) was determined as the percentage number of germinated seeds/total number of seeds (Atik et al., 2007).

Chromosomal Damage and Mitotic Index (MI)

After several days, root tips of germinated *A. cepa* were cut about 1-1.5 cm in length for cytogenetic analysis. Then, they were pretreated with saturated para-dichlorobenzene for 4 hrs, fixed for overnight in ethanol: acetic acid (3:1) solution at room temperature and kept at 4°C in 70% ethanol due to experimental procedures. For the preparation, the root tips were hydrolyzed at 5 N HCl for 20 min, then stained in Feulgen for 1-1.5 hrs, crushed at 45% acetic acid, counted at X500 magnification in the research microscope and microscopic slides were made permanent by mounting in balsame after 24 hrs. The mitotic phases and mitotic aberrations were photographed with a digital camera (Olympus C-5060) mounted on an Olympus CX41 microscope. Mitotic index (MI) was calculated by counting at least 9.000 cells per treatment (approx. 3.000 per slide). MI = number of cells in mitosis / total number of cells observed. Statistical evaluation of all parameters was made by using SPSS program according to DMRT. All experiments were repeated 3 times. The present study has realized in Plant Physiology and Cytogenetic Laboratories of Biology Department in Süleyman Demirel University.

RESULTS AND DISCUSSION

Table 1 shown that, the germination percentage and radicle length of the group III seeds treated with aspartic acid statistically showed the same values as the group I (control) seeds germinated in distilled water medium while their radicle numbers and fresh weight 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

Table 1. Effect of aspartic acid 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^{c}	10.5 ± 0.3^{c}
Group II	27 ± 2.8^{a}	13.5 ± 1.2^{a}	18.4 ± 1.4^{a}	7.1 ± 0.2^{a}
Group III	100 ± 0.0^{c}	59.9 ± 0.4^{c}	46.8 ± 0.5^{d}	11.6 ± 0.7^{d}
Group IV	80 ± 0.0^{b}	16.3 ± 0.4^{b}	24.8 ± 0.2^{b}	9.3 ± 0.2^{b}

*Means with the same letter within the same column are not statistically different at the level 0.05 (±SD). Group I (control) was treated distilled water, Group II was treated 0.125 M NaCl alone, Group III was treated 50 mg L^{-1} dose of aspartic acid and Group IV was treated 50 mg L^{-1} dose of Asp+0.125 M NaCl.

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 plant growth and regulation. 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. 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).

Aspartic acid application markedly mitigated the inhibitive effect of salt stress on the seed germina-

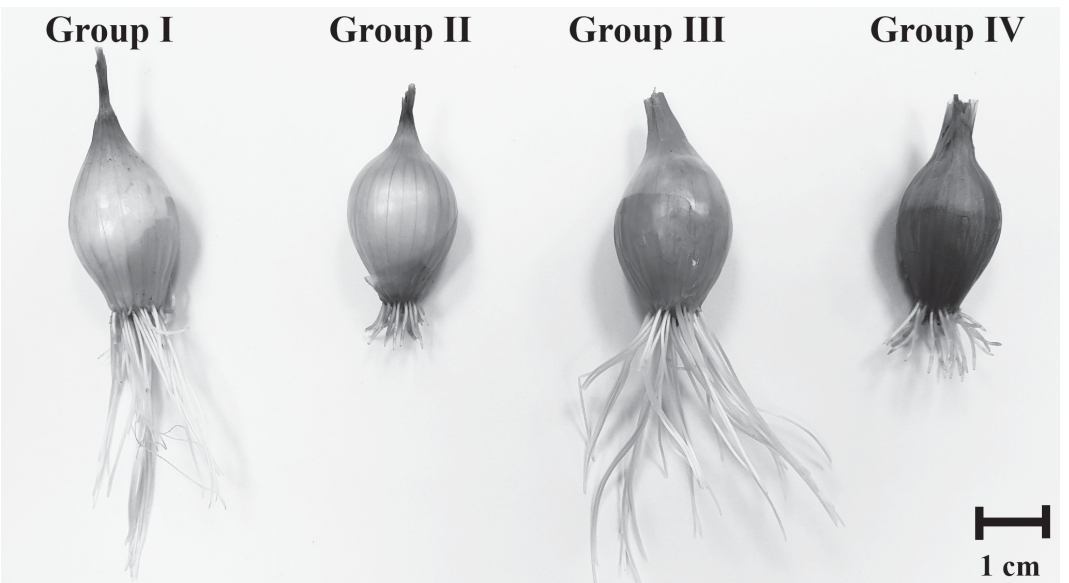

Figure 1. Root tip cells of *Allium cepa* showing germination situations at the end of 7 day. Group I (control): distilled water, Group II: 0.125 M NaCl alone, Group III: 50 mg L^{-1} aspartic acid and Group IV: 50 mg L^{-1} Asp+0.125 M NaCl. Scale bar = 1 cm

tion. The group IV seeds treated with aspartic acid showed 80% germination (Fig. 1). Aspartic acid also continued its success on the radicle length, radicle number and fresh weight. The radicle length, radicle number and fresh weight of the group II seeds grown in 0.125 M salinity were 13.5 mm, 18.4 and 7.1 g, respectively while these values were 16.3 mm, 24.8 and 9.3 g in the group IV seedlings treated with aspartic acid (Tab. 1). No study have been conducted about role of aspartic acid on the seed germination and seedling growth under both normal and saline conditions until now. That aspartic acid alleviates salt stress on the seed germination and seedling growth can be understood from the decrease in the salt's osmotic effects. If necessary the sample display, at 0.125 M NaCl medium, aspartic acid 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 inhibiting lipid photoperoxidation and reactive oxygen species (Blokhina et al., 2003) (Table 2).

So far, there is no reported data relating to effects of aspartic acid on the mitotic activity and chromosomal aberrations in salt stress and non-stress conditions. Therefore, in the present study was carried out to find whether aspartic acid is affecting these parameters in normal and saline conditions or not. The data obtained in this work indicated that mitotic index of the group III seeds germinated in the medium with aspartic acid alone partially showed a decrease (54%) as the group I (control) seeds germinated in distilled water medium while their chromosomal aberrations excessively increased (fifteen-fold) 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 *A. cepa* root-tips. For instance, while the mitotic index and chromosomal aberrations were 5.3% and 1.1% at control (group I), respectively, they were 2.2% and 13.6% respectively, at 0.125 M NaCl concentration. The cytotoxic and inhibitory 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, Asp+NaCl application (group IV) showed a perfectly good performance in ameliorating the negative effects of salinity on the chromosome aberrations (4.3% that is 68% reduction). However, the mentioned amino acid application was ineffective in reducing of salt damage on the mitotic index (3.0 %). Statistically, all values mentioned here are substantially significant (Table 2).

Table 2. Effect of aspartic acid on some cytogenetical parameters of *Allium cepa* L.

Groups	Mitotic index (%)	Chromosome aberration (%)
Group I	*5.3 ± 0.7^{b}	1.1 ± 0.0^{a}
Group II	2.2 ± 0.3^{a}	13.6 ± 1.0^{c}
Group III	2.4 ± 0.3^{a}	15.1 ± 1.7^{d}
Group IV	3.0 ± 0.6^{a}	4.3 ± 0.4^{b}

*Means with the same letter within the same column are not statistically different at the level 0.05 (±SD). Group I (control) was treated distilled water, Group II was treated 0.125 M NaCl alone, Group III was treated 50 mg L^{-1} dose of aspartic acid and Group IV was treated 50 mg L^{-1} dose of Asp+0.125 M NaCl.

The normal stages of mitotic cell division are showed in Figure 2 and also Figure 3 shows the various aberrations observed in the microscopic examination of *A. cepa* root-tip mitotic cells. The chromosomal damages majorly noticed were disorientation at anaphase in this study. Other chromosomal alterations were observed as of bilobulates, metaphase/anaphase with chromosome losses, cells with several lobed, disturbance at prophase, bridge(s) formation in anaphase, vagrant chromosomes and pole deviations in anaphase (Fig. 3).

Chromosomal aberrations induction could affect the fertility, vigour, competitive or yield ability of

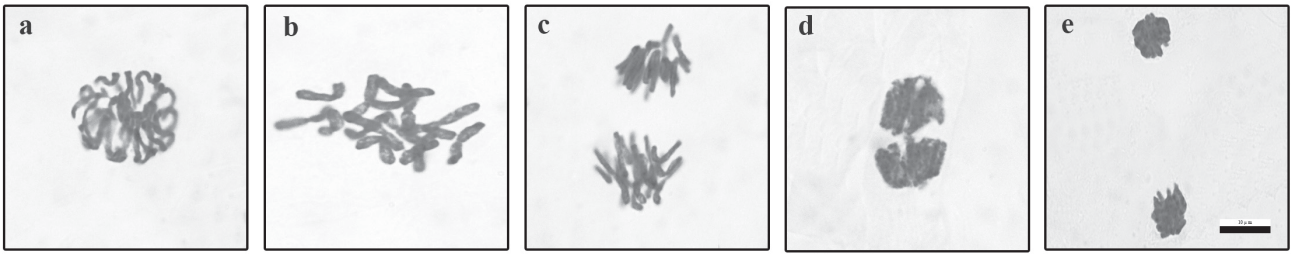

Figure 2. Normal mitosis phases in *A. cepa* root tip meristematic cells, Scale bar = 10 µm (a) prophase (b) 2n = 16 chromosome, metaphase (c) anaphase (d) early telophase (e) late telophase

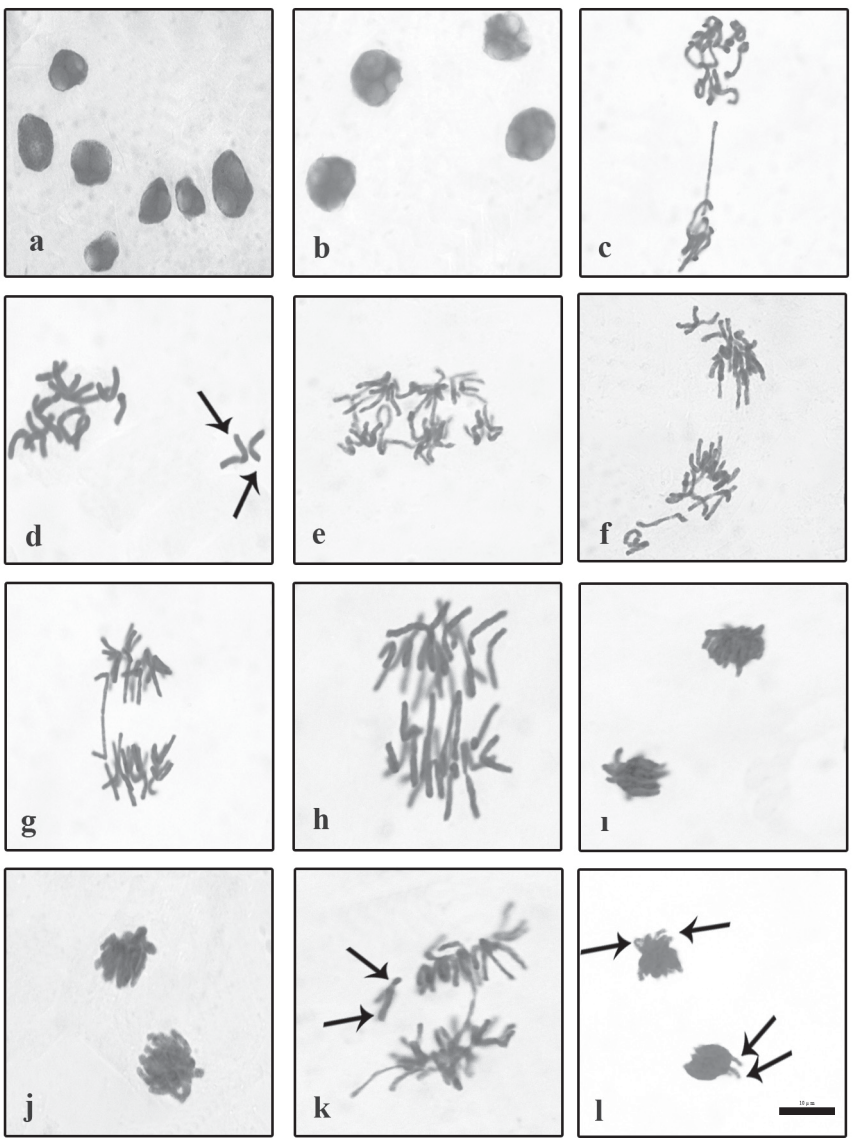

Figure 3. Chromosomal damages; a: bilobulates b: cells with several lobed, c: disturbance at prophase, d: metaphase with chromosome losses=arrows, e-f: disorientation at anaphase, g: anaphase with bridge formation, h: anaphase with bridges, ı-j: pole deviations in anaphase, k: disorientation at anaphase with chromosome losses=arrows, l: pole deviations at anaphase with vagrant chromosomes = arrows (Scale bar = 10 µm)

the exposed plants. CAs, an efficient test to investigate the genotoxic potential of chemical agents, are a change in chromosomal material or exchange in the chromosomal structure resulting from breakage (Leme & Marin-Morales, 2009). According to Akaneme & Iyioke (2008), the presence of bilobulates and cells with severeal lobed (Fig. 2a, b) indicates cytological evidences for the inhibitory effect on DNA biosynthesis. Disturbance at prophase (Fig. 3c) might cause chromosome loss when they can not bind to the spindle and therefore are not separated (Gisselsson et al., 2004). Metaphase/anaphase with chromosome losses (Fig. 3d, k) are typically associated with mitotic spindle malfunction. (Leme & Marin-Morales, 2009). Disorientation at anaphase (Fig. 3e, f) might be due to spindle apparatus disturbance which allows that the chromosomes to spread irregularly over the cell (Luzhna et al., 2013). Anaphase with bridges (Fig. 3g, h) can be resulted by the occurrence of breaks that result in a chromatid fusion at the chromosome end or by the formation of dicentric chromosomes or may be a result of chromosomal rearrangements (Singh, 2003). Pole deviations in anaphase (Fig. ı, j, l) 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 aspartic acid application in both normal and saline conditions on the cytogenetical 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 aspartic acid 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 aspartic acid 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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