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L-Ascorbic acid modulates the cytotoxic and genotoxic effects of salinity in barley meristem cells by regulating mitotic activity and chromosomal aberrations

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Abstract. The objective of the present study was to with all details explain of the efficiency of L-ascorbic acid (L-AsA) also known as vitamin C on cytotoxicity and genotoxicity induced by salt stress in the barley apical meristems. As a result of the statistical analysis salt stress caused a significant ($P \leq 0.05$) decrease in mitotic index of barley seeds depending on concentration increase, while the frequency of chromosomal aberration (CA) increased. In addition, it was determined that mitotic index value was decreased by 46% with 1 µM L-AsA supplementation as compared to control and chromosomal abnormalities were increased by 8.96% as well as. However, in the case of simultaneously application of 1 µM L-AsA and different salt concentrations, the high salt concentrations exhibited an excellent success according to low salt concentrations in alleviating the mitodepressive effect of salt stress. Moreover, the frequency of chromosomal aberrations in the root meristem cells of those seeds with 1 µM L-AsA supplementation germinated at different salt concentrations was substantially reduced compared to own control group (alone 1 µM L-AsA pretreatment). The 1 µM L-AsA pretreatment at the highest salt concentration (at 0.40 M) was showed an excellent success by reducing the frequency of the chromosomal aberrations by approximately 90 %. Different salt concentrations and/or 1 µM L-AsA supplementation caused micronuclei and granulation as well as various chromosomal aberrations in prophase, metaphase, anaphase and telophase.

Keywords: cytotoxicity, genotoxicity, *Hordeum vulgare* L., mitotic index, ascorbic acid, salinity.

INTRODUCTION

Together the global climate change, which is starting to make its presence felt more and more, plants are becoming more frequently subjected to adverse abiotic stresses, such as extreme temperatures, cold, high salinity, and drought, which limiting plant growth and crop productivity. Salinity is one of the major environmental factors that reduce plant productivity

(Tobe et al. 2003; Sabagh et al., 2019). Nearly 20% of the world's cultivated land and also five-hundred thousand hectares of irrigation area in Turkey are threatened by salinity (FAO 2016). Salt stress inhibits or delay growth and development of plants by negatively affects plant growth via oxidative stress, especially ion toxicity, nutritional and hormonal imbalance, and osmotic stress (Parida and Das 2005; Ashraf, 2009; Elsheery et al. 2020a). Moreover, the retardant effects of salinity stress on growth, physiological aspects, productivity and cellular activity were also recorded on other different many plants species (Bargaz et al. 2016; Nassar et al. 2016; Elsheery 2020b; Tabur et al. 2021). However, plants develop highly complex mechanisms for tolerate salinity. Tolerance to salt stress of plants is of three types: osmotic stress tolerance, Na+ or Cl- exclusion, and the tolerance of tissue to accumulated Na+ or Cl- (Munns and Tester 2008; Zvanarou et al. 2020). Since the mechanisms behind salinity are quite complex and difficult to understand, impact of salinity on plants, type and causes of salinity, and salt tolerance strategies of plants are still discussed in level cellular and molecular (Zhu et al. 2016). The common view of many researchers in combating salinity is the development of high salt-tolerant plant varieties. However, this method, which is one of the economical ways to eliminate the negative effects of salinity on plants, shows inconsistency between different crops. Therefore, there is a great scientific burden on researchers to cope with this important environmental stress that also limit crop productivity. For all these reasons, most of the researchers contributed to overcome the disadvantages of salt stress and to develop salt tolerant varieties by using various hormones, plant growth regulators, leaf extracts, vitamins biofertilizer and amino acids (Tabur and Demir 2010 a,b; Mohsen et al. 2014; Çavuşoğlu et al. 2016 a,b; Naser et al. 2016; Mahfouz and Rayan 2017; Farheen et al. 2018; Özmen and Tabur 2020; Tabur et al. 2021).

In recent studies, it has been reported that some vitamins may be effective to alleviate the negative effects of salinity by increase resistance to salt stress (Shalata and Neumann 2001), plant growth and yield quality (El-Bassiouny et al. 2005; Bassuony et al. 2008), seed germination, seedling growth (Emam and Helal, 2008), mitotic activity (Özmen and Tabur 2020) some metabolic changes.

Ascorbic acid (AsA) is a naturalist product that acts as an antioxidant and enzyme and also improves cofactor. It acts as an essential substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide. There are various isomers of ascorbic acid or vitamin C (L-AsA, D-AsA, D-izoAsA). D-AsA and D-isoAsA do not have vitamin C function. Therefore, when ascorbic acid is mentioned, L-ascorbic acid (3-keto-L-gulofuranolaktan) comes to mind from these isomers because of the only isomer with biological activity (Dizlek and Gül, 2007). The stimulatory roles of L-AsA, a minor, water-soluble antioxidant, in plant growth and other developmental processes are well documented (Gallie 2013; Hossain et al. 2017; Gaafar et al. 2020). In plants L-AsA serves as a major redox buffer and regulates various physiological processes controlling growth, development, and stress tolerance. Being a major component of the ascorbate-glutathione (AsA-GSH) cycle, L-AsA helps to modulate oxidative stress in plants by controlling ROS detoxification alone and in co-operation with glutathione. Any fluctuations, increases or decreases, in cellular L-AsA levels can have profound effects on plant growth and development, as L-AsA is associated with the regulation of the cell cycle, redox signaling, enzyme function and defense gene expression (Hossain et al. 2017).

The ascorbic acid concentration increases in plant cells exposed to stress conditions and plays a role in providing tolerance against oxidative stress by playing a role in the direct clearance of O^{-2} and OH^{-}. As a result of enzyme and gene expression analyzes carried out under different abiotic conditions in many plants, it was determined that ascorbic acid-related gene expression levels increased and these increases were given as a defense response against stress. On account of this, it is emphasized that higher L-AsA levels are important to minimize oxidative stress and regulate plant metabolic processes. (Athar et al. 2008, 2009; Akram et al. 2017). The cellular AsA pool size in plants can be regulated by the coordinated action of many related enzymes. Numerous recent studies have confirmed that AsA level increases the tolerance and adaptation of crops to many abiotic stresses such as cold, drought, salinity, heavy metal toxicity and ozone stresses (Xie et al. 2009; Çavuşoğlu and Bilir 2015; Akram et al. 2017; Xu 2017; Sabagh et al. 2019; Gaafar et al. 2020; Nunes et al. 2020; Wang et al., 2020; Chen et al. 2021).

As mentioned above, there are many studies on the effects of AsA on seed germination, seedling growth, plant resistance, plant growth and yield quality, antioxidant enzyme activity, and some biochemical and metabolic changes under various abiotic stress conditions. In addition, it has been known for few decades that AsA plays an important role in plant growth and development by regulating cell division (Smirnoff 1996; Gallie 2013). However, a rather limited number of studies have been found on the response of ascorbic acid to cytotoxicity and genotoxicity caused by various abiotic stresses, including salinity (Barakat 2003; Yu et al. 2014; El-Araby

et al. 2020). For this reason, this work was designed to comprehensively test of the efficiency level of exogenous L-AsA against effects cytotoxic and genotoxic in caused by salt stress in barley meristem cells and to contribute to the gap in the literature. Namely, it is aimed at clarifying to what extent exogenous L-AsA is able to tolerate salt stress, whether it encourages cells to enter the mitosis division, and whether it causes any changes in the structure and behavior of chromosomes.

MATERIALS AND METHODS

The barley cultivar (*Hordeum vulgare* cv. 'Bülbül 89') used in this study was requested from the Field Crops Research Institute, Ankara, Turkey. NaCl and ascorbic acid (L-AsA) used in the experiments were obtained from Merk and Sigma-Aldrich, respectively. Primarily, to prevent fungal contamination, the barley seeds were surface sterilized by immersion in 1% (w/v) NaClO solution for 10 min, rinsed thoroughly five times with sterile distilled water and dried on filter papers at room temperature prior to experimental procedure. The sterilized seeds were divided into two groups and soaked in constant volumes (50 ml) of distilled water (control, C) and L-AsA (1 µM, micromolar) for 24 h at 20 ± 1°C. The solutions were filtered at the end of this pretreatment session and 20-25 barley seeds which uniform sized were placed in Petri dishes covered with two sheets filter papers moistened with 7 ml of distilled water or three different NaCl (0.32, 0.35 and 0.40 M, molar) concentrations. After, Petri dishes were transferred to incubators at constant temperature (20 ± 1 °C) for germination for several days. These salt levels hindering germination of seeds on a large scale and the most proper concentration of L-AsA level in alleviation of the salt inhibition at the germination were determined in a preliminary investigation conducted by us.

To cytogenetic analyses after 3 or 4 days, the root tips reached to 0.5-1 cm were excised, pretreated with a saturated solution of paradichlorobenzene for 4 h at 20°C, fixed with Carnoy's Fluid I (absolute ethanol: glacial acetic acid, 3:1, v/v) for 24 h, and stored in 70% ethanol at 4°C until required. Then, root tips were hydrolyzed in 1 N HCl at 60°C for 15-18 min, stained for 1-1.5 h in accordance with the standard procedure for Feulgen staining, and squashed in 45 % acetic acid (Sharma and Gupta 1982; Elçi and Sancak 2013). After one day, microscopic slides were made permanent in by mounting Canada balsam by alcohol vapor exchange method. The best mitosis phases and aberrances were observed in permanent slides and photographed (100X) with a digital camera (Olympus C-5060) mounted on an Olympus CX41 microscope.

The prepared slides were examined under the microscope at 100X magnification, and mitotic index, i.e. percentage of dividing cells were accounted by counting approximately 15000 cells (three repeat, 5000 per slide) for all per-application. The mitotic index (MI) was calculated using the following the equation:

$$MI\ (\%) = \frac{\text{total number of dividing cells}}{\text{total number of analyzed cells}} \times 100$$

In addition, chromosomal aberrations (CA) occurring at all stages of mitosis during microscopic observation of the slides were calculated according to the following the equation for each per-application as the percentage of 350 dividing cells counted.

$$CA\ (\%) = \frac{\text{total number of abnormal cells}}{\text{total number of dividing cells}} \times 100$$

All experiments were repeated three times. Statistical evaluations of obtained data were actualized using the SPSS 14.0 program and Duncan's multiple range test (Duncan, 1955).

RESULTS

The mitotic index (MI) data obtained from the cytological analysis of barley root tips treated with different NaCl levels and 1 µM L-AsA (vitamin C) are summarized in Table 1. Based on these data, MI gradually drastic reduced with parallel to increasing NaCl levels as compared to the control group. At the highest salt level (at 0.40 M, molar), the mitotic index was reached to the lowest value by reducing from 7.0 ± 1.5 (control, in distilled water) to 1.6 ± 0.07 (77%). In the root meristem cells exposed to 1 µM L-AsA alone, a mitotic index reduction of about 46% was recorded according to control group. When samples with L-AsA treated germinated at different salt levels were compared with their selves control group (L-AsA alone), it was determined that the mitotic index increased statistically a little except for 0.32 M NaCl. 0.35 and 0.40 M NaCl levels exhibited major successful compared as each other the mitotic index values of L-AsA pre-treated and untreated samples at the same salt concentrations (Table 1). Especially, it was recorded that at the highest salt concentration (0.40 M NaCl) the mitotic index value was increases approximately two and a half times (from 1.6 ± 0.07 in control group to 4.0 ± 0.3 in 1 µM AsA).

Table 1. Mitotic index scores and frequency of chromosome aberrations in meristem cells of *H. vulgare* L. exposed to different NaCl concentrations after 1 µM L-AsA supplementation

NaCl (M, mol/L) and L-AsA (µM) Concentrations	Mitotic Index (%)	Chromosome Aberrations (%)
Control (0.00, Distilled Water)	*7.0±1.5^{c}	*0.00±0.0^{a}
1 µM L-AsA	3.8±0.4^{b}	8.96±2.5^{b}
0.32 M NaCl	6.3±0.4^{c}	2.30±1.0^{a}
0.32 M NaCl + 1 µM L-AsA	3.3±0.7^{b}	2.50±2.0^{a}
0.35 M NaCl	2.8±0.1^{b}	8.96±2.5^{b}
0.35 M NaCl + 1 µM L-AsA	4.0±0.2^{b}	1.70±0.6^{a}
0.40 M NaCl	1.6±0.07^{a}	21.2±2.5^{c}
0.40 M NaCl + 1 µM L-AsA	4.0±0.3^{b}	2.00±2.0^{a}

*Values with insignificant difference ($P \leq 0.05$) for each column are indicated with same letters (± Standard deviation). As test solution, 1 µM ascorbic acid (L-AsA) was used. Concentrations of NaCl were 0.32, 0.35, 0.40 M (mol/L). The pretreatment process of seeds was performed by soaking 24 h in constant volumes of distilled water (control) or L-AsA. Different concentrations of salt were added to germination medium. All data were evaluated as three replicates

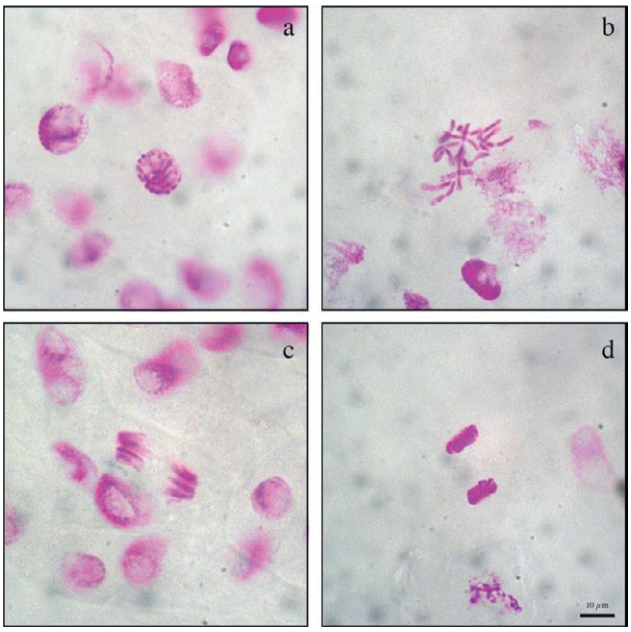

Figure 1. Normal mitosis stages in meristem cells of *H. vulgare* L. germinated in distilled water (Control). **a**- Prophase **b**- Metaphase (2n = 14) **c**- Anaphase **d**- Telophase. Scale bar = 10µm.

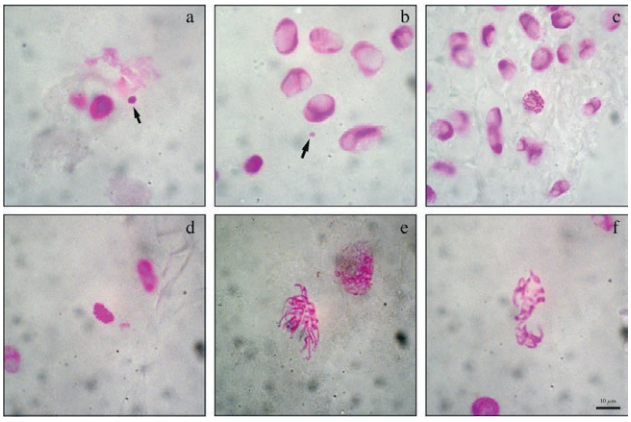

Figure 2. Aberrations observed in pre-prophase and prophase stage in meristem cells of *H. vulgare* L. germinated at different NaCl concentrations after 1 µM L-AsA supplementation for 24 h. **a-b**- micronuclei, **c-d**- chromatin granulation in interphase, **e-f**- disorderly prophase. Scale bar = 10µm.

The chromosomal aberration frequencies data obtained from barley root tips germinated both distilled water and different NaCl levels in the absence or presence of 1 µM L-AsA are summarized in Table 1. In parallel with the increasing salt concentrations, a very high rate of chromosomal aberrations observed in the root meristem cells of barley seeds. That is, while the chromosomal aberration frequency was 0.00±0.0 in the control seeds germinated in distilled water medium, it was recorded as 2.30±1.0 at 0.32 M salinity, 8.96±2.5 at 0.35 M salinity, and 21.2±2.5 at 0.40 M salinity. On the other hand, the frequency of chromosomal aberrations in seeds germinated in salt stress-free medium after 1 µM L-AsA supplementation alone was remarkably higher than that in the control group (distilled water, 0.00 M NaCl) and was also statistically significant. However, the frequency of chromosomal aberrations of seeds germinated at different salt concentrations after 1 µM L-AsA supplementation has exhibited a statistically significant decrease compared to the percentage of seeds treated with 1 µM L-AsA alone. When these values are compared with the frequencies of seeds germinated only at different salt concentrations, although1 µM L-AsA supplementation partially increased the chromosome aberration rate at the lowest salt level studied, it significantly reduced the negative effect of salt stress on this parameter, especially at high salt levels (at 0.35 and 0.40 M salinity). In other words, while the chromosomal aberration rate was 8.96 % at 0.35 M salinity and 21.2 % at 0.40 M salinity, the application of 1 µM L-AsA showed an excellent success, reducing these aberration rates to 1.70% and 2.00%, respectively (Table 1).

As a result of scans in mitosis slides, no abnormality was found in the meristem cells of the control group barley seeds germinated in distilled water and at 20°C, and all stages of mitosis were observed normally (Figure 1). Microscopic images of a wide range of chromosome aberrances observed in the preparations prepared with

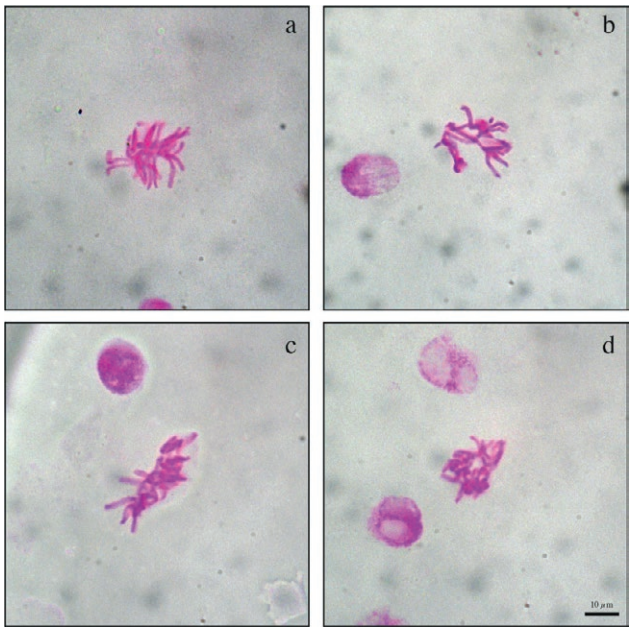

Figure 3. Aberrations observed in metaphase stage in meristem cells of *H. vulgare* L. germinated at different NaCl concentrations after 1 µM L-AsA supplementation for 24 h. **a-b-** uncoiling chromosomes, **c-d-** sticky chromosomes. Scale bar = 10µm.

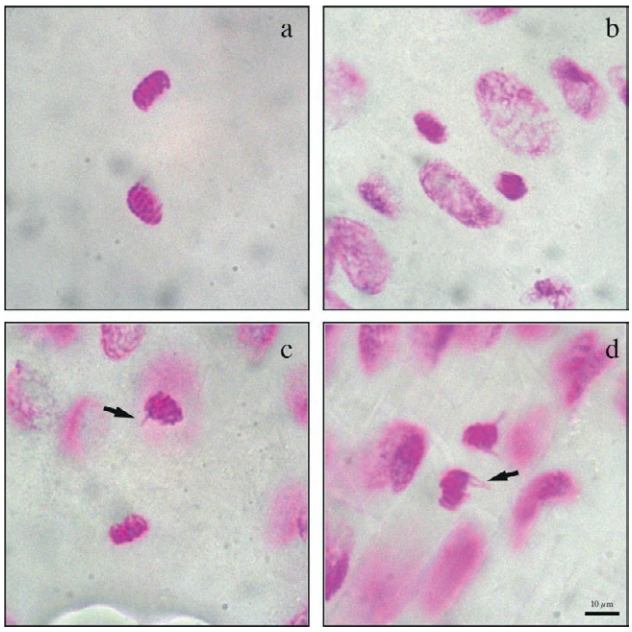

Figure 5. Aberrations observed in telophase stage in meristem cells of *H. vulgare* L. germinated at different NaCl concentrations after 1 µM L-AsA supplementation for 24 h. **a-b-** false polarization in telophase, **c-d-** false polarization in telophase and vagrant chromosomes (arrows). Scale bar = 10µm.

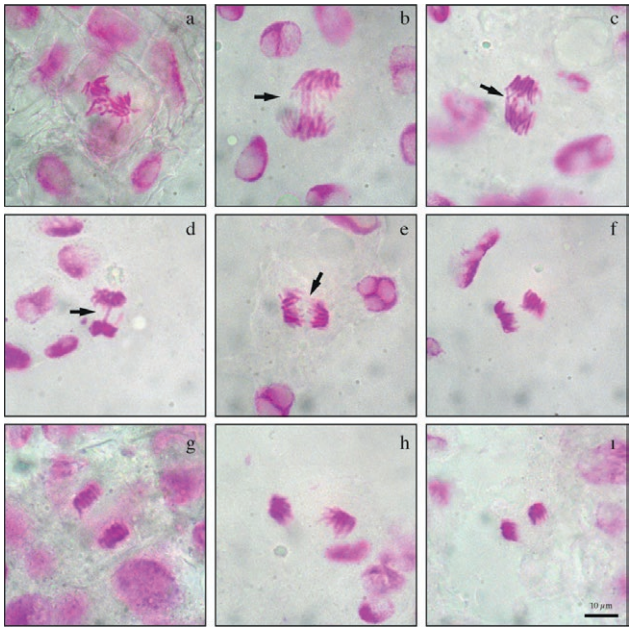

Figure 4. Aberrations observed in anaphase stage in meristem cells of *H. vulgare* L. germinated at different NaCl concentrations after 1 µM L-AsA supplementation for 24 h. **a-** disorderly anaphase, **b-d-** bridges in anaphase (arrows), **e-** laggard chromosome (arrow), **f-g-** alignment anaphase, **h-ı-** false polarization in anaphase. Scale bar = 10µm.

root tips belonging to all other application groups are shown in Figure 2-5. The most common chromosome abnormalities observed in all application were micronucleus, disorderly prophase and anaphase, uncoiling chromosome, sticky chromosome, bridges in anaphase, and false polarization in anaphase and telophase. The abnormalities such as alignment anaphase and vagrant chromosomes were observed in the minimal level.

DISCUSSION

In the present work, effect cytotoxic and genotoxic in the apical meristem cells of barley seeds exposed salt stress of exogenous 1 µM L-AsA supplementation were investigated comprehensive.

Although its mechanism has not been fully explained yet, the effects of salinity stress one of the most important abiotic stresses have been known for a long time by many researchers at cellular and chromosomal levels (Lutsenko et al. 2005; Tabur and Demir 2010 a,b; Pekol et al. 2016; Kiełkowska et al. 2017; El-Araby et al. 2020). All these researchers agree that salt stress causes chromotoxic actions and total inhibition of mitotic processes on meristematic cells, just as in our study. In addition to, Zvanarou et al. (2020) reported

that dividing root meristem cells are more sensitive to NaCl than other tissues since remains in direct contact with abiotic stress factors. However, salt damage extent depends upon plant species, stages of plant development, genotype, salinity concentration, and exposure time (Vicente et al. 2004; Tabur et al. 2021).

To date, many studies have been conducted on the effect of ascorbic acid on morpho-physiological, biochemical and metabolic changes under both normal and various stress conditions using various plant species (Khan et al. 2006; Dolatabadian ve Jouneghani 2009; Fatemi 2014; Mohsen et al. 2014; Gaafar et al., 2020; Nunes et al. 2020; Chen et al. 2021). However, studies on the protective role of exogenous L-AsA supplementation against the cytotoxic effects of various abiotic stresses and its effect on mitotic activity and chromosomal abnormalities, especially against salt stress, are quite insufficient (Barakat 2003; Yu et al. 2014; El-Araby et al. 2020). Therefore, first of all, it was found appropriate to compare the effects of L-AsA during germination in distilled water at 20°C before proceeding to its effects on these parameters under salt stress conditions.

As mentioned in the research findings section, the mitotic index value of barley seeds that were not pretreated with L-AsA (0.00 control, C) was 7.0±1.5, while this value was 3.8±0.4 in seeds that were pretreated. In other words, L-AsA supplementation alone caused a decrease of approximately 46% on the mitotic index compared to the control group (see Table 1). Mitotic index, as known is one of the most important indicators reliably identified the presence of cytotoxicity (Fiskesjö 1985). The decrease of the mitotic index value below 50% compared to the control variant leads to a sublethal effect, while below 22% it can cause lethal effects on test organisms (Mesi and Kopliku 2013). Undoubted, in this case 1µM L-AsA supplementation alone has a potential for sublethal effects. In addition, L-AsA application alone increased the rate of chromosomal aberrations by 8.96% compared to distilled water (see Table 1). As a result of this study, it was revealed that 1µM L-AsA supplementation alone reduced the mitotic index value and had a negative effect on chromosomal aberrations in barley seeds germinated in distilled water environment. Our findings regarding mitotic index and chromosomal abnormalities are in agreement with the study reported in *Allium cepa* by Asita et al, (2017). However, Cenanovic and Durakovic (2016) reported that ascorbic acid treatment at different concentrations (250, 500 and1000 µg/ml) increased the mitotic index in *Allium cepa* root meristems. It is thought that this difference may have occurred depending on the plant species studied and/or the dose and application time of the ascorbic acid used.

As for the effect of L-AsA application on the mitotic index and chromosomal aberrations of barley seeds germinated in saline conditions, the data obtained from our study on the mentioned parameters will be presented for the first time for barley plant. As a result of our literature research, only three previously reported studies were found that were more or less close to the subject. Firstly, Barakat (2003) reported that high salt concentrations significantly reduced mitotic activity and increased chromosomal aberrations in *Allium cepa* L. However, the researcer has determined that the ascorbic acid supplementation significantly increased the mitotic index and reduced chromosomal aberrations by reducing inhibitory effect of salt. Secondly, Yu et al. (2014) emphasized that the application of the AsA (0, 0.5, 1, 2, 4 mM) decreased markedly chromosome aberrations frequency, and increased mitotic index on *Vicia faba* roots exposed to different concentration of Pb $(NO_3)_2$. Finally, El-Araby et al. (2020) has been researched the effects of two concentrations of ASA (50 and 100 ppm) on the cytological parameters of pea seedlings under salinity stress. They reported that ASA (100 ppm) treatments significantly reduced the damaging effect of salinity stress on mitotic index and chromosomal abnormalities percentage. Similarly, also in our study, 1µM L-AsA showed an excellent performance on the mitotic index of barley seeds under high salt stress conditions. For example, 1µM L-AsA supplementation has increased mitotic index by approximately two and a half times at the highest salt stress condition (at 0.40 M salinity), (see Table 1). In addition, L-AsA supplementation under especially high salt stress conditions showed statistically positive effects on chromosomal aberrations in root meristems of barley seeds too. Although the application of 1µM L-AsA alone caused a significant increase of chromosomal aberrations in root meristem cells of seeds germinated in distilled water, in parallel with the increasing of the salt concentrations, the detrimental effect of supplementation 1µM L-AsA has seriously reduced, from 8.96 ± 2.5% abnormal cells (at distilled water, control) to 2.00 ± 2.0% (at 0.40 M). Moreover, while ratio of the chromosomal aberrations in the highest salt level studied (at 0.40 M) was 21.2 ± 2.5%, it was reduced to 2.00 ± 2.0% with the application of 1µM L-AsA. In other words, 1µM AsA application at 0.40 M salinity has shown an excellent success by almost zeroing the detrimental effect of salt stress (see Table 1). That is, we can say that L-AsA application may be more successful in high salt levels than in low salt levels in alleviating the detrimental effect of salt stress on chromosome structure and behaviors. From here, it can be concluded that effective in including the adaptive response to genotoxic stress since L-AsA at

high salt concentrations significantly reduces the clastogenic effects induced by salinity. Also, it is important to point out that L-AsA, known as vitamin C, have the ability to reduce the toxic effect of various genotropic toxicants if used in appropriate doses and at the convenient stage of growth and development. In unstressed conditions, administration of L-AsA alone might have been function as a stimulator, slowing down the mitotic cycle by suppressing the synthesis of proteins required for normal cell division (Tabur et al. 2021). The slowdown in the mitotic cycle might have triggered mitodepressive effects during cell division, thus causing a significant increase (8.96%) of chromosomal aberrations. It has been known for a long time that external stimulatory growth regulator applications are useless and even harmful under normal conditions without stress (Tabur ve Demir 2010a). Therefore, it is not surprising that L-AsA application alone in distilled water reduces the mitotic index and increases chromosome aberrations. Then, we can say that L-AsA supplementation under stress conditions, especially at high salt concentrations (at 0.35 M and 0.40 M salinity), may have accelerated mitotic activity and consequently reduced chromosomal aberrations caused by stress. Undoubtedly, these results supported that exogenous L-AsA may play a protective role against the harmful effect of salt stress on chromosomes by eliminating the mitodepressive effects that occur under stress conditions.

Chromosomal abnormalities that occur spontaneously or as a result of exposure to environmental stresses are indicate the harmful effect of a toxic agent on plant cells (Nag et al. 2013). Many biotic and abiotic toxic agents can promote the occurrence of chromosome aberrations by different mechanisms, including aneugenic (changes in total chromosome number) and clastogenic (changes in chromosome structure) actions. Feretti et al. (2007) sugessted that if toxic ajans cause damage to plant cell chromosomes, they may also be potentially harmful for mammalian cell chromosomes. Micronucleus (MN) assay is accepted as the most effective endpoint to analyze the mutagenic effect of the toxic agents. The large MN in the cell indicates aneugenic effect resulting from chromosome loss while small MN indicates clastogenic effect due to chromosome breaks (Kontek et al. 2007). Briand and Kapoor (1989) have reported that the micronuclei (Figure 2 a, b) are probably the result of vagrant chromosomes and fragments. Dane and Dalgıç (2005) reported that chromatin granulation is related to the inhibition of enzymes and histone proteins. It emphasized by many researchers that several chromatin regulation-related factors, such as histone modification enzymes, linker histone H1, HMG proteins and ATP-dependent chromatin remodeling factors have been functioned in plant abiotic stress responses (Kim et al. 2010; Asensi-Fabado et al. 2017). Chromatin granulation at interphase (Figure 2 c, d), most likely caused to deformation of the nuclear material by toxic agents, might be a consequence of all these reasons and abnormal chromatin condensation and indicative of many abnormalities that may occur in future mitosis phases. Uncoiling chromosomes (Figure 3 a, b) and disorderly prophase (Figure 2 e, f) may be the result of a weak mitotic effect and irregular chromosome contractions (Tabur et al. 2021). Sticky chromosomes (Figure 3 c, d) could be originated from abnormal DNA condensation, abnormal chromosomal wrapping and inactivation of the axes (Asita and Mokhobo 2013). At the same time it has been asserted that such aberrations may be a result of improper folding of the chromatin fibers (Klášterská et al. 1976). According to some researchers, sticky chromosomes are a marker of high toxic effect on chromatin and irreversibility of the change (Fiskesjö and Levan 1993; Türkoğlu 2007). In the current study, all of mitoclassic impacts in anaphase and telophase (Figure 4-5) that form an important portion of chromosomal abnormalities might have been largely resulted from spindle dysfunction. Fiskesjö (1997) have informed that bridges (Figure 4 b-d) are clastogenic effects, both resulting from chromosome and chromatid breaks. According to Tabur and Demir (2010 b) the bridges in anaphase and telophase might have been the result of inversions. Moreover, Bonciu et al. (2018) have asserted that nucleoplasmic bridges originate from dicentric chromosomes or occur as a result of as faulty longitudinal break of sister chromatids during anaphase. The disorganizations in mitosis such as disorderly anaphase (Figure 4 a), fault polarization at ana-telophases (Figure 4 h, ı; Figure 5 a-d), alignment anaphase (Figure 4 f, g) and bridges may be mainly the result of faulty kinetochore attachment or of spindle dysfunction (Rieder and Salmon 1998). Such irregularities constitute a significant portion of chromosomal aberrations. Vagrant (Figure 5 c, d) and lagging chromosomes (Figure 4 e) occurs during the anaphase where one or more chromatids gets detached from the rest of the chromatids and is incapable of moving towards the poles. Patil and Bhat (1992) have suggested that laggard chromosomes could be originate from the failure of spindle apparatus to organize in normal way. Also, the laggard of chromosomes may have occurred due to a weak mitotic impress. It known that salt stress, particularly NaCl caused too many c-mitotic reactions (Fiskesjö 1997). Therefore, increasing salt concentrations may have been reason to the formation of laggard chromosomes at high rates. Briefly, L-AsA alone and/or dif-

ferent salt levels used in our study may have been caused to all these abnormalities mentioned above by triggering the stimulation/ inhibition of enzymes and proteins necessary for the normal cell division, by disturbing the spindle mechanism.

CONCLUSION

In the present work, it has been compared the interactions between the mitotic index and chromosome behaviors of L-AsA under normal and salt stress using barley seeds. As known, the mechanisms by which salinity affections plant growth and development are rather complex and also controversial since a long time. Unfortunately, although the causes of salinity have been characterized, our understanding of the mechanisms by which salinity prevents plant growth is still rather poor. In summary, it was determined that L-AsA supplementation alone significantly reduced mitotic activity (46%) and caused a very high (8.96%) abnormality on chromosome behaviors in this study. In this case, L-AsA supplementation alone can create various types of mutations over time. However, this study supports that exogenous L-AsA pretreatment, especially at high salt concentrations, can eliminate the negative effects of salinity on the mentioned parameters in barley plant. The obtained results in our work may provide new conceptual tools for designing the hypotheses of different salt tolerance in plants and to brighten many contradictions particularly in relation to effects of L-AsA and high salt stress on mitotic activity and chromosomal abnormalities. Surely! Further investigation is needed to confirm these findings. Consequently, surveying the effects of L-AsA on principal metabolic events, which can be directly or indirectly effective on cell division and chromosome configuration will contribute to clarify of this mechanism.

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