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PRODUCTION AND BIOACTIVITY DETECTION OF GIBBERELLIC ACID BY *Gibberella fujikuroi* G5

Received 27.11.2000

Nilüfer CİHANGİR *

Abstract

In this study, *Gibberella fujikuroi* G5 fungus strain was grown in Czapek Dox broth medium. The yield of gibberellic acid was 650 mg/ l . Then, the bioassay of gibberellic acid (GA3) produced by *Gibberella fujikuroi* G5 was carried out for detection of bioactivity. For this purpose, rice seedling test was applied. After the incubation period of 18 days, the GA3 isolated from the culture media was applied to the dwarf rice variety supplied from Tokyo University, Faculty of Agriculture. The shoot elongation increased depending on the dose of GA3 , proving the bioactivity of GA3 obtained by *Gibberella fujikuroi* G5.

Key words: *Gibberella fujikuroi*, gibberellic acid, bioactivity, dwarf rice seedling.

Introduction

Gibberellins are important plant growth regulators obtained from plants and microorganisms. They are widely used in agriculture, nurseries, greenhouses, viticulture etc. The bioactivity of these substances plays an important role in agricultural area.

The production of plant growth promoters such as gibberellic acid represents a priority for the enhancement of agricultural cultures and thus, by extension, for the food industry. This is of particular importance in developing countries.

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The gibberellins are a large family of diterpene acids. They have been found to elicit a variety of responses in higher plants including shoot elongation, sex expression, fruit growth and seed germination (1,2). Of the many existing gibberellins and the most widely known is gibberellic acid. As gibberellic acid (GA_3) is an important growth promoter in plants, world wide demand for this substance is steadily increasing. This growth regulator is used to obtain a variety of economic benefits. Thus, the bioactivity of gibberellic acid is quite important for finding application in the fields mentioned above.

Among the various bioassays for gibberellins (GAs), the micro-drop method using GA deficient mutants of dwarf rice is one of the most useful because of the simple manipulations involved and the high degree of sensitivity of the assay . This method is based upon the elongation response of the second leaf sheath of rice plants to GA3 (3,4).

There is no report in the literature on detection of bioactivity of gibberellic acid obtained from *Gibberella fujikuroi* G5 fungal strain .In the present study, we report the effect of gibberellic acid produced by *Gibberella fujikuroi* G5 on the dwarf rice variety and detection of its bioactivity.

Materials And Methods

Microorganism

The fungus strain *Gibberella fujikuroi* G5 used in this study was obtained from Faculty of Agriculture, Ankara, Turkey. Cultures were maintained on potato dextrose agar slants and subcultured every month, then transferred to storage at 4 ^{o}C .

Media and culture conditions

Czapek- Dox liquid medium was used as a base growth medium (5). The pH of the above media was adjusted to 5.5 followed by sterilization at 120 ^{o}C for 15 min.

Growth and gibberellic acid production were carried out in 250 ml Erlenmeyer flasks containing 100 ml of the medium, followed by incubation at 30 ^{o}C for 18 days in the dark in a rotary shaker (150 rev min -1).

Gibberellic acid extraction and quantification

Samples of filtered culture medium were acidified to pH 2.5 with 0.1 N HCl and were then extracted three times with ethyl acetate. The organic phase was treated with activated charcoal to adsorb pigments and subsequently filtered (6).

The samples were concentrated to dryness and redissolved in methanol. The samples were applied to precoated thin layer chromatography sheets (silica gel 60 F 254 , Merck) and eluted with the mixture of the organic phase of the solvent system : benzene: butanol: acetic acid (30:15:5, v/v/v). The gibberellin spots were revealed with a universal reagent (5 % sulfuric acid in ethanol) and were visualized in an ultraviolet light chamber at 365 nm. Good separation of Gibberellin GA3 (gibberellic acid) and GA7 was obtained. These plates, with an internal standard (GA3 from Sigma chemicals) were read in a scanning densitometer analyzer apparatus to quantify the gibberellic acid concentration.

Dwarf rice assay

The dwarf rice (*Oryza sativa* L. cv. Tan-ginbozu) micro-drop method was used to assay bioactivity. Tan- ginbozu was kindly supplied by Prof. Noburo Murofushi; University of Tokyo, Faculty of Agriculture,Japan. Seeds of the dwarf rice cultivars were sterilized with Benlate (O.1 % , Du Pont, Del.,U.S.A) soaked and germinated in sterilized water for 3 days at 30 ^{o}C . The coleoptils were 0.5 mm in length. After the incubation period of *Gibberella fujikuroi* G5 for 18 days, the GA3 isolated from the culture media was applied to the dwarf rice variety by micro drop method. Authentic GA3 was dissolved in 50 % (v/v) aqueous acetone. Various concentrations of test solution was applied with a microsyringe to the region between the coleoptile and the first leaf of a seedling. The length of second leaf sheath was measured seven days after application (7,8).

Statistical analysis

The correlation between amount of gibberellic acid (μg) and length of second leaf sheath (mm) was determined according to Pearson's correlation test (9).

Results and Discussion

The early descriptions of GA induced elongation, especially those associated with genetic dwarfism. Some researchers presented evidence that gibberellins are active in the control of shoot elongation in Zea mays L. This idea is now supported by similar studies using single gene mutants of several other species e.g. *Oryza sativa* L. (10-12).

As can be seen in Fig 1, the length of the second leaf sheath of the control group has been estimated as 15 mm, consequently gibberellic acid has positive effect on seedling and the length of second leaf sheath increasing in direct proportion with the GA3 concentration. All data were the means of three separate replicates.

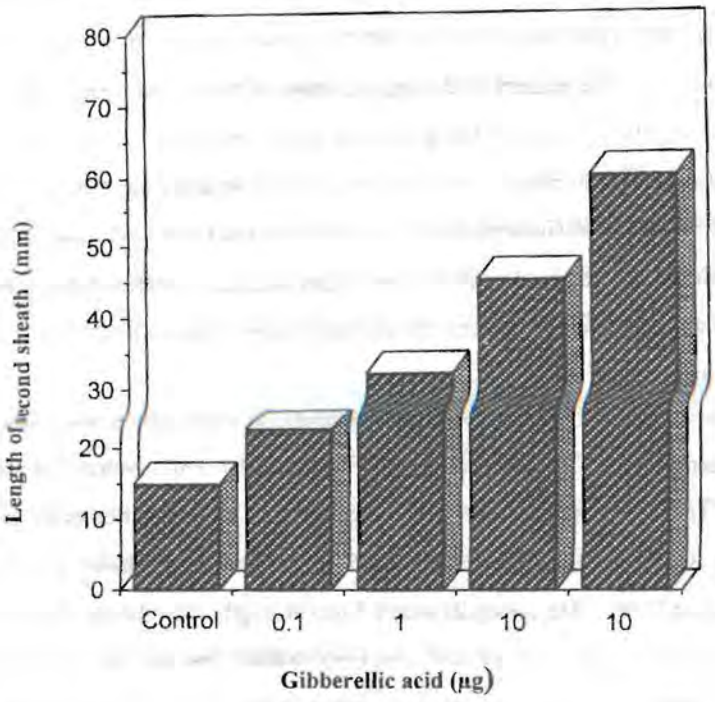

Figure 1. Length of second leaf sheath at various concentrations of GA3 obtained from *Gibberella fujikuroi* G5 culture media. Average values from three cultures are reported for each parameter.

A statistical analysis was carried out to determine the relation between the two variables. For this purpose, Pearson Multiplication of Moments Correlation test was applied. Correlation coefficient between the two variables was determined as $r = 0.716$. Since the correlation was found significant, it was proved that the relation between the amount of GA3 and the length of second leaf sheath was valid. In studies for determining the biological activities of gibberellins (GA_S), divergent physiological effects of these hormone groups were utilized. The method used must be practical and specific for gibberellins This method is the only method sensitive to GA3 which is among the gibberellin types. GA3 can be bioassayed

in the range of from 0.05 microgram up to 100 micrograms (13-15).

In this study, GA3 isolated *Gibberella fujikuroi* G5 culture media was prepared varying between the range of 0.1 to 100 micrograms and applied to the dwarf seeds. Figure 1 shows the effect of gibberellic acid obtained from *Gibberella fujikuroi* G5 culture media on elongation of second leaf sheath of Tan ginbozu. Gibberellic acid (GA3) has significantly increased elongation which correlates with previous results (8, 16).

It is possible that the bioactive compound may be substituted for various hormonal signals, both in the breaking of dormancy mechanisms and in the stimulation of elongation and growth. Results have shown that increasing amount of gibberellic acid effects the length of the second leaf sheath positively. As shown in Figure 1, while the length of second leaf sheath has been estimated as 15 mm in control group, this value has been determined as 60 mm for the sample treated with 100 mg GA3 . As reported previously, gibberellins applied exogenously achieve the stem growth not by repairing the mutant gene but increasing the endogenous gibberellin quantity observed at low levels in dwarf plants to a higher level capable of performing physiological effect (17,18) . Gibberellic acid isolated from *Gibberella fujikuroi* G5 strain culture medium has proved to have a positive effect on seedling showing significant bioactivity . This property provides a great advantage in the utilization of this hormone with agricultural and horticultural purposes. The positive effect of this gibberellic acid on elongation and development may also have important commercial implications. Thus, this research is a promising study for future aspects.

Acknowledgement

I wish to express my sincere thanks to Prof. Noburo Murofushi, Tokyo University, for helpful advice about the method of micro drop assay and for a gift of GA7.

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ORNITHOLOGICAL OBSERVATIONS OF SEYFE LAKE, KIRŞEHIR

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Received 5.12.2000

Abstract

Ornithological observations were made between February 1998-1999 in the Seyfe Lake and its vicinity, two times in each season. The aim of this study is to find out bird species, their populations and bio-ecological importance of the Seyfe Lake. As a consequences of the observations, 107 bird species were recorded and listed in the area. Among the recorded bird species, 66 species are nonpasserine, 41 are passerine and 55 species breed in the area. Tree bird species *(Anser albifrons, Aythya nyroca* and *Falco naumannii*) are in the "*vulnarable*" and 1 bird species *(Circus macrorus)* is in "*lower risk*" categories according to European Red List. Also they are 48 of *"strickly protected fauna species"* and 53 of *"protected fauna species"* according to Bern Convention.

Key Words: Ornithofauna, Seyfe Lake, Kırşehir, Turkey.

Introduction

Seyfe Lake is located in the pit of a tectonic origin in the north-east of the province

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of Kırşehir in Cenrtal Anatolia (Figure 1;coordinates: 38° 18'N, 34° 23'E). The nearby village of Seyfe has lent its name to the lake.The Lake and a 10 700 hectare (ha) surrounding area were declared a Site for the Preservation of Nature on 1970. The lake is also a first degree nature site. A 10 700 ha area has been included in Ramsar List since 1995

Administravely, its falls within the boundaries of the district of Mucur in the Kırşehir Province. As it is in enclosed basin, the Lake has no out-flows that for reason its water is salty. The Lake is fed by the springs coming from the villages of Seyfe and Badıllı, surface springs, surface flows within its own drainage area and precipitation. Discharging is by way of evaporation.

Seyfe Lake is a shallow brakish lake. The water level falls considerably in summer and most of the Lake turns into salty marshes, because the area receives very litlle percipitation. The brooks feeding the Lake largely dry up in summer time due to higly level of evaporation. The area of the Lake varies between 1 500 ha and 7 000 ha depending on the water level. The avarege area is 3400 ha and the maximum water depth is aproximately 2 m. There are extensive pastures and fields around the Lake.

The Lake is bourdered by arable fields in the south and west, while in the north and north-east extensive steppe reaches the Lakes's edge. East of Kızıldağyeniyapan are 1 500 ha of brakish freshwater marshes which provide feeding and breeding habitat for waterbirds. Another important feature of Seyfe Lake are dozen island. The largest island is 1 km long and several metres high, some islands are covered with scrub and grasses, other are barren (1, 2 and 3)

Material and Methods

Bird observations were made between February 1998-February 1999 in the Lake and its surroundings. Observations were carried out at different points and line transects by the vehicle and/or walking. Each points were visited two times in each season. Binoculers (Pentax, 16 x 24, Tasco 7 x 50), monoculer teleskop (Minolta 20-60 x 80, and Vista-80 20-50 x 80) and mechanical numerator were used during the observations and counting (2). Generally, the species have been identified after their recording in field using some ornithological quide books. While carriying out the obsevations in order to find out the species and population density, 1 / 25 000 maps were used (4). For the systematical categorization of bird species, it was followed that proposed by Kiziroğlu, 1989 (5).

Results and Discussion

A list of the birds species and related numbers of observed at Seyfe Lake during the observation period are given in Table 1. As consequences of the observations, 108 bird species have been recorded in study area where they have been intensively using the area for feeding, settling, wintering and breeding. Among these species 66 are non passerine birds, 41 species are passerine birds. And 55 of 107 bird species are breeding in this area exactly which can be determined from nest and juvenil observations, courtship, displaying and agitated behaviour from adults indicating presence of nests or youngs.

Several ornitological observations and midwinter waterfowl cencus were done by various researcher and associations in the area. According to previous researches and counts, this area qualifies for its breeding population of greater flamingos, little egret, red-crested pochard, avocet, spur-wigned plover, mediterranean gull, black headed gull, selender billed gull, gull-billed tern and little tern. (2, 6).

According to previously observations and researches, 187 bird species (including songbirds) have been observed at the Lake and its surroundings. This area is one of the most important feeding, breeding and wintering area for birds not only in Turkey but also in the Europe. The existance of the biggest colonies of waterbirds (especially flamingos) were recorded and is stop-over area for hundreds of thousand of ducks in Autumn. In spring, birds of various species, especially waterbirds, breed on the island in the west of the Lake. In addition, the area an important gathered ground for storks and cranes in the Autumn. (1, 2, 6).

As a result of this study, it is remarkable that white fronted goose (*Anser albifrons*), ferruginous duck (*Aythya nyroca*), little kestrel (*Falco naummanni*) are evaluated as a"Vulnarable (VU)" species; while pallied harrier (*Circus macrorus*) is evaluated as a "Lover Risk: Near Threatened" species according to European Red List (7). According to Bern Convention (8), 48 of "Stricktly Protected Fauna Species". Some of these species observed at the research area are especially significant. The existance of biggest colonies were recorded flamingos (*Phonicopterus ruber* - 1 573 indivudials on July 1998), white fronted goose (*Anser albifrons > 10 000 in number on November 1998 and February 1998).*

Seyfe Lake was declared a SİT in 1989 (23 585 ha), a Nature Reserve in 1990 (10 700 ha) and a Ramsar Site in 1994 (10 700 ha). Currently DSİ (State Hydrolic Works) is engaged in the planning and implementation of the "Seyfe Lake Ecological Conservation Project". This project principally aim to reduce the lake to its original size by diverting the flow from the drainage channels into the Kalaycık Stream (north-east of the area) which subsequently drains into the Kızılırmak River. In order to achieve this 35 km of channel will be dug. As a result,

15 792 ha north and east of the lake will be reclaimed for agriculture, including the 8 000 ha previously lost (9). Most of the breeding islands and all brakish and freshwater marsh in the east can be disappear as a result of the DSİ project. About the eco-ornitological risks associated with the DSİ project are so great that it should be halted immediately and a through environmental impact assessment carried out (2).

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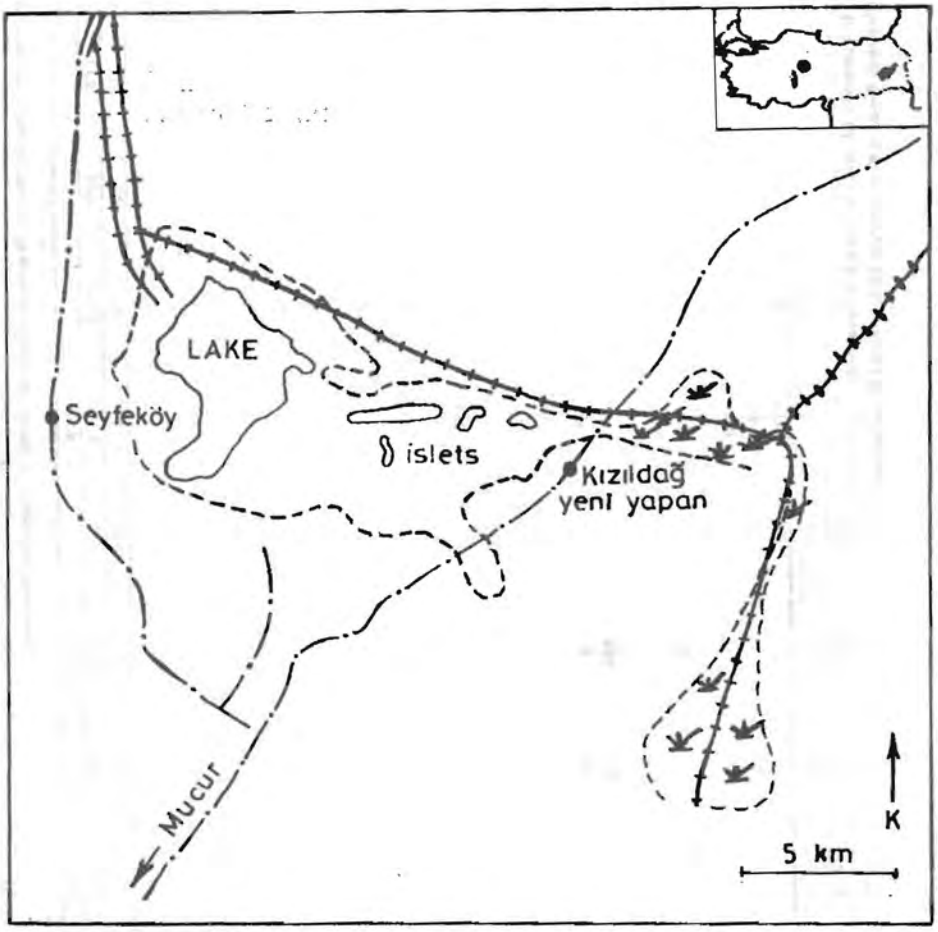

Figure 1. Study area; *(∼): permanent lake area, (- --): maximum lake area, (–·–): main roads, (⫲): main drainage canal, (+++): suggested drainage canal.*

Table 1. The List of Seyfe Lake Birds, Their Protection Status and Monthly Dispersion of Maximal Counted in a Single Day Observation

		1998						1999	PROTECTION STATUS	
		FEB.	APR.	MAY.	JULY	SEP.	OCT.	NOV	FEB	
NO	SPECIES	21	18	16	4	19	24	28	24	E. R. L.	BERN
1	*Podiceps cristatus*				3						PFS
2	***Podiceps ruficollis***				1			1			**SPFS**
3	*Ardea cinerea*								2		PFS
4	*Egretta alba*	3									SPFS
5	*Egretta garzetta*			59							SPFS
6	*Ardeola ralloides*			1							SPFS
7	***Ciconia ciconia***		3	3							**SPFS**
8	*Plegadis falcinellus*			1							SPFS
9	***Phonicopterus ruber***		741	172	1573		15	343			**SPFS**
10	*Anser anser*	>100									PFS
11	*Anser fabalis*	4									PFS
12	*Anser albifrons*		3					> 10 000	> 10 000	VU	PFS
13	***Tadorna tadorna***	6	48	8	29		4	64	3		**SPFS**
14	***Tadorna ferruginea***	5	5		3		250	101	3		**SPFS**
15	***Anas platyrhynchos***	4		4							**PFS**
16	*Anas crecca*				>100						PFS
17	*Anas strepera*	10			2		11				PFS
18	***Anas acuta***	10	12	3							**PFS**
19	***Anas querquedula***	10	15		>100						**PFS**
20	***Anas clypeata***			4	5			38	100		**PFS**
21	*Netta rufina*	10		5	> 100						PFS

*Breeding birds shows bold letter

E.R.L. : According to European Red List (VU: Vulnarable; LR:nt: Lower Ris k, Not Threatened)

BERN: According to Bern Convention (SPFS: Strictly Protected Fauna Species; PFS: Protected Fauna Species)

Table 1. (continue)

NO	SPECIES	1998 FEB. 21	APR. 18	MAY. 16	JULY 4	SEP. 19	OCT. 24	NOV 28	1999 FEB 24	PROTECTION STATUS E. R. L.	BERN
22	*Aythya ferina*		5	5							PFS
23	*Aythya fuligula*				2						PFS
24	***Aythya nyroca***				2					VU	**PFS**
											PFS
25	*Milvus migrans*							1			PFS
26	*Circus cyaneus*	1					1	2			PFS
27	*Circus macrourus*								1	LR	PFS
28	*Circus pygarcus*			1			4		1		PFS
29	***Buteo rufinus***	2		1	6	3	3				**PFS**
30	***Buteo buteo***	1		1					1		**PFS**
31	***Falco tinnunculus***		2	1	3	12	2		1		**SPFS**
32	***Falco naumanni***		5							VU	**SPFS**
33	***Gallinula chloropus***				1				1		**PFS**
34	***Fulica atra***						3	3	15		**PFS**
35	*Grus grus*		14				121				SPFS
36	*Haematopus ostralegus*			3							PFS
37	***Himantopus himantopus***		1	24	42						**SPFS**
38	***Recurvirostra avocetta***			4	12						**SPFS**
39	***Charadrius alexandrinus***		12		12						**SPFS**
40	***Vanellus vanellus***	2	4	9	5		17		1		
41	***Hoplopterus spinosus***		4	6	3		20		2		**SPFS**
42	*Tringa totanus*						1				PFS
43	*Tringa erythropus*						11				PFS
44	*Tringa nebularia*		2				4	38			PFS

Table 1 (continue)

		1998							1999	PROTECTION STATUS	
		FEB.	APR.	MAY.	JULY	SEP.	OCT.	NOV	FEB		
NO	SPECIES	21	18	16	4	19	24	28	24	E. R. L.	BERN
45	*Tringa stagnatilis*			1							SPFS
46	*Tringa hypoleucos*				3				1		SPFS
47	*Philomachus pugnax*		3								PFS
48	*Numenius arquata*			1							PFS
49	*Limosa limosa*				10						PFS
50	*Gallinago gallinago*		82								PFS
51	***Burhinus oedicnemus***				2						**SPFS**
52	*Larus melanocephalus*			2							SPFS
53	***Larus ridibundus***	> 100	50		> 1000		1				PFS
54	*Larus genei*						601				SPFS
55	***Larus fuscus***				2		10		4		
56	***Larus argentatus***		233		> 100						
57	*Chlidonias hybrida*			2							SPFS
58	***Gelochelidon nilotica***		5		> 100						**SPFS**
59	***Columba livia***	10	4		8	6	8	6	> 100		**PFS**
60	***Streptopelia decaocto***	2		2				> 100	10		PFS
61	*Streptopelia turtur*		8								PFS
62	***Athena noctua***	1	1	1	2			1			**SPFS**
63	*Apus apus*		> 100	> 100	> 100						PFS
64	***Alcedo atthis***								1		**SPFS**
65	*Upupa epops*		3	2	4						SPFS
66	*Dendrocopus medius*							1			SPFS

Table 1. (continue)

		1998						1999	PROTECTION STATUS	
		FEB.	APR.	MAY.	JULY	SEP.	OCT.	NOV	FEB		
NO	SPECIES	21	18	16	4	19	24	28	24	E. R. L.	BERN
67	*Melanocorypha calandra*						> 1000		10		SPFS
68	*Melanocorypha bimaculata*		> 100	> 100		3		25			SPFS
69	*Ammomanes cincturus*					2	2				PFS
70	*Galerida cristata*	3	> 100	> 100	> 100	> 100		> 100			PFS
71	*Lullula arborea*				4	2					PFS
72	*Alauda arvensis*		2	> 100	> 100			> 100			PFS
73	*Eremophila alpestris*			2					2		SPFS
74	*Hirundo rustica*		> 100	> 100	> 100	10					SPFS
75	*Riparia riparia*				> 1000						SPFS
76	*Delichon urbica*				> 100						SPFS
77	*Anthus spioletta*				1	2					SPFS
78	*Motacilla flava*										
	M. f. feldeg			1		3					SPFS
79	*Motacilla alba*										
	M. a. alba		1			2					SPFS
80	*Luscinia megarynchos*			1							SPFS
81	*Phoenicurus ochruros*						4				SPFS
82	*Oenanthe oenanthe*		12		4		10				SPFS
83	*Oenanthe hispanica*					1					
84	*Oenanthe isabellina*		1			10	1				SPFS
85	*Muscicapa striata*					2					SPFS
86	*Parus caeruleus*								2		SPFS
87	*Parus major*		2								SPFS
88	*Lanius collurio*		1	1							PFS
89	*Lanius minor*			2							PFS

Table 1. (continue)

		1998							1999	PROTECTION STATUS	
		FEB.	APR.	MAY.	JULY	SEP.	OCT.	NOV	FEB		
NO	SPECIES	21	18	16	4	19	24	28	24	E. R. L.	BERN
90	*Lanius excubitor*					1					PFS
91	*Garrulus glandarius*		2	3							
92	*Pica pica*	3	10	2	4	3	4	10	10		
93	*Corvus monedula*	1	2	2		10					
94	*Corvus frugilegus*								2		
95	*Corvus corone*										
	C. c. cornix	2		2							
96	*Sturnus vulgaris*	> 100	> 100		> 100	10	> 10 000	3	10		
97	*Passer domesticus*	> 100	> 100	> 100	> 100	> 1000	> 100	> 100	10		
98	*Passer montanus*	> 100		10	> 100						PFS
99	*Passer hispaniolensis*		> 100	> 100	> 100		2	1			PFS
100	*Fringilla coelebs*								10		PFS
101	*Carduelis chloris*			2							SPFS
102	*Carduelis carduelis*	2				6	10	2	1		SPFS
103	*Emberiza cia*			1							SPFS
104	*Emberiza hortulana*		1								PFS
105	*Emberiza schoeniculus*							1			SPFS
106	*Emberiza melanocephala*				> 1000						SPFS
107	*Emberiza calandra*	2	> 1000	> 1000	> 1000						PFS

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THE TEMPERATURE EFFECT ON "IN VITRO" POLLEN GERMINATION IN *BRYONIA ALBA* L. (*CUCUCRBITACEAE*)

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Summary

In this study, effects of different temperatures treated during both germination and storage period on "in vitro"pollen germination in *Bryonia alba* were investigated. Results indicated that the optimal incubation temperature for in vitro pollen germination was 25 degrees C and depending upon storage temperature in deep-freeze, germination ratio was either higher or lower than optimum ratio. As regards pollen grains stored under normal room conditions, germination ratio decreased significantly. When the same pollen grains were stored again at different temperatures in deep-freeze, their germination ratio elevated again to optimum or decreased very much.

Key Words: Temperature, pollen germination, Bryonia

Introduction

Several studies on in vitro pollen germination have been done in many of plant species so far. In many of these studies, effects of different physical and chemical factors on pollen germination were investigated. Especially on plants whose pollen germination ratio which is low, interesting treatments for increasing production have been performed (1). In these studies, it is logically suggested that one of the most important physical parameters

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which is successfully treated is temperature, as pollen tube elongation is entirely related with temperature (2,3). Furthermore, according to some authors, heat treatments were more effective than grow regulators in overcoming self incompatibility and yielded higher ratio viable seeds (4). Especially in pollen viability studies, it is claimed that the temperature have undoubtedly an important role in germination process and storage period (3). In other words, both viability and fertilisation ability of pollen grains stored under cold storage conditions increased significantly (5,6). From literatures, only palynological characteristics of *Bryonia alba* L have been investigated (pollen garins, radially symmetrical isopolar, 3-4 zonocolporate, sphaeroidal or oblate-spheroidal, Amb semiangular, microreticulate exine, and circular pores) (7). However neither in vitro pollengermination and temperature nor storage conditions for pollen grains of this species has not been known yet. Moreover in a recent studies, it was revealed that the temperature responses of pollen germination were not always congruent and varied between species, populations and often between morphs (1). In author's opinion, in different species, investigations of pollen germination are required with regard to reach to striking results. The aim of this work is therefore to investigate effects of temperature on both "in vitro" pollen germination and during storage pollen longevity in *Bryonia alba* L..

Materials and Methods

Anthers used in treatments were collected from *Bryonia alba* L. plants in Güllapoğlu Campus of University of Thrace. In order to find the most ideal basal growth medium and conditions with related to germination, pretreatments have been done. So the basal growth medium consisted of 10% sucrose, 0,1 % Boric acid, 0,6 % $CaNO_3$ and distilled water and, it was detected that pollen germination occurred in darkness, at 25 degrees C, and relative humidity 48% (this means normal room conditions). In this work, effects of temperature factor during both germination and storage on pollen germination have been tested. During treatments, all germination ratios obtained were compared with the control, whose ratio is those of anthers freshly collected from nature.

In treatments concerning "effects of germination temperature", three drops of liquid medium were placed on the slide. Fresh anthers of *B.alba* were dissected in the medium with the aid of needles under binocular microscope and then the slide with pollen was placed in petri dishes with a piece of moist paper towel. The assay dishes were put in incubator at 25 degrees C. After 2 hours, medium was stained briefly with aniline Blue 1% (8). From the slide average 1000 pollens were counted under Prior light microscope and germination ratio was calculated by percentage. The temperatures tested were 10, 15, 20, 25 (Control), 30, 35 degrees C. For every test, separate anthers (pollens) were used. Consequently, 6 tests have been done and almost totally 6000 pollen grains were counted. Germination ratios of control

and the test groups were compared using statistical analysis (9) and all values were demonstrated in table.

In investigations related to storage temperature, the methods used were modified by Johansson and Stephenson's procedure (10). So, some of anthers were placed in petri dishes with a piece of moist of paper towel and then stored in deep-freeze (air-dry); the remaining ones were kept in incubator regulated at 25 degrees C and under 48 % relative humidity (normal room conditions). During investigations which were done in deep-freeze, seven different test temperatures (-15,-25,-35,-45,-55,-65,-75 degrees C) were selected and for each temperature, different petri dishes and anthers were used. Anthers were stored in deep-freeze for one month. In the end of this storage period these were put in humidity chamber (48%) for one day. Afterwards germination experiments were done as described above and, in the same way germination ratios were calculated. Consequently seven treatments were done and almost totally 7000 pollen grains were counted. All germination ratios were compared statistically with that of the control.

Considering anthers were kept under normal room conditions, totally three germination experiments were done in the end of respectively second, fourth and sixth months of storage periods. After germination ratio decreased to minimum level, the same pollen grains were stored again at seven different test temperature in deep-freeze (11) and subsequent process for germination experiments and germination ratios were similarly performed, as described above.

Results

A. Effects of temperature applied during germination on "in vitro" pollen germination

The highest germination ratio (35.6) were observed in treatments at 25 degrees C. As regards in all other treatments, it is detected that germination ratios were lower (Table 1). Comparing data of control and all tests in terms of statistic analysis, it is shown that all "t" values were significant, as shown in Table 1.

Table 1: Germination ratios observed at different temperatures during germination and statistical values*

Temperature (°C)	Germination ratio (%)	t values / $P_{(0,05)}$
10	14.6	-9.13 <
15	14.26	-9.56 <
20	12.86	-10.4 <
25 (Control)	35.6	----
30	29.3	-2.8 <
35	36.2	-3.75 <

* t values, <: significant; >, not significant

B. Effects of storage temperature on "in vitro" pollen germination.

1. Effects of storage temperature applied in deep-freeze on "in vitro" pollen germination

The germination ratios of pollen grains stored at –25, -35, –45 and -75 degrees C are approximately equal to or higher than that of control. Especially pollen grains stored at –25 and –35 degrees C have significantly higher germination ratios; as regards ones stored at –15, -55, -65 degrees C of which germination ratios is also statistically lower, as was shown in Table2.

2. Effect of storage temperature on "in vitro"germination of pollen grains stored firstly under normal room conditions and then in deep-freeze.

The pollen grains stored under normal room conditions whose germination ratio decreased significantly, particularly in the end of sixth months (Table 3).

Table 2: Effects of different storage temperatures applied in deep-freeze on the germination ratio and statistical values*

Storage Temperature (°C)	Germination Ratio (%)	T values / $P_{(0,05)}$
Control	35.60	---
-15	11.81	-12.5 <
-25	46.50	0.32 <
-35	52.47	5.5 <
-45	38.75	1.071 >
-55	22.24	-6.086 <
-65	24.27	-4.858 <
-75	33.30	-1.016 >

* t values, <: significant; >, not significant

Table 3: Pollens stored under normal room conditions, of which germination ratios in the end of different periods and statistical values*

Storage temperature (25°C) / time	Germ. ratio (%)	t values / $P_{(0,05)}$
Control	35.6	-----
25°C / 2th Month	34.2	-0.689 >
25°C / 4th Month	22.8	-0.501 >
25°C / 6th Month	15.4	-8.86 <

* t values, <: significant; >, not significant

When the same pollen grains were stored at –45 degrees C, their germination ratio were higher even than that of the control. In the remaining ones stored at other temperatures, germination ratios increased relatively (-55 and –75 degrees C) or not (-25 degrees C) (Table 4). On the other hand, germination ratios of the pollen grains stored at –15 and –35 degrees C decreased reasonably. Comparing germination ratios of control and these test groups, only t value of test at -45 degrees C is not significant, whereas all other ones are significant (Table 4).

Table 4 : Changes in germination ratios of pollens stored firstly under normal room conditions depending upon storage temperature in deep-freeze and statistical values*

Storage Temperature (°C)	Germination Ratio (%)	t values / $P_{(0,05)}$
Control	35.6	----
-15	9.65	-10.88 <
-25	17.19	-8.189 <
-35	9.75	-11.818 <
-45	40.47	1.408 >
-55	28.83	-2.86 <
-65	16.00	-8.289 <
-75	25.71	-3.367 <

* t values, <: significant; >, not significant

Discussion

According to the present results, it was proved statistically that temperatures applied during germination affected certainly pollen germination. Therefore it was observed that the optimal incubation temperature for in vitro pollen germination was 25 degrees C and germination decreased significantly at all other higher or lower temperatures. From literatures, similar results were claimed by other researchers (12-13). In the light of the present and recent results, it is fairly clear that the most convenient temperatures for in vitro pollen germination were 20-25 degrees C and, in general at higher temperatures germination decreased sharply (13-15).

In view of storage temperature, it was shown that temperatures applied affected also pollen germination. Germination of pollen grains stored constantly at –25 and –35 degrees C increased clearly. When all the remaining temperatures were tested, germination ratios were either approximately as that of the control one or decreased. These results obtained were

either approximately as that of the control one or decreased. These results obtained were similar to the following studies reported. In one of these studies, it was suggested that when *Hydrangea* L. pollen grains were stored at – 20 degrees C of which over 36% were germinated (16). Furthermore, in another study, the author demonstrated that cold storage maintained germinability at about 50% for up to 12 months at –20 degrees C (17). In contrast, as noted in the present results, as long as pollen grains stored under normal room conditions germination ability decreased continuously. In the end of 6 months of the storage period, germination ratio of so-called pollen grains was lower as half of those fresh anthers. When the same pollen grains were stored in deep-freeze, depending on storage temperature striking changes were occurred in their germination ratios. Especially in treatments at –45 degrees C germination ratio was higher even than that of the control. These results were supported by works of Inagaki et al. (11). According to these authors, pollen grain dried to 6 % water content showed normal germination frequency the freezing process at -196 and -80 degrees C for 5-6 months. Consequently both present results and recent explanations support the idea that the principal effect of cold storage was to impair pollen viability and, pollen grains stored at room temperature lost viability (10,18).

Buitink et all., in their own work, explained storage temperature and germination relations (19). According to these authors, storage stability is very important, since molecular mobility was found to be inversely correlated with storage stability. With decreasing water content, the molecular mobility reached a minimum, and increased again at very low water content. Minimum mobility and maximum storage stability occurred at a similar water content. This correlation suggest that storage stability might be at least partially controlled by molecular mobility. Furthermore they mentioned that consistent with changes in isotherms with temperature and critical humidity for storage, optimum water contents increased with decreasing temperature (20). In the light of these explanations, it is not astonishing that pollen grains of which germinability decreased relatively if stored at critical temperature in deep-freeze, they reached to optimum viability again, as shown in this study.

In a similar study, researchers tested six different constant temperatures from -5 to -45 degrees C to quantify the effect of air-dry storage environment on pollen longevity, as in the present work (21). As a result, their findings suggested that there is a negative logarithmic relation between longevity and pollen moisture content (% wet basis) and a curvilinear semilogarithmic relation between longevity and temperature. Considering tables in this work, the present findings also appear to be paralleled by the results of these studies. Consequently, very low or extreme storage temperatures are always not suitable; in contrast, sometimes they affected negatively pollen germinability. In addition an important result is that the germination ratios of pollen grains stored constantly in deep-freeze and, pollen grains stored firstly under room conditions and then in deep-freeze are reasonably different. To illustrate this, although pollen grains stored constantly at -35 degrees C of which germination ratio is highest, that of the other group is lowest. So, with changes in storage temperatures response

of the latter group to germination is much variable than the former one. Therefore, it was detected that storage conditions are critical factors for pollen germinability.

In the light of these explanations and the present results, the temperature factor plays an important role in germination process (3,22). Furthermore, impairment of pollen and anther development and timing of pollination by elevated temperature will be an important contributing factor to decrease fruit set in all crops and wild plants with global warming and meteorological variables (23-25). In addition it was proved that at least some of the effects of high temperature had a genetic basis (10). Therefore the relations between pollen and temperature is also very important for losses in yearly agricultural production. From literatures, only in *Cucurbita pepo* L. belonging to Cucurbitaceae family, such a work have been done, but not yet in *Bryonia alba* L. of the same family (10). However it is possible to make different studies and reach striking results, since the pollens are easily obtained and germinated, also because of suitable storage conditions (temperature and humidity). Furthermore as was noted by Lyakh et al., such an approach may also be successfully applied in plant breeding (5). In further studies, the relations between temperature and pollen germination will be investigated with some biochemical treatments.

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BIOBLEACHING OF SEKA-DALAMAN SOFTWOOD KRAFT PULP BY WHITE-ROT FUNGUS *Poliporus versicolor*

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Abstract

Three fungus (*Pleurotus sajur-caju* , *Phanerochaete chrysosporium*, *Poliporus versicolor*) were tested for their ability to bleach softwood kraft pulp under aerobic, agitated conditions. Among these white-rot fungus *Poliporus versicolor* decreased the kappa number from 38.55 to 19.42 and increased brightness from 28 to 32.7 in 15-day treatment.

Key Words: White-rot fungi, Biobleaching, Softwood kraft pulp

1. Introduction

Paper consumption per person is one of the development level criterios of a country. Paper consumption level has been increasing due to modernization and paper demand in printing and cleaning industry in Turkey. Production of high quality white paper has been producing by using chemical bleaching process which require the use of large amount of chemicals like chlorine. Waste of paper plants include large amount of ligninase treated by chlorine is dispose to the environment (1,2). Main part of organic materials in bleaching liquor has weight of high molecular structure. The structure of these compounds are fenolic compounds in type of guaiacol and catechol which are dangerous for the environment (3).

* Bu çalışma Aysun Ergene'nin doktora tezinin bir bölümüdür.

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The use of chlorine in the paper plants has increased due to the demand in high quality white paper. In the same time the sensitivity of the Turkish society to environmental issues has also increased.. This environmental sensitivity is suggesting the use of less chemicals in paper and pulp plants. Although paper and pulp industry invested significant amount of technology, energy and money in reducing the negative effects of chemical waste the effects of paper plants to the environment is still debatable issue.

2. Materials and Methods

A- Microorganisms

Pleurotus sajur-caju (Weisdranweg 4, 3300 Braunschweing, Federal Republic of Germany), *Phanerochaete chrysosporium* ME 446 (US.Dept. of Agriculture Forest Products Lab., Madison, Wisconsin 53705,U.S.A.) and *Poliporus versicolor* (İnönü University, Dept of Biology, Malatya, Turkey) were used in this study. The fungi were maintained on malt dextrose agar (Difco Laboratories) slants(4-6).

B- Pulps

Softwood kraft pulp, produced from pine and spruce, was obtained from SEKA-Dalaman pulp mills. The pulp was manufactured by various conventional or extended cooking methods.

C- Media and cultivation

Mycelia were grown by inoculating 10^8 (1 ml) conidia in 250 mL Erlenmeyer flasks containing 50 ml mycological broth (%1 soytone, %4 D-glucose and % 0.5 cellulose pulp) inoculated flasks were incubated for 5 days at 30 °C with shaking at 100 rpm. After incubation the biomass transferred to second step medium. This medium prepared in 250 ml Erlenmeyer flasks containing 100 ml mycological broth (% 0.2 soytone, %4 D-glucose) and 1:1: 2 cm 3 polyurethane foam for immobilization. inoculated flasks were incubated for 5 days at 30 °C with shaking at 100 rpm. Immobilize fungi on the polyurethane foam inoculated in the 250 ml Erlenmeyer flasks containing 10 ml mycological broth and % 2 unbleached cellulose pulp after five days. Inoculated flasks were incubated for 20 days at 30 °C with shaking at 100 rpm (7).

D- Kappa, Viscosity and Brightness Assays

Kappa number was determined by Micro Kappa Number Method(1). Viscosity was determined TAPPI Official Test Method T 230 and brightness was measured at 457 nm with an Elreho instrument (8,9).

3. Results and Discussion

Unbleached cellulose kraft pulp was inoculated with three different fungi *(Pol. versicolor, Pl. sajur-caju, P. chrysosporium*) and incubated at 30 C for 20 days. During an initial five-day treatment with *Pol. versicolor*, the kappa number of pulp decreased 10.29 point, from 38.55 to 28.26. During the subsequent 15-day treatment kappa number decreased an additional 8.84 point as seen in fig.1.

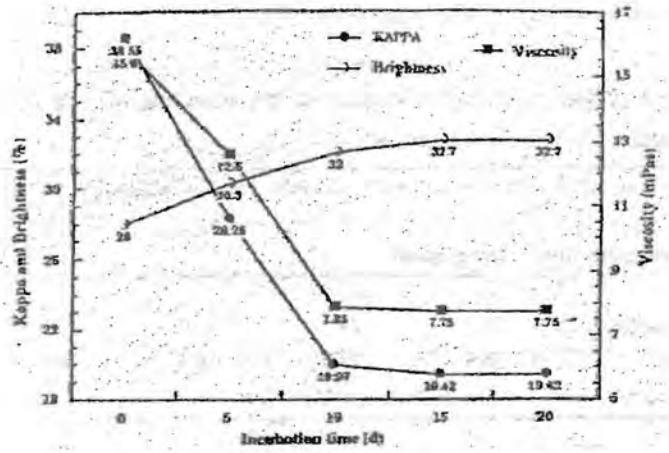

Figure 1. Daily changes of kappa, brightness and viscosity of cellulose kraft pulp which incubated with *Pol. versicolor* after alkaline extraction.

Table 1. The effect of three fungal strains on brightness, kappa and viscosity in unbleached softwood kraft pulp.

	Control (pulp only)	*Poliporus versicolor*	*Phanerochaete chrysosporium*	*Pleurotus sajor-caju*
		Before1% alkaline extraction		
Brightness, % ISO	28	30	30.6	31
Kappa number	38.55	29.99	30.3	28.6
Viscosity,mPaS	15	12.4	13.2	12.4
		After 1% alkaline extraction		
Brightness, % ISO	30.3	32.7	32.3	33
Kappa number	37.34	19.42	21.97	15.06
Viscosity,mPaS	12.6	7.75	7.35	5.75

Samples of unbleached softwood kraft pulp at 2% consistency were first sterilized, second inoculated with each of the three different fungi and than incubated for 15 days with constant agitation. The resulting mixtures of pulp and fungi were homogenized and tested for kappa, viscosity and brightness (Table 1). The *Poliporus versicolor* -treated pulp showed increase in

brightness. The increased brightness with *Poliporus versicolor* was acompanied by a decrease in kappa number. Pulp-fungus contact can efficiently remove and decolorize lignin from kraft pulp (10). However, the viscosity of the pulp decreased appreciably, indicating that cellulose attack had also occurred. Tran and Chambers reported (11) that *P. chrysosporium* can reduce the kappa number of kraft pulp when combined with alkaline extraction. Extraction of the pulps with 1% NaOH following fungal treatment gave increased brightness and kappa number for all fungi. The pulp treated with *Pl. sajor-caju* showed a slighly decreased kappa number after alkaline extraction.

Table 2. Effect of glucose concentration the bleaching of pulp by 15 day treatment with *Poliporus versicolor*

Glucose conc., mM	0 (no glucose)	10	30	50
Brightness, % ISO	29.1	44	40.7	43.4
Kappa	26.8	23.35	18.6	16.6

Different concentrations of glucose were added to the medium. After 15 days treatment, a significant decrease in kappa number was found at higher glucose concentrations (Table 2).

Table 3. Effect of various buffer (0.1M)on the bleaching of pulp by 15 day treatment with *Poliporus versicolor*

	Control (unbufferred)	Dimethyl-succinate	Citrate	Acetate
Brightness, % ISO	29.1	45.9	30.2	31
Kappa	26.8	14.88	25.55	22.79
Final pH, after bleaching	4.6	4.8	5.2	5.1

Various buffers at 4.8 and 0.1 M were added to the pulp in place of water. Dimethylsuccinate buffered pulps was brighter than the unbuffered control after 15-day (Table 3). The rate and extent of kraft pulp bleaching with *Poliporus versicolor* depend on glucose concentration. Glucose addition affects the extent of bleaching.

4. Conclusions

This results show that there is some potential for bleaching SEKA- Dalaman softwood kraft pulp with fungi as a biological process. Combined with conventional chemical bleaching, biobleaching methods can cause less chlorine consumption and also less treat to the environment. This study also may bring chlorine free bleaching for papers.

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THE EFFECTS OF GIBBERELLIC ACID ON DEVELOPMENT OF *Agaricus bisporus* (Lange) Sing.

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Abstract

In this study, the effects of gibberellic acid (GA_3) which was added to the mushroom compost and casing soil were determined on vegetative development, on fruiting initiation and the yield of *Agaricus bisporus* (Lange) Sing.. Addition of GA_3 on both casing and unattended compost soil resulted in a stimulant increase on the initiation of basidiocarps. GA_3 caused to increase the number of basidiocarps and the quantity of harvested product significantly when added to the compost, casing soil and during basidiocarp stage.

Key Words: Gibberellic acid, *Agaricus bisporus* fructifications.

1.Introduction:

Development of the commercial mushroom *Agaricus bisporus* (Lange) Sing. industry, large-scale production and consumption have been taken into consideration for the

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last several decade. Much work has centred on the best strategy of fruiting likewise the economic viability of cultivation systems, type of production technology, microbial and biochemical processes for compost, breeding programmes in addition to pests and diseases of *Agaricus bisporus*. Despite the high commercial value of the mushroom crop, fundamental studies of the physiology and biochemistry of the mushroom are still at an early stage compared with the other crops (1). The effects of growth regulators from seedling to reproductive stage have been studied widely in higher plants; but the information about their effect on *Agaricus*, is meagre and scanty (2). Effect of gibberellin on the mushroom field was detected by Aleksandrov (3). In his preliminary work, author reported that GA_3 has increased the effect on growth and yield of mushroom. Gibberellins were also tested for their effect on growth of *Lentinus edodes* (4-7), *Pholiota destruens* (2,8) and *Calvatia gigantea* (9). Growth promoting substances, in general promote the growth and increase the yield in mushroom (10). Kaur and Lakhanpal (6) who reported that gibberellic acid (20-40 ppm) was the best growth regulator for mycelial growth of *Lentinus edodes*. The effect of growth regulators on sporophore initials of *Lentinus edodes* growing on oak logs was reported by Shukla (7) who showed that treatment with 5 ppm Indol-3-asetic acid (IAA) gave the highest yield of 331.6 g/log, as compared to 126.3 g/log for the control. Similarly 5 ppm Indol butyric acid (IBA) application also resulted in the best sporophore formation (41.3 sporophores/log, as compared to 12.0 sporophores/log for the control. The effect of GA_3 on the growth of *Lentinus edodes* sporophores was reported also by Tan and Chang (5).They have shown that with GA_3, superior mycelial development was obtained compared to the control group. Han et al. (4) reported that immersion of full-grown bags of *Lentinus edodes* in 5 ppm IAA and 10 ppm GA_3 have indicated to produce the highest number of fruit bodies. Chen-Hsioh (10) also studied the effect of growth promoting substances on fruit body development and found that growth-promoting substances promote the growth and increase the yield of mushroom.

This paper reports the effects of gibberellic acid at a concentration of 20 ppm applied to the compost ,casing soil and fructification of *Agaricus bisporus*.

2.Material and Methods

A-Fungal Strain: A tested commercial strain of *Agaricus bisporus* var. U1 was selected in the spawn laboratory of İnelli Mushroom Crop. in İstanbul-Turkey. For preparation of spawn wheat grains were used, during spawning the age of the main culture was 5 days and then they were stored +4 °C.

B-Cultural Conditions Pasteurised, cooled synthetic compost that consist of wheat straw, chicken manure , urea and gypsum was obtained from commercial grower and used as a substrate in which the fungus spawned. Compost was prepared by the method (11) which is widely used in Central Anatolia and formulated as 1.9 % N, 68-70 % moisture content and pH=7.2. For spawn-running, 1 kg of spawned compost was placed in each clear plastic bag (15cm Ø). The plastic bags prepared in this way were kept in a growth room from spawning to casing. The growing conditions were maintained at 24-25 °C, 90-95 % relative humidity

and dark. At the 16th days of vegetative growth, without ventilation, 3 replicates each containing 10 bags, from healthy fully spawn were prepared for control and for each treatment. Then the bags were opened and 2.5 cm thick pasteurised casing soil was spread on the spawn inoculated compost. After surrounding of the casing soil with mycelium, the temperature of the growth room was lowered to 16 °C and full ventilation was supplied until basidiocarp formation.Control of the conditions in the growth room was made by means of a single computer-based system. Throughout the developmental stage; control of air, compost temperature, relative humidity and CO_2 level were performed under indirect light. Initiation of the basidiocarps began 7-10 days after casing. The mature basidiocarps of the same size (5cm Ø) were collected at the same time. After the harvest mushrooms were trimmed following normal commercial practices. Fresh weight yields (g) were determined for each group.

C- GA_3 Application: Gibberellic acid (GA_3) at concentration of 20 ppm was applied as spray and the application for various groups was shown in Table 1.

Table 1. GA_3 application for various experimental groups of *Agaricus bisporus*.

Groups	Compost*	Casing Soil	Basidiocarps **
A	+ +	+	+ +
B	+ +	+	- -
C	+ -	-	- -
D (control group)	- -	-	- -

\+ = numbers of GA_3 applications

\- = no application

* = The interval between the applications of GA_3 was 3 days

** = The interval between the applications of GA_3 was 7 days

D- Statistical analysis: In the statistical analysis the variance analysis were made and the data were calculated as least significant differences (LSD) 5%. The mean and the standard deviation of the quantity and the numbers of fructifications were estimated in the growth period.

3.Results: In the groups of A,B and C all containing GA_3 in the compost, a rapid mycelial development was observed comparing to the control group (D). Similarly, in the groups of A and B, having GA_3 both in compost and casing soil more mycelial density was observed comparing to the control group. In the group of A which contains GA_3 in compost, casing soil and basidiocarps, a certain improvement was found in the quantity of total production and the number of mature fructification in comparison to control group (D). The results related to GA_3 application were summarised in Table 2.

Table 2. The effects of GA_3 sprayed at different developmental stages.

GA_3 applications	Results
a- In compost	Rapid mycel development
b-In compost and casing soil	Density in mycel colonisation
c- In compost, casing soil and basidiocarps	Early development, rapid growth, rich product

The quantity of total product and the number of fructifications during the harvest were shown in Fig. 1 and Fig. 2.

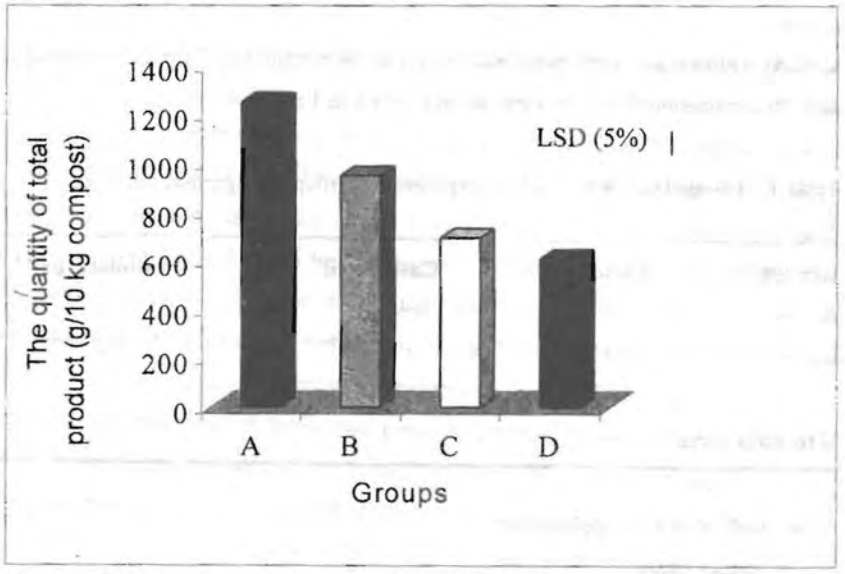

Figure 1. The quantity of total product in survey groups

Fig. 1 shows that total quantity of products are 1250 g, 948 g, 694 g and 607 g in the groups of A,B,C and D (control) respectively. It is clearly seen that the maximum product is obtained in the group of A in which GA_3 applied to the compost, casing soil and as well as at the basidiocarp stage. On the contrary, the least product is obtained in the control group which had no GA_3 treatment.

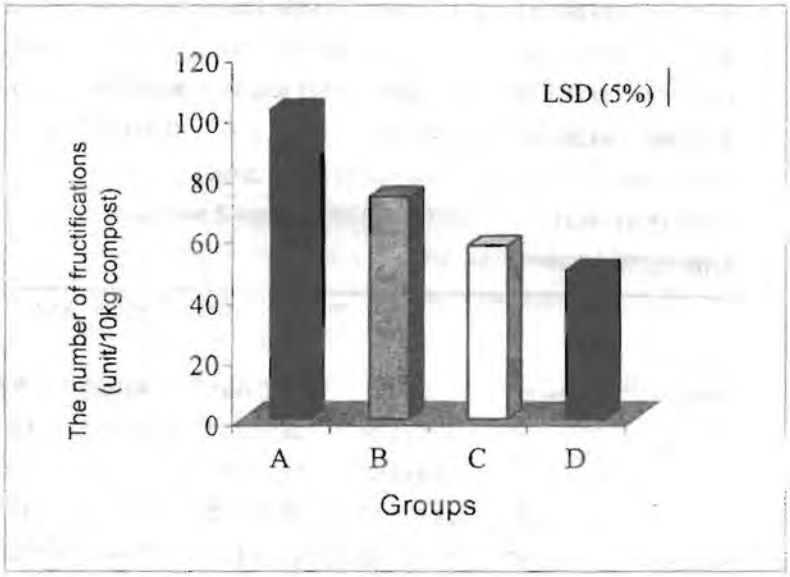

Figure 2. The number of fructifications in survey groups

Fig. 2 also shows the number of total fructification throughout the growth period. The maximum number of fructification is obtained in the group of A. In the experimental groups the max. number of fructifications were found as 102, 73, 57 and 49 for group A, B, C and D respectively. In the groups of B and C although the numbers of fructifications were lower than the group A but were still higher than the control group .During the growth period, the yield in GA_3 treated groups (A,B,C) were harvested in 5 flushes whereas in control group the mushroom collection was completed only in 4 flushes. The interval between the flushes were 7 days. Results of the product quantity and the number of fructification of each flush period were shown in Table 3.

Table 3. The mushroom quantity in each flush and the number of fructification of survey groups

Quantity (g)

Groups	1.flush*	2.flush*	3.flush*	4.flush*	5.flush*	Total**
A	137.6±0.57	534.5±1.00	394.9±1.00	142.3±1.00	40.2±1.00	1250.4±1.00
B	112.4±1.00	76.5±1.52	307.3±1.00	131.3±0.57	20.6±0.57	947.8±1.52
C	82.6±1.00	298.3±0.57	215.4±0.57	90.2±1.00	8.3±0.57	693.6±1.00
D (cont)	64.9±1.00	272.8±1.00	188.2±1.00	81.3±0.57	0.00±0.00	607.3±1.00

LSD* (5%) =0.31

LSD(5%) =2.39**

Number of fructifications (unit)

Groups	1.flush*	2.flush*	3.flush*	4.flush*	5.flush*	Total**
A	21.3±0.57	40.1±1.00	28.6±0.57	8.6±0.57	3.2±0.57	102.4±1.00
B	10.3±0.57	29.2±0.57	25.3±1.00	6.9±1.00	2.3±0.57	72.7±1.00
C	7.4±0.57	24.3±0.57	20.4±0.57	5.3±0.5	1.0±0.57	57.4±1.00
D (cont)	5.6±0.57	22.2±1.00	16.7±1.15	3.6±0.57	0.00±0.00	49.4±1.00

LSD* (5%) =1.63

LSD (5%) =2.27**

4.Discussion: In this study, the effect of gibberellic acid applied at different growth stages to the cultivated mushroom (*Agaricus bisporus*) development was investigated. These effects can be summarised as early development, accelerating fructification development and increasing the number of fructifications. The number of fructifications increased up to 201 %, 149 % and 116 % in the groups of A,B,C respectively in comparison to the control group. The quantity of product is also increased up to 206 %, 156 % and 114 % in the groups of A,B,C as compared to the control group which had no GA_3 treatment.

Gibberellins may have a role in the growth of a *Agaricus bisporus* sporophores was suggested by Aleksandrov (3) who showed that the yield was increased to 236 % and 374 % by applications of GA_3 to sporophores at 5 and 10 ppm concentrations respectively. Our data are somewhat supported by Aleksandrov, although the percentage of increase in our results are lower than his results. This may well be due to the concentration effect.

The positive effect of various hormones on mushroom growth and development has attracted the attention of several investigators.

All these results mentioned above, support directly or indirectly our results obtained with *Agaricus bisporus*.

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EFFECT OF L-ASCORBATE / Cu (II) ON GROWTH OF *Salmonella typhimurium* and *Escherichia coli**

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Abstract

We studied the antibacterial effect of L-ascorbate (L-AsA) / Cu(II) on Gram negative bacteria, *S. typhimurium* and *E. Coli*. Mechanism of antiviral effect of L-AsA / Cu(II) was also established. Effect of different concentrations of AsA+$CuCl_2$ on growth of *E. coli* and *S.typhimurium* were investigated. It was found that inhibitory concentration for these bacteria was 8 mM AsA + 100 μM $CuCl_2$.

Key words : *S. typhimurium, E. coli,* Oxidation products of L-AsA ; Cell wall; Cell membran

Introduction

Antiviral (1-5) and antitumoral (6) effects of L-AsA has been established. In this study we investigated the antibacterial effect of AsACu(II). It has been shown that various and effective oxidation products of AsA occured in the presence of Cu(II) (7).

These oxidation products including free radicals cause breaks in proteins and nucleic acids (8,9). AsA/Cu caused damage on bacterial cell membrane and cell wall. Therefore *E.coli* and *S.thphimurium* were applied to Aqueus Two-Phase System as a whole cell , to determine the changes occured in bacterial cell wall.

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* Bu çalışma Sema Tan'ın doktora tezinin bir bölümüdür.

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Materials and Methods

A- Chemicals, Organisms and Culture Conditions

AsA (Merck) was dissolved in distilled water and sterilized by passing milipore filter. $CuCl_2$ (Merck) stock solution was autoclaved at 121°C for 120 minutes.

E.coli (ATCC 25822) was supplied from Hacettepe Univ. Medical School, Dept. of Microbiology and *S. typhimurium* (Kauff Orij. 209,18.2.1971) was supplied from Institute of Hıfzısıhha (Ankara, Turkey). Bacterial strains were grown on Nutrient Agar plates. They were stored at 4°C and passaged monthly.

Nurtient Broth (NB) was used for overnigt culture. It was autoclaved at 121°C for 120 minutes. Bacterial growth followed by spectrophotometrically at 400 nm.

Overnight culture was prepared by growing bacterial cells in NB at 37°C by shaking 100 rpm. and 8 mM AsA + 100μM $CuCl_2$ were added in the growth media (NB) to show vtheir antibacterial effects.

B- Preparation of Bacterial Membranes

Osmotically sensitive cells (spheroplast) were repared. Spheroplasting of cells by Lysozyme-EDTA (10,11) was performed as follows. Cells were harvested at late logaritmic growth phase (2900xg, 10min, 4°C) and washed once in 10 ml of Tris-HCL buffer containing 20% (w/v) sucrose, pH 8,0 warmed to 37°C. A freshly prepared solution of 60 μg/ml. of lysozyme in 100 mM EDTA was then diluted 10 fold into the suspension of cells at 37°C.

The extend of spheroplasting as a function of time was followed by phase-contrast microscopy and by monitoring susceptibility to celşl lysis as indicated by absorbance at 400 nm. for 1:30 dilutions of cell suspensions into distilled water. After 10 min. of incubation at 37°C, less than 5% of ceels were rods.After an additional 10 min. of incubation the spheroplasting was judged complete. Spheroplassts were centrifugated (2900xg, 10min.).

Cold 10 mM Tris-HCl, 1mM EDTA were added on cell pellet and vortexed. This suspension was added slowly in a 4 fold distilled water inorder to lyse the spheroplast. After lysis, stirred magnetically at 0-2°C for 10 min. and microfuged (2400xg, 0-2°C, 10 min.). Supernatant centrifuged at 50.000xg for 2 hours to get membranes.

The bacterial membranes stored in 30mM Tris-HCl buffer at -20°C.

C-Aqueous Two-Phase Systems

1- Preparation of Polimer Solutions

PEG 8000 and Dextran T-500 were obtained from Sigma. Stock solutions were prepared as Albertsson's (12). Stock solutions of PEG 8000 40% (W/W) and Dextran T-500 20% (W/W) were prepared and sterilized at 110°C for 15 minutes seperately from the growth media. These stock solutions of polymers stored three weeks mostly at 4°C (12).

2- Preparation of Aqueous Two-Phase System

5% Dextran T-500 and 4% PEG 8000 were mixed and settled at 25°C for 1 hour for phase seperation (13).

3- Applying Cells to Aqueous Two Phase System

S. typhimurium and *E. coli* grew in NB till stationary phase and were added to Aqueous Two-Phase Systems as a control. Bacterial cells grew in 8 mM AsA+ 100 μM $CuCl_2$ containing media till stationary phase and were also added to Aqueous Two-Phase System. After adding the bacterial cells to this system, phase system was mixed and settled at 25°C for few hours in order to partitioning of cells.

4- SDS-PAGE

Membranes isoleted from *E.coli* and *S. typhimurium* (control and grown on AsA+$CuCl_2$ media) were applied on SDS-PAGE 10% to show the changes occured in membrane proteins (10,14,15). 60μl membrane sample was put in each well. Samples were boiled for 3 minutes before put on the gel. Runing buffer was composed of Tris-Glisin pH 8.0 .Electrophoresis were run at 220 V for 2 hours. Silver staining method was used to stain protein bands on the gel (16,17).

Results and Discussion

Various concentrations of AsA(1-10 mM) and $CuCl_2$ (50-100μM) were tried to find subtoxic concentration that affect bacterial growth. The effective concentrations determined as 8 mM AsA+100 μM $CuCl_2$ for *E. coli* and *S. typhimurium* (Figure 1, Figure 2). Viable cell counts were also made (Table 1, Table 2).

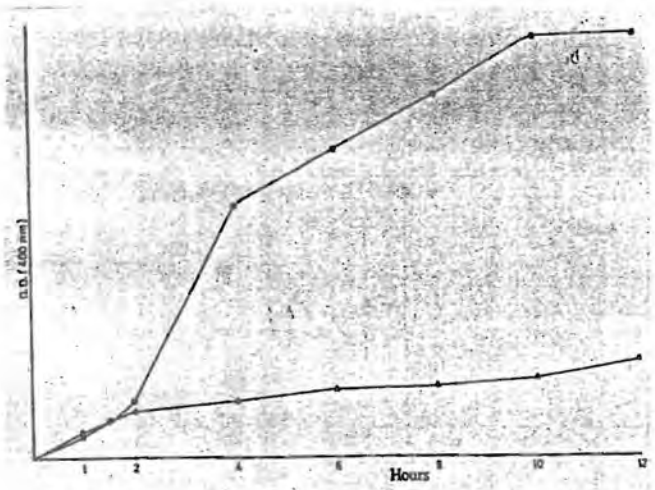

Figure 1. ● Growth curve of *S. typhimurium* (Control)
▲ Growth curve of *S. typhimurium* in the presence of 100 μM $CuCl_2$ + 8 mM AsA
(Values are the average of three experiments)

Table1. Viable cell counts of *S. typhimurium* ($x10^5$)

(Values are the average of three experiments)

Hours	Control	100μM $CuCl_2$+ 8 mM AsA
1	76	12
2	21	18
4	5351	29
6	5995	102
8	9048	462
10	14269	474
12	14280	494

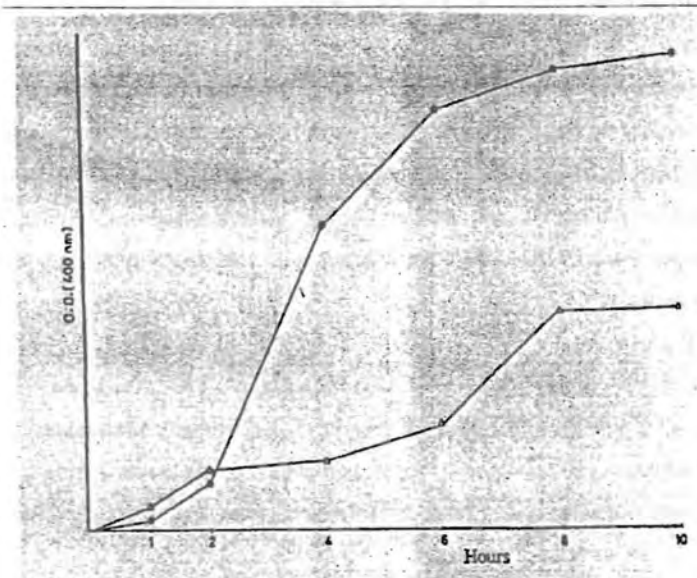

Figure 2. • Growth curve of *E. coli* (Control)
Δ Growth curve of *E. coli* in the presence of 100 μM $CuCl_2$ + 8 mM AsA
(Values are the average of three experiments)

Table 2. Viable ceel counts of *E. coli* ($x10^5$)

(Values are the average of three experiments)

Hours	Control	100μM $CuCl_2$ + 8 mM AsA
1	51	10
2	154	20
4	5459	30
6	6944	137
8	9754	1712
10	13.491	1800
12	20.076	2100

It was reported that bacterial cells partitioned at different phases of Aqueous Two-Phase System depending on the structure of cell walls(18).

We applied *S. typhimurium* and *E.coli* cells on Aqueus Two-Phase System composed of 4% PEG 8000 and 5%Dextran T-500.

We found that control cells grown in NB partitioned at PEG phase and cells grown in AsA/Cu (II) media took place at Dextran phase (Figure 3).

Bacterial membranes were isolated by Lysozyme-EDTA Method to show the effects of AsA/Cu(II) on membranes. The membranes were applied on SDS-PAGE (slab gel) to observe the changes on membrane proteins (10,14). Silver Staining Method was used. We found that some of the soluble protein bands lost in bacterial membranes isolated from bacteria grown in AsA/Cu (II) containing media (Figure 4).

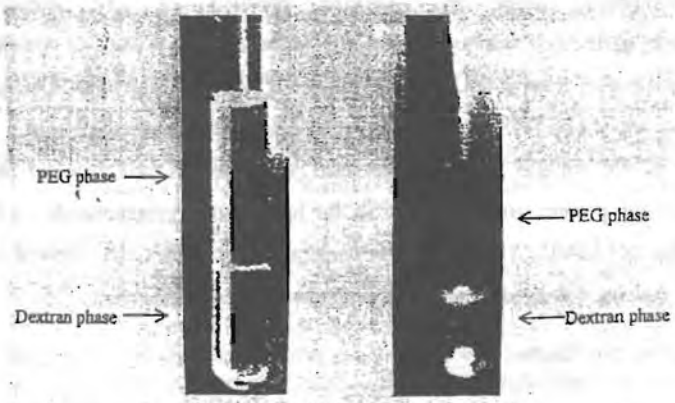

Figure 3 . Partition of *S. typhimurium* (grown in AsA + $CuCl_2$ and control media) in Aqueous Two-Phase System.
Same partition was observed with *E. coli* too.

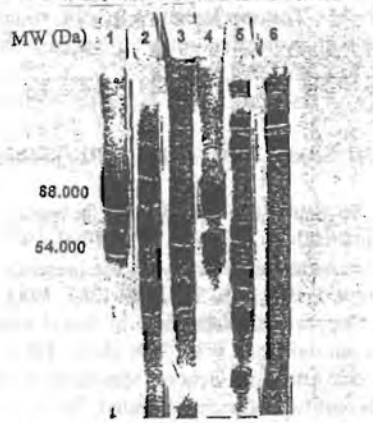

Figure 4 . Gel profile of membrane proteins on 10 % SDS-PAGE gel.
1. Marker proteins
Bovin serum albumin (MW 68.000), Lipase (MW 54.000)
2. *S. typhimurium* (Control)
3. *S. typhimurium* (Medium with AsA + $CuCl_2$)
4. Marker proteins
Bovin serum albumin (MW 68.000), Lipase (MW 54.000)
5. *E. coli* (Control)
6. *E coli* (Medium with AsA + $CuCl_2$)

We investigated the antibacterial effect of AsA/Cu (II) in this study. It has been known that various and effective oxidation products of AsA occur in presence of Cu(II) (19). Therefore we chose $CuCl_2$ in our study.

The subtoxic concentration that affect growth of *E.coli* and *S. tyhphimurium* were established as 8 mM AsA+ 100 μM $CuCl_2$. It has been known that oxidation products of $CuCl_2$ cause breaks in proteins and nucleic acids (8,9). They also leads lipid peroxidation (20-22). We showed the changes caused by oxidation products of AsA on the cell wall in Aqueous Two-Phase System as a whole cell. Control cells having intactlipopolysaccharide layer partitioned in PEG phase. The cells grown in AsA+$CuCl_2$ medium took place in Dextran phase

Lipid peroxidation caused by oxidation products of AsA damaged lipopolysaccharide layer of the cell wall of the bacteria grown in AsA+ $CuCl_2$ containing media. We isolated membranes from control cells and the cells grown in AsA+ $CuCl_2$ media These membranes were applied on SDS-PAGE to show the changes on membrane proteins.

That some of the soluble membrane proteins were lost. It was reported that AsA/Cu(II) cause breaks in protein structure (8,9). So, the loss of some protein bands can be attributed to this effect of AsA/Cu (II) .It can be concluded that AsA/Cu(II) showed its antibacterial effect by causing damage in bacterial membranes and cell wall.

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[1]ULTRASTRUCTURAL STUDIES ON STEM AND LEAF OF ORCHIS ANATOLICA BOISS. AND CYCLAMEN HEDERIFOLIUM AITON. GROWING AT DIFFERENT ALTITUDES IN WEST ANATOLIA

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Abstract:

In this research, structural changes growing naturally at different altitudes in *Orchis anatolica* Boiss. and *Cyclamen hederifolium* Aiton. in West Anatolia have been studied. Results showed that the paranchymatic cell walls of stem and leaves of both *Orchis anatolica* Boiss. and *Cyclamen hederifolum* Aiton. in the high altitude samples are thicker, the osmiophilic granulles in the chloroplasts of the stem cortex cells are more in number and there are small vacuoles in stroma. The endoplasmic reticulum membranes in the stem and leaves cells of *Orchis*'s are generally in the form of circles within one another in the high altitude samples whereas they exist as small fibrous substances in the samples growing at lower altitudes. The mitochondria of the latter samples in *Orchis* and *Cyclamen* are rounded in the mesophyll cells whereas in the former samples they're bigger and fibrous in structure.

Key Words: *Orchis anatolica*, *Cyclamen hederifolium*, Ultrastructure.

Introduction

Our country has a great wealth of plants of economical value. However, these plant species are about to vanish because these are uncontrollably collected from nature for

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local consumption or export (SEZİK 1967, 1984, CEYLAN 1976, GÖKÇEOĞLU and SUKATAR 1985,1986, KIRYAMAN 1988, EKİM et al.1989).

Orchis tubers contain starch (%8-30), sugars (glycose, fructose), nitrogenous compounds and glycomanous mucilage. The substance from *Orchis* tubers is called salep and it is used widely in pharmaceutical sector. Salep tubers have been used as an aphrodisiac in ancient times, especially as a power source to stop the infant and as food resently. These have also been used as balancer in ice-cream production, in central Anatolia (SEZİK 1967, 1984). Salep tubers are taken from wild plants from the ancient times till today (SEZİK 1984).

Cyclamen tubers have emetic, and stimulating effects and contain fats, gum, organic acids and saponyn - type glycozites and these tubers are very much liked and preferred by pigs as a food. In addition, it is stated that the tobacco producers use the boiled essence of these tubers against earth worms (BAYTOP 1984).

The saponozyte, obtained from *Cyclamen* tubers, is called syclamine and it decreases the collesterol level in blood, having anti-tumoral and anti-microbial effects (TANKER and TANKER 1985). The same researchers have reported that these saponozytes in the tubers have diuretically and antiexudatively effective, and also useful for preventing the ringing of ear.

It is a wellknown fact that plants generally grow up and continue to develope under available ecological conditions. Charges in the ecological conditions result in the changing, breaking and even the termination of the vegetative phase of the plant species. Altitudinal variations in Turkiye play an important role in the zonation of various vegetation types vertically. These variations together with exposure and the degree of inclination result in various conditions at even short distances, as well as various formations. Moisture loving plant groups inhabit deep valleys and slopes, where the cracking degree and inclination increase. Exposure has important effects on the distribution of plants. With an increase in altitude, leaf, relative moisture decreases, on the contrary, precipitation, vaporisation and radiation rates increase. In addition wind becomes stranger, daily temperature differentiations increase, besides, vegetation period and termal pedogenesis shortens (KOÇMAN 1989).

In an experiment carried out on *Betula pubescens* L. at various altitudes, it has been reported that at higher places, length of stem is inhibited (SULKINOJA et al.1987). During studies on the structural adaptation of *Trifolium* L. genus at different alpine zones of Central Caucasia, it has been observed that, out of 34 species of *Trifolium* L.; which are widespread in Caucasian flora; only 7 species can survive in the alpine and subalpine zones, 6 species can grow up to 800-1000 m from the sea level in the Precaucasian zone, and 5 species can survive in every part of the mountain (NIKOLAEVSKAYA 1992).

CORRIAS et al. (1991) have reported in *Orchis longicornu* Poiret. that, these species can adapt to local differences and survive in habitats between 0-1500 m altitude. Growth habits of small tubers of *Cyclamen hederifolium* Aiton. under different light and moisture conditions have been investigated and it has been found that the best tuber growth

occurs in wet sites with a little shadow, and tubers are bigger than their original size (GÖKÇEOĞLU and SUKATAR 1985).

Morphological and pharmaceutical studies on *C. hederifolium* have been carried out by TANKER (1965) and regional studies on the anatomical features at generic level have also been done (TANKER and TÜRKÖZ 1984, BEYAZOĞLU 1988).

Morphological and anatomical differentiations seem to be a result of different ecological conditions. Similar observations can also be expected ultrastructurally. For instance, the changes due to lack of water and heat stress in the chloroplast structure on two different variations of *Zea mays* L., have been followed and it is reported that after 7 days of soil-drying, the chloroplast membranes on 13 days-old seedlings get destroyed due to an effect of heat at 45°C ; the chloroplast structure also changes and the thyllakoid format gets swelled, in addition, amount of the osmiophilic globules in the chloroplasts increases (RISTIC et al. 1992).

In a similar study, it has been stated that heat causes morphological changes in mitochondria and In another paper, physiological changes occuring in the bean plant left in ozone for a long period have been examined, and it has been seen that there has been fragmentation in the chloroplast membrane and tonoplast effected by the ozone, and also the weight of leaves declined. In addition, ozone reduced the photosynthetic capacity as well (SANDERS et al.1992).

The similarities and differences between some species of *Calamagrostis* Adanson. genus have been examined under scanning electron microscope and light microscope, their relations with the environmental factors have been investigated. Results have revealed that there are differences in the number of vascular bundles, in the dispersion of scleranchyma cells, gathering of silicium in papillas, thornlike hair, the number of stomata and their distribution, and in the length of hairs. A correlation between morphological, anatomical and ultrastructural features amongst the species and the environmental factors has been put forth (ESCALONA 1991).

In this study, our aim is to put forth ultrastructural changes in *Orchis anatolica* and *Cyclamen hederifolium* such as light, heat, wind and soil which are of great economical importance for Turkiye.

MATERIAL AND METHOD

Orchis anatolica Boiss.and *Cyclamen hederifolium* Aiton. distributed in west Anatolia has been chosen as a subject at our study. The plant samples were collected from the area between the lowlands to the highest peaks of the Mount Nif-Kemalpaşa İzmir (1506 m) and the Mount Spil-Manisa (1514 m).

For ultrastructural observations, 5 mm pieces taken from stem and leaves of the samples were put in the 25% Glutaraldehyde and Cacodylate buffer (PH=7.2), then they were blocked according to Millonig (1961). Thin cross-sections taken from the blocks by OmU_2

sittle marc ultratome were stained with uranylacetate and leadcitrate (REYNOLDS 1963, VENABLE 1965). The grids were analyzed under Jeol 100 CX II electron microscope. Micrographies of were taken.

RESULTS

The structural characteristics of the stem and leaves of *Orchis* and *Cyclamen* from high and lowland areas were studied comperatively using micrographs. *Orchis* stem cross-sections revealed that the cells have a lot of cytoplasm and organelles in the lowland samples are rich (Figure 1).

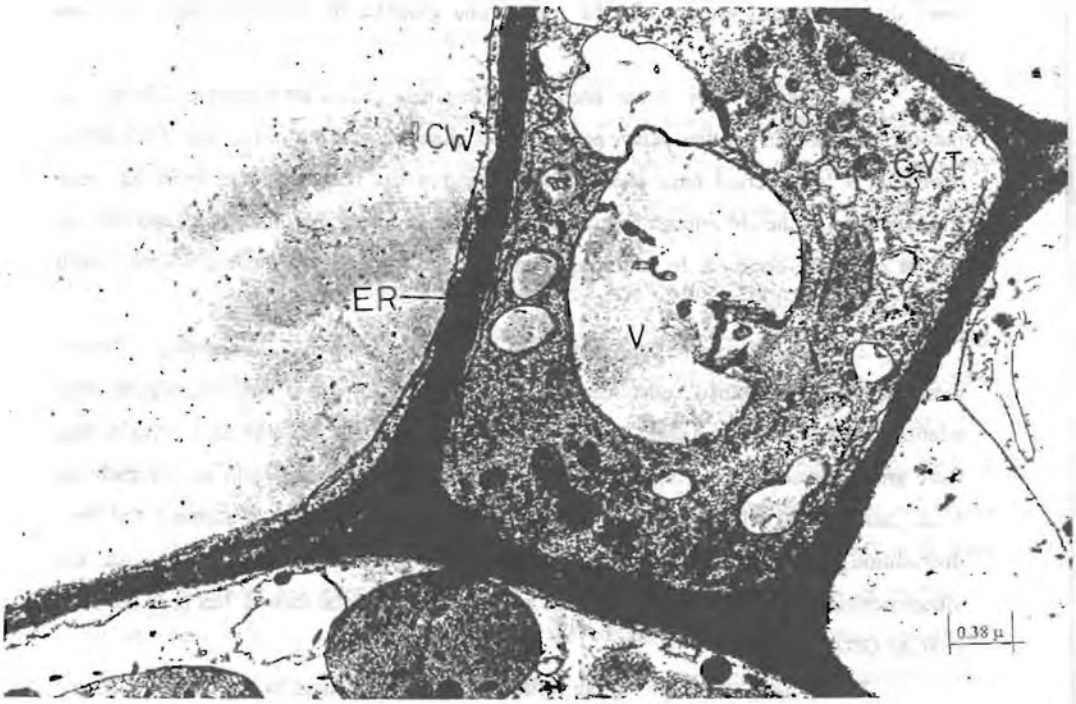

Figure 1. Transverse section of stem cortex cell of *O .anatolica* (850 m) X 1150.
CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, M= Mitochondrium,
ER= Endoplasmic Reticulum, V= Vacuol.

In the high altitude samples, the cell content is less intense than the lowland samples (Figure 2).

Figure 2. Transverse section of stem cortex cell of *O .anatolica* (1310 m) X 11500.
CW= Cell wall, CYT= Cytoplasm, ER= Endoplasmic Reticulum.

No clear difference has been observed in the cells of *C.hederifolium*'s low and high altitude samples (Figures 3,4).

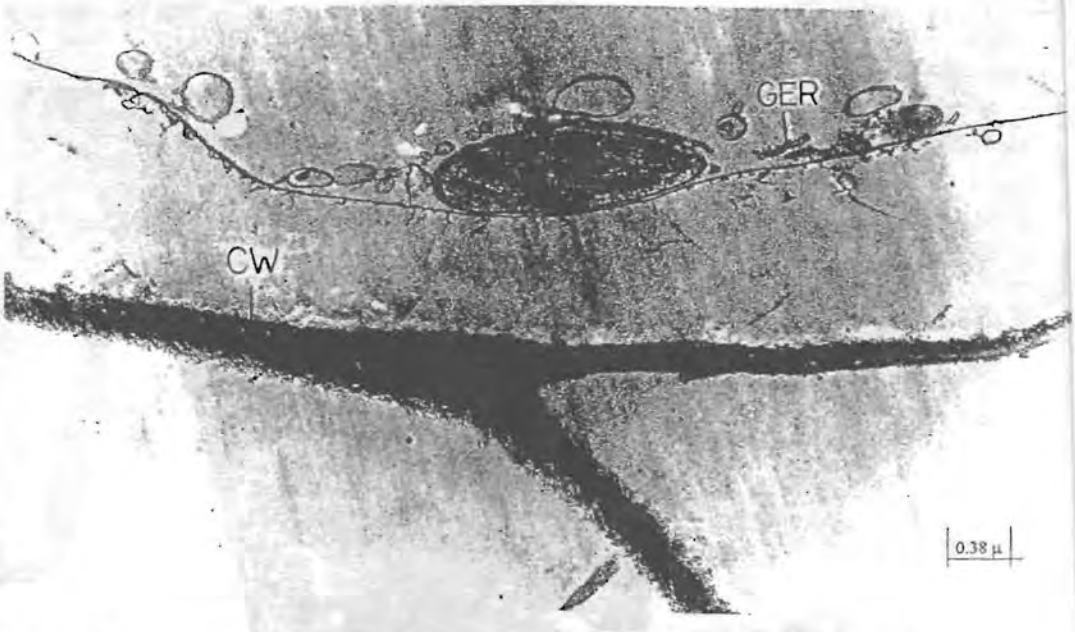

Figure 3. Transverse section of stem cortex cell of *C.hederifolium* (300 m) X 11500.
CW= Cell wall, CHL= Chloroplast, GER= Granular Endoplasmic Reticulum.

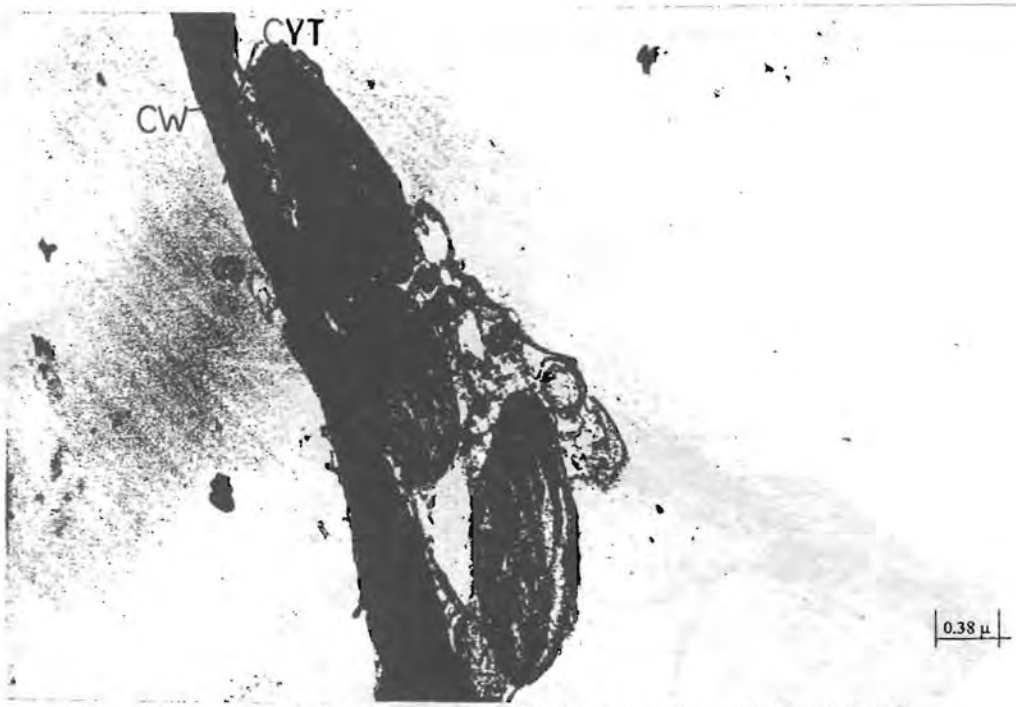

Figure 4. Transverse section of stem cortex cell of *C.hederifolium* (1020 m) X 11500.
CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, M= Mitochondrium.

A comparison of the structural characteristic high and lowland samples shows that in the micrographs of *Orchis* stem cross-sections, the cell wall is thicker in the highland samples (Figures 5,6) and although not that clear, it's the same in the highland samples of *Cyclamen* (Figures 3,4)

Figures 5. The appearance of cell wall and chloroplast structure in the cross-section of *O.anatolica* stem (850 m) X 19090
CW= Cell wall, CYT= Cytoplasm, CHL = Chloroplast, M= Mitochondrium.

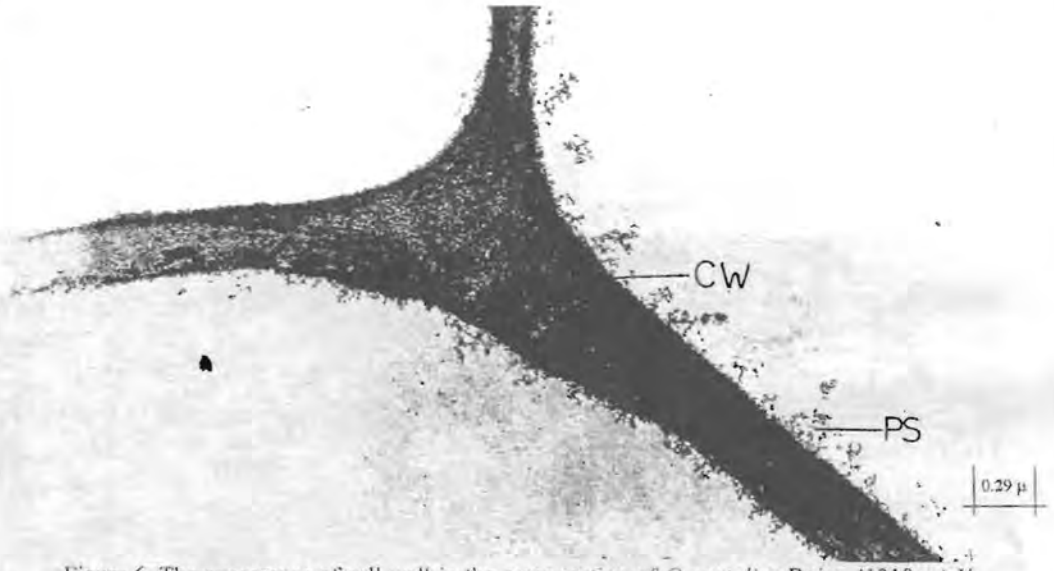

Figure 6. The appearance of cell wall in the cross-section of *O.anatolica* Boiss. (1310 m) X 15180.

CW= Cell wall, PCYT= Peripheric cytoplasm.

When the structure of chloroplast amongst the cytoplasmic organelles is examined, it's observed that in the highland samples of *Orchis*, there are more osmiophilic granulles in the stroma, besides there are small vacuoles which don't exist in the lowland samples (Figures 5, 7).

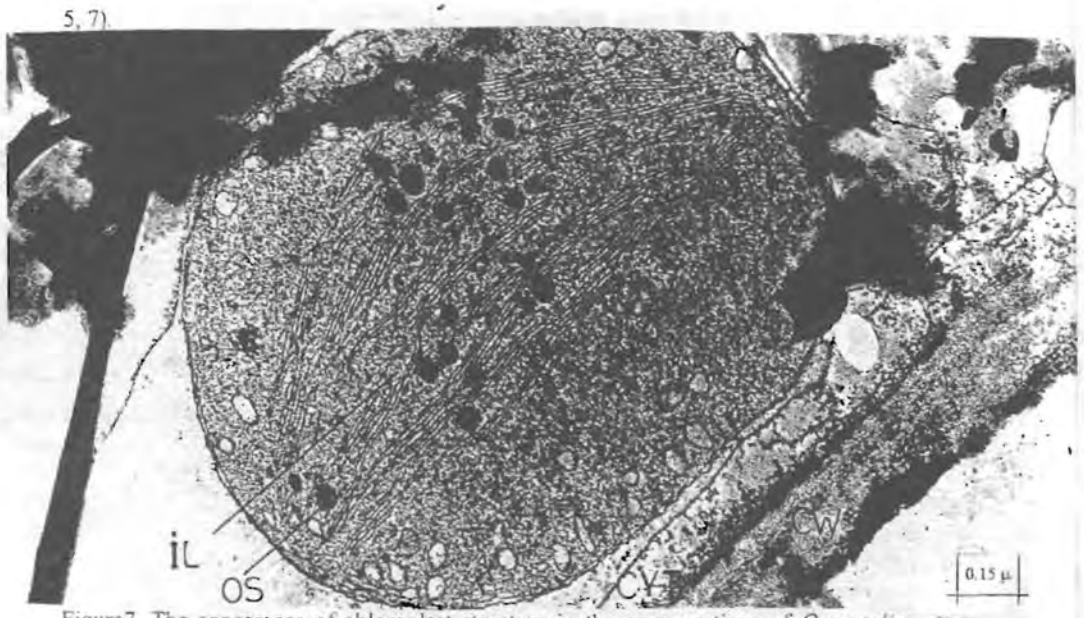

Figure7. The appearance of chloroplast structure in the cross-sections of *O.anatolica* stem (1310 m) X 29900.

CW= Cell wall, CYT=Cytoplasm, CHL= Chloroplast, GR= Granal lamelles, IL= Intergranal lamelles

OS= Osmiophilic granulles, V= Vacuole

In the lowland samples of *Cyclamen*, it has been observed that thyllakoid formation is localized in a certain region of stroma (Fig.8), however there's no such structural difference in the highland samples (Fig. 4)

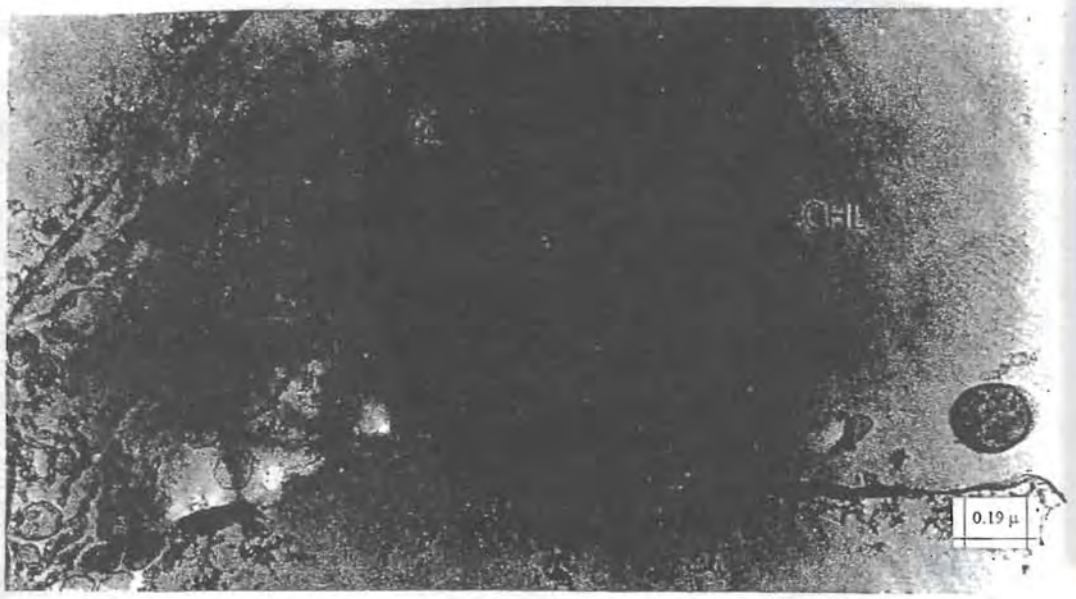

Figure 8. The appearance of chloroplast structure in the stem cross-section of *C.hederifolium* (300 m) X 23000.

CHL= Chloroplast, GR= Granal lamelles, IL= Intergranal lamelles, M= Mitochondrium ,

GER= Granular endoplasmic reticulum, S= Stroma.

When endoplasmic reticulum membranes are examined, it is seen that these organelles are fiberous in structure without granulles (Figure 9) in the lowland samples, whereas they're generally in the form of circles in the highland samples (Figures 2,12).

Figure 9. The appearance of Endoplasmic Reticulum membranes in the cross-section of *O.anatolica* stem (850 m) X 23000.

CW= Cell wall, CYT= Cytoplasm, M= Mitochondrium, ER= Endoplasmic Reticulum, V= Vacuol.

The crosssection of *Cyclamen*'s lowland samples show that endoplasmic reticulum membranes are generally granular, resembling small purses in shape (Figure 8), whereas they're granular but thin and fiberous in the highland samples (Figure 10).

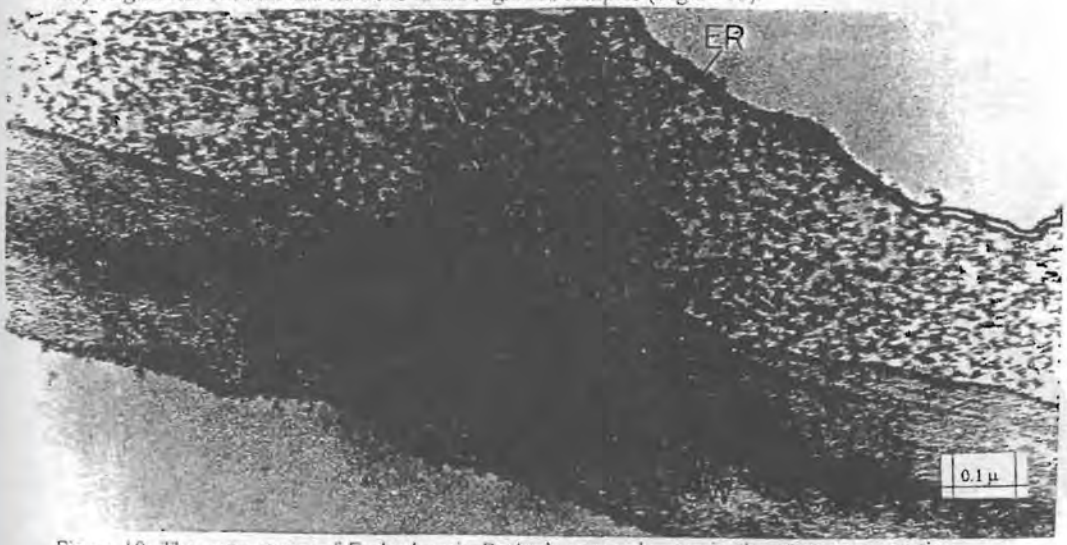

Figure 10. The appearance of Endoplasmic Reticulum membranes in the stem cross-section of *C hederifolium* Aiton.

(1020 m) X 46000. CW= Cell wall, CYT= Cytoplasm, ER= Endoplasmic reticulum.

The micrographs taken from the leaf cross-sections, depict that *Orchis* mesophyll cells have a peripheric cytoplasm (Figure 11) and also in the highland samples they have a similar shape (Figure 12).

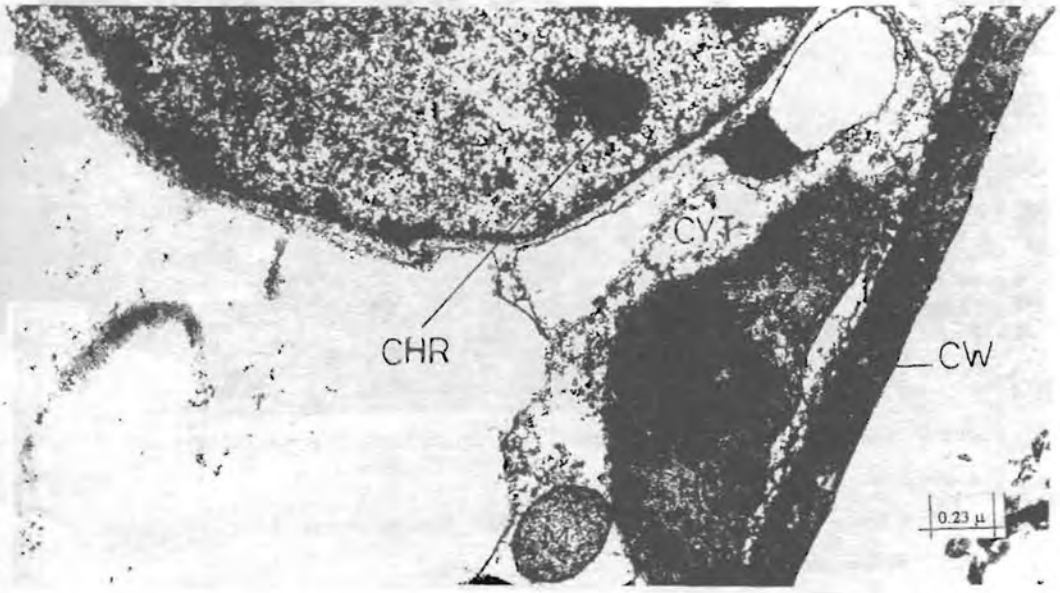

Figure 11. General appearance of the leaf cross-section of *O.anatolica*.(850 m) X 19090.
CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, M= Mitochondrium, N= Nucleus, CHR= Chromatine.

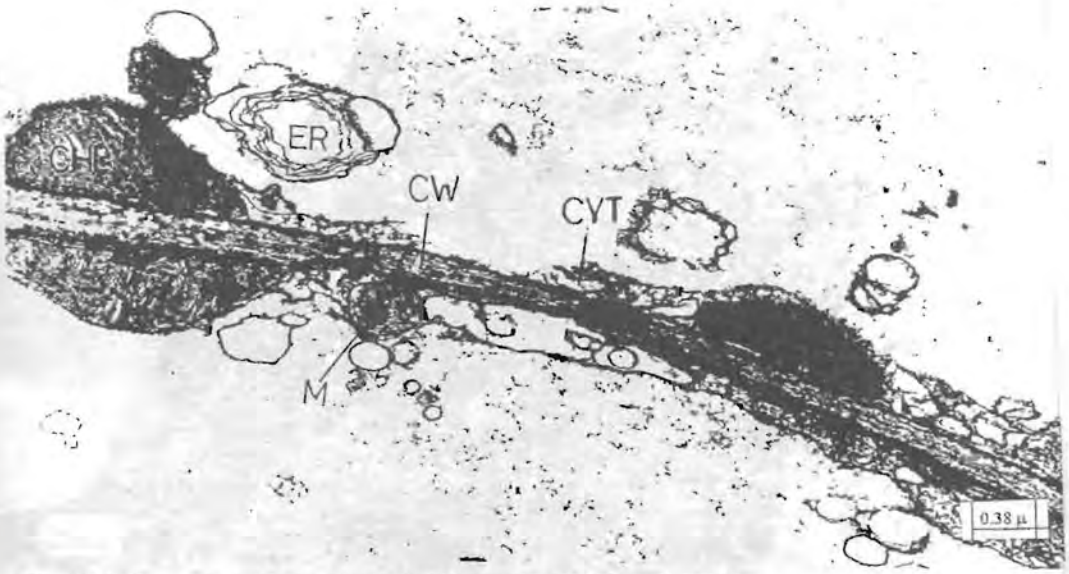

Figure 12. Transverse section of the leaf of *O.anatolica*.(1310 m) X 11500.

CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, M= Mitochondrium, ER= Endoplasmic reticulum.

In the lowland samples of *Cyclamen* leaves, it was observed that cytoplasm is peripheric along the cell wall and chloroplasts are arranged in parallellity to the cell wall (Figure 13). Highland samples show similar structures (Figure 14).

Figure 13. Transverse section of the leaf of *C.hederifolium* Aiton.(300 m) X 11500.

CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, ER= Endoplasmic reticulum.

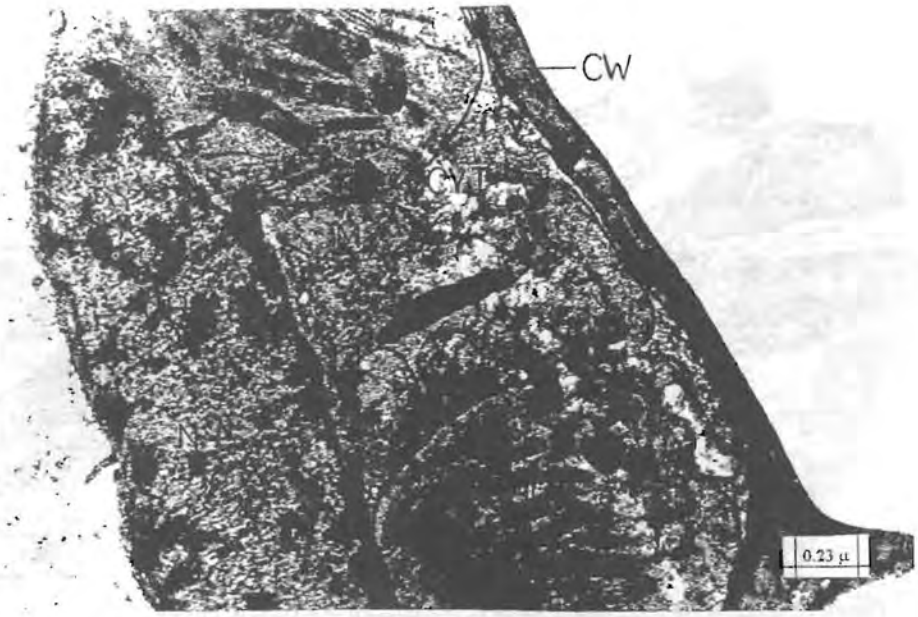

Figure 14. Transverse section of the leaf of *C.hederifolium* Aiton.(1020 m) X 19090

CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, M= Mitochondrium, N= Nucleus, CHR= Chromatine.

In the leaf cross-sections of both materials the cell wall are thicker in the highland samples (Figures 15,16,17,18).

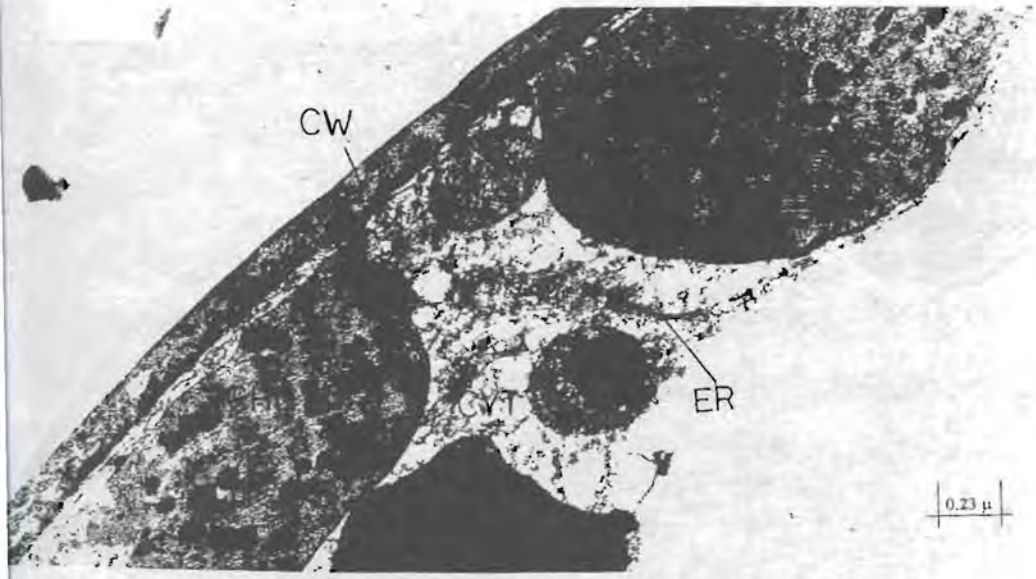

Figure 15. The appearance of cell wall in the leaf cross-section of *O.anatolica* Boiss.(850 m) X 19090.

CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, M= Mitochondrium, ER= Endoplasmic reticulum.

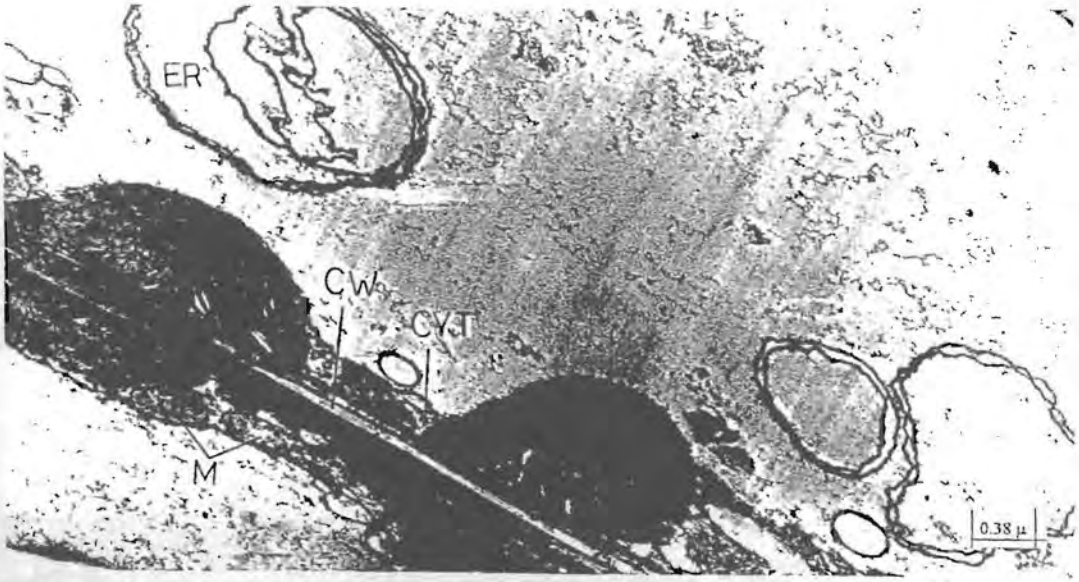

Figure 16. The appearance of cell wall and mitochondria in the leaf cross-section of *O.anatolica* Boiss.(1310 m) X 11500.

CW= Cell wall, CYT = Cytoplasm, CHL= Chloroplast, M= Mitochondrium, ER Endoplasmic reticulum.

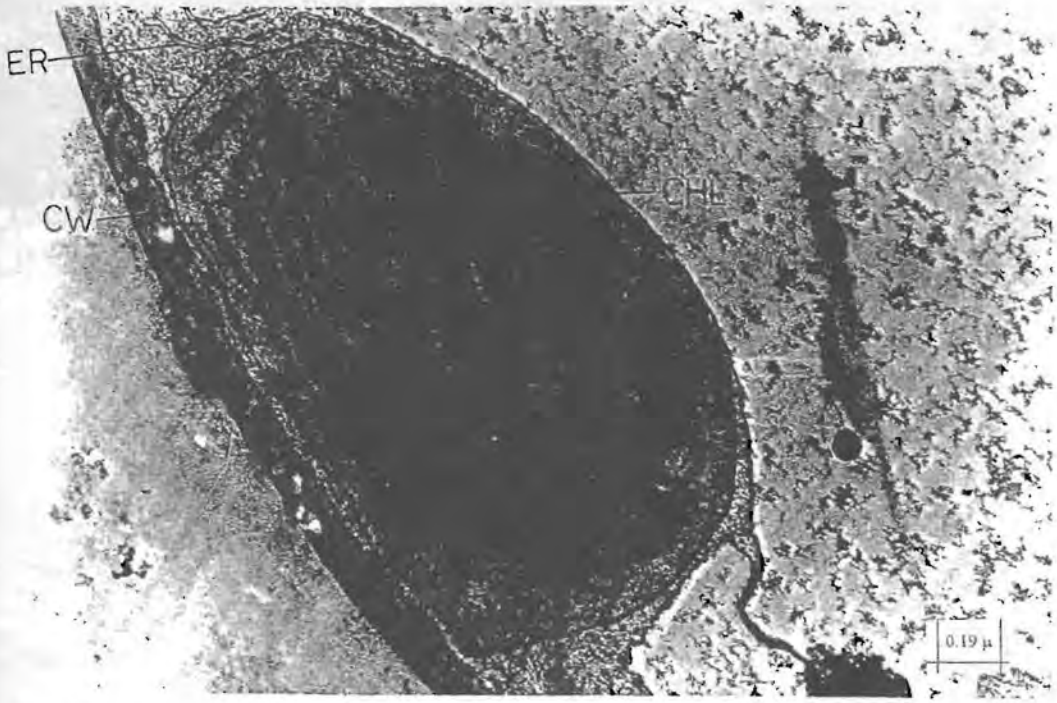

Figure 17. The appearance of cell wall in the leaf cross-section of *C.hederifolium* Aiton.(300 m) X 23000.

CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, ER= Endoplasmic reticulum, GR= Granal lamelles.

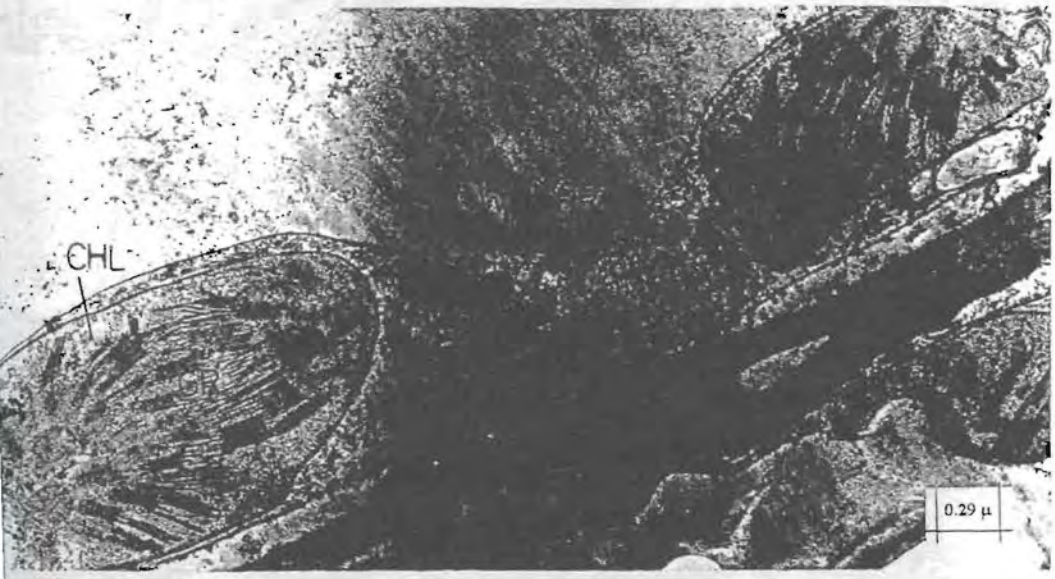

Figure 18. The appearance of cell wall and thyllakoid structures in the leaf cross-section of *C.hederifolium* Aiton.(1020 m)

X 15180. CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, M= Mitochondrium, ER=Endoplasmic reticulum, GR= Granal lamelles.

When the chloroplast structure in the cross-sections was analyzed, no distinct differences were seen in osmiophilic granulles between the high and lowland samples of *Orchis*, there were no vacuoles inside the chloroplasts (Figures 15,16).

There was not any differences in osmiophilic granulles in the *Cyclamen* samples either. However, it was observed that in both high and lowland samples thyllakoid structures show a radial arrangement (Figures 18,19).

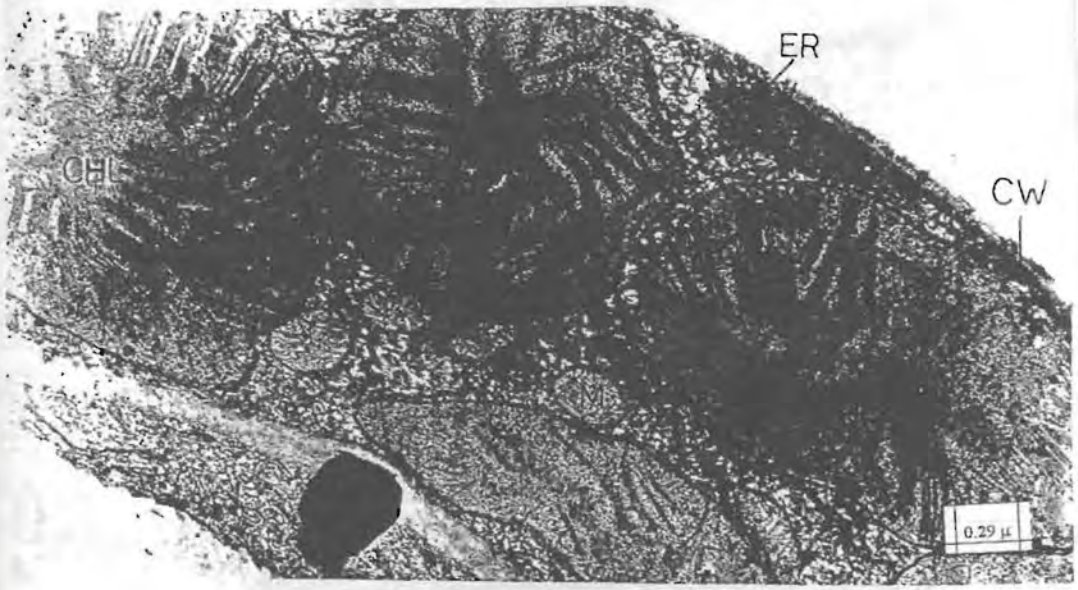

Figure 19. The appearance of thyllakoid structures in the leaf cross-section of *C.hederifolium* Aiton.(300 m) X 15180.

CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, GR= Granal lamelles, M= Mitochondrium,

ER= Endoplasmic reticulum.

This radial arrangement in thyllakoid structures is seen more in the lowland samples (Figure 19), but a neat thyllakoid arrangement which never existed in the lowland materials was observed at the same time with the ones that carry out a radial arrangement in the highland (Figure 20).

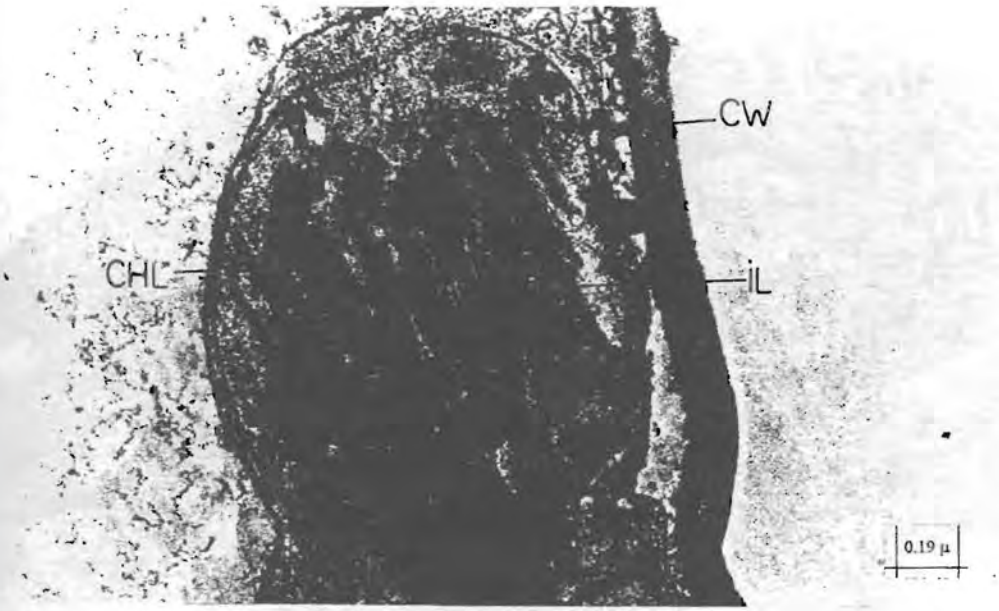

Figure 20. The appearance of neat thyllakoid formation in the leaf cross-section of *C.hederifolium*.(1020 m) X 23000.

CW= Cell wall, CYT= Cyotplasm, CHL= Chloroplast, GR= Granal lamelles, IL= Intergranal lamelles.

Endoplasmic reticulum membranes, just like stem are as fiberous structures without granulles in the lowland samples of *Orchis* (Figure 21) but in the form of circles in the highland samples (Figure 22).

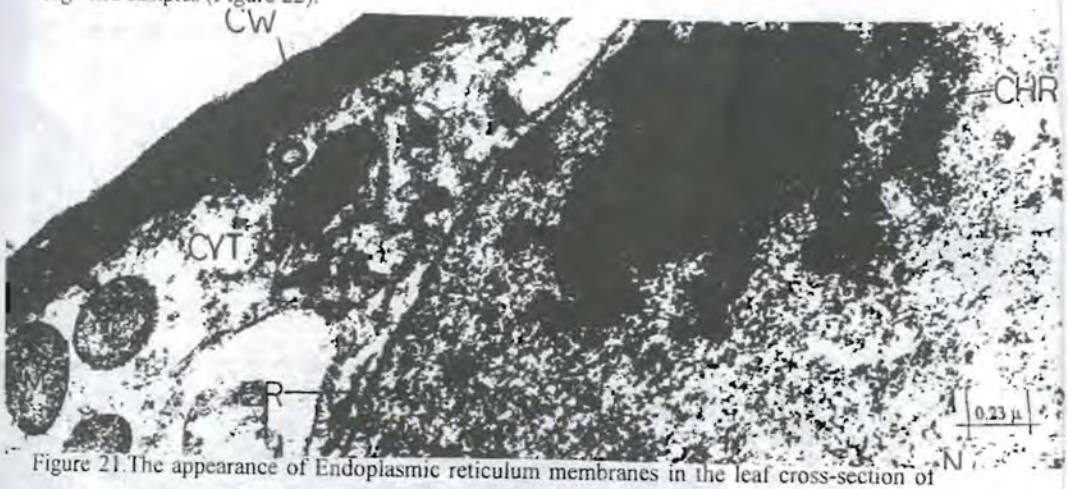

Figure 21.The appearance of Endoplasmic reticulum membranes in the leaf cross-section of *O.anatolica*.(850 m) X 19090.

CW= Cell wall, CYT= Cytoplasm, ER= Endoplasmic reticulum, M=

CHR= Chromatinc.

Figure 22. The appearance of Endoplasmic reticulum membranes in the leaf cross-section of *O.anatolica* Boiss.(1310 m)

X 11500. CW= Cell wall, CYT= Cytoplasm, ER= Endoplasmic reticulum, CHL= Chloroplast,

M= Mitochondrium.

No clear differences were noticed in the endoplasmic reticulum membranes between the low and highland samples of *C. hederifolium* (Figures 18,19). Mitochondria in the lowland samples are generally round (Figure 15) whereas in some of the highland samples there are big and fiberous ones as well as round ones present (Figure 16).

The mitochondria lie inside the cytoplasm in the lowland samples of *Cyclamen* (Figures 19,23), and are clearly elliptic and fiberous in the highland samples (Figure 24).

Figure 23. The appearance of mitochondria in the leaf cross-section of *C.hederifolium* Aiton.(300 m) X 19090.

CW= Cell wall, CYT= Cytoplasm, ER= Endoplasmic reticulum, CHL= Chloroplast, M= Mitochondrium,

N= Nucleus, CHR= Chromatine.

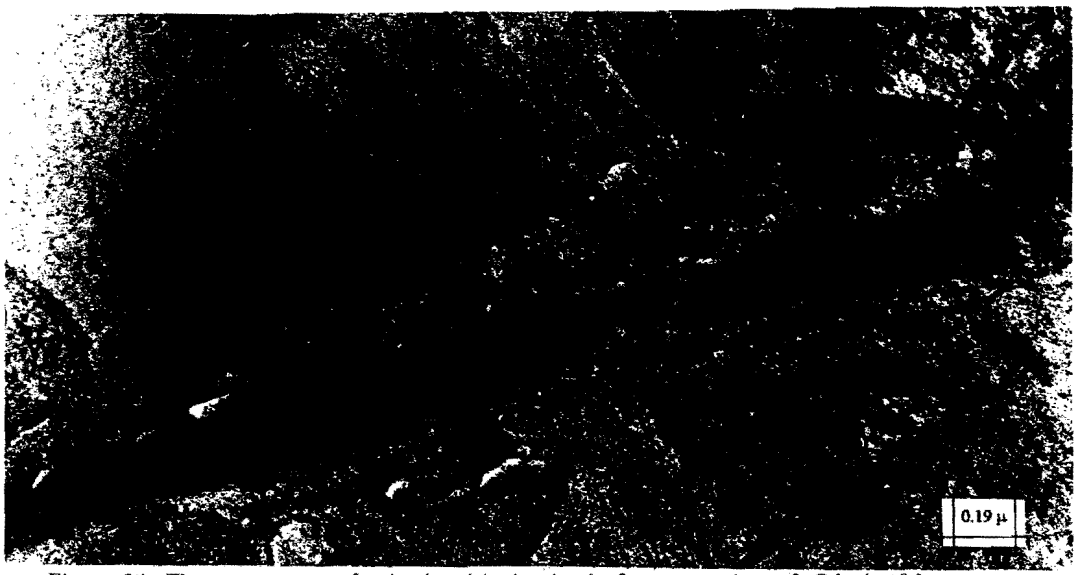

Figure 24. The appearance of mitochondria in the leaf cross-section of *C.hederifolium* Aiton.(1020 m) X 23000.

CW= Cell wall, CYT= Cytoplasm, ER= Endoplasmic reticulum, CHL= Chloroplast, M= Mitochondrium.

In the low and highland samples of both materials, nucleus is in the typical granular form and chromatine material is intense at some parts. No difference is noticed in this organelle (Figures 25, 26, 14, 27).

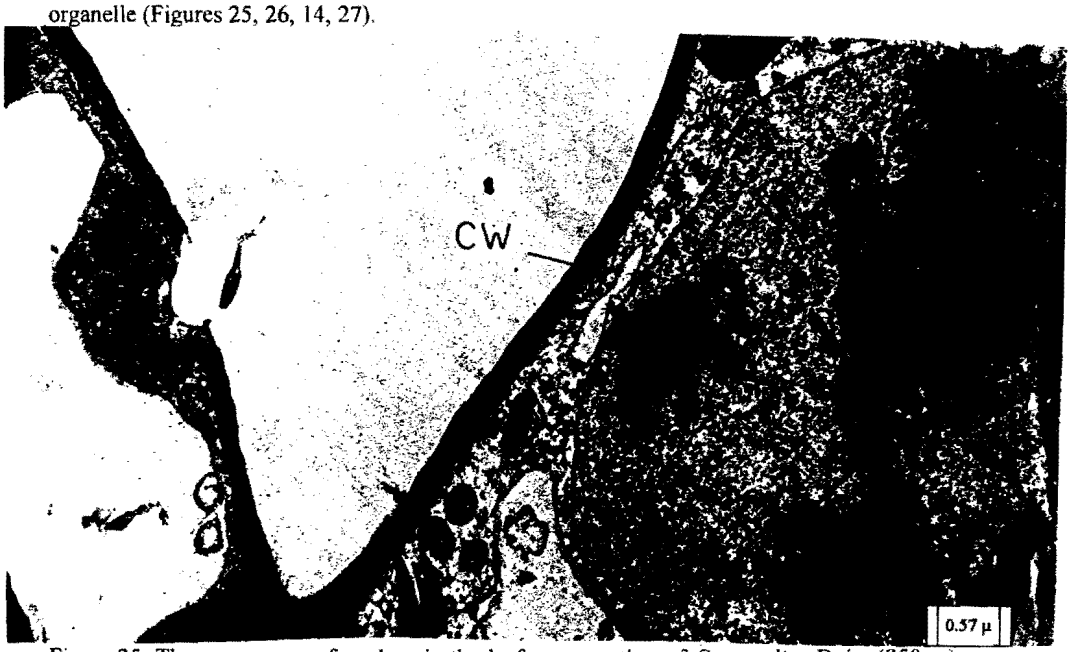

Figure 25. The appearance of nucleus in the leaf cross-section of *O.anatolica* Boiss.(850 m) X 7590.

CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, M= Mitochondrium, N= Nucleus.

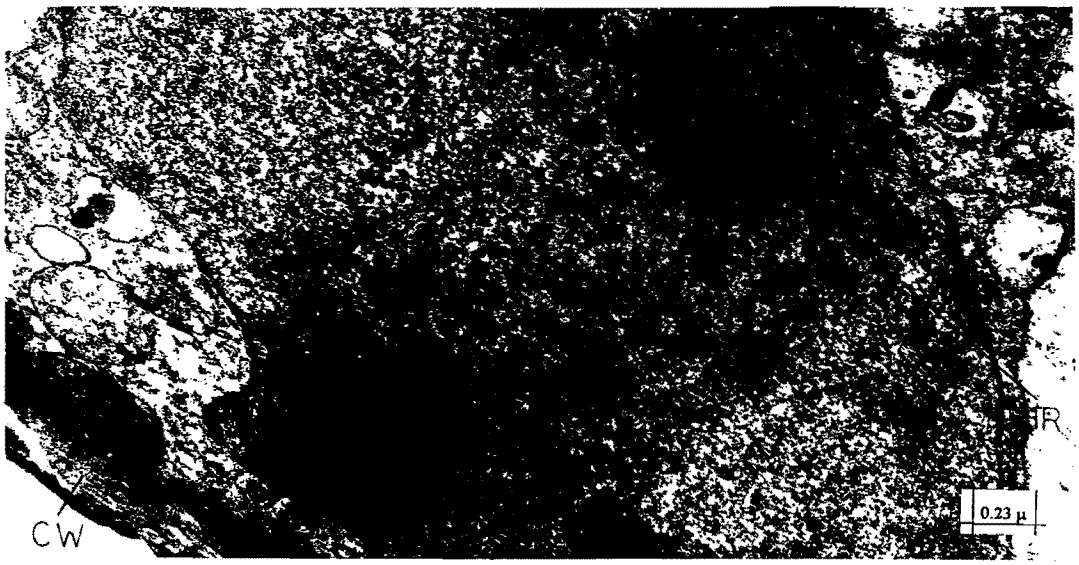

Figure 26. The appearance of nucleus in the leaf cross-section of *O.anatolica* Boiss.(1310 m) X 19090.

CW= Cell wall, CYT= Cytoplasm, CHL= Chloroplast, M= Mitochondrium, N= Nucleus, CHR= Chromatine

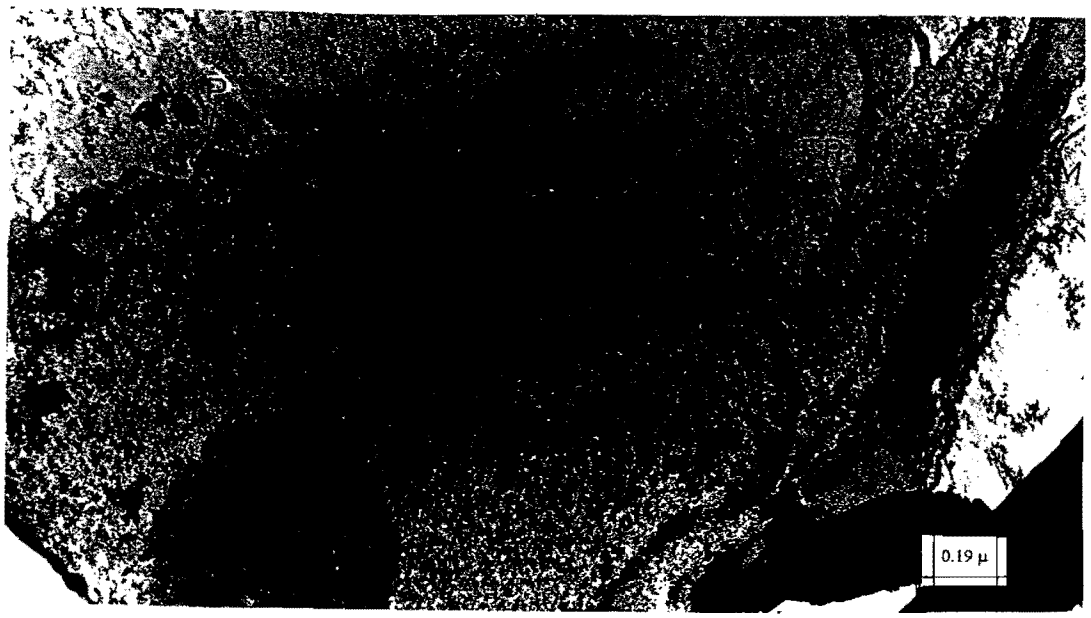

Figure 27. The appearance of nucleus in the leaf cross-section of *C.hederifolium* Aiton.(300 m) X 23000.

CW= Cell Wall, CYT= Cytoplasm, M= Mitochondrium, N= Nucleus, ER= Endoplasmic Reticulum,

NP= Nucleus pore, NO= Nucleolus.

DISCUSSION

It's well-known fact that different species react differently against different environmental factors. Changes in local environmental factors play an important role in the distribution of species together with the climate and soil properties. What determine the existence of plant and animal populations in a certain geographic site, the environmental factors and other plant and animal populations existing there (RICKLEFS 1973).

With change of altitude, many environmental factors also change. For instance, with an increase in the altitude, the amount of precipitation, vaporisation and radiation increase, the effect of wind, daily temperature variations, cloudliness and atmospheric moisture increases too. However as against these heat, relative moisture content or water vapour decrease and vegetation and pedogenesis periods shorten with an increase in altitude (KOÇMAN 1989).

In our study, we tried to determine the ultrastructural changes in *Orchis anatolica* and *Cyclamen hederifolium* which are of great importance to our Country's economy, in relation to the ecological factors like light, heat, wind and soil properties that change according to the altitude.

Although each of these factors changes with the altitude and affects plant life separately, the main permanent differentiations in morphological and anatomical structure occurs as a result of an interaction of these factors with each other (ÖZTÜRK et al. 1989).

According to BJÖRKMAN (1980) the plants have the light potential necessary for assimilation at a low level and they get harmed under strong light.

The micrographs from the stem and leaf cross-sections of *Orchis* and *Cyclamen* showed that the cell walls of the highland samples are thicker in general (Figures 3, 4, 5, 6, 15, 16, 17, 18). JAFFE (1981) states that strength increases in plants with the development of thick-walled cells, this goes in accordance with our results. Similarly, a radial increase occurs in the bean plants when exposed to wind at 4.5 m/s strength. This radial broadening is in linear relation with the decline of distance between the nodiums (HUNT and JAFFE 1980). The increase in the thickening of cell walls in the highland samples of our plants may be explained by increase in the strength of wind at these sites.

The chloroplast structure in the cytoplasmic organelles in stem cross-sections shows more osmiophilic granulles in the *Orchis*'s highland samples, besides there are tiny vacuoles in stroma which don't exist in the lowland samples (Figures 5,7). RISTIC et al.(1992) reported that the peristromium around chloroplast is ruined, thyllakoid structure swells, and too many lipid bodies occur when 45 ^{0}C heat shock is applied to 13 days old seedlings of two different lines of corn after 7 days of soil drying. Total amount of lipids in *Origanum majorana* increased when soil moisture decreased (RHIZOPOULOU et al.1991). When an increase in the soil moisture occurs with the effect of wind in highland areas, an increase of osmiophilic granulles in chloroplasts highland samples can be justified.

Thyllakoid structure is localized in certain regions of stroma (Figure 8), no such structural difference is observed in the highland samples (Figure 4).

According to RISTIC et al.(1992), soil drying and heat cause swelling of the chloroplasts and thyllakoid system and disruption in peristromium. The Cyclamens chosen as experimental material generally grow at oak edges, that is on shad habitats, but the sample we gathered from the lowland is the one that was able to grow up away from the oaks, in the agora. In this aspect, moving from the point of soil drying and great heat together with strong light, such a degeneration in thyllakoid system agrees with the findings of RISTIC and BJÖRKMAN. The presence of olive plantations on the sites of our lowland samples increases the possibility of an occurance of pesticide application. It's probable when the application of pesticide is considered there is such a degenerative effect, because it was reported in one of the studies of MOSTKOVSKA et al.(1991) that degeneration in the chloroplast thyllakoid system took places after 4-9 hours application of 1,10 phenantrolin,which is a photodynamic herbiside.

The chloroplasts in the leaves of our samples showed no important difference.

It was observed that endoplasmic reticulum membranes in the stem and leaf cells of *Orchis* were as circles within each other in the highland samples (Figures 2, 12). Whereas they're as small fiberous structures and non granular in the lowland samples (Figure 9). We think that this can be due to a correlation between non granular endoplasmic reticulum membranes with an increase in the amount of lipid when soil moisture declines.

No typical difference was noticed about the endoplasmic reticulum membranes in *Cyclamen* leaves.

It was observed that mitochondria are as small and circular structures in the leaf cross-sections of lowland samples in both *Cyclamen* and *Orchis* (Figures 15,19,23), however, ones in one of the highland samples are bigger and fiberous in shape (Figure 16, 24). In a study performed by CİRELİ (1967), it has been observed that mitochondria are seen as small granulles in warm climates and as fiberous structures under cool conditions. Heat and gamma radiation to lead to morphological and functional metabolic changes in the newly-growing mitochondria (WANG and LIU 1989). There are distinctive differences between heat and other factors in both high and lowlands, it's thus natural to observe some differences in mitochondria which act as energy stores in the cell. Still we believe that the problem can be better explained with the studies done on the cellular organelles in detail.

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