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Alleviation of Salt-Induced Stress in *Allium cepa* L. by Exogenous Glycine Treatment

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Abstract: In this study, the role of glycine on the mitotic index, chromosome aberrations, micronucleus frequency as cytogenetic parameters and on the seed germination, radicle length, radicle number, fresh weight as physiological parameters in *Allium cepa* L. seeds exposed to saltness were studied. Salinity displayed a significant inhibitory effect on the seedling growth and seed germination of *Allium cepa*. Furthermore, salinity reduced significantly the mitotic index in *A. cepa* root tip cells and ascended number of chromosomal aberrations and frequency of micronucleus which is the simplest indicator, the most effective of cytological damage. On the other hand, the restricting effects of salinity on the seed germination, mitotic activity, chromosomal aberrations and seedling growth were alleviated dramatically in varying degrees by glycine application, but glycine was ineffective in reducing of salt damage on the micronucleus frequency.

Key words: Glycine, Seed germination, Seedling growth, Mitotic index, Chromosomal aberrations, Salt stress

Dışsal Glisin Uygulamasıyla *Allium cepa* L.'da Tuz Teşvikli Stresin Hafifletilmesi

Özet: Bu çalışmada, tuzluluğa maruz bırakılan *Allium cepa* L. tohumlarındaki fizyolojik parametreler olarak tohum çimlenmesi, radikula uzunluğu, radikula sayısı, taze ağırlık ve sitogenetik parametreler olarak mitotik aktivite, kromozomal anormallikler ve mikronükleus sıklığı üzerine glisinin rolü incelenmiştir. Tuzluluk *A. cepa*'nın tohum çimlenmesi ve fide büyümesinde önemli bir inhibe edici etki göstermiştir. Dahası, tuz *A. cepa'*nın kök ucu hücrelerindeki mitotik indeksi önemli ölçüde düşürmüş ve sitolojik hasarın en basit ve en etkili göstergesi olan mikronükleus sıklığı ve kromozomal anormalliklerin sayısını arttırmıştır. Buna karşılık, tohum çimlenmesi, mitotik aktivite, kromozomal anormallikler ve fide büyümesi üzerine tuzun inhibe edici etkileri glisin uygulamasıyla önemli ölçüde azalmış, fakat mikronükleus sıklığı üzerinde glisin tuz hasarının azaltılmasında yetersiz kalmıştır.

Anahtar Kelimeler: Glisin, Tohum çimlenmesi, Fide büyümesi, Mitotik indeks, Kromozomal anormallikler, Tuz stresi

1. Introduction

The reasons for the loss of soil productivity worldwide are the accumulation of partially or completely excess salts in the root zone. Areas under the threat of salinity are 20% of cultivated land in the world, more than 6% of the total area in the World [1]. Especially salinity-affected soils in Turkey are particularly located in Mediterranean Region,

Central North and Central South [2]. Saltness is one of the serious environmental problems causing a decrease in plant growth, reduced crop yield and osmotic stress in semi-arid and arid areas [3]. Saltness affects plant metabolism in many aspects like reduces yield and growth. Excessive salt in the soil solution can adversely influence plant growth either through osmotic inhibition of specific ionic effects or water uptake of roots [4]. Processes like vegetative growth, seedling growth and viability, seed germination, fruitset, flowering are negative influenced by high saltness concentration. Eventually, economical yield and at the same time production quality is reduced [5].

Development of high tolerant varieties is the most effective way to minimize salt damage in plant breeding. So scientists have used diverse vitamins and plant growth regulators to try eliminate or reduce harmful effects of saltness on the seedling growth [6, 7], mitotic activity [8] and seed germination [9]. Additionally, on the best way to breeding, most of the researchers agree that the pyramidization of different beneficial physiological features. But, despite significant endeavors, due to physiologically complex and genetically of this trait, the lack of reliable screening tools and most importantly, the lack of a comprehensive understanding mechanisms behind salt tolerance, results are still disappointing poor [10].

Glycine (Gly) is considered to be especially important as a nitrogen source for plants owing to low carbon-to-N ratio, its low-molecular weight, relatively rapid diffusion rates in soil. Gly is the most common amino acid used in plant uptake studies [11]. Gly, which assists in the formation of normal RNA and DNA constructs in the body, provides the appropriate construction of cellular functions and formations. It is also called a glucogenic amino acid because it provides the glucose needed for energy [12].

Allium cepa, commonly known as onion, was found to be an excellent indicator target for investigations of genotoxicity of many compounds with many privileges such as low cost, ease of use, short test duration and large chromosome size for the study of chromosome aberrations [13]. Although there have been several studies with combination of amino acids in normal conditions. Unfortunately, there is no published study on role of single glycine treatment on the physiological and cytogenetical parameters under especially saline conditions. For this reason, this study is examined the first time whether glycine effected on the seedling growth, seed germination, chromosomal aberrations, micronucleus frequency and mitotic activity of *Allium cepa* L. exposed to non-stress and salt stress conditions.

2. Material and Method

Approximately equal-sized and healthy *Allium cepa* (2n=16) L. seeds were used as test material in this laboratory bioassay. By a preliminary investigation carried out in the present study, glycine concentration was determined as 10 mg L^{-1} and salt (NaCl) concentration was determined as 0.175 M. Glycine obtained from Merck.

Experiments of germination carried out with *A. cepa* seeds that are physiologically and genetically homogeneous. Onion seeds were germinated in an incubator at 20°C in dark field condition. In the assay, the seeds were sterilized by NaClO 2.5% solution for ten minute and washed by ultra-distilled water for 24 h. Twenty seeds per each treatment group allocated into plastic containers. For seven successive days, they were divided into four groups:

- Group I (control) exposed to distilled water
- Group II exposed to alone 0.175 M NaCl
- Group III exposed to a 10 mg L^{-1} dose of glycine
- Group IV exposed to a 10 mg L^{-1} dose of glycine + 0.175 M NaCl

It is assumed that the seeds in plastic containers placed in the incubator for germination should have length of 10 mm. Approximately 7 days after the beginning of the assay: final germination percentages were taken, radicle numbers were recorded and also fresh weights were determined. Entire experiments were repeated three times.

When the roots after several days reached 10.5 mm in length, these roots were excised, pretreated with saturated para-dichlorobenzene for four hrs, fixed in (3:1) ethanol:acetic acid solution for 24 h at room temperature for cytogenetic analysis. These fixed roots were stored in 70 % C_2H_6O, were hydrolysed with 5 N HCl by cold-hydrolysis method for 45 min, were stained in Feulgen for 1-1.5 h at room temperature, smashed in a drop of 45 % acetic acid, squashed then counted (micronucleus cells, mitotic aberrations and mitotic phases) in Olympus CX41 research microscope, they photographed (Olympus C-5060) at X500 magnification [14]. A total of 1000 cells were counted in each application group for the formation of micronucleus (MN).

In addition to the evaluation of the induction of chromosomal aberrations to in the study, mitotic inhibition (MI) approximately 2000 cells per each slides per sample were analyzed. Mitotic index calculated as the number of cells in mitosis divided by the total number of cells × 100%. Statistical analysis was employed followed by Duncan's multiple range test in SPSS [15]. Micronucleus test is based on the criteria of Fenech [16]. According to this:

i. MN should be 1/3 of the cell nucleus or smaller.
ii. MN should be round or oval.
iii. MN membrane should be clearly distinguishable from cell nucleus.

3. Results and Discussion

The results from Table 1 clearly demonstrate that while the germination percentage and radicle length of group III statistically exhibited the same values as group I (control), their radicle number partly decreased, but the fresh weight partly elevated. On the other hand, there are merely a few studies relating to the impacts of glycine on the seedling growth and seed germination in non stress conditions. But, it can't reach a consensus in these studies. Thus, glycine has been reported to increased [17] or inhibited [18] the seedling growth and seed germination. In addition to, there are several studies conducted with multiple amino acids on the effects of glycine on the seedling growth and seed germination under normal conditions. Galindo et al. [18] reported that a mixture of glutamate+aspartate+alanine and single treatments of glycine, asparagine, histidine on the germination rates showed reversal effect in addition, showed reversal effect on root weight using histidine and glycine in lettuce seedlings under normal condition. Liu et al. [17] detected that foliar application of mixed amino acids solution contained seven equal concentrations of Gly+Glu+Gln+Asp+Ala+β-Ala+Asp increased both fresh weight and dry weight according to the control seeds in radish under normal condition. These differences of observations might have resulted from concentrations used, application method, plant species and differences in treatment times.

It has been previously reported that salinity conditions can negatively impact development, growth events in general even and even in halophytes. But, mechanism of the action of saltness has not fully been elucidated to date [19]. It is well known that saltness inhibits seedling growth [20] and seed germination [21]. Saltness showed an inhibitory effects on all growth parameters were re-emphasized with this study. For instance, the control seeds germinated in distilled water after 7 days displayed germination 100%, whereas this value was 23 % in group II seeds germinated at 0.175 M saltness. That is to say, NaCl prevented 77 % the seed germination. Salinity stress can be preventive in many ways. Seed germination can be prevented by causing to change in water situation of the seed, thus prevent water intake [22]. Results of the present study displaying the diminish in water content of the seedlings and fresh weight in salted conditions can be expressed by the inability of roots to receive enough water due to high of osmotic pressure in medium. Saltness prohibitive impact on the radicle number, fresh weight and radicle length might result from reducing protein synthesis, nucleic acid and cell division [23]. Harmfull impact of saltness stress on the seed germination was mitigated markedly by glycine application. In the said salt level, Group IV seeds treated with glycine demonstrated 77 % germination (Figure 1). Glycine continued also its success on all of the growth parameters. In such a way that group II's the length of radicle, radicle number and fresh weight were determined to be 10.3 mm, 12.7 and 7.0 g, respectively, these values were determined as 18.5 mm, 13.7 and 13.2 g in glycine+ NaCl applied group (Table 1). Unfortunately, to date, there isn't extant literature data in connection with effects of glycine on the seedling growth and seed germination exposed to saline conditions.

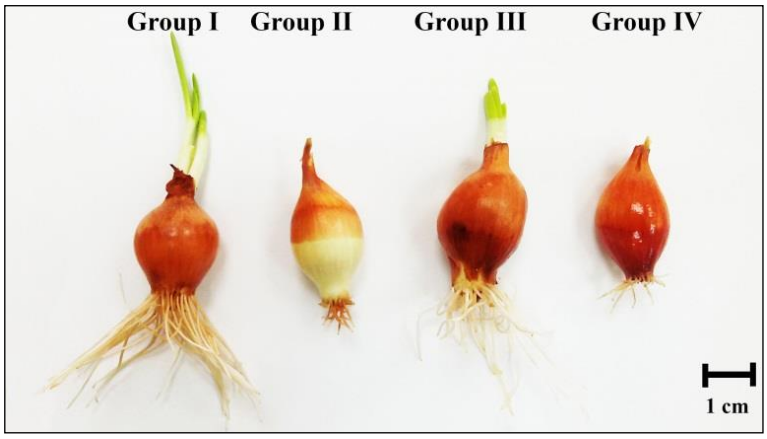

Figure 1. Germination situations showing of *Allium cepa* root tip cells at the end of the seventh day. Group I (control) exposed to distilled water; Group II exposed to 0.175 M NaCl alone; Group III exposed to a 10 mg L^{-1} dose of glycine; Group IV exposed to a 10 mg L^{-1} dose of glycine+0.175 M NaCl

That glycine 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 0.175 M NaCl medium, glycine 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 stimulating mitotic activity of the embryo (Table 2).

Table 1. Efficacy of glycine 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}	63.5 ± 0.5^{c}	63.2 ± 0.6^{d}	14.2 ± 0.8^{b}
Group II	23 ± 2.8^{a}	10.3 ± 0.3^{a}	12.7 ± 0.5^{a}	7.0 ± 0.5^{a}
Group III	100 ± 0.0^{c}	63.0 ± 0.4^{c}	36.5 ± 0.5^{c}	16.0 ± 0.8^{c}
Group IV	77 ± 2.8^{b}	18.5 ± 0.8^{b}	13.7 ± 0.3^{b}	13.2 ± 0.3^{b}

* At the level 0.05 is not significant the difference between values with the same letter in each column (±SD). Control (Group I) exposed to distilled water; Group II exposed to 0.175 M NaCl alone; Group III exposed to a 10 mg L^{-1} dose of glycine; Group IV exposed to a 10 mg L^{-1} dose of glycine+0.175 M NaCl

Çavuşoğlu et al. [6, 7] state that if stress conditions are present in the environment, any plant growth regulator should be added as in the germination process and some growth regulators might cause particularly cell distortions, mitotic irregularities and chromosomal abnormalities even without stress conditions. Even a single study of the effects of glycine on the chromosomal aberrations, micronucleus frequency and mitotic activity has not been performed until now. For this reason, this study was examined the first time whether glycine affected these parameters both normal and salinity conditions. According to results acquired from this experiment, the frequency of micronucleus and chromosome aberration in *A. cepa* root tip mitotic cells (group III) seeds exposed to 10 mg L^{-1} dose of glycine application partly ascended according to ones of control seeds (Table 2).

Table 2. Efficacy of glycine on some cytogenetic parameters of *Allium cepa* L.

Groups	Mitotic index (%)	Micronucleus frequency (%)	Chromosome aberration(%)
Group I	*11.6 ± 1.0^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Group II	1.2 ± 0.2^{a}	13.0 ± 1.0^{c}	17.0 ± 0.4^{d}
Group III	14.1 ± 0.6^{d}	2.0 ± 0.0^{b}	13.1 ± 0.3^{b}
Group IV	8.0 ± 1.3^{b}	11.3 ± 1.5^{c}	15.6 ± 0.7^{c}

* At the level 0.05 is not significant the difference between values with the same letter in each column (±SD). Control (Group I) exposed to distilled water; Group II exposed to 0.175 M NaCl alone; Group III exposed to a 10 mg L^{-1} dose of glycine; Group IV exposed to a 10 mg L^{-1} dose of glycine+0.175 M NaCl

Mitotic index could be used as a biological marker of increse cell growth in measuring the percentage of cells in different mitotic stages. A significant portion of the genetic damage produced by most mutagenic agent constitutes an important part of the chromosomal abnormalities [24]. Some researchers have reported that high salt concentration resuls in total chromosomal abnormalities and mitotic activity inhibition in root tip mitotic cells [25]. Results of the present study clearly show that salt is cytotoxic on mitotic cells of the tested plant. This data showed that saltness according to the control decreased 89 % mitotic index, also has shown an increase number of chromosomal abnormalities and micronuclei. For example, frequency of micronucleus and chromosomal abnormality in the meristem cells of the seeds germinated in distilled water were 0.0 and 0.0 respectively while it were 13.0 and 17.0 at 0.175 M salt. In addition to, simultaneous glycine+NaCl application can be succeeded in alleviating of inhibitive impact of saltness on the mitotic activity and chromosomal aberration but statistically this performance was inefficient in reducing of salt damage on the

micronucleus formation. The cause of micronuclei and these high abnormalities might be due to salinity as mentioned above (Table 2).

The normal and abnormal mitotic phases observed in course of microscopic examination in root tip cells of *A. cepa* show in figure 2 and figure 3. The most frequent abnormalities induced by salt were irregular prophase and micronucleus. The order of chromosomal aberrations was misorientation at ana-telophases with forward chromosome > cells with several lobed nuclei > disorganized anaphase > chromosome loss > unequal separation of chromosome at anaphase stage > pole to pole arrangement at metaphase > splits in nuclei > coagulated nucleus > ring formation > tetranucleate cells > early ball metaphase > vacuolated nucleus at prophase > adhered chromosomes with loop.

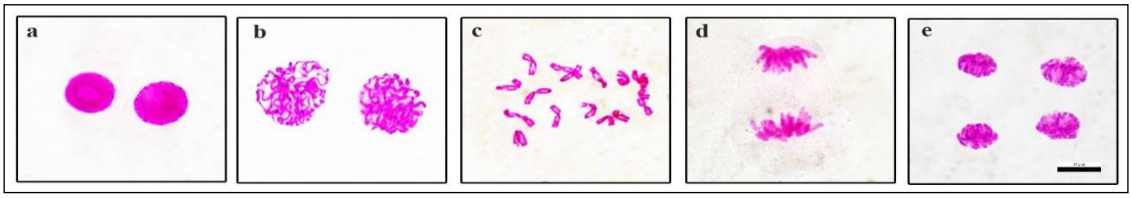

Figure 2. The normal mitosis phases in root tip meristem cells of *A. cepa* Scale bar = 10 µm. a) interphases b) prophases c) metaphase (2n = 16 chromosomes) d) anaphase and e) telophases

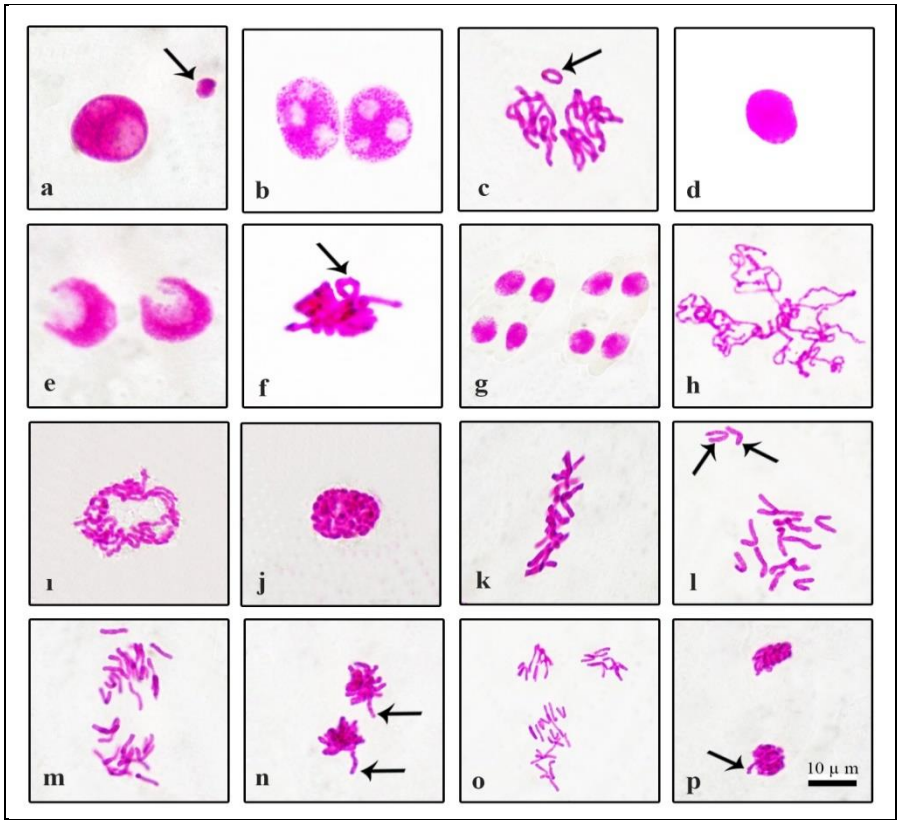

Figure 3. Photomicrograph indicating the presence of chromosomal abnormalities present in cells of *A. cepa*. Scale bar = 10 µm (a) bilobulated nucleus with micronucleus, **(**b) cells with several lobed nuclei, (c) ring formation=arrow, (d) coagulated nucleus, (e) splits in nuclei, (f) adhered chromosomes with loop=arrow, (g) tetranucleate cells, (h) irregular prophase, (ı) vacuolated nucleus at prophase, (j) early ball metaphase, (k**)** pole to pole arrangement at metaphase, (l) metaphase with chromosome losses=arrows, (m) disorganized anaphase, (n) misorientation in anaphase with forward chromosomes=arrows, (o) unequal separation of chromosome at anaphase stage, (p) misorientation in telophase with forward chromosome=arrow

The micronuclei formation (Figure 3a), the most efficient and simplest endpoint for analyzing mutagenic impacts, suggest that constraints in the salt may be closely related to the loss of genetic material. Ring chromosome **(**Figure 3c) is the result of chromosomes loss from telomeric side [26]. Any irregular prophase formation (Figure 3h) might cause unequal distribution of the chromosomes. Forward chromosome (Figure 3n, p) and chromosome loss **(**Figure 3l) are typically alterations associated to the malfunction of the mitotic spindle [13]. Fernandes et al. [27] stated that polynucleated cells (Figure 3g) and lobate nuclei (Figure 3a, b) are resultant of chromosome abnormality, as a consequence of multipolar anaphases, which are associated or not with chromosomal adherence, making the cells inviable. Vacuolated nucleus at prophase (Figure 3ı) resulted in the chromatin lost its stain-ability or appeared as dense granulated and nuclei become vacuolated. Adhered chromosomes (Figure 3f) causes extremely thickened and shortened chromosomes that situated at metaphase and prophase. Thus salt related to organization of chromatin might be regarding impact on the chemical and physical properties of DNA, protein or both and leading to abnormal chromatin folding [28]. Hoseiny Rad et al. [29] stated that ball metaphase (Figure 3j) results from the complete destruction of spindle fibres and a subsequent clumping of chromosomes into a tight ball.

4. Conclusions and Comment

There is no literature data on role of glycine application on the cytogenetic and physiological parameters examined exposed to salinity conditions. Therefore, the results of this study have been particularly reported for the first time in salinity conditions. As a conclusion, this study showed that glycine can significantly increase activations like the seedling growth and seed germination under salt or normal conditions. But, mechanisms in which salt restricts growth are controversial and complex. They can also vary by cultivar and species. So far, an universal mechanism has not been established. In spite of characterized salinity causes, the understanding of the salt prevent mechanisms plant growth remains weak. It is for this reason with further work is needed to learn more knowledge about the efficacy of glycine on the cell cycle, cell division and molecular metabolism of germination. In summary, this study to design salt tolerance hypotheses in plants can serve to provide new conceptual tools.

References

[1] F. A. O. (2015, February 20) . FAO land and plant nutrition management [Online]. Available: http:www.fao. org/ag/agl/agll/spush.

[2] K. Haktanır, A. Karaca, and S. M. Omar, *The prospects of the impact of desertification on Turkey, Lebanon, Syria and Iraq* (Book style). Springer Science-Business Media, Dordrecht, 2012, pp. 139–154.

[3] N. Çiçek, and H. Çakirlar, "The effect of salinity on some physiological parameters in two maize cultivars," *Bulg. J. Plant Physiol.,* 28(1-2), 66-74, 2002.

[4] E. Yildirim, A. G. Taylor, and T. D. Spittler, "Ameliorative effects of biological treatments on growth of squash plants under salt stress," *Scı. Hortic.,* 111, 1-6, 2006.

[5] R. K. Sairam, and A. Tyagi, "Physiology and molecular biology of salinity stress tolerance in plants,". *Curr. Scı.,* 86, 407–721, 2004.

[6] D. Çavuşoğlu, S. Tabur, and K. Çavuşoğlu, "The effects of Aloe vera L. leaf extract on some physiological and cytogenetical parameters in *Allium cepa* L. seeds germinated under salt stress," *Cytol.,* 81(1), 103-110, 2016.

[7] D. Çavuşoğlu, K. Çavuşoğlu, and S. Tabur, "The effects of Black cumin (*Nigella sativa* L.) seed extract on the seed germination, seedling growth, mitotic activity and chromosomal abberations of *A. cepa* L. under saline condition," *ARPN.,* 13(5), 50-57, 2018.

[8] S. Tabur, and K. Demir, "Cytogenetic response of 24-epibrassinolide on the root meristem cells of barley seeds under salinity," *Plant Growth Regul.,* 58, 119–123, 2009.

[9] M. M. Emam, and N. M. Helal, "Vitamins minimize the saltinduced oxidative stress hazards," *Austral. J. Basic Appl. Scı.,* 2, 1110–1119, 2008.
[10] M. Zhu, S. Shabala, L. Shabala, Y. Fan, and M. X. Zhou, "Evaluating predictive values of various physiological indices for salinity stress tolerance in wheat," *J. Agron. Crop Scı.,* 202(2), 115-124, 2016.
[11] T. Näsholm, K. Kielland, and U. Ganeteg, "Uptake of organic nitrogen by plants," *New Phytol.*, 182, 31-48, 2009.
[12] E. Aldemir, "Bazı bakır (II) kelatlarının askorbik asidin oksidasyonundaki katalitik etkilerinin spektrofotometrik incelenmesi," M. S. thesis, Institute of Science, Yıldız Teknik Ünv., Istanbul, Turkey, 2007.
[13] D. M. Leme, and M. A. Marin-Morales, "*Allium cepa* test in environmental monitoring: a review on its application," *Mut. Res.,* 682, 71–81, 2009.
[14] P. C. Sharma, and P.K. Gupta, "Karyotypes in some pulse crops," *Nucleus,* 25, 181-185, 1982.
[15] D. B. Duncan, "Multiple range and multiple F tests," *Biometrics*, 11, 1-42, 1955.
[16] M. Fenech, W. P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi, and E. Zeiger, "Human micronucleus project: Detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures,". *Mutat. Res.-Genet. Tox. En. Muta.*, 534(1), 65-75, 2003.
[17] X. Q. Liu, Y. S. Kim, and K.S. Lee, "The effect of mixed amino acids on nitrate uptake and nitrate assimilation in leafy radish," *Korean J. Env. Agric.*, 24 (3), 245-252, 2005.
[18] J. C. G. Galindo, A. Hernάndez, F. E. Dayan, M. R. Tellez, F. A. Macías, R. N. Paul, and S. O. Duke, Dehydrozaluzanin C, "A natural sesquiterpenolide, causes rapid plasma membrane leakage," *Phytochem.*, 52, 805-813, 1999.
[19] C. Ghoulam, and K. Fores, "Effect of salinity on seed germination and early seedling growth of sugar beet (*Beta vulgaris* L.). seed," *Scı. Tech.,* 29, 357–364, 2001.
[20] M. Y. Ashraf, G. Sarway, M. Ashraf, R. Afaf, and A. Sattar, "Salinity induced changes in alpha amylase activity during germination and early cotton seedling growth," *Biol. Plantarum*, 45, 589–591, 2002.
[21] I . Demir, K. Mavi, M. Özçoban, and G. Okçu, "Effect of salt stress on germination and seedling growth in serially harvested aubergine (*Solanum melongena* L.) seeds during development," *Israel J. Plant Scı.,* 51, 125–131, 2003.
[22] R. M. Ali, "Role of putrescine in salt tolerance of *Atropa belladonna* plant," *Plant Scı.,* 152, 173-179, 2000.
[23] S. J. Roy, S. Negrão, and M. Tester, "Salt resistant crop plants," *Curr. Opin. Biotechnol.*, 26, 115-124, 2014.
[24] F. Kaymak, "Cytogenetic effects of maleic hydrazide on *Helianthus annuus," Pak. J. Biol. Scien*, 8 (1), 104–108, 2005.
[25] S. Radic, M. Prolic, M. Pavlica, and B. Pevalek-Kozlina, "Cytogenetic effects of osmotic stress on the root meristem cells of *Centaurea ragusina* L.," *Environ. Exp. Bot.*, 54, 213-218, 2005.
[26] N. Khanna, and S. Sharma, "*Allium cepa* root chromosomal aberration assay: a review," *Indian J. Pharm.Biol. Res.*, 1, 105-119, 2013.
[27] T. C. C. Fernandes, D. E. C. Mazzeo, and M.A. Marin-Morales, "Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide," *Pest. Biochem. Physiol.*, 88, 252–259, 2007.
[28] A. A. El-Ghamery, M. A. El-Kholy, and M. A. Abou El-Yousser, "Evaluation of cytological effects of Zn^{2+} in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L.," *Mutat. Res.*, 537, 29–41, 2003.
[29] R. M. Hoseiny, A. A. Aivazi, and S. Jagannath, "Cytogenetic and biochemical effects of imazethapyr in wheat (*Triticum durum*)," *Turk. J. Biol.*, 35, 663–670, 2011.