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Effects of Dosages and Application Periods of EDTA on Morphological and Cytogenetic Characters of Barley (*Hordeum vulgare* L.) Seedlings

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Abstract: Ethylenediaminetetraacetic acid (EDTA) has many applications in plant research in addition to other applications, such as chelation and medical therapy. The morphological and cytogenetic effects of EDTA on barley seedlings were investigated in the present study. Seeds of *Hordeum vulgare* L. were treated with different doses of EDTA (30, 60, 90, 120 and 150 mmol/l) and 2 different application periods (20 and 22 h). Except mitotic index, all of the measurements for the investigated characters were carried out on days 7 and 14.

EDTA treatments affected germination rates in nearly all treatments. In general, EDTA treatments decreased the root, leaf and coleoptile lengths, but it had the greatest effect on coleoptile length. Increasing the EDTA dosages and treatment periods also decreased the mitotic index compared to the untreated control. The inhibitory effects of EDTA dosages on the mitotic index indicate that EDTA can have genotoxic and mutagenic effects on barley seedlings.

Key Words: EDTA, *Hordeum vulgare*, mitotic index

Farklı Doz ve Sürelerde Uygulanan EDTA'nın Arpa Fidelerinin Morfolojik ve Sitogenetik Karakterleri Üzerine Etkisi

Özet: Etilendiamintetraasetik asit (EDTA) bitki araştırmalarında birçok kullanım alanına sahip olan bir kimyasaldır. Bu çalışmada, EDTA'nın arpa fideleri üzerindeki morfolojik ve sitogenetik etkileri incelenmiştir. *Hordeum vulgare* L. tohumları farklı dozlarda (30, 60, 90, 120 and 150 mmol/L) ve farklı sürelerde (22 ve 24 saat) EDTA uygulamasına maruz bırakılmışlardır. Mitotik indeks dışında, incelenen karakterlere ait ölçümler 7. ve 14. günde gerçekleştirilmiştir.

EDTA uygulaması hemen tüm dozlarda ve her iki uygulama süresinde tohum çimlenmesi üzerine önemli etkiye sahip olmuştur. Genelde, EDTA uygulaması arpa fidelerinde primer kök uzunluğu, koleoptil uzunluğu ve yaprak uzunluğunu azaltmış, ancak bu etki en fazla koleoptil uzunluğunda görülmüştür. EDTA dozları ve ugyulama süreleri, kontrolle karşılaştırıldığında, mitotik indeks üzerine azaltıcı bir etkiye sahip olmuştur. Yüksek EDTA dozlarının mitotik indeksi engelleyici etkisi, EDTA'nın arpa fideleri üzerinede genotoksik ve mutagenik etkilere sahip olabileceğini göstermektedir.

Anahtar Sözcükler: EDTA, *Hordeum vulgare*, mitotik indeks

Introduction

There are many chemical mutagens affecting living organisms. Many of these chemicals have a clastogenic (chromosome-damaging) effect on plants by mediating reactive oxygen-derived radicals (1). These effects are mediated spontaneously or artificially. Chemical mutagens contains substances such as base analogs, antibiotics, alkalizing agents, and derivatives of nitrogen.

In this study, barley seeds were treated with ethylenediaminetetraacetic acid (EDTA). Barley (*Hordeum vulgare* L.) is a monocotyledonous plant that belongs to the family Gramineae (Poaceae). Because of its ecological tolerance, barley can grow in harsh conditions. The chromosome number of barley is 2n = 14 (2).

EDTA can be used as a chemical mutagen in addition to its other usages. EDTA and its alkaline salts are used

as food preserving substances, for purification of antibiotics and as a regulatory factor in plant growth (3). It was determined that EDTA causes chromosome aberrations in *Tradescantia paludosa*, *Drosophila* sp. and *Hordeum vulgare*, and mitotic abnormalities in the root cells of onion (3). Redei (4) reported that treatments of 20 and 50 mmol/l EDTA dosages at 25 °C for 12 h cause mutation in *Arabidopsis thaliana*. EDTA is used in pot and field experiments to enhance the heavy metal uptake of plants (5). Synthetic chelating agents (such as EDTA) bind to metals or minerals such as lead, mercury, iron, arsenic, and aluminum. EDTA and other chelating agents are used in soils and in nutrient solutions to increase the solubility of metal cations and the translocation of lead into shoots.

The sap exudation rates of phloem can be increased by treating the cut surface with EDTA (6). Urquhart and Joy (7) evaluated the phloem exudation technique in their study of amino acid translocation in *Pisum sativum* L. seedlings by using EDTA. They determined that the sap exudation rate of phloem from cut petioles of fully expanded leaves was enhanced by EDTA (20 millimolar disodium salt) treatment.

Pre-applied dosages of EDTA (100 and 200 ppm for 24 h) used as a dormancy-breaking agent were not effective in *Carica papaya* L. (8). EDTA treatments, pre-applied on seeds as 0.001 M with or without GA3 for 15 min at 50 °C and 500 ppm for 24 h at 30 °C, were not effective in breaking the seed dormancy in *Citrus aurantifolia* (9).

In this research, the effects of EDTA on morphological characters (germination rates and root, leaf and coleoptile lengths, and mitotic index (MI)) of barley seedling were investigated. As a cytogenetic test, the MI is extensively used both in vivo and in vitro to examine the genotoxic effects in a short time and to evaluate mutagenic effects of agents in different environments. The MI assay is used to characterize proliferating cells and to identify compounds that inhibit or induce mitotic progress. Compounds that inhibit mitotic progress result in an increase in the MI of the population.

Materials and Methods

EDTA treatments

Dry dormant seeds of barley were treated with 30, 60, 90, 120 and 150 mmol/l of EDTA and distilled water was used as a control for 20 and 22 h at 21 °C.

Germination

The untreated (control) and treated barley seeds were soaked in distilled water for 1 h at room temperature. Then each group of seeds was separately transferred on wet Whatman paper in petri dishes and incubated for 24 h at 21 °C for studying the MI. To observe the effect of EDTA on morphological characters, germination was carried out for 7 and 14 days at room temperature. The sample size consisted of 20 seeds for observing the effects of EDTA on morphological characters. Germinated seeds were transferred from petri dishes to sands for sieving and washing with distilled water. After a certain time, root tips were cut (1-1.5 cm length) to observe the MI.

Fixation and Preparation

The root tips were pre-treated in saturated aqueous solution of α-monobromonaphthalene for 2 h at 4 °C and fixed in pure glacial acetic acid for 30 min. Thereafter, samples were rinsed and transferred to 70% alcohol and stored in a refrigerator until used. The root tips were taken from 70% alcohol and hydrolyzed in 5 N HCl for 20 min at room temperature and then stained in acetocarmine solution for 1 h. To prepare the acetocarmine solution, 45% glacial acetic acid was melted and cooled to 50 °C and then 1 g of acetocarmine was added and this mix was melted for 10 min. Chromosome spreads were made by using the squash technique (10).

Examination of Morphological and Cytogenetic Characters

Germination percentages of seeds were determined on days 7 and 14. The lengths of roots, leaves and coleoptiles were measured in centimeters. Frequency of mitosis was determined by counting the number of dividing cells among the 500 cells in the control and treated groups.

Statistics

Analysis of variance was used to analyze the data obtained from EDTA treatments. Least significant differences tests were applied to analyze the differences among the groups. The significance level was 0.05.

Results

The Effects of EDTA on Germination

In general, EDTA treatments decreased the germination rates. Differences in germination rates between EDTA treatments and the control group were statistically significant ($P < 0.05$, Table 1), but differences among dosages higher than 90 mmol/l were not ($P > 0.05$). The lowest germination rate (15%) was observed when 120 mmol/l EDTA was applied for 20 h (Table 1). With respect to average germination rates, the difference between 20 and 22 h EDTA application periods was not statistically significant (Table 1).

The Effects of EDTA on Growth of Seedlings

Generally, the effects of EDTA treatments on root length were statistically significant ($P < 0.05$). In particular, increasing dosages of EDTA up to 90 mmol/l decreased the root length, but the effects of dosages higher than 90 mmol/l were not statistically significant (Table 2).

EDTA treatments affected leaf length ($P < 0.05$) in all applications except treatments of 20 h on day 7 of the germination period. Effects of EDTA dosages higher than 60 mmol/l at 7 days and higher than 90 mmol/l at 14 days on leaf length were not statistically significant (Table 2).

EDTA treatments affected coleoptile length in both 20 and 22 h application periods and on days 7 and 14 of the germination periods. The lowest coleoptile length observed was 0.71 cm with 150 mmol/l EDTA treatment in the 20 h application period and 0.86 cm with 150 mmol/l M EDTA treatment in the 22 h application period. Effects of dosages higher than 90 mmol/l were not statistically significant in either 20 h or 22 h application periods (Table 2).

The Effects of EDTA on Mitotic Index

The increasing dosages of EDTA apparently decreased MI in both 20 h and 22 h application periods. The differences among all groups were statistically significant ($P < 0.05$) with respect to MI in all treatments. MIs of the embryonic roots of barley were 5.6%-18.6% in the 20 h application period and 3.2%-19.4% in the 22 h application period (Table 3). The highest MI was 18.6% in the control group and the lowest was 5.6% in the 150 mmol/l dosage in the 20 h application period. The highest MI was 19.4% in the control group and the lowest was 3.2% in the 150 mmol/l dosage in the 22 h application period (Table 3). The difference between the 20 and 22 h application periods was statistically significant in terms of the MI ($P < 0.05$).

Discussion and Conclusion

In the present study, the effects of EDTA on germination rates, lengths of embryonic root, leaf, coleoptile and MI of barley (*Hordeum vulgare* L.) were

Table 1. Rate of seed germination for seed treated with EDTA.

EDTA (mmol/L)	Germination rate (%)			
	Day 7		Day 14	
	20 h exposure	22 h exposure	20 h exposure	22 h exposure
0 (control)	85 a	85 a	100 a	80 a
30	55 b	75 a	90 ab	60 b
60	40 bc	70 a	75 b	50 b
90	30 cd	30 b	45 c	30 c
120	15 d	40 b	45 c	25 c
150	25 cd	45 b	35 c	20 c
Average	45	58	65	44

Values within a column with different letters are significantly different (LSD, $P < 0.05$).

Table 2. Effects of different doses of EDTA on root, leaf and coleoptile lengths.

Exposure time	Doses (mmol)/l	Average length (cm)					
		Root		Leaf		Coleoptile	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
20 h	0 (control)	3.6 a	19.2 a	2.1 a	13.5 a	3.1 a	5.2 a
	30	2.0 ab	13.4 b	1.7 a	10.2 b	2.1 ab	3.6 b
	60	0.8 ab	11.5 b	0.3 a	8.3 b	1.1 bc	3.2 b
	90	0.6 b	6.4 c	0.4 a	4.3 c	0.9 c	1.7 c
	120	0.5 b	6.4 c	0.2 a	3.9 c	0.5 c	1.7 c
	150	0.5 b	4.7 c	0.3 a	2.8 c	0.7 c	1.3 c
22 h	0 (control)	8.3 a	8.6 a	6.1 a	7.4 a	3.6 a	3.2 a
	30	8.2 a	8.4 a	4.2 ab	6.3 a	2.8 ab	2.5 ab
	60	7.9 a	6.3 a	3.0 b	6.3 a	2.2 bc	1.9 bc
	90	2.9 b	2.7 b	2.5 b	2.5 b	1.7 c	1.0 c
	120	2.8 b	1.9 b	2.3 b	1.9 b	1.5 c	0.9 c
	150	1.6 b	1.1 b	1.2 b	1.9 b	1.4 c	0.9 c

Values within a column of each exposure time with different letters are significantly different (LSD, $P < 0.05$).

Table 3. Mitotic index of seeds exposed to different concentrations of EDTA.

Exposure time	EDTA (mmol/l)	Total no. dividing cells	Average MI (%)
20 h	0 (control)	93	18.6 a
	30	66	13.2 b
	60	61	12.2 c
	90	41	8.2 d
	120	32	6.4 de
	150	28	5.6 e
	Average		10.7
22 h	0 (control)	97	19.4 a
	30	59	11.8 b
	60	51	10.2 bc
	90	40	8.0 c
	120	24	4.8 d
	150	16	3.2 d
	Average		9.6

Total numbers of dividing cells were determined by counting among the 500 cells for each treatment.
Values within a column of each exposure time with different letters are significantly different (LSD, $P < 0.05$).

investigated. Different dosages of EDTA (30, 60, 90, 120 and 150 mmol/l) decreased the germination rates of barley seeds. However, Mazaheri (11) reported that dosages of EDTA (50, 70 and 90 mmol/l) applied for 18 h did not affect germination rates of *Arabidopsis thaliana* seeds. The conflict between the results probably occurred because of the lower dosages and shorter application periods applied by Mazaheri (11).

When EDTA dosages were increased, the numbers of mitotically dividing cells significantly decreased. Fishbein et al. (3) reported that EDTA decreased mitotic division via chromosome aberrations in the bean and barley. They also reported the occurrence of mitotic abnormality in onion root cells. These results correlated with our results. Redei (4) reported that 20 and 50 mmol/l dosages of EDTA for 12 h at 25 °C increased mutation frequency in *Arabidopsis thaliana*. Our results also showed that both increasing dosages of EDTA and longer application period decreased the MI. It was reported that EDTA had different mutagenic effects on different plants (3,4,12). The results of this study will form a baseline for subsequent research in this area.

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