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CONTENTS

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THE KARYOTYPE ANALYSIS OF *Lilium martagon* L. (LILIACEAE) WITH B CHROMOSOME

A. Nihal GÖMÜRGEN*, Haşim ALTINÖZLÜ*

Received 29.05.2003

Abstract

In this study, karyotype of *Lilium martagon* L. with B chromosome collected from İstanbul, Beykoz - is given for the first time. Its chromosome number has been determined as 2n = 24 + 1B. The karyotype of *L. martagon* is characterized by one metacentric (pair1) with the centromere at the median region; one submetacentric (pair2) with the centromere at the submedian region; three subterminal (pair 7, 9 &11) with the centromere at the terminal region; seven terminal (pair 3-6, 8, 10&12) with centromere at the terminal region and has one B chromosome, additionally. As a consequence, this karyotype has the idiogrammatic formula: 1 m + 1 sm + 3 st + 7 t + 1B chromosome.

Key words: Karyotype analysis, B chromosome, *Lilium martagon*

Introduction

The genus *Lilium* L. (Liliaceae) ranged from Europe and Asia (until the high mountains of Philippines) to North America - has approximately 100 species (1). According to the flora of Turkey (2), the genus *Lilium* L. has 6 species in Turkey.

While this genus includes many beautiful ornemantal flowers, they are also widely cultivated in the world. Commercial breeding studies continue on the economically important *Lilium* species, and studies on the production of interspecific hybridization takes attention (3,4).

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Although there are some karyological studies on *Lilium* species (5-8), the karyology of *Lilium martagon* L. with B chromosome distributed in Turkey has not been studied. As it is very well known, a lot of plants carry B-chromosomes (9-12). Kamari & Atelari reported B chromosomes at two species of *Stenbergia* (13). In Federow 81 species chromosome numbers were given, 15 of them have carry 1-2 B chromosomes (14). The term B-chromosome has priority for a distinct class of chromosomes with a number of definite characteristics over and above the normal zygote complement. It is also possible to determine that B- chromoseomes are extra or supernumary chromosomes addition to normal chromosome complement (A chromosomes), and generally they are not homologous with A-chromosomes (10). Schulz-Schaffer states that the possible origin of B-chromosomes is partly or completely heterochromatic (15). B-chromosomes are also mentioned in prior studies to obtain artificial polyploid plants (16).

In this study, it is shown that *Lilium martagon* L. - distributed at İstanbul, Beykoz - carries B-chromosome and the results of karyotype analysis of this population are presented for the first time.

Material and Methods

Seeds and samples of *Lilium martagon* L. - which were collected and determined by Haşim Altınözlü from A2 İstanbul; Beykoz, 100 m, under *Castanea sativa* and its open places in May 2001 -, were used in this study.

Seeds were germinated at room temperature (20 ^{0}C) on moist filter paper in Petri dishes. Actively growing root tips reaching 0.5 cm length were pretreated for 4 hours with 8-hidroxiqunioline, and fixed with GAA for 30 minutes. Root tips were also hydrolyzed with 1 N HCl for 10 minutes at 60 ^{0}C, and finally stained with Feulgen. The detection of the homologous chromosomes and the determination of their position in the karyograms were carried out following the method proposed by Levan *et al.* (17). The measurements obtained from ten - long and short arm length - metaphase plates allowed the construction of the idiograms of the taxon. Photographs were taken by the combination of "Olympus CH 40 microscope", and "Diagnostic Instruments" digital camera. Permanent slides stored in the Department of Biology, Hacettepe University, Ankara.

Results and Discussion

Chromosome numbers has found as 2n = 24 + 1B at the root tip cells of *Lilium martagon* L. collected from İstanbul, Beykoz (fig.1).

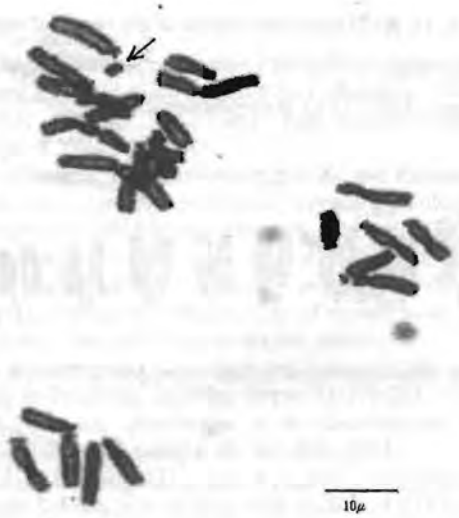

Figure 1: Metaphase chromosomes of *Lilium martagon* L. (2n = 24 + 1B)

L.martagon L. has a long chromosome set. There is a difference in total chromosome lengths between the first and last pairs of the chromosome complement, which vary from 8.08 μm to 19.43 μm, and the length of B chromosome is 2.5 μm (Table 1).

Table 1: Morphometric data on chromosomes of *Lilium martagon* L. (2n = 24 + 1B)

Chromosome	Chromosome length (μm)±SD			Arm ratio	Centromeric index	Relative	Centromere
Pairs	Long arm	Short arm	Total	r=L/S	i=100xs/c	length (%)	position
1	9.98±0.09	9.45±0.06	19.43±0.07	1.05	48.74	13.61	m
2	9.65±0.04	4.77±0.02	14.42±0.04	2.03	33.06	10.09	sm
3	13.21±0.08	1.16±0.04	14.37±0.04	11.43	8.05	10.06	t
4	12.42±0.05	1.36±0.01	13.77±0.02	9.14	9.88	9.65	t
5	11.55±0.07	1.65±0.04	13.20±0.03	6.99	13.00	9.25	t
6	10.53±0.06	1.17±0.03	11.69±0.03	9.01	9.99	8.19	t
7	8.91±0.10	1.65±0.02	10.56±0.03	5.40	15.66	7.39	st
8	9.42±0.03	0.61±0.04	10.03±0.07	15..34	6.12	7.03	t
9	8.91±0.04	1.01±0.04	9.93±0.07	5.40	15.64	6.95	st
10	7.77±0.05	1.11±0.08	8.88±0.07	7.00	13.00	6.22	t
11	6.77±0.02	1.65±0.04	8.42±0.02	3.84	22.85	5.89	st
12	7.11±0.05	0.97±0.07	8.08±0.07	7.32	10.01	5.66	t
B			2.50±0.01				

The karyotype of *L. martagon* was characterized by one metacentric (pair 1) with the centromere at the median region; one submetacentric (pair 2) with the centromere at the submedian region; three subterminal (pair 7, 9 &11) with the centromere at the terminal

region; seven terminal (pair 3-6, 8, 10 &12) with centromere at the terminal region, and has one B chromosome, additionally (fig. 2, fig. 3). Accordingly, this karyotype has the idiogrammatic formula: 1 m + 1 sm + 3 st + 7 t +1 B chromosome.

Figure 2: Karyogram of metaphase chromosomes of *Lilium martagon* L. (2n=24 + 1B)

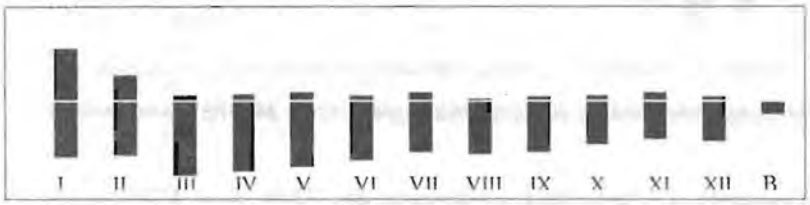

Figure 3: Idiogram of metaphase chromosomes of *Lilium martagon* L. (2n = 24 + 1B)

Lily species have an exceptionally large genom size (5) and all of them are diploid (2n=2x=24), except for *L. lancifolium* for which triploids also occur (6). Our research material has diploid chromosome set and has long chromosomes, and its longest chromosome length is 19.43 μ. Karyotype analysis of *L.martagon* - distributed in North East Anatolia , Turkey - was made by İnceer *et al.* (8). Fernades gives chromosome number as 2n = 24, 24 + 1 - 3 B (1). Our results obtained on the root-tips meristems confirms the earlier reports of İnceer *et al.* and Fernades (8,18) . Our research material contain B chromosome like at the study of Fernandes However, İnceer *et al.* (8) have reported the chromosome number of this species as 2n = 24 for the material collected from the North East Anatolia and have gave choromosome set almost same as in our study. They have not reported the existence of B chromosomes (8). B -chromosome frequencies change between populations in same species (18,19). Fernandes reported 1-3 B chromosomes at *L. martagon* (18). As a consequence, it should be emphasized that *Lilium martagon* L. - distributed at Istanbul, Beykoz - has one B chromosome, and the occurrence of B chromosome in *Lilium martagon* L. - distributed in Turkey -, and karyology of this *Lilium* species has been reported for the first time in this paper.

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Hacettepe Journal of Biology and Chemistry
Volume 33 (2004), 7-21

IMMUNOLOCALIZATION OF FIBRONECTIN AND TRANSFORMING GROWTH FACTOR-β1 IN THE THYROID, PARATHYROID AND ADRENAL GLANDS OF MALE RATS TREATED WITH CARBENDAZIM

Evrim A. KOÇKAYA*, Sibel HAYRETDAĞ*, Nurhayat BARLAS* and Güldeniz SELMANOĞLU*

Received 31.06.2003

Abstract

The aim of this study is was is to evaluate the immunolocalization for fibronectin and transforming growth factor-β1 in adrenal, thyroid and parathyroid glands of rats treated with carbendazim. The Swiss albino male rats were exposed to 0, 150, 300 and 600 mg/kg/day carbendazim doses by gavage for 3.5 months. The thyroid, parathyroid and adrenal glands of rats killed by cervical dislocation were removed and paraffin blocks were prepared. Then, the tissue sections stained by the PAP method were examined. The immunreactivity for fibronectin and transforming growth factor-β1 were increased in adrenal, thyroid and parathyroid glands in 300 and 600 mg/kg/day groups depending on advancing doses.

Key Words: Carbendazim, endocrine glands, TGF-β1, FN

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Introduction

Carbendazim (MBC = methyl 2-benzimidazolecarbamate) is a systemic fungicide which is used widely to prevent and control plant diseases caused by fungi. As carbendazim binds to tubulins, it affects microtubule formation and mitosis (1). In many reports, it has been shown that carbendazim causes to impair liver function, haematopoiesis, reproduction and hormone levels in various mammals with actions ascribed to microtubule disturbances during cell divisions (2-4). Carbendazim that uses widely to prevent and control plant diseases caused by various fungi in Turkey.

Fibronectin (FN), one of the major constituents of extracellular matrix (ECM), is important in cell migration, cell adhesion, cell spreading, cell differentiation, and growth and tissue repair (5).

Enhanced FN expression has been reported after tissue injury and in a variety of lung disorders (6), suggestive of its role in injury and repair processes (7). FN is a known chemo attractant for several cell types which are involved in the wound healing process, like fibroblasts, endothelial cells and macrophages.

Transforming growth factor (TGF-β) is a family of multifunctional proteins that regulate hepatocyte proliferation, and biosynthesis of ECM (8). The different biological functions of TGF-β1 have been the subject of several recent studies (9-11). TGF-β1 can either stimulate or inhibit cellular proliferation and/or differentiation, depending on the status of the target cells. TGF-β1 influences a number of specific functions of adrenocortical cells in several animal species (12). Among growth factors, TGF-β1 is considered as the most important fibrosis promoting cytokine (11).

TGF-β1 increases biosynthesis of ECM, and decreases the production of tissue inhibitor of the metalloproteinase's (9,10). TGF-β1 also increases the production of ECM by stimulating the synthesis of matrix components, inhibiting matrix degradation, and increasing the expression of integrins (13,14). It has been suggested that TGF-β1, which probably plays an essential role in embryogenesis, tissue remodelling, and repair, as well as in abnormal cell proliferation, exerts some of its effects by regulating the production of ECM (15).

Previous studies showed that TGF-β and FN expression in different tissues were increased with exposure to various xenobiotics, or in dissimilar diseases (8, 16). There are no available data about the effects of carbendazim on immunolocalization of FN and TGF-β1 that the productions affected by different thyroid, parathyroid and adrenals

hormones in endocrine glands and endocrine system of mammals. Therefore, it is thought that some functions (as hormone production) of certain endocrine tissues such as thyroid, parathyroid and adrenal glands or the histological organizations of these tissues and the TGF-β and FN productions related to histopathological changes in these tissues may be altered with treated carbendazim treatment.

The aim of this study is to explain the effects of carbendazim that disturbed the histological organization of adrenal, thyroid and parathyroid glands, on TGF-β and FN immunolocalization in these endocrine organs. There is no information about this subject in literature. We have focused on to explain of the mechanism of histopathological changes observed these endocrine glands by carbendazim exposure in rats.

Materials & Methods

Animals

Swiss albino male rats (*Rattus rattus*) weighing 200-250 g were used during the experiment. The rats were provided from the Production Centre of Experimental Animals in Hacettepe University, Ankara, Turkey. They were fed with commercial pellets (The Production Centre of Experimental Animals in Hacettepe University) and water *ad libitum*. Laboratory conditions were maintained at 25±2 °C and 68±4 % relative humidity during the study.

Chemicals

Carbendazim, purity 98% was purchased from Agro-San Company, Kırklareli, Turkey. Antibodies that specifically recognized polyclonal anti-human FN and monoclonal anti-mouse TGF-β1, goat anti-rabbit IgG, PAP complex, goat serum, Tris-phosphate buffer (pH 7.2-7.6), 3,3-diaminobenzidine were obtained from Sigma (Diesenhofen, Germany).

Experimental Design

After a week of adaptation period, the animals were randomly divided to five groups 10 ats each. Carbendazim suspended in corn oil was daily applied to rats by gavage for 15 weeks. The doses were chosen of, 150, 300 and 600 mg/kg/day carbendazim. Two control groups were made. One group was chosen as the control group which was nourished with tap

water and laboratory diet. A second group was treated an equivalent amount of pure corn oil and served as an oil control group.

Unlabelled peroxidase-antiperoxidase (PAP) method

The immunreactivity for FN and TGF-β1 were evaluated in tissue sections of thyroid, parathyroid and adrenal glands of the rats in all groups. The rats were killed by cervical dislocation. The tissue samples were fixed in Bouin solution and embedded in paraffin wax and cut at 4 μm. The tissue sections were then deparaffinized and stained using the PAP method and then the sections were counterstained with Haematoxylin and mounted. At the same time, negative control sections were also stained. Negative control sections were treated without any primary antibodies. All of the sections were evaluated by two different observations. The sections of thyroid, parathyroid and adrenal glands were examined for FN and TGF-β1 immunreactivity at light-microscopic level. The labelling intensities defined as (-) negative; (±) faint; (+) weakly positive; (++) positive; (+++) clearly positive, were evaluated.

Results

As no differences were observed between the control and oil control groups and the comparisons were made between normal control and treatment groups.

Table 1 shows the intensity of FN and TGF-β1 immunreactivity in various regions of thyroid, parathyroid and adrenal glands sections of rats in control and treatment groups. There were no significant differences in the intensity of the staining for FN and TGF-β1 of 150 mg/kg/day group compared with the control samples. However, significant differences were found in the intensity of the staining for FN and TGF-β1 between control and 300 mg/kg/day and 600 mg/kg/day groups. The grade of the immunreactivity for FN and TGF-β1 were especially high in tissue sections of rats in 300 mg/kg/day and 600 mg/kg/day groups. Although, FN was weakly positive especially at the capsule region of the adrenal glands of the control group, the cortex of the adrenal glands were stained faint positive and no positive reaction was observed in the medulla (Figure 1). At the same regions of adrenal glands of the 600 mg/kg/day group clearly positive staining for FN was observed (Figure 2).

When the FN immunolocalization in control thyroid and parathyroid sections were investigated, the intensity of FN was seen weakly positive (Figure 3-4). However, the capsules of thyroid and parathyroid glands of rats in 300 mg/kg/day and 600 mg/kg/day groups were shown clearly positive staining for FN (Figure 5-6). Positive staining for FN was also observed in the connective tissue and the basal membranes of thyroid epithelium.

Table 1. The distribution of TGF-β1 and FN in the adrenal, thyroid and parathyroid glands of control and treatment groups[a]

Parameters	Groups (mg/kg/day carbendazim)	Tissues				
		Adrenal			Thyroid	Parathyroid
		Capsule	Cortex	Medulla		
FN	0	+	±	-	+	+
	150	+	±	-	++	++
	300	++	++	±	++	++
	600	+++	+++	+	+++	+++
TGF-β1	0	±	±	±	+	+
	150	±	±	-	+	+
	300	+	±	-	++	++
	600	++	+	+	++	++

[a]Intensity of labelling defined as : (-), negative; (±), faint; (+), weakly positive; (++) positive; (+++), clearly positive.

When the TGF-β1 immunreactivity was investigated in adrenal gland of rats in the control group, it was found that capsule, cortex and medulla showed faint staining (Figure 7). In the cortex, capsule and medulla of adrenal glands of rats in 150 mg/kg/day and 300 mg/kg/day groups, same staining pattern was seen. In 600 mg/kg/day group, especially in the capsule region of adrenal glands showed positive staining for TGF-β1 (Figure 8). However, cortex and medulla region was showed only weak positive staining for TGF-β1.

TGF-β1 immunreactivity was weakly positive in the thyroid and parathyroid of rats in control (Figure 9-10) and 150 mg/kg/day groups. On the other hand, in 300 mg/kg/day and 600 mg/kg/day groups, thyroid and parathyroid sections exhibited positive staining for TGF-β1 (Figure 11-12).

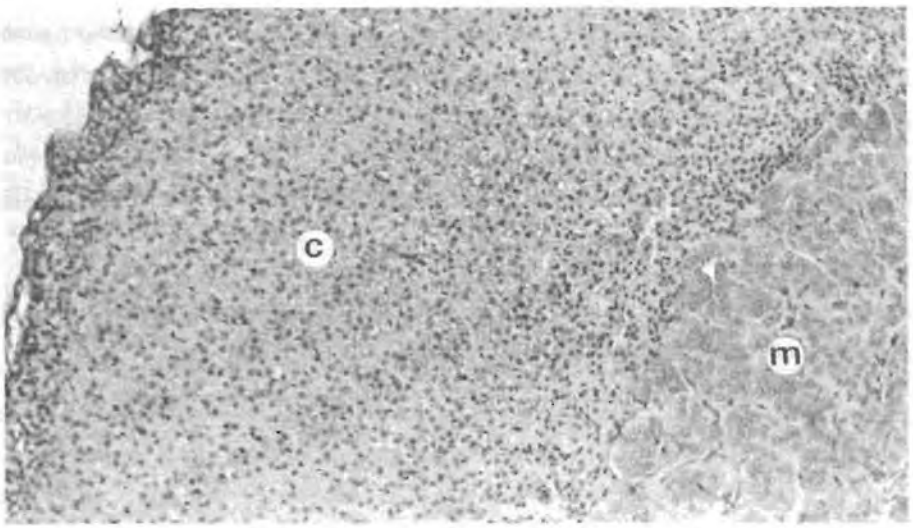

Figure 1. Control rat's adrenal gland. No FN-positive reaction in medulla (m) and faint FN-positive reaction in cortex (c). X180

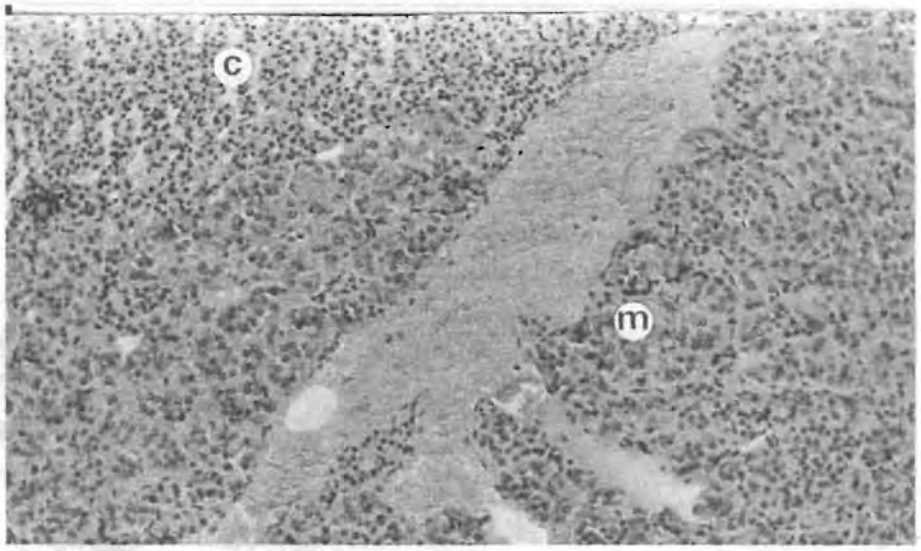

Figure 2. Treated with 600 mg/kg/day carbendazim rat's adrenal gland. Clearly FN-positive reaction in cortex (c) and medulla (m). X180

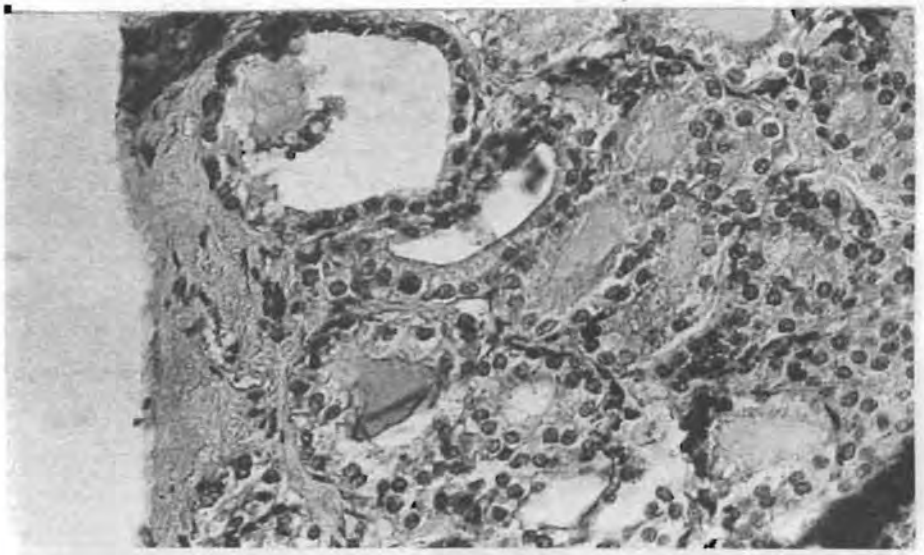

Figure 3. Control rat's thyroid gland. Faint FN-positive reaction in thyroid gland. X450

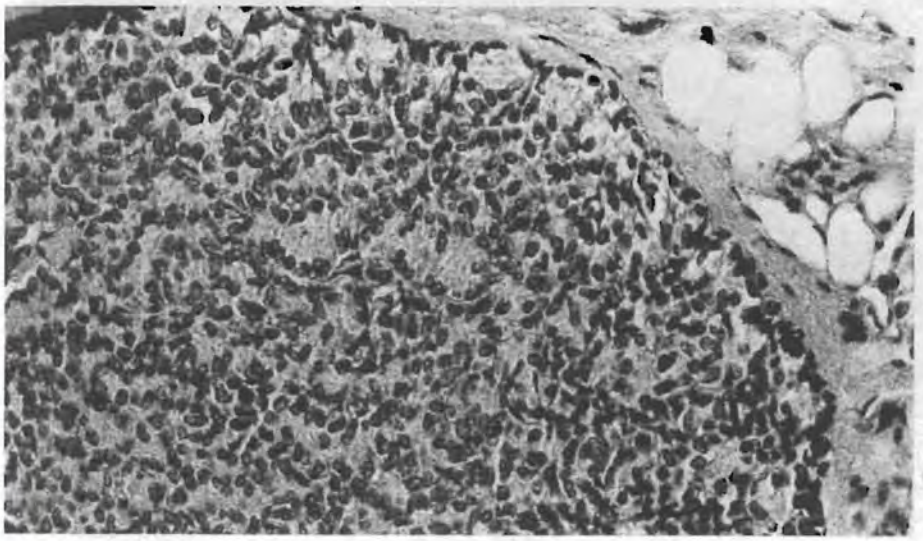

Figure 4. Control rat's parathyroid gland. Faint FN-positive reaction in thyroid gland. X450

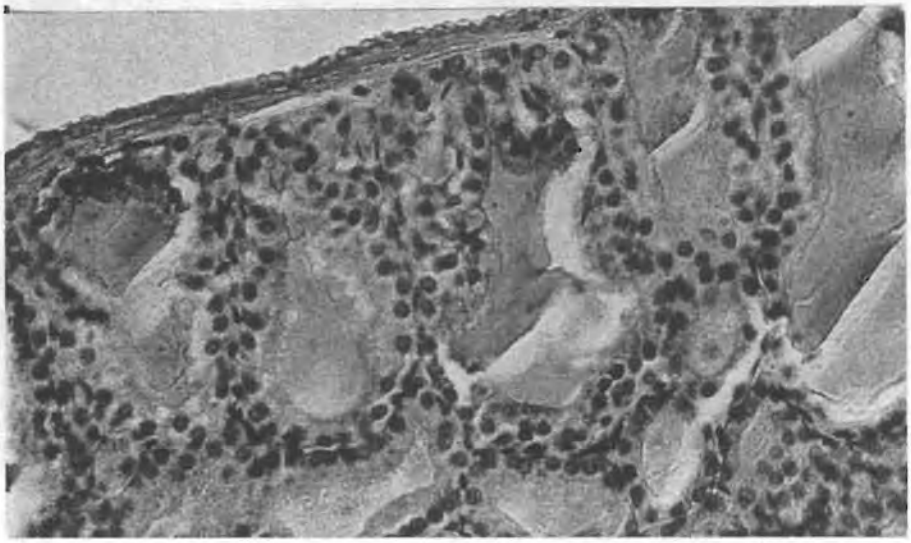

Figure 5. Treated with 600 mg/kg/day carbendazim rat's thyroid gland. Clearly FN-positive reaction in thyroid gland. X450

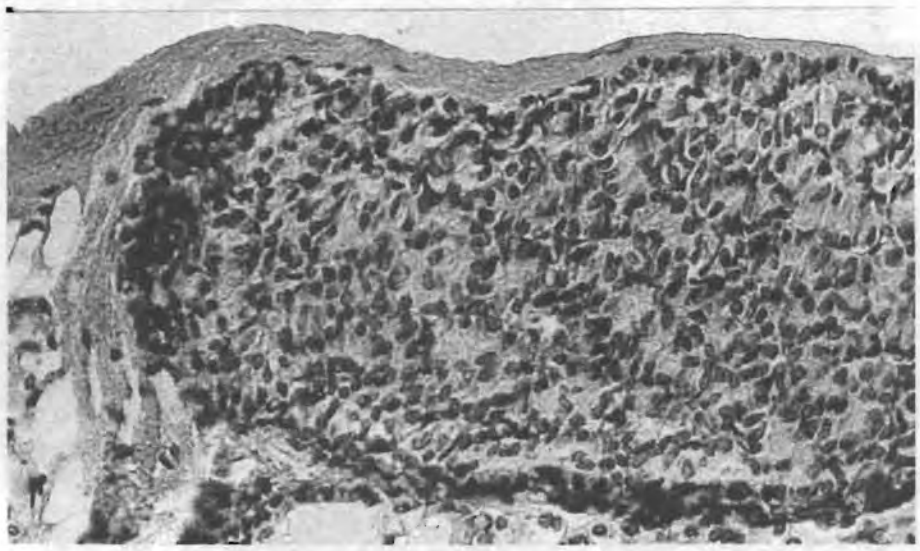

Figure 6. Treated with 600 mg/kg/day carbendazim rat's parathyroid gland. Clearly FN-positive reaction in parathyroid gland. X450

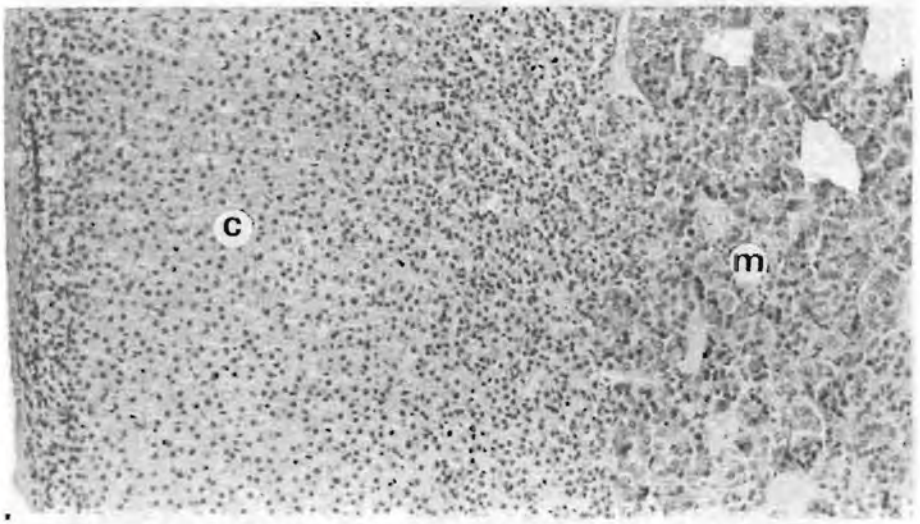

Figure 7. Control rat's adrenal gland. Faint TGF-β1 positive reaction in, cortex (c) and medulla (m). X180

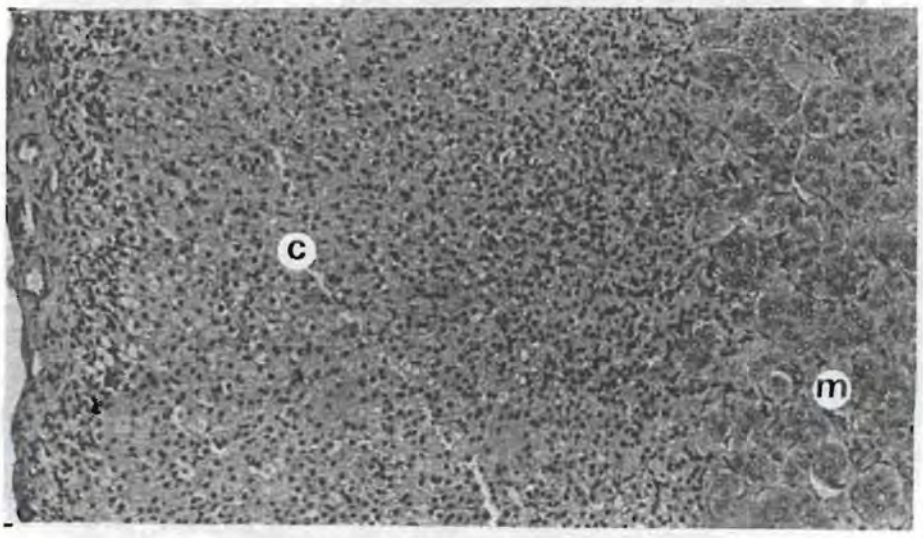

Figure 8. Treated with 600 mg/kg/day carbendazim rat's adrenal gland. Weakly TGF-β1 positive reaction in cortex (c) and medulla (m). X180

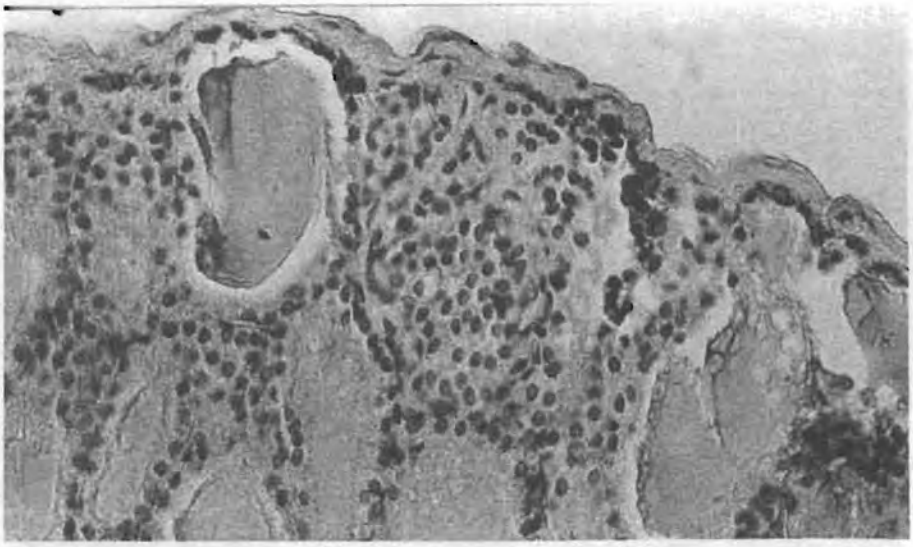

Figure 9. Control rat's thyroid gland. Weakly TGF-β1 positive reaction in thyroid gland. X450

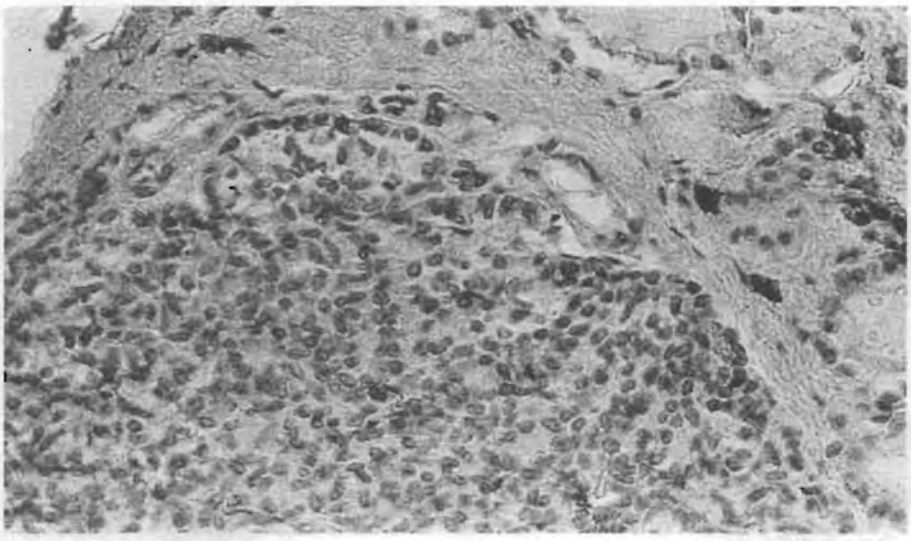

Figure 10. Control rat's parathyroid gland. Weakly TGF-β1 positive reaction in thyroid gland. X450

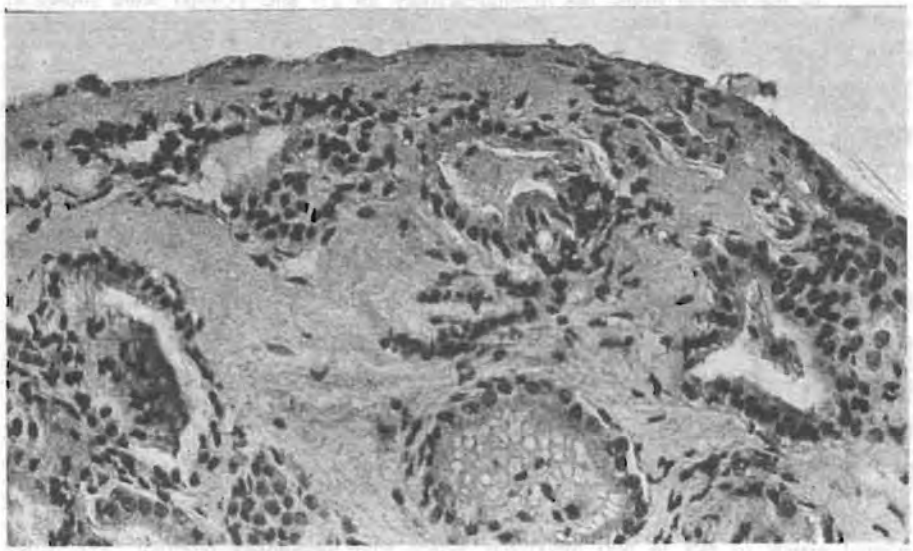

Figure 11. Treated with 600 mg/kg/day carbendazim rat's thyroid gland. Positive TGF-β1 reaction in thyroid gland. X450

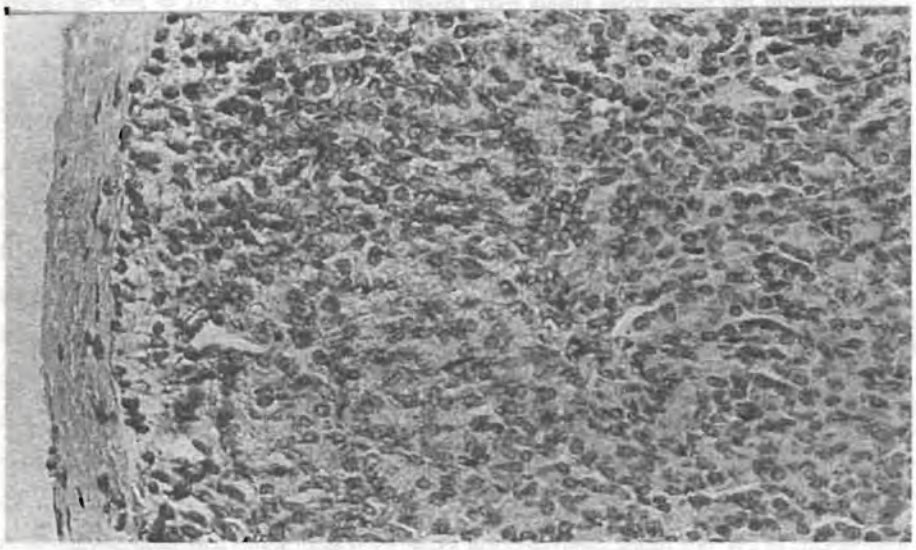

Figure 12. Treated with 600 mg/kg/day carbendazim rat's parathyroid gland. Positive TGF-β1 reaction in parathyroid gland. X450

Discussion

In the present study, the immunreactivity for FN and TGF-β1 were shown in the thyroid, parathyroid and adrenal glands of rats treated with various carbendazim doses. The degree of intensity of FN and TGF-β1 immunreactivity was correlated with advancing doses as previously reported and histopathologies in thyroid, parathyroid and adrenal glands (17).

Although, many researches have been made on the effects of some toxic materials (such as cyclosporine and $HgCl_2$) on immunolocalization of TGF-β1 in different tissues, no data have been reported concerning the effects of pesticides on this cytokine (11, 18, 19). In our study, the staining intensity for TGF-β1 in adrenal glands of rats in control group showed similarities to the findings reported by Judd (20). The histopathological changes such as cellular degenerations, high amount of lipid droplets in the tissue sections and blood vessel dilation, and significant increases in the amount of macrophages in the medulla region of adrenal glands of rats in 600 mg/kg/day group were previously reported (17). These histopathological changes resulted in an increase in the immunolocalization of TGF-β1 in these regions. TGF-β1 is a key cytokine whose actions in promoting the production and deposition of ECM are essential for normal tissue repair following injury (21). Thus, the present study may be an important contribution to investigations in this area. The cells of injured thyroid and parathyroid glands were strongly stained for TGF-β1 in 300 mg/kg/day and 600 mg/kg/day groups compared with control group. Normally, TGF-β1 is biologically inactive, being activated through proteolysis activator with the tissue injury. That is, TGF-β1 expression is correlated with both development and degree of injury.

Barlas and colleagues (17) reported that 300 and 600 mg/kg carbendazim caused the changes in thyroid and parathyroid histology such as some follicles joined, expanded and contained abundant basophilic cytoplasm, congestion, lymphoid cell infiltration and enlargement of interstitial tissue between follicles, cellular degeneration. While the FN immunolocalization in the sections of thyroid and parathyroid glands of rats in control group showed similarities to the findings reported by Burgi-Saville and colleagues (22,23), in the thyroid and parathyroid tissues of rats of 300 and 600 mg/kg/day groups increasing positive staining intensity for FN and the amount of interstitial connective tissue were observed. The biosynthesis of FN, one of the components of ECM, increased from 1 month to 24 months in rat thymus with regard to grown interstitial connective tissue (24). These increases may be caused by the augmentation of interstitial connective tissue of thyroid and parathyroid glands. Usenko and colleagues (16) also reported that the amount of FN was increased in thyroid gland after the radioactive iodine exposure, and Bhalla and colleagues (7) reported that FN

biosynthesis increased in lungs of rats exposed to 1 ppm ozone for 3 hours. It is often concluded that FN production by macrophages increases after human and animal exposures to asbestos, nickel, cobalt chloride, cadmium chloride, and O_3 (25, 26). We previously reported that 300 and 600 mg/kg/day carbendazim doses caused significant increases in the amount of macrophages (17). As a result, FN biosynthesis was correlated with increased quantity of macrophages in thyroid and parathyroid tissues of rats treated with carbendazim.

TGF-β1 increases the production of ECM by stimulating the synthesis of matrix components such as FN, collagens, inhibiting matrix degradation via decreasing production of metalloproteinase's (11, 13, 14). These findings are also consistent with previous reports and our results. Also, the deposition of FN component of ECM gradually progressed in the course of time accompanying the development tissue injury. The TGF-β1 and FN immunreactivity are increased throughout the tissue repair. TGF-β is a multifunctional peptide that is produced by activated mononuclear cells, by leukocytes that infiltrate injured tissues, and by platelets. At the same time, TGF-β possesses the capacity to control the productions of many components such as FN and collagen of the ECM. The increases that observed because of histopathological changes in the TGF-β1 and FN immunreactivity in the thyroid, parathyroid and adrenal glands of rats treated with carbendazim are related to the repair of tissue injury which was caused by carbendazim treatment. As a conclusion, the results may help to explain the injury mechanisms resulted from carbendazim.

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EFFECT OF SALT STRESS ON PHOTOSYNTHETIC RESPONSES IN MAIZE (*Zea mays* L.)

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Abstract

Effects of salt stress on photosynthetic activities in two maize (*Zea mays* L., var. *indendata*, C.6127 and DK.623) cultivars were investigated. Plants were grown for 20 days in the controlled growth room. 0, - 0.1, - 0.3 and - 0.5 MPa salinized culture solutions were applied to plants. Salt treatments caused decreasing amounts of total chlorophyll, chlorophyll a and b, and chlorophyll a/chlorophyll b ratio in C.6127 cultivar. But, in DK.623 cultivar all amounts of pigments were increased with increasing salt treatment levels. Salinity caused a decline in photosystem II (PS II) activity in both two cultivars. PS II activity was found to be sensitive in C.6127 than DK.623 at the all salt treatment.

Key Words: Maize, photosynthetic pigments, photosystem II activity, salt stress

Introduction

Salinity caused a decrease in growth and photosynthesis of higher plants. Growth rate of plants is generally reduced by salinity even at low salt concentration. The reduction in growth is the consequence of several physiological responses including modification of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, carbon

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allocation and utilization (1,2). Kingsbury et al. (3) have suggested that the reduction in plant growth by salt stress is plausibly due to osmotic stress on the specific ions or metabolic processes ranging from absorption of nutrients to enzyme activation and inhibition. The degree of salinity is considerably important among the environmental factors affecting the photosynthetic apparatus. Salt stress results in a decrease in chlorophyll content (4) and the reduction of photosystem II (PS II) activity (5,6). The ratio of light harvesting pigment complex (LHC) chlorophyll to photosystems chlorophyll increases with the increasing degree of salinity and the observed photosynthetic activity drops. Of all photosynthetic functions, PS II activity is known to be the most sensitive to salt stress (7).

The changes in photosynthetic parameters (PS II activity, net photosynthetic rate, intercellular [CO_2], stomatal conductance, etc.) could potentially be used as a screening method for salinity tolerance in plants, because the more tolerant cultivars are expected to exhibit fewer disturbances in the photosynthetic processes when growing under salinity (8).

The aim of this study was to determine the changes that could be induced by NaCl and $CaCl_2$ in the some photosynthetic parameters (pigment contents and PS II activity) of maize cultivars grown in Turkey as a second yield.

Material and Methods

Plant Material and Experimental Conditions

In this study the registered maize (*Zea mays* L. var. *indentata*, C.6127 and DK.623) cultivars, which are grown as a second yield in the Southeastern Anatolia Region (SAP) of Turkey (9), were used.

The selected seeds were surface sterilized in 1 % sodium hypochlorite solution for 8 min (10). Then seeds were washed with distilled water three times, and were wetted with culture solutions with different osmotic potentials for 12 h. In the experiment, ½ strength Hoagland's solution was used as the main culture solution. Salinized culture solutions at different osmotic potential were prepared by adding a mixture of NaCl and $CaCl_2$ in a 2:1 molar ratio to the main culture solution (11). It was decided salt levels by means of results obtained preliminary experiments. Five seeds were planted in each pot containing perlite. A completely randomized block design was used with five replicates. The experiment was carried out in controlled growth room at 25±1 °C, with 16 h daylength and with a relative humidity of 60 %±5. All conditions were maintained constant for the growth period. Plants were irrigated regularly every two days with culture solutions during the experiment (20

days). To determine effects of salt treatment on maize cultivars were measured two photosynthetic parameters:

Chlorophyll Content

Leaf extracts were prepared according to Krishnaraj et al. (12) and chlorophyll determinations were done as described by Arnon (13). The correction for calculations was done according to Porra et al. (14). Chloroplasts were isolated the shortest time as possible as and in cold (0°C). 2 g leaf tissue was cut in to small pieces and homogenized in a mortar with 3 ml buffer/g leaf tissue for 20 s. The isolation buffer consisted of 50 mM Hepes (pH 7.5), 400 mM Sorbitol, 5 mM $MgCl_2$, 10 mM KCl, 1mM EDTA, and 0.1 % BSA. Then the homogenat was filtered through 4 layers miracloth and centrifuged at 3500 rpm for 6 min. The resulting pellet was resuspended in 4 ml isolation buffer containing 50 mM Hepes (pH 7,5), 50 mM Sorbitol, and 50 mM $MgCl_2$. The samples were centrifuged at 13000 rpm and 0°C for 10 min. (Beckman Model L-2 Ultracentrifuge). And then pellet was resuspended in 0.4 ml suspending buffer and stored in ice cold and dark.

PS II activity

PS II activity in isolated thylakoid membranes was determined by modifying the method of Chetti and Nobel (15). It was based on decreasing in absorbance of dichlorophenolindophenol (DCPIP) as a using electron acceptor at 590 nm. The thylakoid membranes containing 20 µg chl were mixed reaction mixture contained 40 mM Hepes (pH 7.5), 5 mM $MgCl_2$, 2 mM K_2HPO_4, 10 mM KCl, and 10 µM DCPIP. The reaction mixture was illuminated with mechanism that prepared according to Kyle and Zalik (16). The measurements were made in a Jenway 6105 UV/Vis. spectrophotometer at 25°C. At the 590 nm spectrophotometric absorbances were recorded total 75 s interval 15 s. The slope of graph used for calculating inhibition of PS II activity. The change in PS II activity was interpreted as inhibition of PS II. The equation of % inhibition compared with controls was given below:

% Inhibition= (1 - slope of stressed/slope of control) X 100

Statistical Analysis

Statistical analyses of data were performed using Statistica Programme. Duncan's Multiple Range Test was used to determine significant differences of means at a 5 % level.

Results

Effects of salt treatments with different osmotic potential on chlorophyll contents and Chla/Chlb ratios in leaf tissue of cultivars are presented in Figure 1. In C.6127 cultivar, all pigment contents and Chla/Chlb ratio were decreased, but in DK.623 cultivar, it increased significantly with increasing the salt treatment. However, it was found that the difference between two cultivars was significant, except at - 0,3 MPa osmotic potential of salt level (Figure 1a, b, c and d).

Effect of salinity at different osmotic potential on inhibition of photosynthetic activity of leaf tissue in maize cultivars was shown Figure 2. Photosynthetic inhibition of cultivars increased with increasing the salt levels. It was determined that the difference between salt treatment levels in C.6127 was significant, whereas was not, except control in DK.623.

Discussion

Environmental conditions need to be suitable for seeing normal growth and development in plants. Environmental stresses affect physiological and biochemical reactions in plants. Therefore, especially salinity causes a reduction both in growth and net photosynthesis of higher plants. The composition and function of the photosynthetic apparatus of plants may undergo in response to salinity (17).

Krishnaraj et al. (12) reported that chl contents of wheat varieties increased with increasing salt treatment (0 to 2 % Na_2SO_4) and demonstrated alterations in total chl contents that were directly proportional to changes chl a content, whereas chl b content alterations were comparatively low. Chl a and b contents increased significantly in salt tolerant variety as the salt concentration increased but no significant variations were seen in the salt susceptible variety at high salt concentration. Although salinity causes increase in chl content, Morales et al. (17) suggested that the relative photosynthetic pigment composition appeared to be unchanged by salinity. Similar results were determined in present study. Chl contents and chl a/chl b ratios of maize cultivars affected differently from salinity (Figure 1). In DK.623 chl content and chl a/chl b ratio showed an increase significantly, whereas C.6127 decreased compared with its controls. Increasing chl content and and chl a/chl b ratio in DK. 623 suggested probably that the mechanism of chl synthesis could be undamaged. But, a decrease in chl content and chl a/chl b ratio of C.6127 cultivar might have been presence of deterioration in mechanism.

PS II activity was examined *in vitro* used DCPIP as a reductant. Salt treatments affected differently PS II activity of maize cultivars (Figure 2). In C.6127 cultivar at – 0.1, -0.3, and – 0.5 MPa stress conditions the increases in the inhibition of PS II activity compared to control were about 20 %, 28 %, and 45 %, respectively. Whereas in DK.623 cultivar of inhibition of PS II activity increased only about 25 % at – 0.1 MPa compared to control (Figure 2). These results may demonstrate that C.6127 is more sensitive to salt than DK.623.

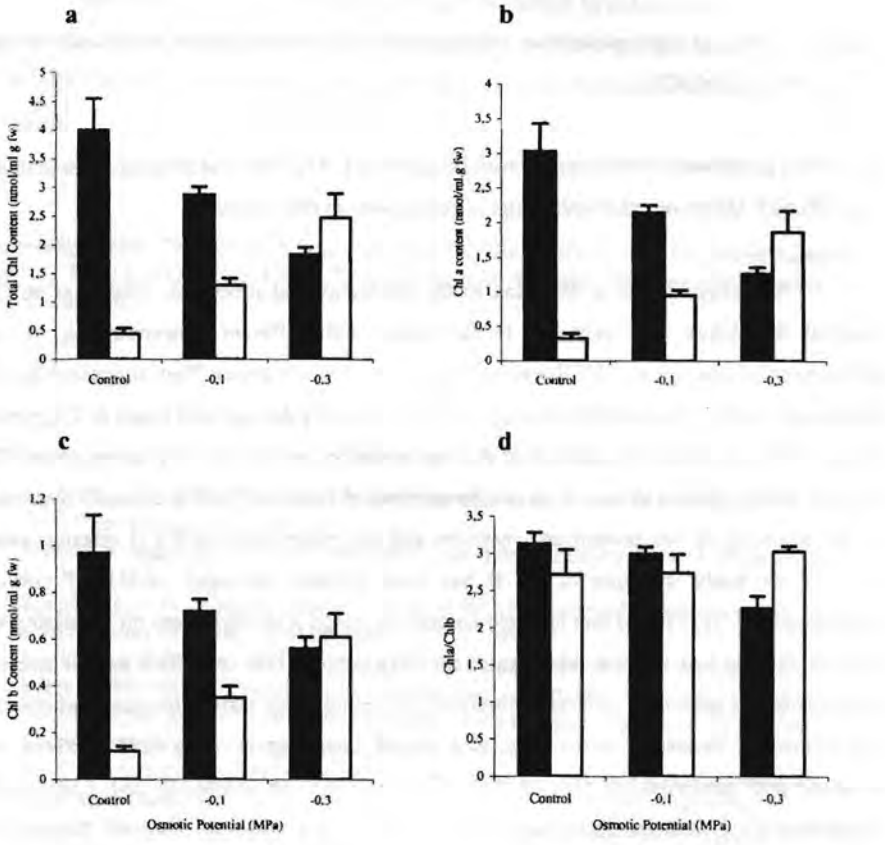

Figure 1. Effect of salt treatment on total chlorophyll (a), chlorophyll a (b) and chlorophyll b contents (c) (nmol/ml g fw) and Chla/Chlb ratios (d) of maize cultivars, (■) C.6127 and (□) DK.623 (Did not obtain enough material at the - 0.5 MPa)

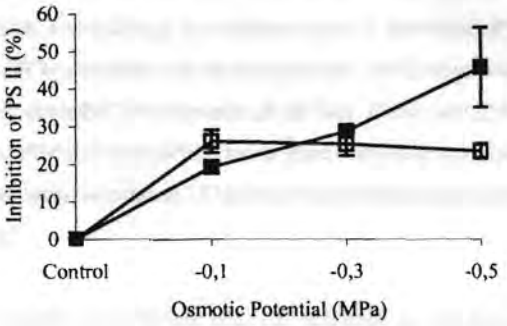

Figure 2. Effect of salt treatment on inhibition of PS II activity (%) of maize cultivars, (■) C.6127 and (□) DK.623

According to measured parameters, it may be said that C.6127 has less protective mechanisms than DK.623. Moreover, photoinhibition is maybe seen in this cultivar.

Salt stress causes a reduction in the photochemical processes. Mishra et al. (18) reported that wheat was exposure to salt stress with different concentrations, *in vivo* photochemical efficiency of PS II was not affected by the salt stress. They suggested that the steady-state level of photoinhibition might be determined by damage and repair of D1 protein. However, Tiwari et al. (19) established that salt stress decline the rate of photosynthesis. The 32 kDa protein appears to have a rapid turn-over rate in repair of PS II is critically dependent on the synthesis of this protein and insertion and integration into the PS II reaction center (20). In our study, structure of PS II has been probably damaged in C.6127 cultivar. Krishnaraj et al. (12) found that total chl content increased with dependent on increasing salt concentration in salt tolerant wheat cultivar. They suggest that the effect of salt stress is primarily on the amount of quinone. Moreover, it is thought that cations increase hydrophobic interactions of thylakoid membranes, this causes changing in water-lipid surfaces and structure and conformation of membrane. In addition, van Rensburg and Krüger (21) determined PS II activity decreased as well as leaf water potential declined. Kaiser (22) suggested that inhibition of photosynthesis in tissue slices and protoplasts is more closely related to cell volume than water potential. Exposure to mild water deficits, above 70 %, primarily causes limitation to CO_2 uptake because of stomatal closure. When stomata close as a result of such root signals, photosynthesis is limited in cells that are not actually suffering any greater water deficit than well-watered plants (23). In our study, the osmolality increased, whereas relative water content decreased with increasing stress in both maize cultivars (24).

In this study, although the method used for measuring PS II activity was different and some technique possibilities were absent, our findings gave same message with findings of summarized above. The photosynthetic activities of both maize cultivars responded to stress differently.

In summary, we proposed that salt treatments affected negatively photosynthetic activity of C.6127 than DK.623. In DK.623, chl content and chl a/chl b ratio increased and PS II activity only decreased at - 0.1 MPa osmotic potential compared to C.6127. PS II activity of DK.623 was probably steady-state in salt stress to not damage thylakoid membrane system. Therefore, DK.623 is more tolerant against salt stress conditions compared to C.6127. We consider that, DK.623 is a cultivar to study salt tolerance at biochemical level due to its ability to withstand salt stress conditions.

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PHYTOPLANKTON AND ZOOPLANKTON STRUCTURE OF SULTAN MARSHES IN CENTRAL ANATOLIA

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Abstract

In this study, the focus is on the planktonic organisms which were identified during the field studies conducted between August 1993-October 1994. The overall zooplankton density was low during winter. However Copepods started to become abundant in the late summer and fall periods while rotifers were abundant in spring. From the Sultan Marches, a total of 26 Rotifera, 3 Cladocera and 2 Copepoda species have been identified. Among phytoplanktonic organisms a total of 175 species were identified. The effects of water quality variables which depend on the seasonal distribution and the succession of the zooplankton taxa, as well as the interrelation with phytoplanktonic organisms, were also discussed.

Key Words: Phytoplankton, Zooplankton, Water quality, Seasonal distribution.

Introduction

Although a relatively large number of publications dealing with the Turkish rotifer fauna are available Dumont & De Ridder, (1); Ustaoğlu & Balık, (2-4) Emir,(5-8) Segers et al. (9); Akbulut, (10); Altındağ and Yiğit, (11); relatively few representatives of Sultan Marshes Özesmi, (12).

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Sultan Marshes is located in a 14000 ha basin within Develi, Yahyalı and Yeşilhisar towns in Kayseri province. Its surface area has been changing greatly due to precipitation regime. The altitude of the marshes is 1170 m above sea level and the coordinates are 38 ° 20' N-25° 17 ' E (13). Sultan Marshes have two major distinct ecological habitats, the freshwater and brackish water systems. The wetland has freshwater swamps at its north and south sides (salinity 0.1 ‰) and there is a moderately salty lake, the Yay lake, between the two (salinity 0.2-0.3 ‰). The average depths of Yay Lake and Sultan Marshes are 0.3 m and 1.5 m, respectively. Yay Lake, which is the biggest lake of the basin, is situated on the North side of the marsh. During summer while the water level of this lake gradually decreases, its salinity increases. To the north east of Yay lake, there is Kepir Marshes which shows the characteristics of a freshwater habitat. Due to the agricultural activities, the water levels of this area greatly decreases during summer and sometimes it completely dries. Çöl Lake, which is located in the same basin, has salty water and its rate of salinity change between 0.15-0.20 ‰. This lake generally remains dry for four or five months in a year (Fig. 1).

Sultan Marshes is one of the largest and most important wetland systems in Turkey. The system has some water regulation problems in terms of quality and quantity. Excess irrigation water carrying the residue of pesticides and fertilisers is channelled into the brackish water and freshwater marshes. The objective of this study is to evaluate seasonal distribution of the planktonic organisms and bio-diversity according to water quality parameters and chlorophyll *a*.

Materials and Methods

Samples were collected from August 1993 to October 1994. Phytoplankton and zooplankton samples were taken from the surface water with a tow net of 20 cm mouth diameter and 55 µm nylon mesh. In order to obtain a reasonable estimate of the lake population three stations have been sampled. Samples were preserved in 4 % formalin solution and sub-samples examined microscopically. Planktonic organisms were counted with an inverted microscope Lund et al (14).

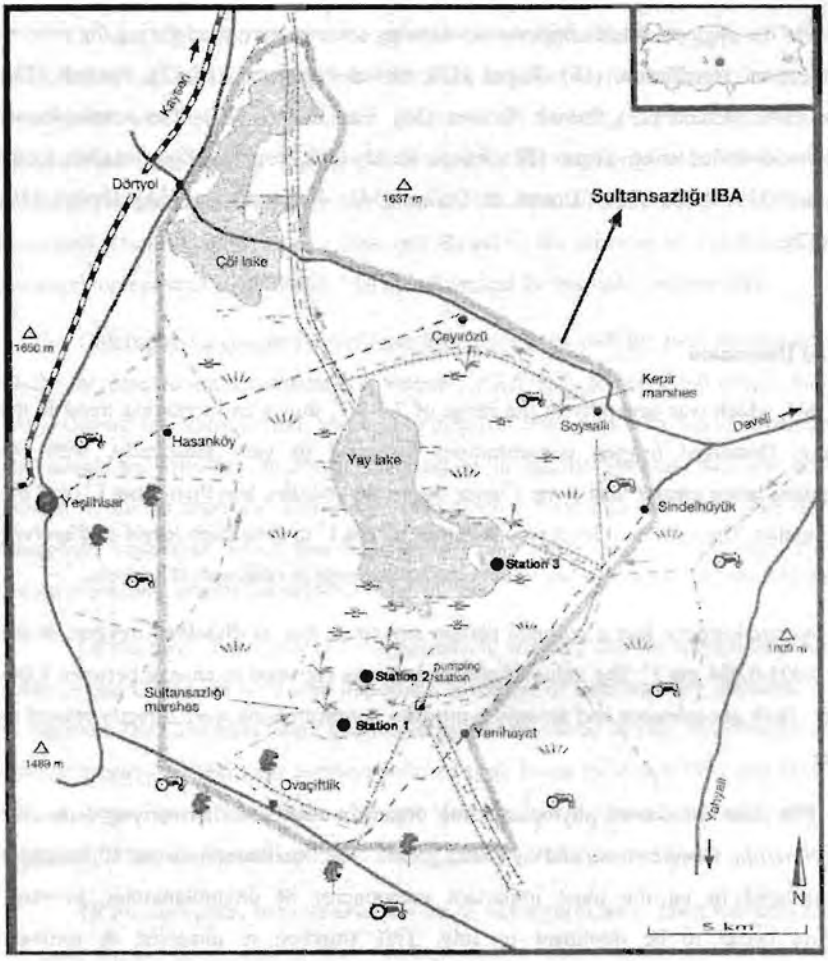

Figure 1. Study Area and Sampling Stations (Yarar and Magnin, 1997).

During the sampling period, dissolved oxygen, conductivity, salinity, pH value, temperature and Secchi depth were measured on the sampling stations using portable instruments (YSI 33 SCT meter, YSI 51 B Oxygen meter, pH meter). The data of total phosphorus and total nitrogen, which are the most important parameters to determine the trophic level, were taken from DSI, the institute which has been analysing these parameters in the last decade. Chlorophyll-a concentrations were analysed according to Youngman (15).

For the phytoplanktonic organisms following sources were used during the process of identification: Hutchinson, (16), Foged (17); Huber Pestolozzi (18-22), Hustedt (23), Komarek. (24), Prescott (25), Patrick, Reimer (26), Van Heurck (27). The zooplanktonic species were identified using Segers (28), Ranga. Reddy (29), Nogrady, Pourriot (30), Koste (31), Koste (32) Kiefer (33), Dusart, & Defaye (34), Korovchinsky (35), Herbst, (36) Kolisko (37).

Result and Discussion

pH, which was generally in the range of 7.1-9.7, shows an increasing trend in the late spring. Dissolved oxygen concentrations appeared to vary seasonally, with the concentrations being greater than 5 mg l^{-1} over the winter months, less than 4 mg l^{-1} over the summer months. The minimum level recorded was 1.7 mg l^{-1} in June.High levels of dissolved oxygen, up to 9 mg^{-1}strongly correlate with the sharp increase in chlorophyll *a* levels.

Nitrate-nitrogen had a seasonal pattern similar to that of dissolved oxygen, in the range of 0.001-0.004 mg l^{-1} The value of phosphorus was recorded to change between 0.04-0.22 mgl^{-1}. Both phosphorous and amonium-nitrogen concentrations were directly related to each other.

The most dominated phytoplanktonic organism was Bacillariophyta, especially *Synedra*, *Navicula*, *Gomphonema* and *Cymbella* genera. The Bacillariophyta and Chlorophyta were considered to be the most important components of phytoplanktonic biomass. Cryptophyta began to be dominant in July. This situation is observed in summer. Bacillariophyta exhibited pronounced variability during the spring period. Chlorophyta was the co-dominant division in terms of species diversity, amoung them *Scenedesmus*, *Monoraphidium* were the most abundant groups during the summer. Euglenophyta and Dinophyta observed smaller fractions of the total algal biomass (Fig. 2).

During the study Cyanophyta was not a dominant group among the total phytoplanktonic organisms. But in this group *Microcystis* and *Anabaena* were in high numbers. In the Cyanophyta, *Nodularia* was abundant organisms especially in the 3rd station where salt content was high. According to Hutchinson (16) this genera was adapted to waters with high salt content.

It was observed that some genera of phytoplanktonic organisms with wide distribution in eutrophic water were also dominant in Sultan Marshes. Especially *Synedra* and *Cryptomonas* were the most abundant genera in all counting periods. Hutchinson (16), reported these genera to be indicator organisms in the eutrophic lakes. However, in the marshes phytoplanktonic organisms like some species of *Navicula*, *Scenedesmus*, *Peridinium*, *Microcystis*, *Oscillatoria* were also observed. Based on the schemes of Hutchinson (16), the phytoplankton community of Sultan Marshes is typical for eutrophic waters (39).

Chlorophyll a concentrations have been correlated with the total phytoplankton and with the increase during the summer (1. Station r^2= 0.678; 2. Station r^2= 0.914; 3. Station r^2= 0.846). During the study period, mean chlorophyll *a* level was 6.03 mg/l. Phytoplanktonic algae which are effective in primary production in aquatic systems, had low population densities in Sultan marshes. However, chlorophyll *a* concentrations have also decreased. Macrophyte vegetation which has high population density in Sultan marshes, may have affected planktonic organisms negatively (39).

Of the three major groups of zooplankton, Rotifera was the numerically dominant, Cladocera and Copepoda were also important. Members of Rotifera were abundant in spring and summer. The Cladocera, were dominated by *Simocephalus vetulus*, *Scapholoberis kingii*, *Bosmina longirostris* and their numbers reached peak levels in March 1993 and May to July 1994. A large spring population did not develop either due to the late start to the phytoplankton growing season, or due to an increase in predation pressure.

Of the copepods, only calanoids were of any significance. Their numbers reached a peak in spring and early summer, then declined rapidly; and remained low for the rest of the year. Especially *Acanthodiaptomus denticornis* was widespread in Sultan marshes. Copepods may be carnivores, omnivores or detrital feeders (39) and therefore they may not have been feeding on the phytoplankton community.

On the other hand rotifers were dominant in spring and early summer (Fig. 3), in this groups *Brachionus calyciflorus*, *Keratella quadrata*, *Lecane luna*, *Synchaeta pectinata* and *Hexarthra fennica*, were dominant especially in freshwater system. These zooplanktonic organisms also are considered as indicators of eutrophic waters (38).

In Mid-July, after increase of the zooplankton population, *Synedra* increased numerically especially at the 2nd station while *Cryptomonas* began to be the dominant organisms at the end of July. This situation can be attributed to the decreasing predation

pressure of the zooplanktonic organisms on the phytoplanktonic organisms. Sultan Marshes has a huge potential for phytoplankton growth. The marshes had a spring increase in diatoms and small flagellates, as it is common in many temperate lakes (39).

Zooplanktonic and phytoplanktonic biomasses were generally high in freshwater system. But biological diversity was decreased in salt water (Fig. 2, 3). However, *Nodularia* and *Peridinium,* which are important organisms in salt water, were dominant in the salty 3rd station (Yay Lake).

In Sultan Marshes, eutrophication is effected by intensive agricultural impact and other factors. Water which is coming from irrigated areas by drainage channel flows to the basin. Besides, there are three villages and some industrial activities in the basin. Freshwater and salt water parts were polluted by the sewage and wastewater (40).

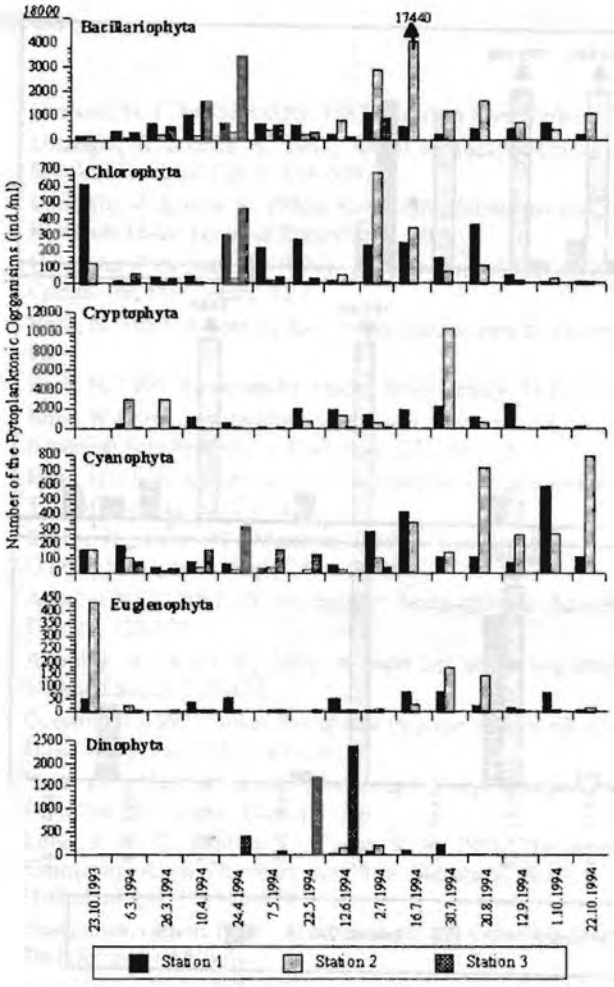

Figure 2. Seasonal Distribution of the Phytoplanktonic Organisims of Sultan Marshes (Ind./ml).

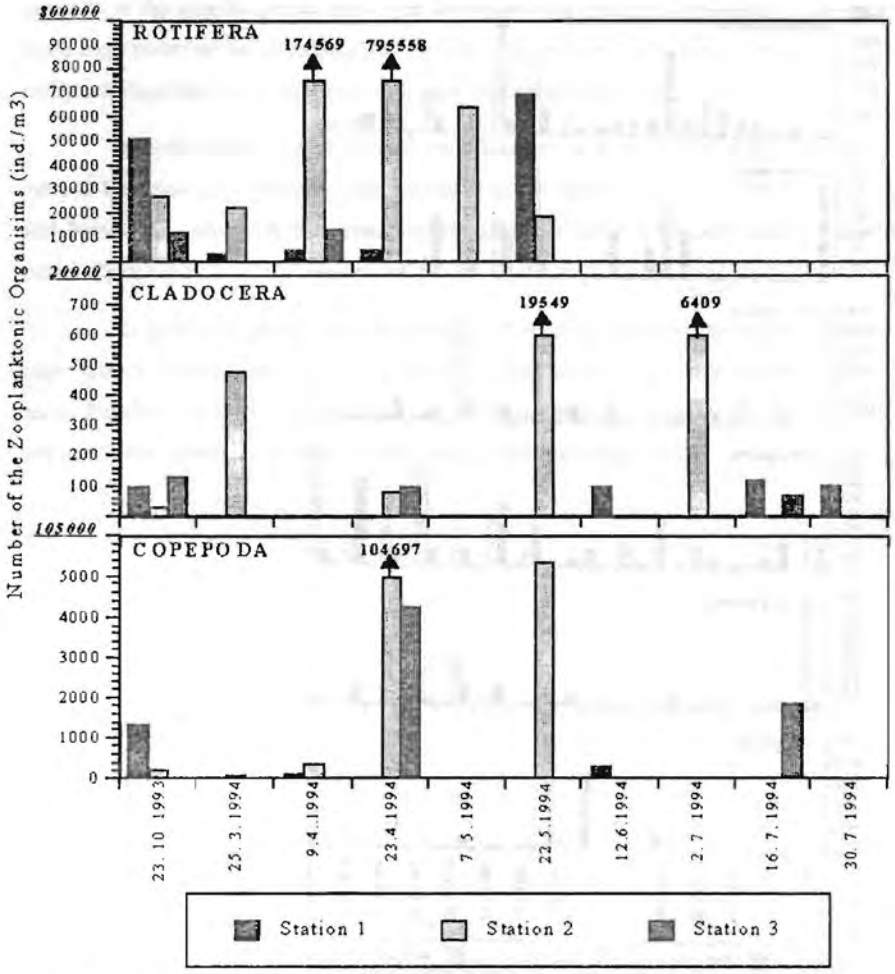

Figure 3. Seasonal Distribution of the Zooplanktonic Organisims of Sultan Marshes (Ind./m3).

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BACILLARIOPHYCEAE (DIATOM) SPECIES: NEW RECORDS FOR TURKEY FROM THE SALT LAKE BASIN

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Abstract

Twenty-two new records for Turkish diatom flora were determined from the Salt Lake Basin. Five wetlands in the basin were investigated (Salt Lake, Hirfanlı Dam Lake, Tersakan Lake, Uyuz Lake and Çöl Lake). Meanwhile, of new-recorded taxa *Scoliopleura* and *Cyclostephanos* are recorded for the first time on genus level in Turkey.

Key Words: Salt Lake Basin, Plankton, Diatom, New Record

Introduction

The Salt Lake Basin is an important area having very distinctive features in terms of wetlands ecosystem. There are many watery areas with salty, brackish and fresh water ecosystems. Diatoms living in the Salt Lake, Hirfanlı Dam Lake, the Tersakan Lake, Çöl Lake and Uyuz Lake were studied.

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The Salt Lake and Tersakan Lake represent salty ecosystem, Çöl and Uyuz Lakes have brackish water ecosystem, and Hirfanlı Dam Lake, fresh water ecosystem.

This study provides 22 diatom taxa that were determined in the Salt Lake Basin which are new records for Turkish diatom flora. These taxa are described with photographs and their distribution in the lakes and their ecological requirements covered in the study.

Material and Method:

In order to reveal the diatom flora in the study (the Salt Lake Basin), 18 field trips were made between January 1998 and October 1999.

Samples were collected using plankton net (with 10µm, 33µm, 55µm diameter) from the selected stations in the lakes (Figure 1). Then they were put into plastic pots (250 cc) for genus identification. Planktonic samples were fixed by 4% formaldehyt. Furthermore water samples taken from 30cm depth (250ml) were centrifuged in the laboratory and were concentrated. Diatoms attached to stones and plants (ephiphilic and ephilythic) were collected by scratching. Those attached to sediment were collected by means of glass tubes (1,2-m height and 0,8 cm diameter) (1).

The samples were brought to the laboratory and regular preparats made by boiling in acid and were identified by means of microscope (2).

The following literatures were used in identification of the species; 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22. Some checklist algal floras of freshwater in Turkey were investigated (23, 24). The maps of the Salt Lake Basin were taken from Magnin and Yarar (25).

The photographs of the genus identified were taken using Nikon Labophat 2.

General information about the wetlands in the Salt Lake Basin is given on Table 1.

Table 1. General Description of the Wetlands

Wetlands	Tuz Lake	Hirfanlı Dam Lake	Tersakan Lake	Çöl Lake	Uyuz Lake
Georaphical Coordination	38 ° 45′ N 33° 22′ E	39° 10′ N 33° 39′ E	38 ° 35′ N 33° 06′ E	39 ° 18′ N 32° 54′ E'	39 °15′ N 32° 57′ E
Surface area (ha.)	190 000	26 000-30 000	6400	1500	15
Altitute (m.)	905	851	910	1045	1185
Max. Depth. (m.)	1.5	70	1	0.7	1.5
Station Number	3	3	2	1	1
Salinity Classification	Salty	Freshwater	Salty	Brackish	Brackish

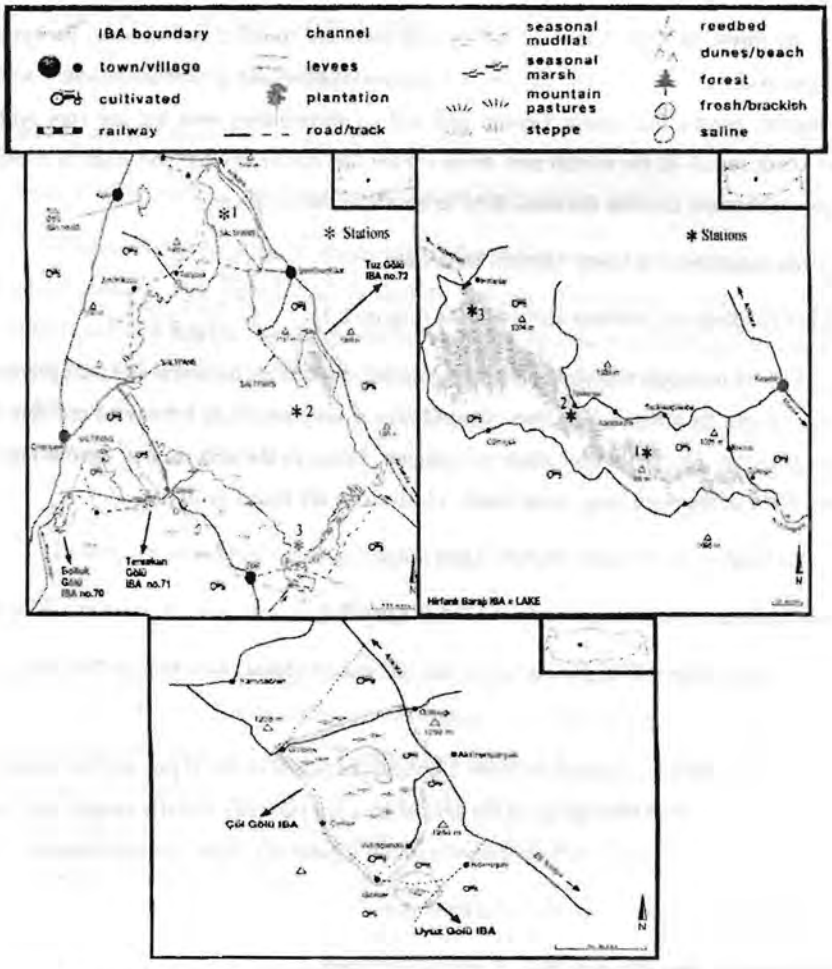

Figure 1. The lakes and stations that were studied in the Salt Lake Basin

Results and Discussion

New records for Turkish diatom flora are given below.

1. ***Navicula margalithii*** Lange-Bertalot 1985 (Figure 2.1)

Frustul is linear and lancelot; its end is in the shape of dagger. Its height is 30-70 µm and its width is 8-10 µm. Raphe lateral, axial region is very narrow and linear. Its central region is very small and asymmetric. In the middle of frustul, stria is radial sequential and it is parallel to ends. Message among striae in this part becomes narrow. There are 9-10 striae in 10µm. This type does not have a common distribution. They are mostly found in the salty water in central Europe where soluted items are widespread.

Its location in the basin; Hirfanlı Dam Lake

2. ***Navicula menisculus*** var. ***menisculus*** Schumann 1867 (Figure 2.2)

Its frustul is wide lancelolat and its end parts are rounded and narrow. Its height is15-50 µm, width is 7-12 µm. Its raphe is in the shaoe of fiber and is near to the shell. Axial part is regular, narrow and linear. Central part and its surrounding area are not very wide. Striae is a little radial. In the central part, striae are parallel to one another and interval among them becomes narrow towards the ends. 8-12 striae are found in 10 µm .

Its location in the basin: Hirfanlı Dam Lake

3. ***Navicula porifera*** var. ***porifera*** Hustedt 1944 (Figure 2.3)

Frustul is widely rhombik, elliptic or rhombik–lanceolat. Its height is 11-22 µm and width is 6-12 µm. Its raphe is like fiber. Central area is very small; its horizontal crosscut is wide and irregular. On the central striae is a stigma. Striae in the central area have a radial range and some of them are long, some small. 13-16 striae are found in 10 µm.

Its location in the basin: Hirfanlı Dam Lake

4. ***Navicula cohnii*** (Hilse) Lange Bertalot 1985 (Figure 2.4)

Stauroneis cohnii Hilse 1860; *Navicula mutica* var. *cohnii* (Hilse) Grunow in Van Heurck 1880

Its shell shape is elliptical or linear elliptical. Its height is 10-30 µm, and its width is 6-12 µm. Its raphe is fiber like. Striae in the central area are perfectly radially ranged and 15-20 striae are found in 10 µm. This is cosmopolite and it's generally found in small waters.

Its location in the basin: Hirfanlı Dam Lake

5. ***Navicula geoppertina*** (Bleisch) H. L. Smith 1874 (Figure 2.5)

Stauroneis geoppertina Bleish in Rabehn., 1861

Frustul may be elliptical lanset, rombik-lanset and flat elliptical; the end point may be like hook. Its height is 10-65 µm, its width is 6-15 µm. Raphe is fiber like; axial area is very narrow or wide and occasionally flat. Central area lasts toward the end point. Striae have radial range toward the end points. 18-24 striae found in 10 µm.

Its location in the basin: Hirfanlı Dam Lake

6. *Scoliopleura peisonis* Grunow 1860 (Figure 2.6)

Frustul may be linear elliptical and slightly convex or parallel ends. The end points are slightly round and its height is 35-80 µm, its width is 10-18 µm. Raphe has "S" shapes. Axial area is very thin and the central area is wide and rounded. Striae are straight in relation to raphe. A total of 12-16 striae are found in 10 µm.

It is rarely found and is seen in brackish waters.

Its location in the basin: The Salt Lake

7. *Cymbella ancyli* Cleve 1902 (Figure 2.7)

Gomphocymbella ancyli (Cleve) Hustedt 1930; *Gomphocymbella ruttnerii* Hustedt 1913

Frustul is dorsiventral. Its dorsal side is flat and its ventral side slightly convex, flat or slightly concave. The central part is slightly potbelly. The shape of shell is elliptical-lanceolat. It is like a mallet. The end points are rounded. Its height is 28-45 µm and its width 7-9 µm. Raphe has a ventral position. Axial area is flat and narrow. There is no central area. In the end points striae exhibit a strong radial range. 10-20 striae are found in 10 µm. Its distribution area is wide. They are found in the oligo-eutrof lakes, even in calcareous and salty lakes.

Its location in the basin: Hirfanlı Dam Lake

8. *Amphora inariensis* Krammer 1980 (Figure 2.8)

The shape of shell is dorsiventral and dorsal part is convex, ventral part is either flat or slightly concave and it is potbelly in the middle part. Its end point is slightly curved towards ventral part. Its height is 10-28 µm and its width is 3-6 µm. Raphe is flat, and axial area narrow. Hyaline area is near to the end point. The end points of striae are wide and narrow near the ends. A total of 15-17 striae are found in 10 µm. Points on the striae are not clear. It is a cosmopolite type.

Its location in the basin: Hirfanlı Dam Lake

9. *Amphora subcapitata* (Kisselev) Hustedt 1959 (Figure 2.9)

Amphora veneta var. *subcapitata* Kisselev 1922

Cells are wide and elliptical from side view. Its height is 45-80 µm and its width is 9-13 µm. Frustul is lanceolat. Dorsal part is convex and ventral part is slightly concave and it is potbelly in the middle part. Its end points are stubby and rounded. Raphe is either flat or slightly curved and near to ventral. Axial area is narrow, and the central part is small and semi-rounded. Striae are radial towards the ends. 13-20 striae are found in 10 µm. It is reported that it is found in salty waters and in the waters of which electrical conductivity is high.

Its location in the basin: The Salt Lake

10. *Gomphonema pseudoaugur* Lange-Bertalot 1979 (Figure 3.1)

Frustul is either oval or lanseolat. Its height is 25-55 µm and width is 7-10 µm. Raphe has slightly lateral position. Axial area is narrow and linear; the central area is very small. 9-12 striae are found in 10 µm. It is widespread in mezotrophic and autropic lakes.

Its location in the basin: The Salt Lake

11. *Gomphonema olivaceum* var. *staurophorum* Pantocsek 1889 (Figure 3.2)

(*Gomphonema salinarum* var. *staurophorum* (Pantocek) Cleve 1894

The shape of shell is rombik, linear and lanceolat. The central area is nearer to one end point. Its height is 20-45 µm and its width is 3-10 µm. Raphe has slightly lateral position and it is flat. Striae are radially arranged. A total of 9-11 striae are found in 10 µm. It is widespread in waters which are slightly salty or which have high electrical conductivity.

Its location in the basin: Hirfanlı Dam Lake, The Salt Lake

12. *Caloneis lauta* Carter & Bailey-Watts 1981 (Figure 3.3)

Frustuls are linear. Their end points are either parallel or slightly concave. Its height is 25-48 µm and its width is 6-9 µm. Raphe is strongly curved. Axial area may be either narrow or partly wide. The central area is very wide and linear. Striae are parallel in the middle part of frustul and are radially ranged towards the end points. 13-18 striae are found in 10 µm.

Its location in the basin: Hirfanlı Dam Lake

13. *Mastogloia braunii* Grunow 1863 (Figure 3.4)

Frustul has convex end points. Its tip is rounded. Its height is 32-95 µm and its width is 14-28 µm. Raphe is curved and has lateral position. In the proximal there is central pore. And there is a split in distal. Axial area is narrow; the central part is small. The middle part of shell is lateral and in the shape of "H" due to hyaline. Range of striae is radial towards

the end points. A lot of 15-20 striae are found in 10μm. It is a cosmopolite type. It is common in brackish water sources. It might be that this type is found in the waters of which electrical conductivity is high.

Its location in the basin: Hirfanlı Dam Lake, Tersakan Lake

14. *Rhopalodia constricta* (W. Smith) Krammer 1987 (Figure 3.5)

Epithemia constricta W. Smith 1853; *Rhopalodia musculus* var. *constricta* (W. Smith) H. & M. Peragallo 1897-1908; *Rhopalodia gibberulla* var. *constricta* (W. Smith) Karsten 1899

Frustul is flat wide elliptical. Tips are parallel and convex. The end point of shell is rounded. It dorsal side is strongly convex and its ventral side is flat. Its height is 24-75 μm and its width is 9-18 μm 3-6 costas are found in 10 μm. 15-20 striae are found in 10 μm. It is a cosmopolite type and a brackish form. It is rarely found in the waters where electrical conductivity is high.

Its location in the basin: The Salt Lake

15. *Rhopalodia operculata* (Agardh) Håkansson 1979 (Figure 3.6)

Frustulia operculata Agardh 1827; *Cymbella operculata* Agardh 1830; *Epithemia minuta* Hantzsch 1863

Frustul is dorsiventral. Its height is 18-52 μm and its width is 13-26 μm. Fibular cells total 3-6 in 10 μm. The number of striae is 16-18 in 10 μm. It is a cosmopolite type. Its density is not very high in the interior waters. It is generally found in the waters with high electrical conductivity, and in thermal and mineral waters.

Its location in the basin: The Salt Lake, Uyuz Lake

16. *Nitzschia flexoides* Geitler 1968 (Figure 3.7)

Frustul is linear. Tip is sigmoidly curved. It is more specifically curved in relation to *Nitzschia sigmoidea*. Its height is 40-103 μm and its width is 3-5 μm. The number of costa is 7-13 in 10 μm. Striae are not clear.

Its location in the basin: The Salt Lake, Tersakan Lake, Çöl Lake

17. *Nitzschia linearis* var. ***tenuis*** (W. Smith) Grunow 1880 (Figure 3.8)

Nitzschia tenuis W. Smith 1853; Nitzschia tergestina (Kützing) 1844) Ralfs in Pritchard 1861

Frustuls are flat lanceolat and become narrow towards the end point. The difference between their height and with is very high. Its height is 34-228 μm and its width is 2-8 μm. Raphe is strongly exantric. 8-17 fibulas are found in 10 μm. The number of stria is 28-41 in 10 μm. Costa is very thin.

Its location in the basin: Hirfanlı Dam Lake

18. *Surirella brightwelli* W. Smith 1853 (Figure 3.9)

Surirella ovalis var. *brightwellii* (W. Smith) H. & M. Peragallo 1897-1908

Apical cell is heteropol. Frustuls are oval and rounded. One of the apical corners is widely rounded. The other one is stubby and the end points are rounded. Its height is 15-80 µm and its width is 10-45 µm. Costas do not form wing structure. 30-45 costae are found in 10 µm. The number of stria is 14-19 in 10 µm.

Its location in the basin: Hirfanlı Dam Lake, The Salt Lake, Uyuz Lake, Çöl Lake

19. *Diatoma moniliformis* Kützing 1833 (Figure 3.10)

Diatoma tenuis var. *moniliformis* Kützing 1833

Frustuls are isopol elliptical and partly convex. Tip is wide and rounded in the shape of hook. Its height is 8-40 µm and its with is 2-5 µm. The number of costa is 7-12 in 10 µm. Axial area is very narrow. It is common among green algae.

Its location in the basin: The Salt Lake

20. *Cyclotella bodanica* var. ***affinis*** (Grunow) Cleve-Euler 1951 (Figure 4.1)

Cyclotella comta var. *affinis* Grunow in Van Heurck 1882

In the middle of shell is a big areolar structure. Its diameter is 28-80 µm. All of striae have the same height and thin, radial. 13-15 striae are found in 10 µm.

Its location in the basin: Hirfanlı Dam Lake, The Salt Lake, Tersakan Lake, Uyuz Lake

21. *Cyclotella rossi* Håkansson 1990 (Figure 4.2)

Discoplea oligactis Ehrenberg 1854; *Cyclotella oligactis* (Ehrenberg) ralfs in Pritchard 1861; *Cyclotella comta* var. *oligactis* (Ehrenberg) Grunow in Van Heurck 1882

Cells exist individually and do not form colony. Frustuls are rounded. In the middle of shells there are structures with the star shape. Its diameter is 5-18 µm. Central part of shell is not regular. It is cosmopolite. They live in lakes and rivers in a pelagic region.

Its location in the basin: Hirfanlı Dam Lake

22. *Cyclostephanos novaezeelandiae* (Cleve) Round 1982 (Figure 4.3)

Stephanodiscus novaezeelandiae Cleve 1881

Frustuls have the shape of a drum. They may exist individually or may form colony. Frustuls are rounded, concentrated waves. Top of shell is radial punctual. Diameter of cell is 7-40 µm.

Its location in the basin: Hirfanlı Dam Lake, The Salt Lake

A total of 150 taxa are identified in the study area of which 22 are new records for Turkey. It raises the importance of the basin. Of new-recorded taxa *Scoliopleura peisonis* Grunow and *Cyclostephanos novaezeelandiae* Cleve are recorded for the first time on genus level in Turkey. The other new taxa have wide spread distribution in European Countries.

Ecological variety of the Salt Lake Basin provides a wide habitat for some species. However, it has some limitations for other species. Freshwater species have a significant distribution in the basin, while salty water species are found in the Salt and Tersakan Lakes. Of new-recorded species, nine are from only fresh water ecosystem (Hirfanlı Dam Lake), 7 from salty water ecosystem and 7 from both fresh water and salty water systems. Thus, most of these new-recorded species adapt to salty water.

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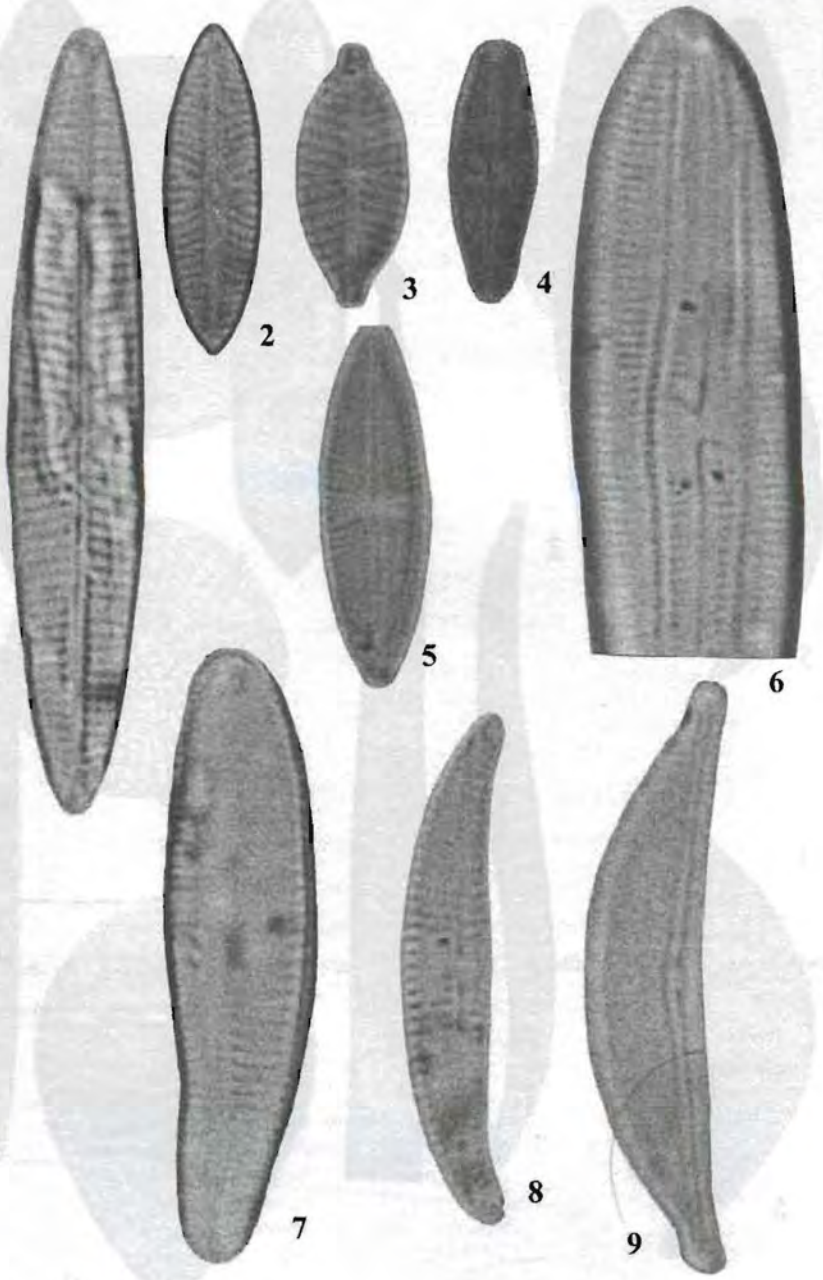


1, 2, 3, 4, 5, 6, 7, 8, 9

Figure 2. 1. *Navicula margalithii* 2. *Navicula menisculus* var. *menisculus* 3. *Navicula porifera* var. *porifera* 4. *Navicula cohnii* 5. *Navicula geoppertina* 6. *Scoliopleura peisonis* 7. *Cymbella ancyli* 8. *Amphora inariensis* 9. *Amphora subcapitata* (Scales 10 µm)

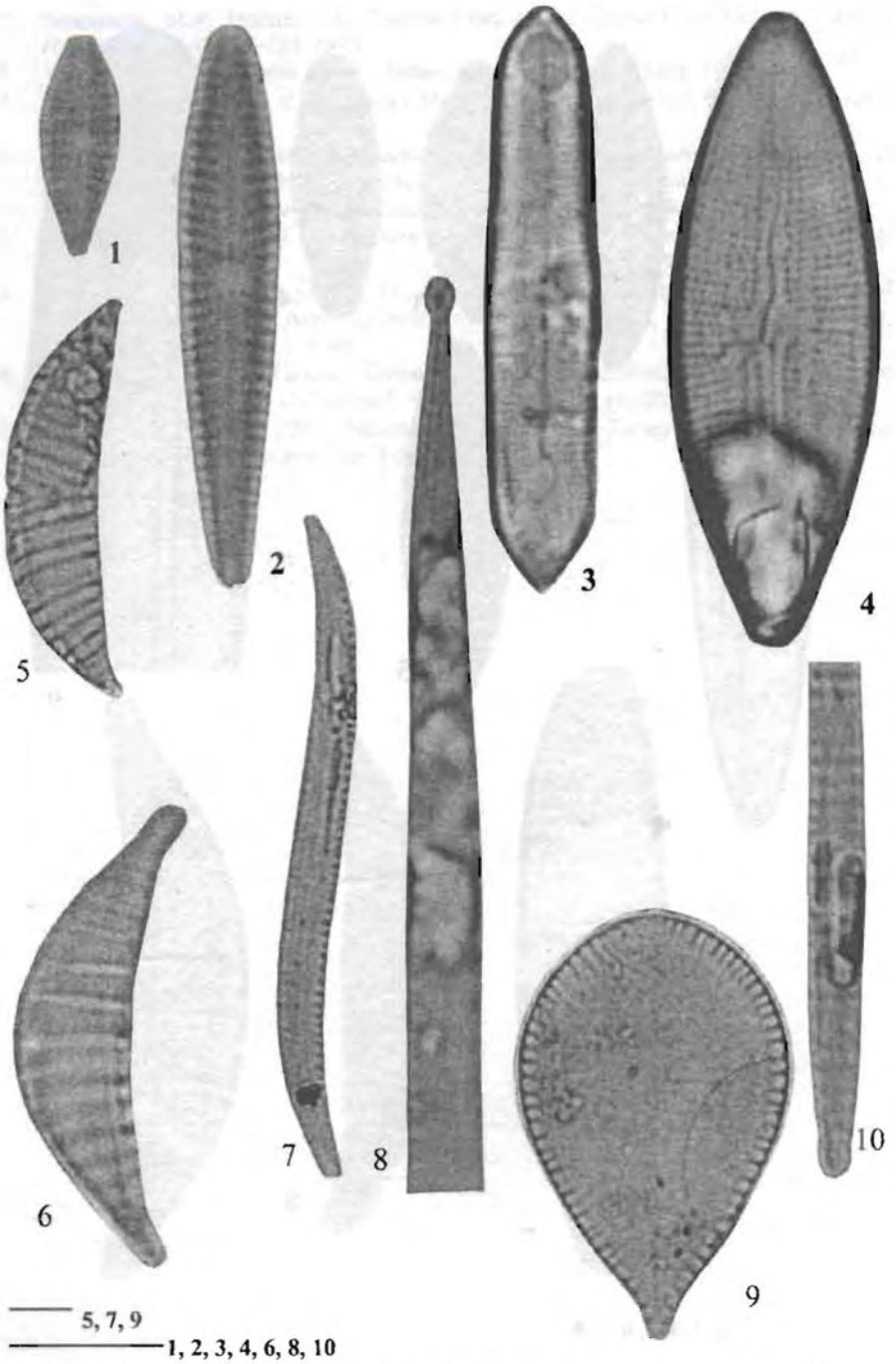
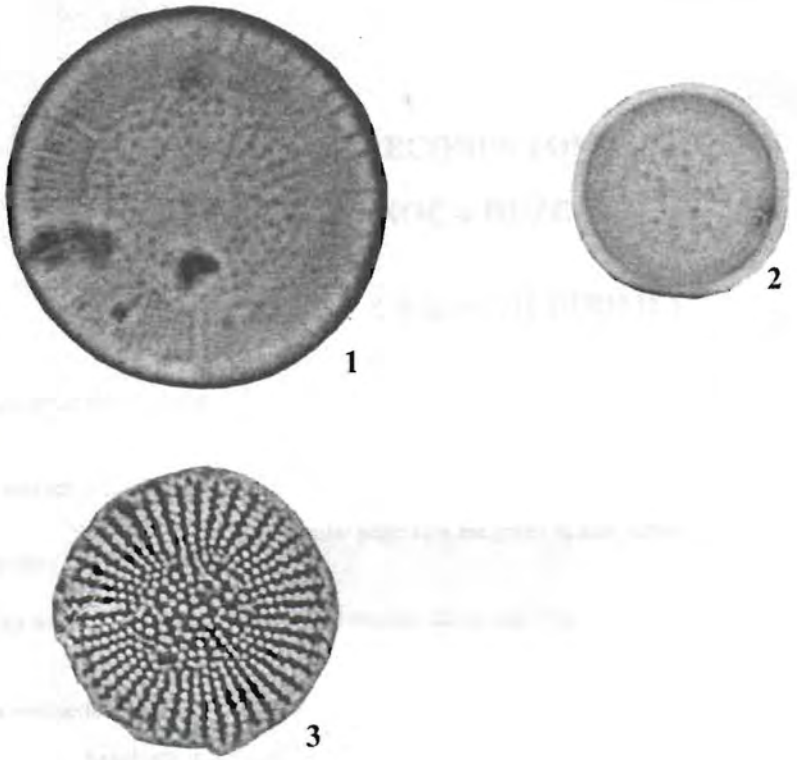


Figure 3. 1. *Gomphonema pseudoaugur* 2. *Gomphonema olivaceum* var. *staurophorum* 3. *Caloneis lauta* 4. *Mastogloia braunii* 5. *Rhopalodia constricta* 6. *Rhopalodia operculata* 7. *Nitzschia flexoides* 8. *Nitzschia linearis* var. *tenuis* 9. *Surirella brightwelli* 10. *Diatoma moniliformis* (Scales 10 μm)



1, 2, 3

Figure 4. 1. *Cyclotella bodanica* var. *affinis* 2. *Cyclotella rossi* 3. *Cyclostephanos novaezeelandiae* (Scales 10 μm)

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NEW FLORISTIC RECORDS FOR A3 SQUARE (AKÇAKOCA-DÜZCE)

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Abstract

In this research, 73 vascular plant taxa are given as new record collected for A3 square (Akçakoca-Düzce).

Key words: New square records, A3 square, Akçakoca-Düzce.

Introduction

Akçakoca is a district of Düzce and placed in west Blacksea region. It is in Euro-Siberian phytogeographical region and A3 square according to Davis' squares system (1). The research area surrounded by naturel borders: Blacksea exists on the north, Kaplandede mountain lies at the south and parallel to the sea and the west border is Melen stream and the east border is Kocaman stream (2).

This plant taxa were collected during a research that named "*Flora and Ethnobotany of The Akçakoca District (Düzce)*" between 2001-2003 (3).

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Materials and Methods

Plant materials were identified according to *The Flora of Turkey and The East Aegean Islands* basically (1,4-6) and Yıldırımlı's chorology papers were checked for the new square records (7-18). Brummit et al.'s book was used for authors' names (19). The collected plant materials are deposited at the Herbarium of Hacettepe University (HUB) and Yıldırımlı Otluk.

Results

PAPAVERACEAE

Glaucium leiocarpum Boiss

A3 DÜZCE: Akçakoca, the border of Alaplı district, around Kocaman, in scruby, 0-1 m, 23.7.2002, AD 1928-BT.

BRASSICACEAE

Lepidium virginicum L.

A3 DÜZCE: Akçakoca, around Şifalı Su, forest pathway, 200 m, 10.6.2001, AD 1453.

Thlaspi alliaceum L.

A3 DÜZCE: Akçakoca, around Şifalı Su, clearing of the forest, 200-250 m, 10.5.2003, AD 2281; between Doğancılar and Çiçekpınar Villages, south side, in scruby, 20 m, 10.5.2003, AD 2247.

Erophila verna (L.) Chevall. subsp. **praecox** (Stev.) Walters

A3 DÜZCE: Akçakoca, around Doğancılar, in scruby, 5-10 m, 15.3.2003, AD 2114.

Cardamine lazica Boiss. & Bal.

A3 DÜZCE: Akçakoca, Kurukavak Village, around Yayladere, near the fountain, 650 m, 7.6.2003, ŞY 28636-AD; around Şifalı Su, towards Kaplandede mountain, mixed deciduous forest (*Fagus orientalis Lipsky-Castanea sativa Miller-Carpinus betulus L*), 250-500 m, 6.6.2003, ŞY 28595-AD. Euxine element.

Maresia nana (DC.) Batt.

A3 DÜZCE: Akçakoca, Tahirli Village, along the stream, under *Fagus orientalis* group, 1-25 m, 19.4.2001, AD 1203.

Sisymbrium irio L.

A3 DÜZCE: Akçakoca, Subaşı Village, roadsides, in scruby, 50 m, 10.5.2003, AD 2274.

VIOLACEAE

Viola canina L.

A3 DÜZCE: Akçakoca, between Melenağzı and Nazımbey Villages, cultivated *Corylus* area, edge of hedge, 1-50 m, 7.4.2001, AD 1063; between Doğancılar and Çiçekpınar Villages, cultivated *Corylus* area, meadow slopes, 1-50 m, 24.2.2002, AD 1600; Edilli Village, cultivated *Corylus* area, stream banks, 1-5 m, 19.4.2001, AD 1177.

PORTULACACEAE

Portulaca oleracea L.

A3 DÜZCE: Akçakoca, bazaar, cultivated plants, 1-10 m, 23.7.2002, AD 1886-BT.

CARYOPHYLLACEAE

Minuartia imbricata (M.Bieb.) Woronow

A3 DÜZCE: Akçakoca, summit of Kaplandede mountain and around the tomb, mixed deciduous forest, 110-1150 m, 7.6.2003, ŞY 28661-AD. Euxine element.

Cerastium pumilum Curtis

A3 DÜZCE: Akçakoca, Edilli Village, cultivated *Corylus* area, stream banks, 1-5 m, 19.4.2001, AD 1171; Akkaya stream, picnic area, cultivated *Corylus* area, 5 m, 22.4.2003, AD 2182; Armutlu Village, cultivated *Corylus* area and mixed deciduous forest, 75 m, 18.5.2001, AD 1113.

Dianthus armeria L. subsp. **armeria**

A3 DÜZCE: Akçakoca, the border of Alaplı district, around Kocaman, roadside, in scruby, 1-40 m, 10.6.2001, AD 1403. Euro-Siberian element.

POLYGONACEAE

Polygonum hydropiper L.

A3 DÜZCE: Akçakoca, Kurugöl Village, towards Sarıyayla Village, cultivated *Corylus* area, hedges, 450-550 m, 26.10.2002, AD 2016-ŞY.

CHENOPODIACEAE

Chenopodium album L. subsp. **album** var. **microphyllum** (Boenn.) Aellen

A3 DÜZCE: Akçakoca, Cumayanı forest, 5 m, 28.10.2002, AD 2085-ŞY.

Atriplex tatarica L. var. **tatarica**

A3 DÜZCE: Akçakoca, Edilli Village, coastal sands, 1-50 m, 29.9.2001, AD 1412.

Atriplex hastata L.

A3 DÜZCE: Akçakoca, Edilli Village, coastal sands, open grounds, 1-50 m, 29.9.2001, AD 1511.

AMARANTHACEAE

Amaranthus patulus Bertol.

A3 DÜZCE: Akçakoca, around Fakıllı cavern, groves, in scruby, 50 m, 27.10.2002, AD 2047-ŞY.

Amaranthus spinosus L.

A3 DÜZCE: Akçakoca, Melen stream banks, edges of cultivated *Zea mays* area, 1-50 m , 29.9.2001, ŞY 27226-AD.

MALVACEAE

Alcea setosa (Boiss.) Alef.

A3 DÜZCE: Akçakoca, around Kocaman, towards Akçakoca, meadow slopes, 1-40 m, 30.6.2002, AD 1704. Mediterranean element.

FABACEAE

Vicia laxiflora Brot.

A3 DÜZCE: Akçakoca, around Dereköy, cultivated *Corylus* area, 50-75 m 10.5.2003, AD 2292. Mediterranean element.

Vicia cuspidata Boiss.

A3 DÜZCE: Akçakoca, around Doğancılar, meadow, 20-30 m, 10.5.2003, AD 2198. Mediterranean element.

Lathyrus saxatilis (Vent.) Vis.

A3 DÜZCE: Akçakoca, around Doğancılar, meadow, 20-30 m, 10.5.2003, AD 2219. Mediterranean element.

Trifolium uniflorum L.

A3 DÜZCE: Akçakoca, around Doğancılar, meadow, 20-30 m, 10.5.2003, AD 2194A. Mediterranean element.

Trifolium mesogitanum Boiss.

A3 DÜZCE: Akçakoca, Nazımbey Village, cultivated *Corylus* area, edge of hedges, 40 m, 30.6.2002, AD 1728; around Doğancılar, meadow, 1-50 m, 9.6.2001, AD 1368. Mediterranean element.

Trifolium clusii Godr. & Gren.

A3 DÜZCE: Akçakoca, Tahirli Village, stream banks, under *Fagus orientalis* group, 1-25 m, 19.4.2001, AD 1210.; Küpler Village, stream banks, cultivated *Corylus* area, 350-400 m, 7.6.2001, AD 1255; around Doğancılar, meadow, 15 m, 29.6.2002, AD 1666.

Trifolium lucanicum Gasp.

A3 DÜZCE: Akçakoca, around Doğancılar, meadow, 20-30 m, 10.5.2003, AD 2234.

Medicago littoralis Rohde ex Loisel

A3 DÜZCE: Akçakoca, Tahirli Village, stream banks, under *Fagus orientalis* group, 1-25 m, 19.4.2001, AD 1205.

Dorycnium hirsutum (L.) Ser.

A3 DÜZCE: Akçakoca, Edilli Village, cultivated *Corylus* area, stream banks, 1-5 m, 19.4.2001, AD 1183. Mediterranean element.

Scorpiurus muricatus L. var. **subvillosus** (L.) Fiori

A3 DÜZCE: Akçakoca, Çiçekpınar Village, around mine of sand, meadow, stream banks, 30 m, 29.6.2002, AD 1642; around Doğancılar, meadow, 20-30 m, 10.5.2003, AD 2190. Mediterranean element.

ROSACEAE

Potentilla detommasii Ten.

A3 DÜZCE: Akçakoca, around Doğancılar, meadow, 1-50 m, 9.6.2001, AD 1362.

ONAGRACEAE

Epilobium minutiflorum Hausskn.

A3 DÜZCE: Akçakoca, Kurukavak Village, along the road, 350-450 m, 22.7.2002, AD 1865-BT; Nazımbey Village, cultivated *Corylus* area, edge of hedges, 40 m, 30.6.2002, AD 1730. Euro-Siberian element.

GROSSULARIACEAE

Ribes alpinum L.

A3 DÜZCE: Akçakoca, around Şifalı Su, towards Kaplandede mountain, mixed deciduous forest, 250-500 m, 6. 6. 2003, ŞY 28607-AD; Gebekese Village, along the road, cultivated *Corylus* area, 75-90 m, 9.6.2001, AD 1325.

R. aff. biebersteini Berl. ex DC. Berland. ex DC.

A3 DÜZCE: Akçakoca, around Şifalı Su, towards Kaplandede mountain, mixed deciduous forest, 250-500 m, 6. 6. 2003, ŞY 28605-AD. Euxine element.

APIACEAE

Aethusa cynapium L.

A3 DÜZCE: Akçakoca, Kurugöl Village, towards Sarıyayla Village, cultivated *Corylus* area, hedges, 450-550 m, 26.10.2002, AD 2013-ŞY; towards Sarıyayla Village, forest, 350-450 m, 26.10.2002, AD 2033-ŞY. Euro-Siberian element.

Anethum graveolens L.

A3 DÜZCE: Akçakoca, near Paşalar Village, roadsides, cultivated *Corylus* area, 40-75 m, 11.5.2003, AD 2330. (Cultivated), (Turkish name "dere otu").

CORNACEAE

Cornus sanguinea L. subsp. **sanguinea**

A3 DÜZCE: Akçakoca, around Doğancılar, opposite of hazelnut factory, in scruby and groves, 1-50 m, 30.9.2001, AD 1584.

ASTERACEAE

Sigesbeckia orientalis L.

A3 DÜZCE: Akçakoca, Kurugöl Village, towards Sarıyayla Village, cultivated *Corylus* area, hedges, 450-550 m, 26.10.2002, AD 2001-ŞY.

Carpesium abrotanoides L.

A3 DÜZCE: Akçakoca, Kurugöl Village, towards Sarıyayla Village, cultivated *Corylus* area, hedges, 450-550 m, 26.10.2002, AD 2020-ŞY.

Aster subulatus Michaux

A3 DÜZCE: Akçakoca, Çiçekpınar Village, on the way of the water depot, by path, cultivated *Corylus* area, 150-200 m, 29.9.2001, AD 1464-ŞY.

Chrysanthemum segetum L.

A3 DÜZCE: Akçakoca, between Hasançavuş and Melenağzı Villages, cultivated *Corylus* area and under the mixed deciduous forest, 10-20 m, 30.6.2002, AD 1723. Mediterranean element.

Leucanthemum vulgare Lam.

A3 DÜZCE: Akçakoca, Karatavuk Village, along the road, slopes, cultivated *Corylus* area, 400-450 m, 22.7.2002, AD 1880-BT. Euro-Siberian element.

Artemisia vulgaris L.

A3 DÜZCE: Akçakoca, Cumayanı forest, 5 m, 28.10.2002, AD 2089-ŞY.

Carthamus lanatus L.

A3 DÜZCE: Akçakoca, around Doğancılar, meadow, 15 m, 29.6.2002, AD 1648; Dilaver Village, Dere quarter, stream banks, stony places, 100-150 m, 22.7.2002, AD 1796-BT.

Taraxacum microcephaloides Soest

A3 DÜZCE: Akçakoca, Armutlu Village, cultivated *Corylus* area and deciduous forest, 75 m, 18.5.2001, AD 1107A; Tahirli Village, stream banks, under *Fagus orientalis* group, 1-25 m, 19.4.2001, AD 1208A.

Taraxacum scaturiginosum G.E.Haglund

A3 DÜZCE: Akçakoca, the border of Alaplı district, along the Kocaman stream, mixed deciduous forest, 1-50 m, 29.9.2001, AD 1555-ŞY; Tahirli Village, stream banks, under *Fagus orientalis* group, 1-25 m, 19.4.2001, AD 1208; Edilli Village, cultivated *Corylus* area, stream banks, coast, 1-5 m, 19.4.2001, AD 1176; Melen stream banks, marshy ground and meadow, cultivated *Corylus* area, 15-20 m, 15.3.2003, AD 2137; Melenağzı Village, along the road, cultivated area, 10-15 m, 15.3.2003, AD 2149.

Taraxacum buttleri Soest.

A3 DÜZCE: Akçakoca, between Doğancılar and Çiçekpınar Villages, roadsides, stream banks, cultivated *Corylus* area, meadow slopes, 1-50 m, 24.2.2002, AD 1608 A.

Crepis paludosa (L.) Moench

A3 DÜZCE: Akçakoca, Kurugöl Village, towards Sarıyayla Village, cultivated *Corylus* area, hedges, 450-550 m, 26.10.2002, AD 2021-ŞY; Edilli Village, near the sea, open grounds, forest, 1-50 m, 29.9.2001, AD 1497-ŞY; Kurukavak Village, cultivated *Corylus* area, roadsides, 550 m, 7.10.2002, AD 2097-ŞY. Euro-Siberian element.

Crepis bithynica Boiss.

A3 DÜZCE: Akçakoca, around Doğancılar, meadow, 20-30 m, 10.5.2003, AD 2216 Euro-Siberian element.

Crepis reuterana Boiss. subsp. **reuterana**

A3 DÜZCE: Akçakoca, Melenağzı Village, Melen stream banks, towards Nazımbey Village, in scruby, meadow, cultivated *Corylus* area, 1-50 m, 11.5.2003 AD 2318. Mediterranean element.

Crepis vesicaria L.

A3 DÜZCE: Akçakoca, Karaburun Village, in scruby, under the forest, 50-60 m, 11.5.2003, AD 2305; around Doğancılar, meadow, 20-30 m, 10.5.2003, AD 2215.

PRIMULACEAE

Anagallis arvensis L. var. **parviflora** (H. Hoffm. & Link) Ces.

A3 DÜZCE: Akçakoca, Karaburun Village, in scruby, under the forest, 50-60 m, 11.5.2003, AD 2299. Mediterranean element.

EBENACEAE

Diospyros kaki L.

A3 DÜZCE: Akçakoca, Sarıyayla Village, roadsides, 150-200 m, 500-550 m, 26.10.2002, AD 2036-ŞY. (Cultivated), (Turkish name "Trabzon hurması").

STYRACACEAE

Styrax officinalis L.

A3 DÜZCE: Akçakoca, south of Armutlu Village, mixed deciduous forest, 75 m, 18.5.2001, AD 1164.

APOCYNACEAE

Vinca major L. subsp. **hirsuta** (Boiss.) Stearn

A3 DÜZCE: Akçakoca, between Dilaver and Küpler Villages, cultivated *Corylus* area and deciduous forest, 200-300 m, 18.5.2001, AD 1152.

CUSCUTACEAE

Cuscuta campestris Yuncker

A3 DÜZCE: Akçakoca, around Yalılar, coastal sands, 0-2 m, 23.7.2002, AD 1973-BT.

BORAGINACEAE

Myosotis ramosissima Rochel ex H.Schult. subsp. **ramosissima**

A3 DÜZCE: Akçakoca, new Düzce road, roadsides, 100-200 m, 10.5.2003, AD 2254.

Symphytum officinale L.

A3 DÜZCE: Akçakoca, near Paşalar Village, roadsides, cultivated *Corylus* area, 40-75 m, 11.5.2003, AD 2327. Euro-Siberian element.

Symphytum tuberosum L. subsp. **nodosum** (Schur) Soó

A3 DÜZCE: Akçakoca, Paşalar Village, slopes, cultivated *Corylus* area, mixed forest, 1-10 m, 19.5.2001, AD 1224; between Melenağzı and Nazımbey Villages, cultivated *Corylus* area, edge of hedges, 1-50 m, 7.4.2001, AD 1040; new Düzce road, Çiçekpınar Village, roadsides, 40-75 m, 10.5.2003, AD 2300. Rare spreading. (VU). Euro-Siberian element.

SCROPHULARIACEAE

Verbascum densiflorum Bertol.

A3 DÜZCE: Akçakoca, Melenağzı Village, Melen stream banks, meadow, 1-25 m, 30.6.2002, AD 1740. Euro-Siberian element.

Misopates orontium (L.) Raf.

A3 DÜZCE: Akçakoca, The border of Alaplı district, around Kocaman, roadsides, meadow, 1-40 m, 10.6.2001, AD 1402; Melenağzı Village, Melen stream banks, marshy grounds, meadow, 1-40 m, 30.6.2002, AD 1710.

Linaria iconia Boiss. & Heldr.

A3 DÜZCE: Akçakoca, around Dereköy, cultivated *Corylus* area, 50-75 m 10.5.2003, AD 2294. Endemic. (LC). Irano-Turanian element.

Cymbalaria longipes (Boiss. & Heldr.) A. Chev.

A3 DÜZCE: Akçakoca, centrum, Yeni quarter, 1-5 m, 8.10.2001, AD 1596-ŞY. Mediterranean element.

LAMIACEAE

Ajuga chamaepitys (L.) Schreb. subsp. **palaestina** (Boiss.) Bornm.

A3 DÜZCE: Akçakoca, Çiçekpınar Village, stream banks, 20-30 m, 29.6.2002, AD 1639.

Lamium purpureum L. var. **aznavourii** Gand. ex Azn.

A3 DÜZCE: Akçakoca, summit of Kaplandede mountain and around tomb, mixed deciduous forest, 110-1150 m, 7.6.2003, ŞY 28673-AD; Yukarı quarter, around the graveyard, 45 m, 6.4.2003, AD 2166; new Düzce road, Çiçekpınar Village, roadsides, 40-75 m, 10.5.2003, AD 2118. Endemic. (CR). Euxine element.

Galeobdolon luteum Huds. subsp. **luteum**

A3 DÜZCE: Akçakoca, around Şifalı Su, clearing of the forest, 200-250 m, 10.5.2003, AD 2284. Euro-Siberian element.

EUPHORBIACEAE

Euphorbia villosa Waldst. & Kit. ex Willd.

A3 DÜZCE: Akçakoca, Edilli Village, meadow slopes, 1-50 m, 29.9.2001, AD 1529, ŞY; Çiçekpınar Village, around mine of sand, meadow, stream banks, 30 m, 29.6.2002, AD 1645. Euro-Siberian element.

Euphorbia exigua L. var. **retusa** L.

A3 DUZCE: Akçakoca, between Çiçekpınar and Doğancılar Villages, stream banks, near the gravel washing foundation, soil mass, 5-20 m, 10.6.2001, AD 1434.

RUBIACEAE

Galium album Mill. subsp. **pycnotrichum** (Heinr.Braun) Krendl

A3 DÜZCE: Akçakoca, forest of Kestane bayırı, along the road, 250 m, 27.10.2002, AD 2054, ŞY; Kurugöl Village, roadsides, slopes, 450-550 m, 9.6.2001, AD 1340. Euro-Siberian element.

MONOCOTYLEDONEAE

LILIACEAE

Ornithogalum sphaerocarpum Kerner

A3 DÜZCE: Akçakoca, Kurugöl Village, roadsides, slopes, 450-550 m, 9.6.2001, AD 1344.

JUNCACEAE

Luzula sudetica (Willd.) DC.

A3 DÜZCE: Akçakoca, south of Melenağzı Village, mixed deciduous forest, 20 m, 7.4.2001, AD 1085; around Doğancılar, meadow, 20-30 m, 10.5.2003, AD 2193. Euro-Siberian element.

POACEAE

Elymus elongatus (Host) Runemark subsp. **elongatus**

A3 DÜZCE: Akçakoca, the border of Alaplı district, around Kocaman, coastal sands, 0-1 m, 23.7.2002, AD 1939-BT.

Bromus madritensis L.

A3 DÜZCE: Akçakoca, towards Alaplı, around Shell, roadsides, 20 m, 8.6.2003, ŞY 28739-AD; towards Alaplı, roadsides, around Karayolları, 10 m, 8.6.2003, ŞY 28768-AD.

Digitaria ischaemum (Schreb. ex Schweigg.) Mühlenb. var. **asiatica** (Tzvelev) Tzvelev

A3 DÜZCE: Akçakoca, Edilli Village, clearing of the forest, 1-50 m, 29.9.2001, ŞY 27215-AD.

Discussion

During the research on the flora of Akçakoca district (Düzce), 73 species are determined as new record for A3 square. These belong to Euro-Siberian (17 species), Mediterranean (12 species) and Irano-Turanian (1 species) phytogeographical regions. *Anethum graveolens* and *Diospyros kaki* are cultivated.

Three species are endemic or rare spreading (20-21): *Lamium purpureum* var. *aznavouri* is growed under *Fagus orientalis*. *Linaria iconia* was known from the square B 3, 4, 5 and C 4, 5. *Cymbalaria longipes* was known C 3, 4 square. Antropogenic effect can be play a role spreading of these two species. Another species is *Symphytum tuberosum* that is not endemic but rare spreading. This species is growed on the west shore between city center and Melenağzı Village, mostly occurs in the scruby area. Gönen (22) recorded *Amaranthus spinosus* firstly for Turkish flora from square C5, and also is collected secondly by us from Akçakoca. Both of these localities are near the cultivated *Zea mays* area.The reason for this can be the mixture of *Zea mays*' seeds that comes from foreign countries and *Amaranthus spinosus*' seeds.

Acknowledgements

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POLLEN MORPHOLOGY OF THE THREE POMOID GENERA x *MALOSORBUS* Browicz, *MESPILUS* L., AND *ERIOLOBUS* (Ser.) Roemer (*ROSACEAE*)

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Abstract

x *Malosorbus* Browicz is a hybrid genus of *Malus* Mill. and *Sorbus* L. The genera *Mespilus* L. and *Eriolobus* (Ser.) Roemer are closely related to this hybrid genus. *Malus sylvestris* Mill. and *Sorbus torminalis* (L.) Crantz are the parent species of x *Malosorbus florentina* (Zucc.) Browicz. Pollen morphology of the parent species, the hybrid species, *Mespilus germanica* L. and *Eriolobus trilobatus* (Poiret) Roemer has been studied under both light and scanning electron microscopes. Morphological features of the pollen grains have been measured and photographed. The palynological data have been evaluated with respect to the taxonomic viewpoint.

Key words: Pollen, *Rosaceae*, x *Malosorbus*, *Mespilus*, *Eriolobus*, Turkey.

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Introduction

The *Rosaceae* is a large and diverse family with 7 tribes in Flora of Turkey (1). The tribe *Pomeae* contains 12 genera, most of which have economically important woody species, used either as ornamental or fruit tree (2, 3).

The genus x *Malosorbus* Browicz is given as a hybrid of the genera *Malus* Mill. and *Sorbus* L. The hybrid genus has one species, x *Malosorbus florentina* (Zucc.) Browicz and it distinctly differs from the parent species *Malus sylvestris* Mill. and *Sorbus torminalis* (L.) Crantz *var. torminalis* in morphology. *Mespilus germanica* L. and *Eriolobus trilobatus* (Poiret) Roemer are other morphologically close genera to the hybrid genus.

Pollen morphology of Rosaceae has been studied by many workers (e.g. 4, 5a and 5b, 6, 7, 8 and 9) but these studies have been concentrated mainly on the members growing in western Europe, the USA and Canada. In Turkey, however, there are few pollen morphological works on Rosaceae pollen (e.g. 10, 11), none of which covers the members of Pomeae.

The aims of our study are to examine the pollen morphology of the hybrid genus and other relatives mentioned above, and to question whether there are significant variations in pollen of these closely related taxa.

Material and Methods

The pollen materials were obtained from the collections of the second author (AAD) deposited in the herbarium of Hacettepe University (HUB). The voucher specimens are listed in Table 1.

Material for light microscopy (LM) was prepared by the method of Wodehouse (12) and examined and measured using a Nikon SE microscope (Table 2). Photographs were taken with a Nikon FDX-35 camera connected to a Nikon Eclipse E 600 microscope. The measurement are based on 22-50 pollen grains for polar (P) and equatorial (E) axes, and for the longest axis of the outline of pollen in polar view (L) and the distance between 2 colpi at poles (t) and 1-10 for the size of apertures (colpi and pori) and 10 for the thickness of exine and intine.

Statistical treatments of the measurements of P, E, L and t were made using the formula by Sokal and Rohlf (13).

Table 1. Specimens investigated.

Mespilus germanica	A1 Kırklareli: Demirköy, Kadınkule Pass, 41°47' N, 27°42' E, 570 m, 9 v 2002, *AAD* 8775.
Sorbus torminalis	A1 Kırklareli: between Manyetik alan-Demirköy, 41° 45' N, 27° 41' E, 790 m, 9 v 2001, *AAD* 8764.
Malus sylvestris	A4 Karabük: Safranbolu, İnceçay village, 41° 24' N, 032° 43' E, 643 m, 5 iv 2002, *AAD* 10464.
***x*Malosorbus florentina**	A2 Bilecik: around Taşcılar village, 40° 13' N, 29° 53' E, 600 m, 5 v 2001, *AAD* 8650.
Eriolobus trilobatus	B1 Balıkesir: İvrindi, Soğanbükü cemetry, 39° 37' N, 27° 33' E, 190 m, 6 v 2001, *AAD* 8661.

Material for scanning electron microscopy (SEM) was mounted directly onto double-sided cellotape and coated with gold before evaporation and examined with a Jeol JSM-5600. The measurements of sculpturing elements of exine were made on the SEM microphotographs. The palynological terminology follows Punt et al. (14).

Results

General characteristic under LM

The main palynological characteristics of the taxa examined are summarized in Table 2. Selected LM photographs are given in Figure 1. A general description can be given as follows: Pollen grains are radially symmetrical, isopolar, tricolporate, occasionally tetracolporate or syncolporate. Their shape varies from oblate to spheroidal. The shape in polar view is mostly circular to semiangular. The polar area index (PAI) ranges from 0.13 to 0.21. The colpi (ectoapertures) are usually long and are usually covered by granular membrane. Opercula are present in *Malus sylvestris* and *Eriolobus trilobatus*. The pori (endoapertures) are somewhat rectangular in outline with rounded angles. Their mean dimensions is from 10x8.75 μm to 28x24.7 μm. Exine pattern is usually indistinctly striate-perforate under LM.

Table 2. Measurements and characteristics of pollen of some Pomeae taxa.

TAXA	POLLEN TYPE	POLLEN SIZE (μm) P			E			POLLEN SHAPE	AMB
		M	SD	V	M	SD	V		
Mespilus germanica	tricolp, occasionally tetracolp, syncolp	33.45	±0.5	30-37.5	48.25	±0.6	45-52.5	o	circular-semiang
Sorbus torminalis	tricolp, occasionally tetracolp, syncolp	26.50	±0.6	25-30	32.35	±0.9	27.5-37.5	so	circular-semiang
Malus sylvestris	tricolp, occasionally tetracolp, syncolp	31.17	±1.3	22.5-37.5	34.75	±1.6	22.5-42.5	s	circular-semiang
x*Malosorbus florentina*	tricolp, occasionally tetracolp, syncolp	21.8	±0.6	25-32.5	34.3	±0.8	30-37.5	so	circular-semiang
Eriolobus trilobatus	tricolp, occasionally tetracolp, syncolp	27.05	±0.6	25-32.5	34.3	±0.9	30-37.5	so	circular-semiang

Abbreviations: **AMB:** the outline of pollen in polar view, **circ-semiang:** circular-semiangular, **E:** equatorial axis, **M:** mean value, **O:** oblate, **P:** polar axis, **s:** spheroidal, **SD:** standart deviation, **so**: suboblate, **syncolp:** syncolporate, **tetracolp:** tetracolporate, **tricolp:** tricolporate **V:** variation.

Table 2 continued.

TAXA	L (µm)			t (µm)			PAI	colpi (µm)		pori (µm)	
	M	SD	V	M	SD	V	(t/E)	Clg	Clt	Plg	Plt
Mespilus germanica	45.7	±0.6	42.5-47.5	10.5	±0.3	7.5-12.5	0.21	31.5	14.25	28	24.7
Sorbus torminalis var. *torminalis*	32.75	±0,6	30-37.5	4.4	±0.5	2.5-7.5	0.13	21.8	11.3	12.6	11.1
Malus sylvestris	31.4	±0.9	27.5-37.5	5.32	±0.3	5-7.5	0.15	27.8	13.4	10	8,75
x*Malosorbus florentina*	33.45	±0.5	30-35	6.07	±0.5	2.5-7.5	0.17	23.1	7.77	15.4	14
Eriolobus trilobatus	33.5	±0.6	30-37.5	5	±0.7	3-6	0.14	24.2	13.8	18.8	15.1

Abbreviations: Clg: length of colpus, **Clt:** width of colpus, **E:** equatorial axis, **L:** the longest axis of the outline of pollen in polar view, **M:** mean value, **PAI:** polar area index, **Plg:** length of pore, **Plt:** width of pore, **SD:** standart deviation, **t:** the distance between two colpi at poles, **V:** variation.

Table 2 continued.

TAXA	operculum	exine thickness (μm)	intine thicknes (μm)	exine pattern in SEM
Mespilus germanica	absent	1.35	1.47	rugulate-microperforate
Sorbus torminalis var. *torminalis*	absent	1	0.55	coarsely striate-perforate
Malus sylvestris	present	1	0.65	coarsely striate-microperforate
x*Malosorbus florentina*	absent	0,97	0.65	finely striate-microperforate
Eriolobus trilobatus	present	1	0.72	coarsely striate-perforate

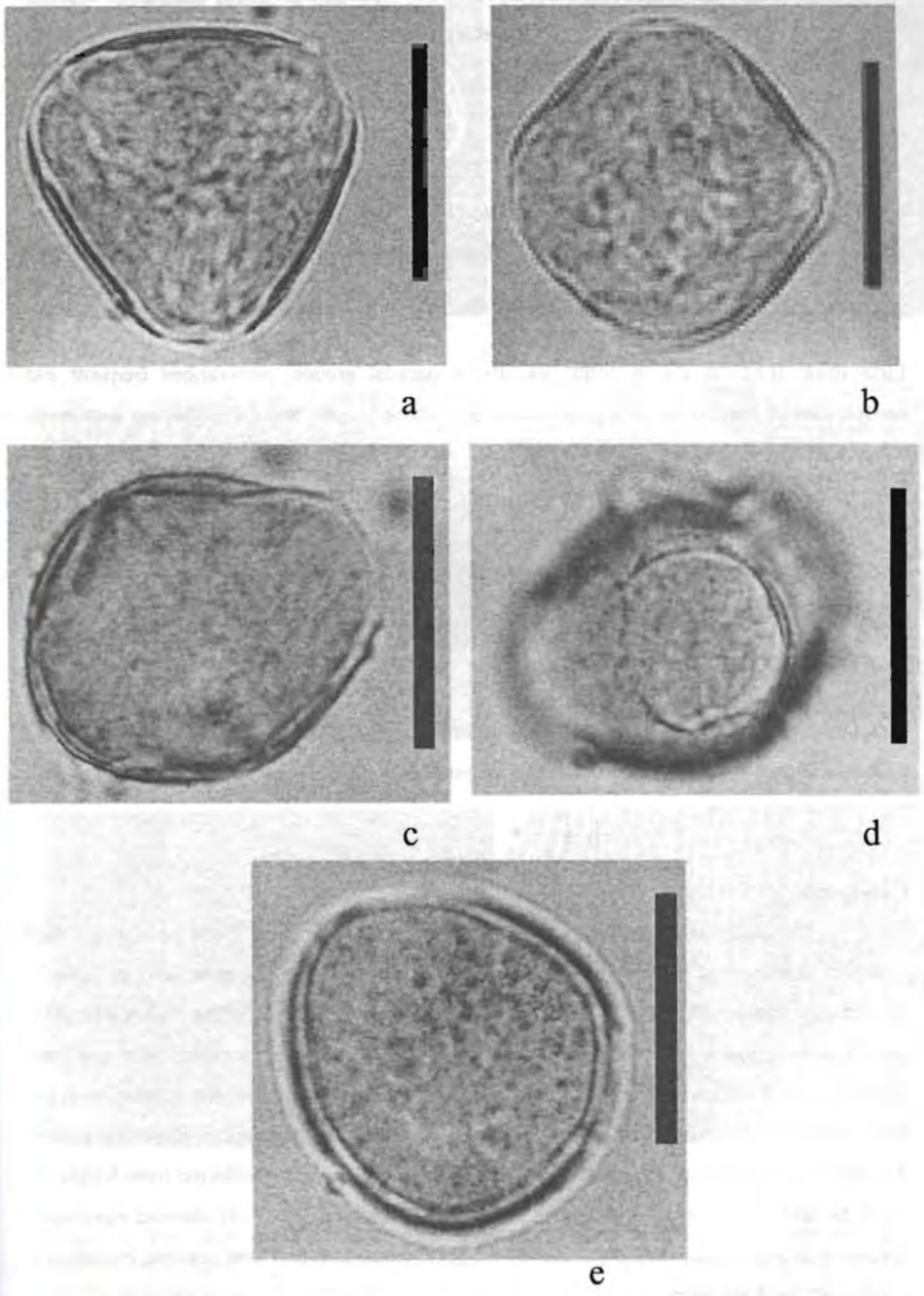

Figure 1. The LM photographs of selected Pomeae taxa studied. **a-b** *Eriolobus trilobatus*: **a.** polar view, tricolporate pollen, **b.** tetracolporate pollen. **c-d** x*Malosorbus florantina*: **c.** equatorial view at optical section, note the protruding pore on right. **d.** endoaperture-pore **e.** *Malus sylvestris*: indistinct striate- perforate exine pattern. Scale bar=30 μm.

Exine patterns in SEM

The exine patterns of the taxa studied show variations in SEM. Four types of exine pattern could be defined based on the width and orientation of the lirae and the size of the tectal perforations:

1-Rugulate-microperforate

Lirae thick, usually exceeding 0.50 μm in width and short, arranged in an irregular pattern, perforations 0.5 μm long or less. Found in *Mespilus germanica* (Figure 2 a-b).

2-Coarsely striate-perforate

Lirae thick, 0.12-0.6 μm in width, usually in parallel groups, perforations frequent and conspicuous at least some of which exceed 0.5 μm in length. Shown by *Sorbus torminalis* var. *torminalis* (Figure 2c) and *Eriolobus trilobatus* (Figure 2d).

3-Coarsely striate-microperforate

As 2, but perforations inconspicuous, less than 0.25 μm long or less. Found in *Malus sylvestris* (Figure 2e).

4-Finely striate-microperforate

Lirae finer, 0.12-0.3 μm in width, perforations inconspicuous, 0.06-0.09 μm long. This pattern is shown by *Malosorbus florentina* (Figure 2f).

Conclusion

This study has revealed that the taxa of Pomeae examined could be distinguished based on characters of general morphology and of exine pattern. As can be seen in Table 2, *Mespilus germanica* differs from the other taxa in many respects, including pollen size, PAI, pore diameter, pollen wall thickness and exine pattern. It has larger pollen size and pore diameter and thicker wall. In addition, PAI which is 0.21 μm separetes the species from the other taxa, also noted by Eide (1981) as 0.01 for the northwest European Rosaceae pollen. Rugulate-microperforate exine pattern found in *Mespilus germanica* collected from Kırklareli could be taken as an another diagnostic character. However, Byatt (4) showed significant variations in exine pattern of specimens of *M. germanica* from different regions, including a specimen from Kastamonu.

The other four taxa of Pomeae examined are similar in general pollen morphology, especially in size. But they could be distinguished by exine characteristics.

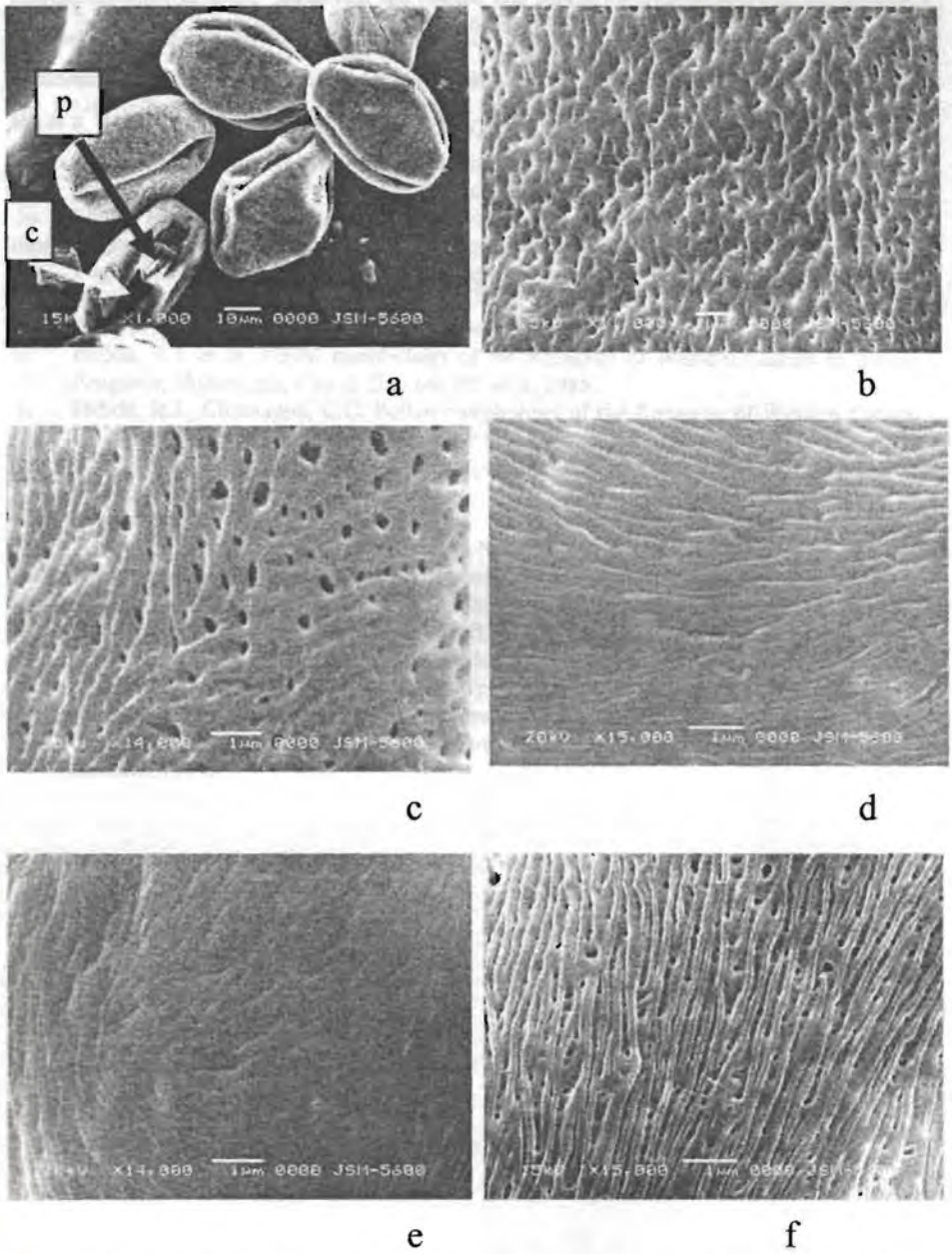

Figure 2. The SEM microphotographs of the taxa studied. **a-b.** *Mespilus germanica* **a.** A general view of pollen grains, arrows-c: colpus, p: pore. **b.** exine pattern. **c.** exine pattern of *Sorbus torminalis var. torminalis*. **d.** exine pattern of *Malus sylvestris*. **e.** exine pattern of **x** *Malosorbus florantina*. **f.** exine pattern of *Eriolobus trilobatus*.

Malus sylvestris is placed in type 3 while x *Malosorbus florentina* is placed in type 4. x *Malosorbus* is a hybrid between *Malus* and *Sorbus* but it is rather different from the parents in SEM. In both *Sorbus torminalis* var. *torminalis* and *Eriolobus trilobatus* pollen has patterns in type 2 characterized by coarse lirae arranged in parallel groups and conspicuous perforations. On the other hand, in the latter operculum is present, but in the former it is not present.

In conclusion, pollen morphology of the hybrid genus x *Malosorbus* differs from the other taxa, and the distinction of the genus is supported by this study.

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THE RELATIONSHIP BETWEEN THE EFFECTS OF THE COLD PRETREATMENTS OF BUDS ON ABSCISIC ACID CONTENT OF ANTHERS AND *IN VITRO* ANDROGENESIS OF PEPPERS (*Capsicum annuum* L.)

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Abstract

Buds of *Capsicum annuum* L. cultivar Malatya were treated cold at +4°C for periods of 48 and 96 hours. The anthers were cultured in the Murashige and Skoog (MS) medium with 4 mg l^{-1} Naphthalene acetic acid (NAA) and 1 mg l^{-1} Benzyladenine (BA), 0.25% activated charcoal. The Abscisic acid (ABA) analyses were performed in anthers taken from the buds that were seperated from the plant (control) and in their cold treated samples at +4°C for 48 and 96 hours and in either their samples that incubated 50 days. The ABA amount of the anthers that had cold treated were lower than the control. The embryo formation was obtained from the control anthers not subjected to cold pretreatments (12.5%).

Keywords: Capsicum annuum L., androgenesis, *in vitro*, abscisic acid.

Introduction

Anther culture is a technique for the production of double haploids in cultivated plants. This method also has a practical application in the acceleration of breeding program. However, the yield has remained extremely low in many cases. The method can only become useful for applied agricultural projects when the haploids are produced in large numbers. Consequently, many efforts are being made to increase the yield of anther cultures.

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It has been shown that the additions of activated charcoal (Anagnostakis 1974, Tıpırdamaz and Ellialtıoğlu, 1998) to the culture medium and pretreatment of whole buds or anthers (Sunderland and Dunwell, 1977; Morrison *et al.*, 1986; Tokuma et al., 1994) can increase embryo production.

ABA is a hormone which has an inhibitory effect on the tissue culture and also as well as on intact plants. The detailed literature information about the physological role and mechanism of effect of ABA was reported in the research of Topçuoğlu (1987). In the anther studies made with different plant species, it has been claimed that the ABA found in the anthers could prevent embryogenesis and that this negative effect could be removed with the addition of activated charcoal to the culture medium (Johansson, 1983) and by applying cold shock treatments to the buds (Johansson and Eriksson 1977, Johansson *et al.*, 1982).

In this paper, the effects of cold shock treatment of buds in the endogenous ABA content of anthers and on pollen embryogenesis in pepper were investigated.

Materials and Methods

Capsicum annuum L. cultivar Malatya were used in the present study. Donor plants were grown under field conditions. Flower buds with anthers containing uninucleate microspores at the first mitotic stage were harvested. The stages of the microspores were determined by comperative studies between the bud size and microspore development (Özkum Çiner and Tıpırdamaz, 2002).

Flower buds were placed in flask and stored in a refrigerator at +4°C for 48 and 96 hours for cold pretreatments. The control buds were not subjected to cold conditions. Before isolating anthers, the buds were sterilized with a 20% commercial bleach for 15 minutes and then washed 3 times in sterile distilled water. The anthers were placed in MS medium (Murashige and Skoog, 1962) containing 3% (w/v) sucrose, 0.8% (w/v) agar, with 0.25% activated charcoal and 4 mg l^{-1} NAA and 1 mg l^{-1} BA. The anthers were placed in petri dishes and kept illuminated continously at +29°C for 3 months. The Abscisic acid (ABA) analyses were performed in anthers taken from the buds that were seperated from the plant (control) and in their cold treated samples at +4°C for 48 and 96 hours and in either their samples that incubated 50 days.

The ABA was analyzed as methyl ester (ABA-Me) with the method used by Andersson *et al.* (1978). A sample of 1 gr anther was homogenized in 50 ml cold methanol (redistilled) at -18 °C for 4 h. The extract was filtered, evaporated to 2 ml and chromatographed on a combined Celite-PVP-Sephadex LH 20 column. As eluent 0.05 M phosphate buffer (pH 3.0) was used. The column (280x11 mm internal diameter) consisted of an upper layer of 110 mm Celite, a middle layer of 25 mm PVP and a lower layer of 240 mm Sephadex LH 20. The fraction 50-70 ml was collected, extracted with 2x20 ml diethyl ether and evaporated to a small volume. Preperation of diazomethane and methylation of extract were done according to Topçuoğlu (1987). After methylation, extract was evaporated to dryness and then dissolved in 100 µl ethyl acetate. The identification of ABA was made in this sample with combined gas chromotography-mass spectrometry. A complete mass spectrum was obtained and shown to be identical with that of a methylated Standard (±) cis-trans- ABA (Sigma). The quantification of ABA was made on a Chrompack model 438 A, gas chromotography equipped with a 1% OV 17 column (1.5 m) and an electron capture detector (ECD) (CP-SIL-5CB). For this purpose the sample was diluted 100 times. The operating conditions were: oven beginning temperature, 85 °C, final, 200° C, dedector temperature, 230 °C, injector temperature, 200° C; carrier gas, nitrogen at a flow-rate of 40 ml/min.

Results and Discussion

The effect of cold shock treatments on the ABA contents of anthers as well as the relationship between the endogenous ABA contents of anthers and pollen embryogenesis were investigated. The ABA amounts in the anthers of buds of *C. annuum* L. that had been cold treated at +4°C for 48 and 96 hours and the control anthers were chemically analyzed. These analyses showed that the control anthers (without cold treatment) contained 2.90 ng g^{-1} ABA at the beginning of the experiment. The ABA amounts of the anthers cold treated at +4°C for 48 and 96 hours were 2.58 and 2.04 ng g^{-1} respectively.

The ABA amounts in the anthers were also analyzed at the 50th day of the culture. The ABA contents of the anthers were reduced to 2.56 ng g^{-1} in the control group and to 2.20 and 1.81 ng g^{-1} in the cold treated anthers for 48 and 96 hours, respectively, as shown in Table 1. Cold treatments reduced the ABA contents of the anthers in both sample times for the analyses; at the beginning of the culture and at the 50th day of the culture. The differences between the 48 and 96 hours treatments were not statistically significant. The decreases in the ABA values in the cold treatments compared with the control values, both at the beginning of the culture and at the 50th day of the culture, were statistically insignificant for the cold treatments at +4°C for 48 hours, but were statistically significant for the cold treatments at

+4°C for 96 hours. When compared to ABA values of the sample times, at the 50th day of the culture were determined lower (Table 1).

Table 1. The ABA contents (ng g^{-1} fresh weight) of anthers samples of *C. annuum* L. cv. Malatya taken at different stages period of cold treatment.

Time of samples taken	Control	48 hours	96 hours
Beginningof the culture	a*2.90 ± 0.18 A**	a 2.58 ± 0.06 AB	a 2.04 ± 0.19 B
50th day of the culture	a 2.56 ± 0.30 A	a 2.20 ± 0.04 AB	a 1.81 ± 0.28 B

* The same letters in each column do not differ significantly at the 1 % level ,
** The same letters in each row do not differ significantly at the 1 % level according to Duncan test results

It has been known that the number of embryos produced by anther cultures could be increased if the pollen exposed to some kind of shock treatment before cultivation. The most common method is a cold treatment of the buds before the anters are placed in the medium (Sunderland and Dunwell, 1977; Sunderland and Roberts, 1979, Morrison *et al.* 1986). The mechanism of this effect has not yet been clarified. Our results showed that in general the cold treatments reduce the ABA content of the anthers. These results display a similarity with the other results which determined the cold treatment has decrease effect on the ABA contents of the anthers (Johansson *et al.*, 1982, Tıpırdamaz and Ellialtıoğlu, 1998).

In our study the lower ABA amounts at the 50th day of the culture are probably due to the loss of vigor in the anthers and inhibition of the ABA as well as the other metabolites (Johansson *et al.*, 1982).

On the other hand a considerably higher number of embryos (12.5%) were produced in the anthers cultivated in the control medium. On the other hand, 1-2 % embryos were obtained from the anthers which treated cold. In spite of the effects of cold shock treatment, a number of pollen grains are forced to divert from the normal path of development and start developing embryos (Bajaj *et al.* 1977). In our study the cold pretreatments decreased the embryo formation and creates a negative effect in pepper anther cultures. We were able to obtain embryos mostly from the control groups. This results are similar to the result of study of Tokuma et el. (1994). It is thought that the existence of ABA in the control anthers is not at a level which would prevent embryogenesis. It has been suggested in the same manner by Johansson *et al.* (1982), Tıpırdamaz and Ellialtıoğlu (1998) that the preventive effect of ABA on pollen embryogenesis only appears when the ABA is above a specific level of concentration.

In conclusion, in our study cold pretreatments of buds decreased ABA content in anthers but had no possitive effect on embryo formation in pepper anther culture of Malatya cultivar under the used experimental conditions. This results showed that there are other factors apart from the ABA which are influencing embryogenesis in pepper anther cultures.

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EVALUATION OF GENOTOXIC POTENTIAL OF MERİÇ DELTA BY USING *UMU* TEST SYSTEM

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Abstract

SOS inducing activity in samples obtained from the Meriç delta was evaluated with the *umu* test system using *Salmonella typhimurium* TA1535/ pSK1002, *S. typhimurium* NM1011 (overexpressed nitroreductase) and *S. typhimurium* NM2009 (overexpressed O-acetyltransferase) strains. The Meriç delta is highly polluted especially in the spring and summer at Ergene origin in Turkey and at Enez at the downstream locations. However, results also confirm that the pollutants are not derived from nitroarenes but from other organic compounds. In view of these results, a high increase in pollution in the spring and the summer in the Meriç delta is caused by the active industrial and agricultural wastes from Turkey and Bulgaria which should be seriously considered.

Keywords: Genotoxicity, *umu* test, nitroarenes, surface water.

Introduction

The widespread occurrence of mutagenic and carcinogenic substances in river water is a serious problem. Early warning systems of environmental hazards have become

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increasingly important in recent years. There have been many studies on the genotoxicity of surface water in various countries (1,2). A number of short-term bacterial systems have been introduced for determining the genotoxic potential of aquatic ecosystems. In recent years, the umu test system has been used for monitoring the genotoxicity of waste water in some of the countries (3,4,5).

Nitroarenes are widespread in the environment and some of them are reported to be potent mutagens and carcinogens (6,7). As nitroarenes are important risk factors for humans, the study concerning their genotoxicity is of great importance.

Early studies on the Meriç delta revealed that the water source of the delta is highly affected by the industrial and agricultural pollutants from Turkey and Bulgaria (8,9,10). As the Meriç delta is the only water source used for irrigation in the region, we aimed to screen the genotoxic potential of the delta.The genotoxicity of the organic concentrate from water samples was tested with the *umu* test by using *S. typhimurium* TA1535/pSK1002, NM1011 and NM2009 strains.

Materials and methods

Chemicals, tester strains and rat liver S9 supernatant

The chemicals used were obtained from the following sources: Glucose-6-phosphate, NADP, O-nitrophenyl-β-D-galactopyranoside (ONPG), sodium dodecyl sulfate (SDS), 4-nitroquinoline-N-oxide (4-NQO) (CAS No: 55-57-5; purity: >97%) from Sigma (USA); ampicillin and chloramphenicol from Fluka; dimethyl sulfoxide (DMSO) (CAS No: 67-71-0; purity: 99%), chloroform, β-mercaptoethanol, methanol (CAS No: 67-56-1; purity: ≥ 99,5%), acetone (CAS No: 67-64-1; purity: ≥99%) and dichloromethane (CAS No: 75-09-2; purity: ≥ 99,9%) from Merck; benzene (CAS No:71-43-2; purity: 99%), diethyl ether (CAS No: 60-29-7; purity: ≥95%) from BDH; Tris (hydroxymethyl) aminomethane and 3-methylcholanthrene (3-MCT) (CAS No: 56-49-5) from ICN (USA). Benzene was chosen as a solvent for 3-MCT. 4-NQO was used as a positive control for the parental strain without S9, NM1011 and NM2009 strains. 3-MCT (11) was used as a positive control for the parental strain with S9 because of restrictions on Aflatoxin B_1 import.

Salmonella typhimurium TA1535/pSK1002, NM1011 and NM2009 strains were kindly provided by Dr. Yoshimitsu Oda (Osaka Prefectural Institute of Public Health, Osaka, Japan). The tester strains used in the *umu* test are constructed by introducing a vector plasmid pACYC184 carrying only nitroreductase (NR) gene (NM1011), only O-acetyltransferase (O-AT) gene (NM2009) into the parental strain *S.typhimurium* TA1535/pSK1002 harboring *umuC*'-'*lacZ* fusion gene (12,13). The cultures were stored at –70 °C and the genetic markers of the strains were checked before mutagenicity performance tests (14).

The S9 (microsomal) fraction used in the *umu* test was prepared from livers of male Sprague-Dawley rats pretreated with 3-methylcholanthrene and phenobarbital according to Ames et al. (14).

Sample collection and preparation

Three sampling stations were chosen according to their pollution levels: Meriç (origin in Bulgaria; unpolluted), Ergene (origin in Turkey; exposed to polluting sources) and Enez (after the crossing point of Meriç and Ergene rivers) in the Meriç delta (Figure 1).

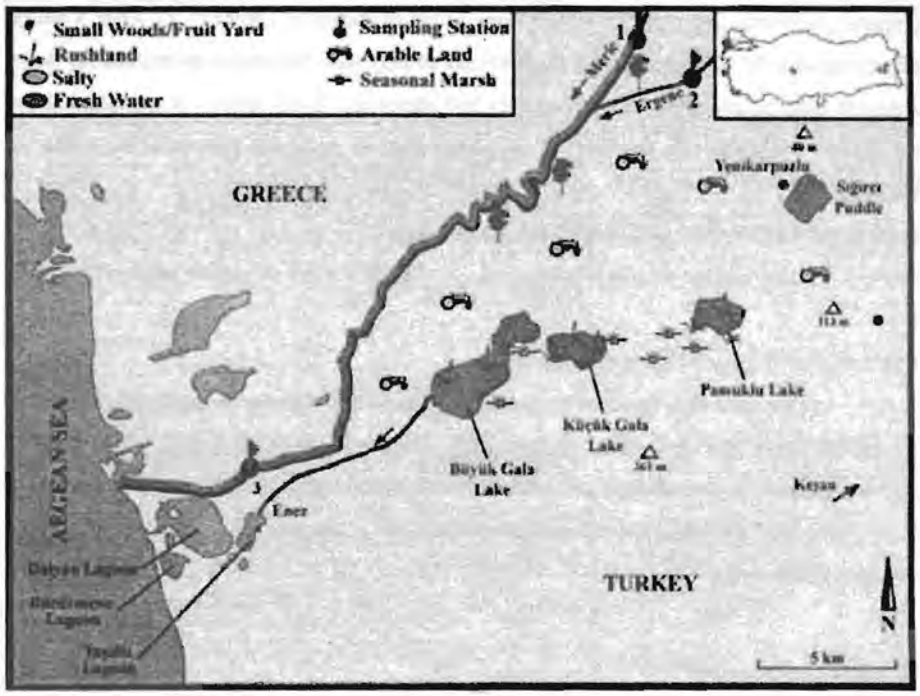

Figure 1. The locations of the sampling stations. Water samples were collected at Meriç (1), Ergene (2) and Enez (3).

The samples were taken from the sampling stations for each seasonally and throughout the year. The extraction of dissolved organics was performed by adsorption on XAD-2 and XAD-4 resins using 5L of water (15,16). Half of each sample taken from each station was run through a XAD-2 column after adjusting the pH to 10 and the other half was run through an XAD-4 column without pH adjustment (pH ≅ 7) at a flow rate of 10 mL/min according to Sayato et al (17). After removing residual water in the column by blowing nitrogen gas, the organic pollutants in the column were consecutively eluted by using 100 mL each of dichloromethane, acetone, diethyl ether and methanol. Each fraction collected was evaporated to dryness in a water bath. Subsequently, all the residues were dissolved in 3 mL DMSO and the maintained concentration factor was observed to be 1600 which was kept

constant throughout the study. 150 μL of this sample extract was used for each assay in the mutagenicity test.

Mutagenicity assay

The *umu* test was performed as recommended by Oda et al. (18). Overnight cultures of bacterial strains were prepared in Luria broth medium. The overnight culture was diluted 50-fold with TGA medium and was incubated at 37 °C until the absorbance at 600 nm of the bacterial culture reached 0.25-0.30. The bacterial culture obtained, was divided into 2 mL fractions, nine in total. Over the prepared nine fractions, 150 μL of the sample concentrate prepared previously and other chemicals were added as shown in Tables (1,2). After 5 hours of incubation at 37 °C with shaking, the bacterial density was determined by measuring A_{600}. After the preincubation with organic extract and chemicals, β-gal activity was measured by the method of Miller (19). The level of enzyme activity in units was calculated according to the equation;

β-gal activity (U) = 1000 (A_{420} – 1.75 x A_{550}) / t.v.A_{600}

where t is the reaction time in minutes and v is the volume of culture used in the assay (mL).

Evaluation of β-galactosidase expression

In the *umu* test, two-fold or greater than two-fold increase in β-gal activity above the control level was defined as positive (11). Nakamura et al. (20) have classified the chemicals as potent, intermediate and weak inducers in accordance with their ability to induce β-gal expression. The three classes increase *umuC* gene expression by about six-, three- and two-fold, respectively.

Results and Discussion

Tables 1 and 2 show the effect of sample extracts of spring, summer, autumn and winter respectively, on the induction of the *umuC* gene in the SOS response in *S.typhimurium* TA1535/pSK1002 both in the presence and absence of the S9 fraction and in NM1011 and NM2009 strains without S9. These strains do not require metabolic activation by liver microsomal enzymes (18).

The mutagenic and genotoxic effects of several nitroarenes arise following the reduction of nitro groups in bacterial or mammalian cell systems where they are tested (21,22). It has been suggested that NR and O-AT catalyze key steps in the mutagen activation of nitroarenes and the strains which overexpress NR and/or O-AT might be particularly sensitive to the genotoxic activities of these chemicals (23,24).

In the present study, the spring sample extract taken from Ergene station caused an increase in β-gal activity in the parental strain without S9 and in NM1011 and in NM2009 strains (Table1), but the increase was not high enough to consider the sample as a weak

Table 1.

The induction of *umuC* gene in *S. typhimurium* TA1535/ pSK1002, NM 1011 and NM 2009 strains by the spring and summer sample extracts [a].

Seasons	Chemicals	Concentration		TA 1535/ pSK 1002		NM 1011	NM 2009
		Amount added/assay		S9 (+)	S9 (-)		
		(μL)	(μmol/L)	β-gal (units)	β-gal (units)	β -gal (units)	β -gal (units)
Spring	Control (DMSO)	150		10 ± 1	14 ± 1	141± 9	145 ± 12
	Control (benzene)	10		11 ± 1	-	-	-
	4-NQO		26.2	-	60 ± 5	1041 ± 54	1632 ± 49
			39.4	-	66 ± 5	1067 ± 69	1000 ± 37
	3-MCT		5.6	8 ± 1	-	-	-
			11.2	18 ± 1	-	-	-
	Meriç extract [b]	150		7 ± 1	5 ± 1	52 ± 10	80 ± 15
	Ergene extract [b]	150		9 ± 2	24 ± 2	211 ± 25	282 ± 22
	Enez extract [b]	150		10 ± 1	11 ± 2	67 ± 6	109 ± 8
Summer	Control (DMSO)	150		29 ± 2	36 ± 2	252 ± 8	143 ± 4
	Control (benzene)	10		20 ± 4	-	-	-
	4-NQO		26.2	-	265 ± 60	3139 ± 308	1688 ± 272
			39.4	-	419 ± 91	2799 ± 188	1315 ± 166
	3-MCT		5.6	22 ± 3	-	-	-
			11.2	29 ± 5	-	-	-
	Meriç extract [b]	150		22 ± 3	24 ± 2	73 ± 6	100 ± 15
	Ergene extract [b]	150		40 ± 3	43 ± 4	178 ± 5	216 ± 7
	Enez extract [b]	150		37 ± 5	81 ± 6[c]	429 ± 12	226 ± 28

[a] Each value is the mean ± S.D. of four replicates from each of 2 separate experiments.

[b] Concentration factor is 1600.

[c] Weak mutagen.

Table 2.

The induction of *umuC* gene in *S. typhimurium* TA1535/ pSK1002, NM 1011 and NM 2009 strains by the autumn and winter sample extracts [a].

Seasons	Chemicals	Concentration		TA 1535/ pSK 1002		NM 1011	NM 2009
		Amount added/assay		S9 (+) β-gal	S9 (-) β-gal	β -gal	β -gal
		(μL)	(μmol/L)	(units)	(units)	(units)	(units)
Autumn	Control (DMSO)	150		31 ± 1	39 ± 2	240 ± 25	209 ± 10
	Control (benzene)	10		20 ± 1	-	-	-
	4-NQO		26.2	-	626 ± 21	2090 ± 130	1522 ± 233
			39.4	-	850 ± 78	1933 ± 128	811 ± 91
	3-MCT		5.6	22 ± 1	-	-	-
			11.2	23 ± 2	-	-	-
	Meriç extract [b]	150		27 ± 1	22 ± 1	136 ± 6	109 ± 6
	Ergene extract [b]	150		30 ±1	23 ± 1	131 ± 3	143 ± 6
	Enez extract [b]	150		33 ± 2	38 ± 1	215 ± 14	193 ± 4
Winter	Control (DMSO)	150		85 ± 8	109 ± 7	204 ± 5	183 ± 14
	Control (benzene)	10		63 ± 3	-	-	-
	4-NQO		26.2	-	1251±194	2350 ± 222	3286 ± 228
			39.4	-	1661±171	1694 ± 80	2283 ± 93
	3-MCT		5.6	78 ± 19	-	-	-
			11.2	60 ± 8	-	-	-
	Meriç extract [b]	150		84 ± 4	147 ± 27	189 ± 19	208 ± 11
	Ergene extract [b]	150		81 ± 6	128 ± 17	199 ± 15	236 ± 12
	Enez extract [b]	150		80 ± 6	141 ± 10	210 ± 4	191 ± 12

[a] Each value is the mean ± S.D. of four replicates from each of 2 separate experiments.

[b] Concentration factor is 1600.

inducer according to Nakamura et al (20). There was no induction of *umuC* gene expression in the parental strain with S9. The organic extracts obtained from the stations of Meriç and Enez did not cause any induction in any of the strains tested. Instead, the extract caused a decrease in β-gal activity compared to the control.

The organic pollutants in summer sample taken from Enez station at the downstream location induced *umuC* gene expression in all of the strains. However the induction in the parental strain (without S9) was high enough to define the extract as a weak inducer (Table 1). The induction of *umuC* gene expression was found to be almost the same for both parental and the nitroarene- sensitive strains. The high NR avtivity in NM1011 and high O-AT activity in NM2009 did not significantly increase the genotoxic activation of the organic extract compared with the parental strain, suggesting that the pollution in Enez station is not derived from nitroarenes but is due to the presence of other organic compounds. Previous studies show that several nitro chemicals which are not genotoxic in NR and O-AT deficient *S. typhimurium* strains have been found to be genotoxic in strains which overexpress NR and O-AT (18). The organic extracts obtained from the stations of Meriç and Ergene caused no induction in any of the strains tested; instead they caused a decrease in β-gal activity compared with the control.

None of the extracts obtained from the sampling stations in autumn and winter induced *umuC* gene expression in any of the strains tested (Table 2).

While the organic extracts obtained from the Meriç station in the spring, summer and autumn caused a significant decrease of the β-gal activity in all the tested strains (Table 1,2), the sample from Ergene station showed decrease both during the summer (Table 1) and autumn (Table 2), the sample from Enez only during the spring (Table 1). The response in the *umu* test and the bacterial growth can be used to assess both genotoxic and toxic properties of compounds, enabling the detection of promutagenic and mutagenic compounds found at low concentration in water samples. 4NQO which is used as a positive control in the test system shows an induction of β-gal activity in the parental strain without S9. While in the parental strain there is a dose dependent induction of β-gal activity, in the strains with high NR, O-AT or NR plus O-AT, 26,2 μmol/L 4NQO causes high induction but 39,4 μmol/L 4NQO causes much lower induction (Table1,2), suggesting that the high levels of NR and O-AT activities in these strains increase their sensitivity towards 4NQO application (25).

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THE ANTIMICROBIAL EFFECT OF GARLIC (*Allium sativum* L.)

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Abstract

The *in vitro* antimicrobial effect of garlic which was harvested in Turkey was examined. To evaluate whether garlic extract possessed antimicrobial activities, two analytical methods; 1) the agar-well diffusion technique 2) minimal inhibitory concentration (MIC) and minimum lethal concentration (MLC) were used. *Escherichia coli, Bacillus anthracis, Pseudomonas aeruginosa and Candida albicans* were used as clinical isolates. The data suggest that garlic has potentially useful agent against Gram-positive and Gram-negative bacteria and yeast like *C. albicans*.

Key Words: Garlic, *Allium sativum* (L.), antimicrobial effect, Allicin

Introduction

Thousands of plants worldwide are used in traditional medicine for bacterial infections (1). Conventional drugs usually provide effective antibiotic therapy for bacterial infections but there is an increasing problem of antibiotic resistance and a continuing need for new solutions.

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Although natural products are not necessarily safer than synthetic antibiotics, some patients prefer to use herbal medicines. Thus healthcare professionals should be aware of the available evidence for herbal antibiotics (2).

Garlic has been used as a spice, food and traditional medicine for over 4000 years (3). Garlic have been reported to reduce blood lipids and decreased risk of gastric cancer by an effect on *Helicobacter pylori* which is associated with gastric cancer (4, 5, 6), and to have antibacterial (5, 7, 8), anticancer (5, 9, 10), antiprotozoal, antifungal and antiviral (11), antioxidant (12), anti-flawmatory, immunomodulatory, hypoglycemic and hormone-like effects (8), hypocholesteremic effects (3) and modulate cardiovascular system because of its ability to lower serum cholesterol and immune system (11), and have protective effects against stroke, coronary thrombosis, artherosclerosis, platelet aggregation and vascular disorders (3).

Allicin is the main antimicrobial component in garlic (2, 13). Alliin was found to be the stable precursor that is converted to allicin by the action of an enzyme termed alliinase, or alliin-lyase (E.C.4.4.1.4.), a pyridoxal 5-phosphate-dependent glycoprotein (Figure 1) (11, 14). During crushing or cutting of the garlic clove, the enzyme alliinase which is present in usually high amounts in garlic cloves to yield allicin and other thiosulfinates that are the source of the characteristic odor of garlic (8, 14). The amounts of alliin and allicin present in different strains of garlic were studied by numerous investigators. The various amount of alliin and allicin was detected ranging from 2.8 to 7.7 mg/gram found in Romanian red (15).

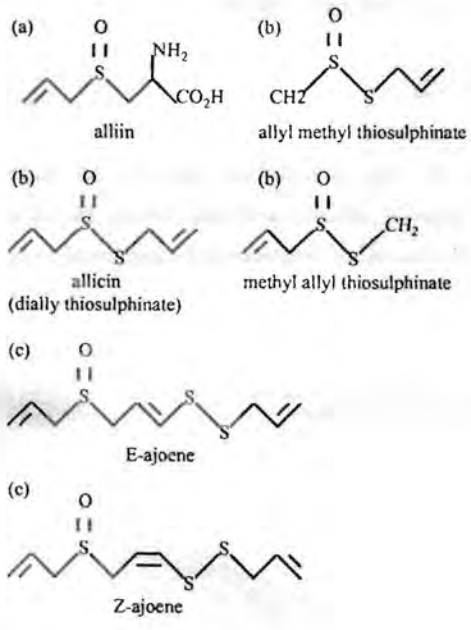

Figure 1. Chemical structure of compounds found in (a) garlic cloves, (b) garlic clove homogenates, or (c) oil-macerates of garlic cloves (13).

This study reported here investigated the antibacterial and anticandidal effect of garlic which was harvested in Turkey. The following aspects were evaluated: 1-) The antibacterial activity of aqueous garlic extract against four different clinical isolates of medicinally important organisms. 2-) The Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of aqueous garlic extract.

Material and Methods

Preparation of garlic extract;

Garlic bulbs were purchased directly from local grocery stores in Turkey. Garlic was peeled and mashed in a blender. Fifty grams of the garlic pulp was agitated with 100 ml of sterile distilled water for 60 minutes. The paste so obtained was refrigerated for two hours and then squeezed through gauze to remove the larger tissue particles, was collected and centrifuged for 15 minutes in 4500 rpm at 25°C. The supernatant was decanted and filtered with Whatman filter paper (16).

Microorganisms and Medium;

All tested microorganisms were obtained from Bacteriology laboratory of Ankara Numune Hospital. *Escherichia coli, Bacillus anthracis, Pseudomonas aeruginosa* and *Candida albicans* were used as a clinical isolates for testing the antimicrobial activity of garlic extract.

Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) were used as the growth media. TSB was used for the determination of MIC and MLC values. TSA was used for the diffusion assays (17). Sabouraud's broth and Sabouraud dextrose agar were used to determine MIC and MLC values of *C. albicans* (13).

Determination of antimicrobial activity of garlic

Agar diffusion method

An agar-well diffusion technique was used to determine the antimicrobial activity of garlic extract. Agar plates were prepared from 20 ml of seeded TSA medium to which cooled to 45°C before 1ml inoculation of cultured organisms ($1x10^6$ cell/ml). When the seeded agar solidified, six evenly spaced, 8 mm diameter wells were cut in a circular fashion into each plate. Each of these wells was filled with 0.2 ml of different dilutions of garlic extracts (17, 18). One well of each plates was charged with 1:1 dilution (undiluted garlic extract) and one well was left empty, as a control.

Plates were incubated overnight at 37°C. The clear zones of inhibition were measured using a ruler under a magnifying lens (17).

Determination of MICs and MLCs

The basic analytical system to evaluate the potential of nutraceuticals to affect the antibiotics of antimicrobial susceptibility of the indicator microorganisms was the MIC clinical method. The *in vitro* minimal inhibitory concentrations (MICs) and minimum lethal concentration (MLCs) of the aqueous garlic extract against different microorganisms were determined by using dilution method (6, 17, 19).

The extract was diluted in Tryptic Soy Broth in two fold dilutions series ranging from 1:2 to 1:256. 0.2 ml of each cultures ($0.2x10^6$ organisms/ml) was added to 10 ml liquid medium. The mixture was spread over the agar surface and after 24 hours at 37°C, the cultures were examined for growth (20). The lethal concentration was defined as the lowest concentration of garlic that achieved 99.9% kill when compared to counts of the original inoculum (0.2 ml/$1x10^6$ organisms) (16, 19).

All values were determined in dublicate and repeated twice.

The pH value of garlic extract was measured at 25°C with Orion pH meter.

Results

To evaluate whether garlic extract possessed antimicrobial activities, two analytical methods were used: 1. The agar-well diffusion technique 2. MIC and MLC clinical methods.

Table 1 shows the zones of inhibition responses for a representative of the different concentrations of garlic extract. The arbitrary values, as a percentage of the garlic extract dosage, were a function of the extract concentration: 1/1, ½, ¼, 1/8, 1/16, 1/32 and 1/64 respectively. Measured zone diameters ranged from no inhibition zones for *P. aeruginosa* to 31 mm for *C. albicans* at undiluted 1/1 concentration of garlic extract. No inhibition zones were observed for the clinical isolates of *P. aeruginosa* at any concentration.

The pH of garlic extract was 6.2 (Orion pH meter, model 230 A).

Table 1. The zones of inhibition responses for a representative of the different concentrations of garlic extract at agar-well diffusion method.

	undiluted	**1/2**	**1/4**	**1/8**	**1/16**	**1/32**	**1/64**
Escherichia coli	20	17	15	13	8	2	-
Bacillus anthracis	27	16	12	12	10	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Candida albicans	31	27	25	19	16	13	5

The Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of aqueous garlic extract for all the microbial cultures are presented in Table 2. *C. albicans* was particularly sensitive to the effects of the garlic extract (MIC=1/32, MLC=1/16). However, clinical isolate of *P. aeruginosa* was strongly resistant to the garlic extract as seen at agar-well diffusion experiments above and only undiluted concentration was effective (MIC and MLC=1/1).

Table 2. The Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) of aqueous garlic extract.

	MIC dilution	MLC dilution
Escherichia coli	1/4	1/2
Bacillus anthracis	1/32	1/8
Pseudomonas aeruginosa	undiluted	undiluted
Candida albicans	1/32	1/16

As compared the other isolates, *B. anthracis* isolate was found as a second sensitive organism to the aqueous garlic extract (MIC=1/32, MLC=1/8). MIC and MLC values for *E. coli* were determined 1/4 and 1/2, respectively.

Discussion

Garlic was found to have antimicrobial effect on clinical isolates of different microorganisms. Four different genera of microorganisms are predominantly responsible for inflammation in patients with superficial wounds.

P. aeruginosa is well known by its strong resistance against many diverse classes of antibiotics (21). It is therefore no wonder that the garlic extracts used in experiments had no inhibitory effects on *P. aeruginosa*. No inhibition zone was observed at agar-well diffusion experiment and only undiluted garlic extraction was determined as MIC and MLC values for *P. aeruginosa*. These results agreed with previous studies made by Ankri and Mirelman (14). The reasons for this resistance are unclear. But, Ankri and Mirelman proposed that hydrophilic capsular or mucoid layers prevent the penetration of the allicin into the *P. aeruginosa*.

The most important point in this work was that the garlic extracts proved to be highly effective in inhibition to *C. albicans* and *B. anthracis* which causes the diseases. These effects were probably due to some inhibitor molecule(s) in garlic.

Considerable amounts of effect was detected when the organism tested was *E. coli*. At agar-well diffusion experiment, *E. coli* was found fairly sensitive to the garlic extract (Table 1). However, the MIC and MLC values for *E. coli* were not as good as *C. albicans* and *B. anthracis*.

It was showed by Ozolin et al., (22), Ankri and Mirelman (14) and Feldberg et al., (23) that *E. coli* RNA polymerase, in its alpha-subunit, contains a single sulfhydryl group which was shown to react with the monomercuric derivative of fluorescein, a specific reagent for thiol groups and RNA polymerase could also be a target for allicin. Hughes and Lawson (13) observed that Allicin was less active than gentamycin against *E. coli*. Sasaki et al., (20) found that 1 % garlic powder solution had a strong anti *E. coli* 0-157 activity and anti 0-157 substances in garlic resisted to heat treatment.

C. albicans, the most common pathogenic yeast, is usually resistant to most antibacterial and antifungal drugs (11, 16). The maximum zone of inhibition (31mm) and the highest dilution factor for MIC and MLC (1/32 and 1/16 respectively) were found for clinical isolates of *C. albicans* in our study.

Yoshida et al., (24) and Hughes and Lawson, (13) presented that E-and Z-ajoene were found active against *C. albicans*, but were not as active as the dialkyl thiosulphinates. The mode of action of allicin on the fungal cells has not yet been elucidated but it is assumed that the inhibition of certain thiol-containing enzymes in the microorganism by the rapid reaction of thiosulfinates with thiol groups was the main mechanism involved in the antibiotic effect by Rabinkov et al. (12). Ankri and Mirelman, (14), Sivam, (8) and Hughes and Lawson (13) suggested that Allicin was more active than the methyl allyl and allyl methyl thiosulphinates for *C. albicans*. They found that Allicin was similar in activity to nystatin against *C. albicans*. Ghannoum (25) found that the presence of garlic affected the structure an integrity of the outer surface of the membrane of *C. albicans*. The fungi were more sensitive to allicin, methyl allyl and allyl methyl thiosulphinates, and garlic than were *E. coli* and *S. aureus* (13). Our findings are agree with previous studies on the inhibition of *C. albicans* made by Rode et al. (16), Yamada and Azuma (26) and Ankri and Mirelman (14).

Nisin and garlic act synergistically against *Listeria monocytogenes* (1). Gupta and Viswanathan (27) found a synergistic effect of allicin with Streptomycin or Chloromphenicol against *Mycobacterium tuberculosis*. On the other hand, no synergitic or antagonistic effect was observed between garlic and amoxicillin, clarithromycin or methionidazole on *H. pylori*.

But a synergitic effect was found for the combination of garlic and Omeprazole by Jankers et al. (5).

The structural differences of the bacterial strains may also play a role in the bacterial susceptibility to garlic constituents. Ghannoum (25) and Sivam (8) suggested that lipid content of the membranes would have an effect on the permeability of allicin and other garlic constituents.

These *in vitro* experiments suggest the use of an aqueous extract of garlic as a potentially useful agent against Gram-positive and Gram-negative bacteria and yeast-like *C. albicans*.

In conclusion, garlic possessed in vitro antimicrobial activity against different clinical isolates. More research must be done to assess the value of garlic as an alternative to chemical food preservatives, especially in foods in which the garlic flavor would be an added bonus. There is also potential usage of garlic with some antibiotics to have additive and synergitic effect.

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PCR BASED RFLP ANALYSIS OF AN INTERGENIC SPACER REGION IN cpDNA OF SOME *AEGILOPS* AND *TRITICUM* SPECIES

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Received 17.08.2004

Abstract

Restriction fragment length polymorphism analysis of some *Aegilops* and *Triticum* species were performed in this study. An intergenic spacer region of chloroplast DNA (cpDNA) was digested by five different restriction endonuclease enzymes. Obtained fragments were investigated on agarose gel electrophoresis technique. Phylogenetic relationships among *Triticum* and *Aegilops* species were discussed, based on the resultant data.

Key Words: cpDNA, phylogenetic relationship, RFLP, wild wheats (*Triticum* and *Aegilops*)

Introduction

Economically, the grass family is the most important family of flowering plants including cereal grains as well as many forage crops (1). Wheat makes up the genus *Triticum* of the grass family *Gramineae* (2). Wheat has been cultivated for food since prehistoric times. The wheats known today are cereals that evolved in the Middle East through repeated hybridizations of *Triticum* spp. with members of a closely related grass genus, *Aegilops*. This evaluation was accelerated by an expanding geographical range of cultivation and by human selection, and had produced bread wheats as early as the sixth millennium BC (3). Because of

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the economical importance of this crop the relationships among species in the tribe have been extensively investigated.

The chloroplast genome has been a major focus in studying plant evolution and plant genetics (4; 5; 6; 7; 8; 9). Conservation of gene content and relatively slow rate of nucleotide substitution in protein-coding genes has made the chloroplast genome ideal for studying plant evolutionary history (10). Despite its conservative nature, as revealed by genome size, restriction fragment analysis and nucleotide sequencing, a number of mutations in the chloroplast genome have been observed. These include inversions (11; 12), rearrangements of gene order (13) and insertions/deletions (14), as well as base substitutions.

Restriction fragment analysis of chloroplast DNAs has become a powerful new means for studying phylogenetic relationships among related plant species (15; 16; 17). Most studies of chloroplast, however, have concentrated on coding regions. As a result, great deal is known about evolution of chloroplast genes and genome structure but little about the evolution of noncoding sequences of the chloroplast genome (18).

In this study an intergenic region of seven *Triticum* and *Aegilops* species were investigated. This is a hypervcraible region and these species harbor different types of length mutations at this position. The purpose of the investigation was to estimate the evolutionary divergence between these species.

Materials and Methods

Aegilops and *Triticum* species (*Ae. tauschii*, *Ae. speltoides* var. *ligustica*, *T. monococcum* var. *boeoticum*, *T. urartu*, *T. dicoccoides*, *T. turgidum* var. *dicoccon*, *T. aestivum*) used in this study were obtained from a field study at Southeastern Anatolia region and from International Center For Agricultural Research In The Dry Areas (ICARDA). Total DNA was isolated following the procedure of Doyle and Doyle (19) from fresh leaf materials that were frozen by liquid nitrogen. The collected DNA was used directly for PCR amplification. Amplification reaction was carried out with primer SU-1 (TCGTGCCTTTAGTAGGCTTAG), located at 3254. position of *psaI* gene, and primer SU-2 (TCGGATTGCCTGGTATTCCAC) located at 4807. position of ORF 230. Each PCR was performed in a 100μL reaction mixture containing 1μg of total DNA extract, 10μL of 10XPCR buffer (100mM Tris-HCl, pH 8.8, 500mM KCl, %0.8 Nanidet P40) supplied with the enzyme, 50μM each of dNTPs, 2mM $MgCl_2$, 100 pmol of each primer and 2.5 U of Taq DNA Polymerase (MBI, Fermentas). The reaction volume was completed to 100μL by PCR grade water. Thermal cycling was carried out in a Biometra thermal cycler. The reaction was 30 cycles of 1 min at 94°C, 1 min at 60°C, and 2 min 30 s at 72°C and a final elongation cycle of 7 min at 72°C. PCR products were fractionated in a 1 % agarose gel and samples were stored at -20°C. The amplification products were digested with *EcoR* I, *Bcl* I, *Hsp92* I, *Taq* I

and *EcoR* V enzymes. Digestion reactions were performed in 20μL reaction mix containing 5μg of amplification product, 2μL of 10Xrestriction buffer, 5 U of restriction enzyme and water. Resultant fragments were separated in 1.5 % agarose gels. Gels, which were screened by SYNGENE, Gene Genius image analyzer system, were analyzed by Cross Checker Fingerprint analysis software v2.9 (20). By resultant data, phenograms were plotted by NTSYSpc V2.10 (21).

Results and Discussion

The region of chloroplast genome bounded by *psaI* gene and ORF 230 was amplified by PCR reaction. Results were given in Fig. 1.

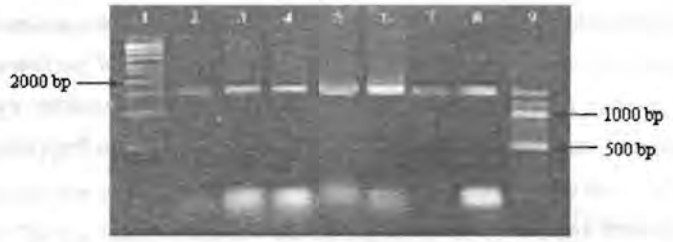

Figure 1. The PCR results. (1) 1 kbp DNA Ladder (2) *T. monococcum* var. *boeticum* (3) *T. urartu* (4) *T. dicoccoides* (5) *T. turgidum* var. *dicoccon* (6) *Ae. tauschii* (7) *Ae. speltoides* var. *ligustica* (8) *T. aestivum* (9) 100 bp DNA Ladder.

PCR products were digested by five restriction endonucleases. In all experiments *T. aestivum* was used as a control group, because, in a previous study, this region's sequence analysis was performed by Ogihara et al. (22). First enzyme used for digestion was *EcoR* I. Only one band (E1) was generated at the end of digestion (Fig. 2). There is only one recognition site of *EcoR* I present for this region in all species and the resultant fragments were almost same sized so that they could not be screened separately by agarose gel electrophoresis. This finding correlates with the results of a previous study performed by Ogihara et al. (22).

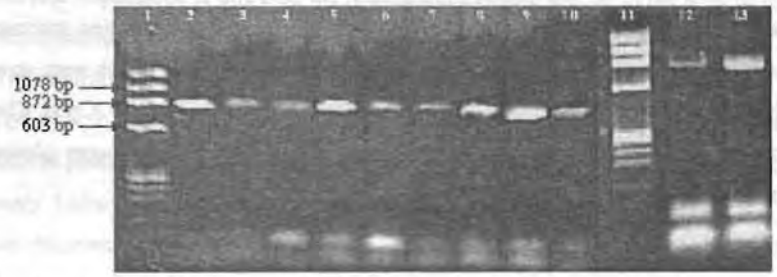

Figure 2. *EcoR* I digestion. (1) ØX 174/*Hae*III (2, 3) *T. monococcum* var. *boeoticum* (4, 5) *T. urartu* (6, 7) *Ae. tauschii* (8, 9) *T. turgidum* var. *dicoccon* (10) *Ae. speltoides* var. *ligustica* (11) ØX 174/*Hae*III (12) *T. dicoccoides* (13) *T. aestivum*.

Second enzyme used for digestion was *Bcl* I. Three bands were generated by this digestion (B1-B3) There is no recognation site of *Bcl* I for this region in *T. aestivum* as shown by Ogihara *et al.* (22). There is no recognition site for this region in all other species (B1) except *T. turgidum*. *T. turgidum* has one recognition site and showed two fragments (B2, B3) (Fig. 3).

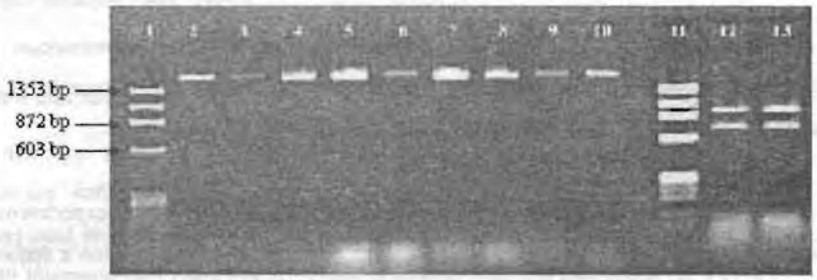

Figure 3. *Bcl* I digestion. (1) ØX174/*Hae*III (2, 3) *T. monococcum* var. *boeoticum* (4, 5) *T. urartu* (6) *T. aestivum* (7) *T. dicoccoides* (8, 9) *Ae. tauschii* (10) *Ae. speltoides* var. *ligustica* (11) ØX174/*Hae*III (12, 13) *T. turgidum* var. *dicoccon*.

Third enzyme was *Hsp92* I. Five bands (H1-H5) were generated by digestion (Fig. 4). There is only one recognation site of *Hsp92* I for this region in *T. aestivum* as shown by Ogihara *et al.* (22) and showed two bands (H2, H5). *Ae. tauschii* also showed two bands (H3, H4). *Ae. speltoides* var. *ligustica*, *T. turgidum* var. *dicoccon*, *T. dicoccoides* and *T. urartu* have no *Hsp92* I recognition site for this region (H1)

Figure 4. *Hsp92* I digestion. (1) ØX174/*Hae*III (2, 3) *T. monococcum* var. *boeoticum* (4) *Ae. speltoides* var. *ligustica* (5) *T. urartu* (6, 7) *T. turgidum* var. *dicoccon* (8, 9) *Ae. tauschii* (10) *T. aestivum* (11) ØX174/*Hae*III (12, 13) *T. dicoccoides*

Another enzyme used for digestion was *Taq* I. Six bands (T1-T6) were generated by digestion (Fig. 5). According to Ogihara et al. (22) there is five *Taq* I recognition site for this region in *T. aestivum*. But we screened only three bands in *T. aestivum and T. turgidum* var. *dicoccon* (T1, T3, T5). But the total length of this restriction bands were smaller than the amplification size so we can predict that the unscreened bands were too small for agorose gel to detect. There is three recognition site of *Taq* I for this region in *Ae. tauschii, Ae. speltoides* var. *ligustica, T. monococcum* var. *boeoticum T. urartu* and *T. diccoccoides*. But two of the resultant fragments were almost same sized so that they were screened as one band (T2). Two other bands were generated as a result of this digestion for these species (T4, T6).

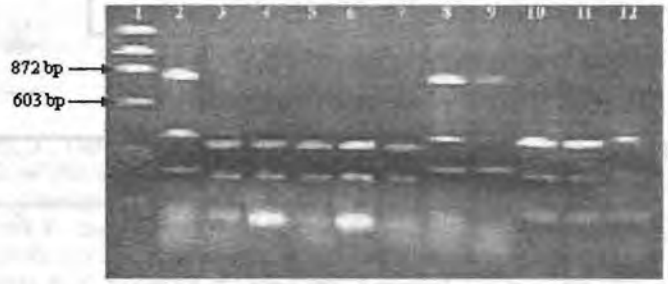

Figure 5 *Taq* I digestion. (1) ØX174/*Hae*III (2) *T. aestivum* (3) *T. monococcum* var. *boeoticum* (4, 5) *T. urartu* (6, 7) *T. dicoccoides* (8, 9) *T. turgidum* var. *dicoccon* (10, 11) *Ae. tauschii* (12) *Ae. speltoides* var. *ligustica*

Last enzyme used for digestion was *EcoR* V. Same sized two fragments were generated by digestion (Ec1) (Fig 6). According to Ogihara et al. (22) there is one digestion site for *T. aestivum*. But the resultant fragments were almost same sized so they were detected as one band. All of the other species showed the same banding pattern with *T. aestivum*.

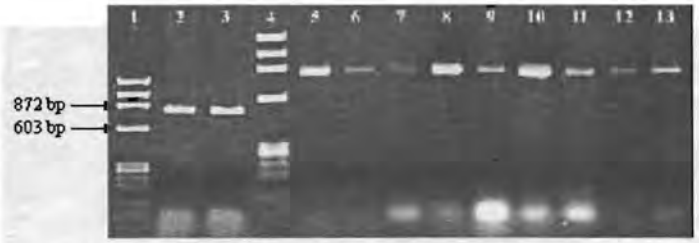

Figure 6. *EcoR* V digestion. (1) ØX174/*Hae*III (2, 3) *T. monococcum* var. *boeoticum* (4) ØX174/*Hae*III (5,6) *T. urartu* (7,8) *T. dicoccoides* (9) *T. turgidum* var. *dicoccon* (10) *T. aestivum* (11) *Ae. speltoides* var. *ligustica* (12, 13) *Ae. tauschii*

According to data obtained from these results, a statistical analysis was performed by using NTSYSpc V2.10 program (21). A phenogram was constructed by using Unweighted Pair-Group Method Using Arithmetic Averages Method (UPGMA) (Fig. 7).

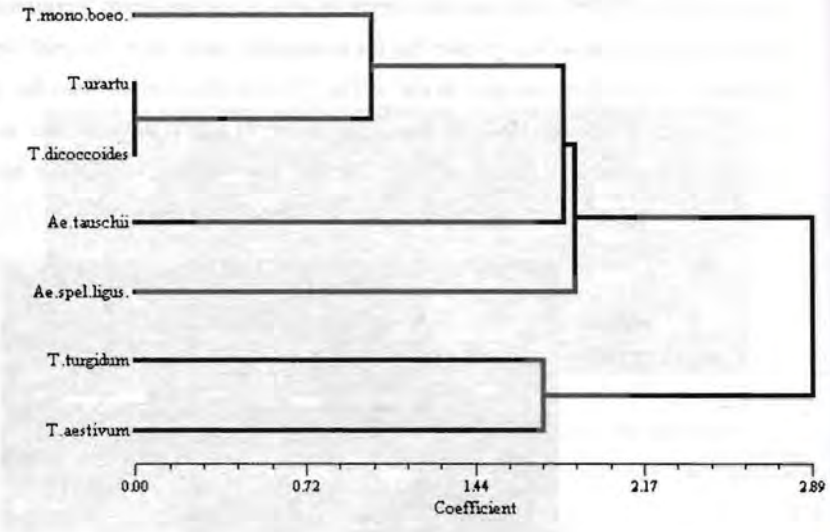

Figure 7. Phenogram constructed by UPGMA method.

According to this phenogram *T. turgidum* (AABB genome) and *T. aestivum* (AABBDD genome) were clustered together as a separate group and connected to the cluster formed by other species. This agrees with the fact that *T. turgidum* and *T. aestivum* have the same B genome; the latter species originated from hybridization of *T. turgidum* with *Ae. tauchii* (23; 24). Other species formed four different groups among them and these groups were connected to each other. The cluster formed by *T. urartu* (AA genome) and *T.*

dicoccoides (AABB genome) was connected to the cluster formed by *T. monococcum* var. *boeoticum* (A^mA^m genome). On evidence of a previous study it appears that the wild allotetraploid emmer wheat *T. dicoccoides* arose by amphyploidy between the wild diploid wheat *T. urartu* and a diploid or diploids with genomes similar to those of the present members of the *Aegilops* genus. The cultivated diploid *T. monococcum* is unlikely to have been the A genome donor, because archaeological data, although not comprehensive, show no evidence of its existence before the tetraploids (25). This cluster was grouped with *Ae. tauschii* (DD genome) and the cluster formed by these species was grouped with *Ae. speltoides var. ligustica* (SS genome). In a previous study, correlating with our findings, *Ae. squarrosa* find to be the only example of a D-type cytoplasm, was found to be unique (26). This large group was connected with the cluster formed by *T. turgidum* and *T. aestivum*. This also agrees with the chloroplast DNA phylogenetic studies, which showed that the *Ae. speltoides* chloroplast genome is closer to that of *T. turgidum* than to that of any other *Triticum* species (13).

Recent studies have shown that differences in chloroplast DNA restriction patterns can provide important clues concerning evolutionary relationships among plant species (15, 16, 26, 27, 28). In addition, strong inferences can be made concerning interspecific relationships and extend of the intraspecific divergence within wheat cytoplasms (29).

Although a significant amount of information is already available, many aspects of wheat evolution remain unknown or require independent verification (30). Our study has shown that RFLP analysis of noncoding regions of chloroplast DNA provides important information for systematic and genetic relationships among these species.

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THE EFFECTS OF A SHORT-TERM MICROWAVE EXPOSURE ON THE LIFE SPAN OF *Drosophila melanogaster*

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Abstract

Electromagnetic fields are a foreign substance to living organisms. The experiments with adult *Drosophila* demonstrated that the mean life-span of a triple mutant of *Oster* (*bw:st p*^{p}) strain might be altered by pulsed square wave (10 GHz). However in the other single mutants of *white (w)* and *multiple wing hair(mwh)* the mean life span has not changed. Further research is necessary in order to clarify the effects of EM fields in *Drosophila.*

Key Words: *Drosophila melanogaster,* life span, electromagnetic field, microwave frequency.

Introduction

The increased use of microwave (MW) sources by various agencies will continue to generate interest in biological effects of exposure to these sources. Therefore, we wanted to investigate the effect of MW fields on *Drosophila* life span. It is known that various stress factors such as environmental temperature as well as ionizing irradiation are effective on adult longevity (1). It may be accepted that the MW irradiation is a stress factor and could also influence longevity of adult *Drosophila*, when it is applied for a short period.

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It is also known that MW frequencies which is widely utilized nowadays, causes water molecule vibration and in the end elevate temperature of the organisms. A slight increase of temperature, may cause to shorten life span to some extend. Although the specific effect of MW field on living things is not certainly known yet, there are some work which studied chronical effect of MW irradiation on experimental animals. Among them some researchers reported that such treatments reduced life span of rats (2) and others found that the similar treatment prolonged life span of rats (3) although insignificantly. It was also recorded that a MW dose of 2375 MHz (megahertz) on *Drosophila melanogaster* males, resulted in shortening of life span in comparison to the controls (4).

In addition, if *D. melanogaster* eggs are treated by the low frequencies of electromagnetic (EM) field, the viability rate of this embryonic stage against its controls was lowered. In 1994, it was showed that the MW power intensity affects the longevity of mice (6). According to this report the power intensity of 2,45 GHz 10 mW/cm^2 MW shortened life span of mice significantly. The same power intensity of 3mW/cm^2 MW frequency prolonged life span; however this increase was not significant.

As it is seen from the publications summarized above, the specific effects of EM fields is not clearly known, and its effects are not found always at the same direction. On the other hand, pulsed MW effect on *Drosophila* life span is not known either. For these reasons, it was thought that it was necessary to study the effect of pulsed MW frequency (10 GHz) applied for various periods, on the life span of both wild type and mutant strains of *D. melanogaster*.

Materials and Methods

a. Life span experiments

In this work, the *Oregon* wild type and 3 mutants of *D.melanogaster* was used. The mutants were as following: i) A *white* eyed mutant on the 1st chromosome; ii) the *mwh* (*multiple wing hair*) mutant on the 3rd chromosome; iii) a triple mutant named as *Oster* which has three different genes (*bw:st* p^p: *bw* stands for brown color eye, *st* stands for *scarlet* eye color and p^p an allel of *pink peach* eye color) (7). For each experimental group approximately a total of 100 flies (1-3 days old adults) were collected. For the treatment by electromagnetic field each vial contained a hundred individuals. Treated flies were transferred to tubes (2,5 x 7,5 cm) each having 10 flies, containing the standard medium described by Bozcuk (8). The control group of each type of strains were treated similarly as the experimental group except they did not have MW irradiation. All flies were kept in a CT room at 25 ± 1 C^0 and

relative humidity of 50-60% and in 12 + 12 hrs dark and ligh periods throughout experimental procedure. Flies were transferred twice weekly into vials containing fresh medium so long as the last fly was dead. During each transfer death flies were recorded.

b. Exposure to electromagnetic field

All treatments were conducted through a electromagnetic source which is a pulsed (modulated) square wave (1 kHz), approximately 5 mW (lowered power) and 10 GHz. During the MW treatment, flies were taken into empty tubes (2,5 x 7,5 cm) without medium. The experimental glass tube was 1 m away across the antenna and stabilized by a rectangular holder. The dosage used has been determined according to previous publications, and to our pilot experiments.

The control groups were in the same lab for the same period as its experimental group, and again were in the empty tubes locked in a metal cubbord, roughly 8 meter far away from EM field source.

Each group contained 100 flies and irradiated by a MW of 0,0156 Watt/m^2 power intensity and SAR = 9,8 mW/kg. The duration of ionizing radiation was only once and for a period of 5 hrs continuously and 3 hrs + 3 hrs discontinuously – 30 minutes resting period in between. During the 30 minutes resting period the flies were kept in vials containing fresh meal. All the treatment were conducted in empty vials.

c. Statistics

The variance analyses among the mean life span of the experimental groups as well as significant tests between them were analized by ANOVA using SPSS 10.0 program.

Results

The summarized data concerning mean life span of *Oregon* w.t. flies irradiated 3+3 hrs and 5 hrs together with their controls were given in Table 1. The mean life span data of mutant strains *white* and *mwh* were given Table 2 and 3 respectively. For all the three strains it was found that the differences between the mean longevity of the treated groups against their respective controls are not significant at all.

Table 1. Mean Life Span Data of Wild Type Strain *Oregon* in *Drosophila melanogaster* Exposed to 10 GHz Microwave for 5 Hours (Continuous), 3+3 Hours (With Half An Hour Break) and The Non-Exposed Control Groups.

Group No.	Group Name	Genotype and Sex	Number of flies	Mean life span (days) ± S.E.
1	C 1	*Oregon* F	101	64,79 ± 1,57
2	C 2	*Oregon* M	101	51,46 ± 1,13
3	E-3+3 hours	*Oregon* F	105	70,57 ± 1,47
4	E-3+3 hours	*Oregon* M	77	55,42 ± 1,19
5	E-5 hours	*Oregon* F	101	67,89 ± 1,32
6	E-5 hours	*Oregon* M	94	52,94 ± 1,12

C: Control (Non Exposed) group, E: Exposed group, S.E.: Standard error, M: Male, F: Female. (The statistic comparisons between the respective means are not significant).

Table 2. Mean Life Span Data of Mutant Strain *white* in *Drosophila melanogaster* Exposed to 10 GHz Microwave for 5 Hours (Continuous), 3+3 Hours (With Half An Hour Break) and The Non-Exposed Control Groups.

Group No.	Group Name	Genotype and Sex	Number of flies	Mean life span (days) ± S.E.
1	C1	*white* F	83	54,59 ± 1,82
2	C2	*white* M	97	59,59 ± 1,49
3	E-3+3 hours	*white* F	89	56,78 ± 1,78
4	E-3+3 hours	*white* M	97	59,90 ± 1,24
5	E-5 hours	*white* F	80	56,26 ± 1,91
6	E-5 hours	*white* M	85	60,96 ± 1,43

C: Control (Non Exposed) group, E: Exposed group, S.E.: Standard error, M: Male, F: Female. (The statistic comparisons between the respective means are not significant).

Table 3. Mean Life Span Data of Mutant Strain *mwh* in *Drosophila melanogaster* Exposed to 10 GHz Microwave for 5 Hours (Continuous), 3+3 Hours (With Half An Hour Break) and The Non-Exposed Control Groups.

Group No.	Group Name	Genotype and Sex	Number of flies	Mean life span (days) ± S.E.
1	C1	*mwh* F	95	70,87 ± 2,43
2	C2	*mwh* M	89	67,96 ± 2,11
3	E-3+3 saat	*mwh* F	81	77,51 ± 2,11
4	E-3+3 saat	*mwh* M	88	64,94 ± 1,88
5	E-5 saat	*mwh* F	93	79,78 ± 2,25
6	E-5 saat	*mwh* M	90	63,56 ± 1,85

C: Control (Non Exposed) group, E: Exposed group, S.E.: Standard error, M: Male, F: Female. (The statistic comparisons between the respective means are not significant).

As it is observed, the mean life spans, and their statistical comparisons of the autosomal triple mutant of *bw:st* p^{p} strain and its controls treated for a period of 3+3 hrs and 5 hrs were shown on Table 4. The survival curves of this *Oster* strain were shown in Figure1.

Table 4. Mean Life Span Data of Mutant Strain *bw:st* p^p in *Drosophila melanogaster* Exposed to 10 GHz Microwave for 5 Hours (Continuous), 3+3 Hours (With Half An Hour Break) and The Non-Exposed Control Groups.

Group No.	Group Name	Genotype and Sex	Number of flies	Mean life span (days) ± S.E.	Significant differences of the means
1	C1	*bw:st* p^p F	92	63,11 ± 1,19	
2	C2	*bw:st* p^p M	95	45,34 ± 1,09	
3	E-3+3 hours	*bw:st* p^p F	98	51,92 ± 1,30	1-3**
4	E-3+3 hours	*bw:st* p^p M	90	51,31 ± 1,15	2-4*
5	E-5 hours	*bw:st* p^p F	96	64,61 ± 1,06	1-5
6	E-5 hours	*bw:st* p^p M	96	48,57 ± 0,88	2-6

C: Control (Non Exposed) group, E: Exposed group, S.E.: Standard error, M: Male, F: Female, *: p<0,05, ** : p<0.001.

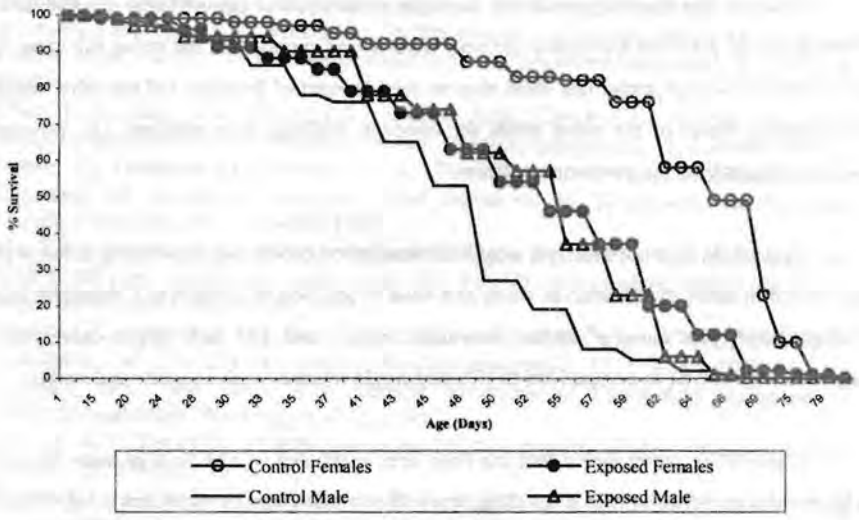

Figure 1. Survival Curves for the *bw:st* p^p Mutant Males and Females Exposed to 10 GHz Microwaves for 3+3 Hours (With Half an Hour Break) and Non-Exposed Control Flies.

As it is noticed, the differences between the groups treated for 5 hrs and their respective sex controls are insignificant for both sexes, however the result for those 3+3 hrs groups is significant. It is indeed noticed that the mean life span of the females treated with electromagnetic field (with a mean of 51,92 days) against the mean of their pertinent controls (untreated; 63,11 days) is shortened. On the contrary, the mean life span of treated males (51,31 days) against their respective controls (45,34 days) is increased.

The significance tests showed that these differences are significant for females at the level of p<0,001, and for males at the level of p<0,05.

Discussion

We know that there is a relationship between genotype and environmental stress factors, and the interaction between these effects upon the life span of animals (see for example 9). In order to understand the pulsed effect of electromagnetic field on *Drosophila Oregon* w.t. and three mutants, this investigation was undertaken.

One of the first observation was that the mean life spans of *D.melanogaster* w.t. strain of *Oregon*, and two mutants *white* and *mwh* were not affected by the MW fields in comparison to their own pertinent controls. There were no significant changes between the mean life spans of treated males and sham males, treated females and sham females of *Oregon* w.t. and this conclusion was also similar for *white* mutant sexes and as well as *mwh* mutant sexes.

However the obtained results for the triple mutant (*Oster* strain) *bw:st* p^{p} were quite different from the previous mentioned groups. Here it was shown that the mean life span of exposed female *bw:st* p^{p} strain has lived shorter than its control females. On the other hand, for the exposed males of the same strain the opposite findings were realized, i.e. exposed males lived longer than the pertinent controls.

First of all, it is not clear yet why MW irradiation causes life shortening in *bw:st* p^{p} strain, but not in other strains such as *white* and *mwh* in addition to *Oregon* w.t. However it is clear immediately that *bw:st* p^{p} mutant is a triple mutant and EM field might cause more damage to this genotype in comparison to w.t. and single mutants such as *white* and *mwh*.

It should be remembered that the flies were irradiated in EM field at once for 3+3 hrs (30 minutes in between two 3 hrs treatment). If this short expose produces a significant decrease or increase in the life span of adults, that means that some genetic or structural damage might be induced in the cells of the organisms. It was assumed that EM fields are a foreign substance to living organisms, therefore it should cause some damage to living systems. The damage might be in the cells (mitochondria, cell membrane etc) in the chromosomes or genes. Some superoxide radicals might also be induced and this in the end changes nucleotide bases of DNA or RNA. It is also possible that EM fields might produce thermal influence on the cell content.

All these might explain the reduced or prolonged life span of *Drosophila* adults.

However relatively little is known with any certainty about other possible effects of non ionizing EM fields on living things and much more research is necessary to improve our knowledge on the actual problem (10-13).

The recent publication reminds the possibility of affecting the authors of the paper, due to the fact that researchers are supported by a mobile telephone firm (14).

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THE EFFECT OF SALT STRESS ON LIPID PEROXIDATION AND ANTIOXIDATIVE ENZYME ACTIVITIES IN TWO CUCUMBER CULTIVARS

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Abstract

The effects of salinity on possible changes of the activities of major antioxidative enzymes such as superoxide dismutase (SOD: EC 1.15.1.1), ascorbate peroxidase (APX: EC 1.11.1.11), catalase (CAT: EC 1.11.1.6) were investigated and lipid peroxidation measured as Malondialdehyde (MDA) content in leaves of cucumber cultivars differing in salt tolerance. Two cultivars of cucumber were grown under the controlled conditions in perlit culture and irrigated with Hoagland nutrient solution for a period of 10 days. After this period seedlings were treated with 0, 50, 100 and 150 mM NaCl solutions for 14 days. Level of MDA content of Çengelköy was lower, but activities of SOD, APX and CAT were higher than in Beith Alpha.

Keywords: Salt stress, *Cucumis sativus* L., malondialdehyde, antioxidative enzymes.

Introduction

Salinity in soil or water is one of the major stresses and, especially in arid and semi arid regions, can severely limit crop productivity (Shannon, 1998).

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The generation of active oxygen species (AOS) is a common response to environmental stress conditions, such as salinity, drought, high and low temperatures, metal toxicity and air contaminants (Luna et al., 1994, Gossett et al., 1994).

Salt stres also causes stomatal closure, which reduces the CO_2 to O_2 ratio in leaves and inhibits CO_2 fixation. The conditions increase the rate of activated oxygen species (AOS). The AOS are produced during normal aerobic metabolism by the interaction between O_2 and electrons leaked from electron transport chains in choloroplasts and mitochondria. These cytotoxic activated oxygen species can seriously disrupt normal metabolism through oxidative damage of lipids, proteins and nucleic acids. Since internal O_2 concentrations are high during photosynthesis, choloroplasts are especially prone to generate activated oxygen species that include superoxide (O_2^-) and hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen (O_2). Also AOS cause membran lipid peroxidation, reducing membran fluidity and selectivity. Lipid peroxidation measured as malondialdehyde (MDA) (a product of lipid peroxidation) content is considered to be indicator of oxidative damage (Luna et al., 2000). Plants synthesize and store antioxidants such as ascorbic acid (ASC), reduced glutathione (GSH), α-tocopherol and carotenoids to remove AOS. (Asada and Takahaski, 1987).

Plants differ in their ability to remove and scavenge AOS. The differences in plant protection mechanisms determine their tolerance to stress conditions associated with AOS toxicity. Comparing the mechanisms of antioxidant production in salt tolerant and salt sensitive plants, which were containing high concentrations of antioxidants show considerable resistance to the oxidative damage caused by the activated oxygen species (Garratt et al., 2002). In *Gossypium*, salt tolerant cultivars had lower MDA levels and higher antioxidant activity than sensitive cultivars (Gossett et al., 1994). Recently, it has been reported that salt tolerant species appear to have a higher degree of protection against salinity induced oxidative stress than the more sensitive species and that it resulted from higher antioxidant system activity and less lipid peroxidation (Bor et al., 2003). However, in view of considerable variations in the protective mechanisms aganist activated oxygen species in different plant species, further work is required to establish the general validity of this phenomenon in salinity tolerance. Ashraf and Haris (2004) suggested that if natural variation in the traits for these protective mechanisms occur, it might provide the basis for a breeding program with antioxidant compounds or enzymes of the protective pathway as indicators of salinity tolerance.

It has been known for many years that there are large differences in salt tolerance between species of crop species (Mc Kersie and Leshem, 1994). Cucumber (*Cucumis sativus* L.), an important crop produced in Turkey, is moderately susceptible to salinity stres

especially at germination and seedling stage. There is not much information about effects of salinity on cucumber species in the current literature with the exception of few studies (Chartzoulakis, 1990; Abd-Alla et al., 1992; Lechno et al., 1997).

The aim of this study was to determine the effects of salt stress on the activity of antioxidative enzymes and the lipid membrane peroxidation in two cucumber cultivars differing in salt tolerance.

Materials and Methods

Two cucumber (*Cucumis sativus* L.) cultivars (Çengelköy, Beith-Alpha) were used as plant material which were compared and considered "Beith-Alpha" less salt tolerant than "Çengelköy" (Baysal et al., 2004). The experiment was carried out in a greenhouse under the controlled conditions (25± 2°C temperature, 50-60 % relative humudity, 16h light /8h dark photoperiod and 100 μmol.m^{-2}s^{-1} light intensity) in perlit culture.

Two cultuvars of cucumber were germinated and grown in perlit culture. At germination stage, the seeds were irrigated with ½ Hoagland nutrient solution for a period of 10 days. After this period, the cucumber seedlings at the cotyledon stage were treated with 0, 50, 100 and 150 mM NaCl solutions. To avoid osmotic shock, NaCl concentrations were increased gradually by 50 mM every day until the desired concentration was reached. After 14 days (exclusive of the addition ones) of salt treatment, the plants were harvested and analyzed to measure the physiological parameters. Fresh weight (FW) and dry weight (DW) (after drying at 70°C for 48 h) of leaves were measured.

For enzyme assays, frozen leaf samples were frozen in liquid nitrogen and extracted with ice-cold 50 mM phosphate buffer including 0,1 mM na-EDTA. All enzymes assays were based on the method of Cakmak and Marshner (1992) and Cakmak (1994).

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in 1 g leaf fresh weight according to Lutts et al.(1996). The concentration of MDA was calculated from the absorbance at 532 nm (correction was done by subtracting the absorbance at 600 nm for unspecific turbitidity) by using extinction coefficient of 155 mM^{-1} cm^{-1}.

Each experiment was carried out with three replications. The differences between the treatments as well as the three species were tested using SPSS statistical programme.

Variance analysis of the results was performed and the mean differences were compared with least significant differences (LSD) at 5% level.

Results and Discussion

All used NaCl concentrations caused a significant reduction in fresh and dry weight of two cucumber cultivars. FW and DW of leaves gradually decreased with an increase in NaCl concentrations (Figure 1). The percentage reductions in leaf FW and DW were greater at 150 mM NaCl and the highest reductions were obtained for the Beith Alpha.

Lipid peroxidation levels in leaves of the two cucumber cultivars, measured as the content of MDA, are given in Figure 2. The presence of NaCl in rooting medium induced an increase in MDA contents in leaves of cultivars. The percentage of increase in leaves was greather at 100 and 150 mM NaCl for Beith Alpha. For Çengelköy, only 150 mM NaCl treatment caused a significant ($p< 0,005$) increase in MDA content. The lower level of MDA in leaves of Çengelköy than Beith Alpha suggests that it may have better protection aganist oxidative damage under salt stress. Cellular membran integrity evaluated by electrolyte leakage and lipid peroxidation, measured as MDA, appeared to be more reliable indices of salt tolerance (Lutts et al., 1996; Luna et al., 2000; Bor et al., 2003).

Enzymic defenses include SOD, CAT and peroxides enzymes which are expected to limit production of free radicals. SOD is reported to play an important role in cellular defense aganist oxidative stress. SOD enzyme converted superoxide into H_2O_2 which is further scavenged by CAT and various peroxidase. AP is also play a key role by reducing H_2O_2 (Asada, 1997). In this study, there were striking differences in antioxidant enzyme activities between the two cultivars with increasing NaCl concentration. Salt treatment caused a significant increase in SOD activity in leaves of two cucumber cultivars. But the percentage of increase in SOD activity was higher than in Beith Alpha in all salt treatment comparison with the control plants (Figure 3). A significant increase in SOD activity could increase the ability of the leaves to scavenge $O_2^{\cdot}$ radicals, which could cause membrane damage. APX uses ascorbate as the electron donor for the reduction of H_2O_2 and is well known to be important in the detoxification of H_2O_2. In our study, APX activity significantly increased with increased NaCl concentration in leaves of Çengelköy. However, in Beith Alpha, 100 and 150 mM NaCl caused a significant increase in APX activity in comparison to the control plants. APX activity was highest in Çengelköy and reached 439% of the control at 150 mM NaCl (Figure 4). For the two cucumber cultivars, NaCl induced an increase in CAT activity in leaves (Figure 5). 100 and 150 mM NaCl caused, respectively, 539 and 647 % incerase in CAT activity in comparison with the control plants. But in Beith Alpha only 150 mM NaCl caused an important increase in CAT activity which reached 129% of the control. These

results may be suggested that CAT and AP which of both responsible for detoxification of H_2O_2, are probably equally important in the detoxification step in Çengelköy. Likewise the results of our study, in several study many crops with high levels of antioxidants have been repoted to have a greater resistance to the oxidative damage (Garratt et al., 2002; Mittova et al., 2002; Bor et al., 2003). On the other hand, some contrast results about antioxidant defense system were mentioned by other researches (Lechno et al., 1997; Dionisiosese and Tobita, 1998).

In conclusion, the higher SOD, CAT and APX activities and the lower degree of membrane damage, as indicated by low MDA content, observed in NaCl-treated plants of relative tolerance of Çengelköy. This result confirms the relationship between salt tolerance and antioxidant defense system.

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Figures

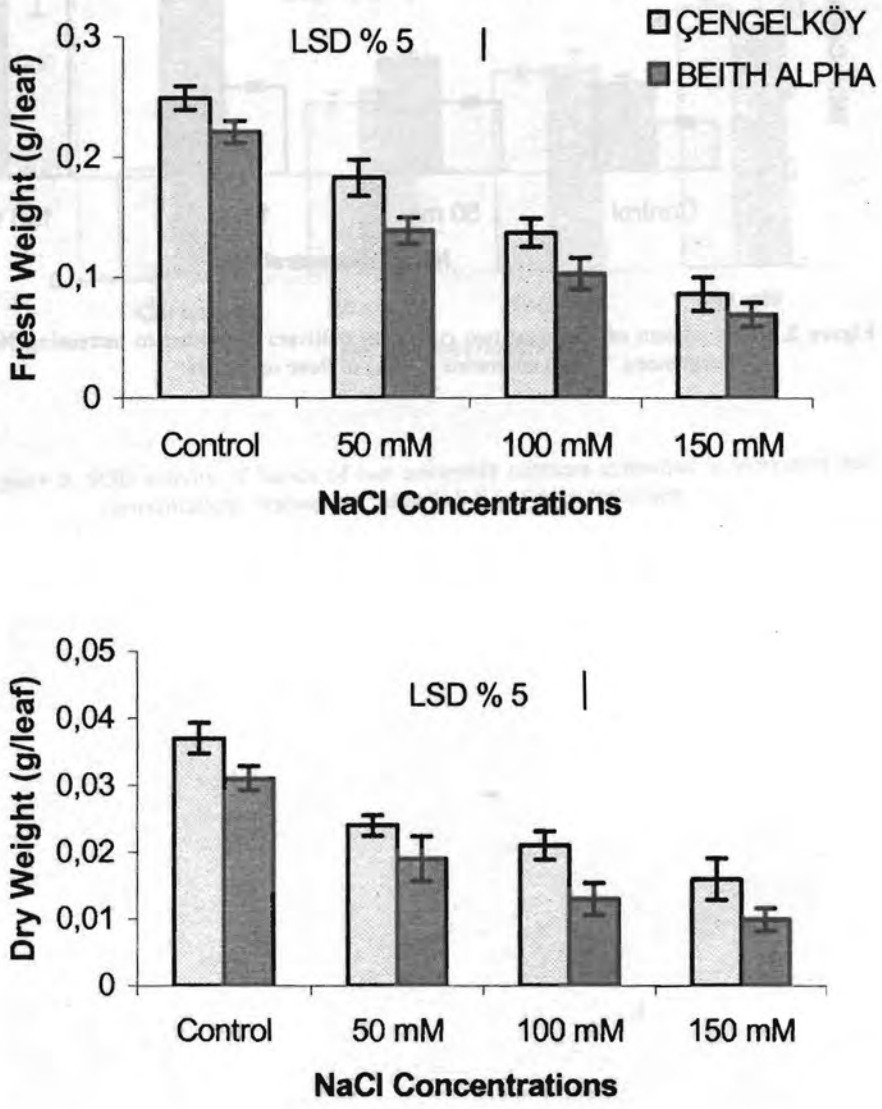

Fig. 1. FW and DW of leaves of two cucumber cultivars submitted to increasing NaCl concentrations. Values are means (±S.E.) of three replicates.

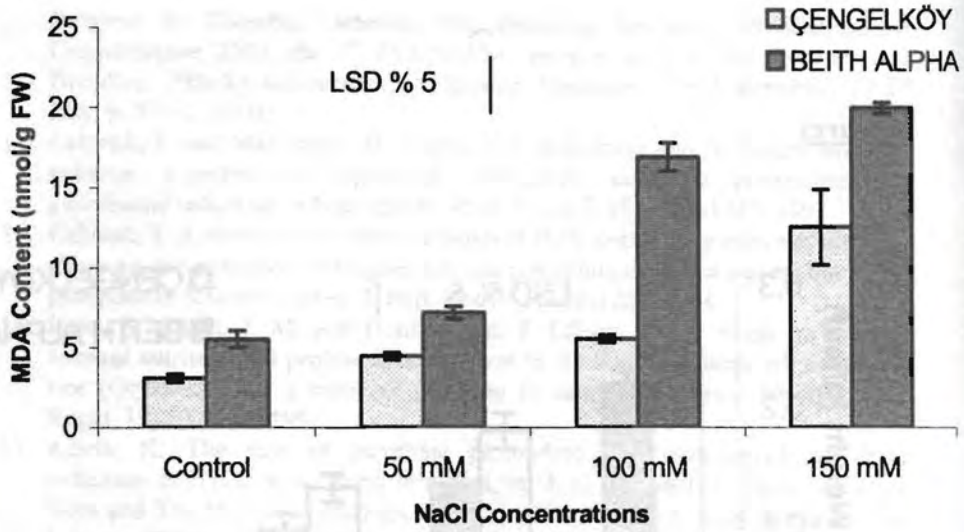

Figure 2. MDA content of leaves of two cucumber cultivars submitted to increasing NaCl concentrations. Values are means (±S.E.) of three replicates.

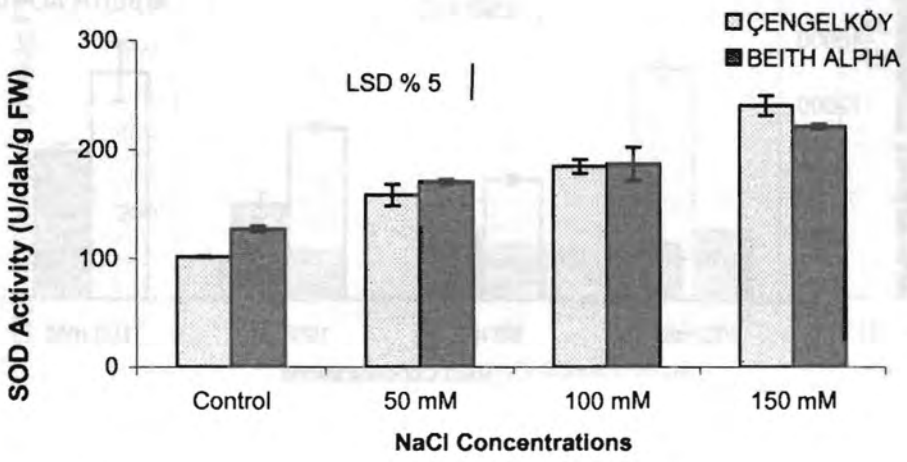

Figure 3. SOD activity of leaves of two cucumber cultivars submitted to increasing NaCl concentrations. Values are means (±S.E.) of three replicates

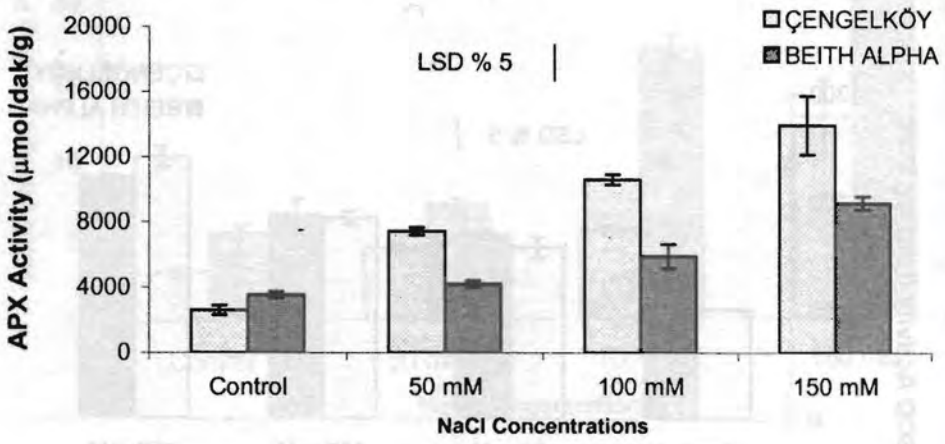

Figure 4. APX activity of leaves of two cucumber cultivars submitted to increasing NaCl concentrations. Values are means (±S.E.) of three replicates.

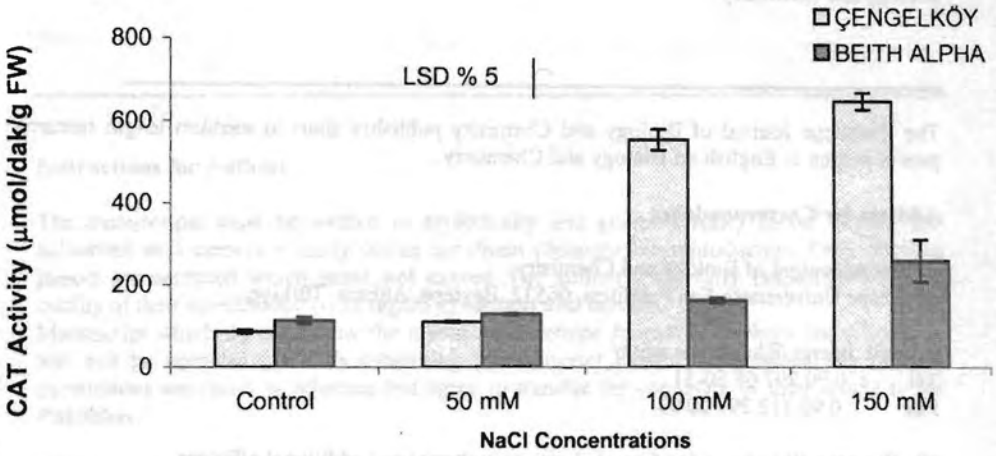

Figure 5. CAT activity of leaves of two cucumber cultivars submitted to increasing NaCl concentrations. Values are means (±S.E.) of three replicates.

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