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Physio-biochemical responses of registered bread wheat (*Triticum aestivum* L.) genotypes to drought stress: Variations in antioxidant parameters and photosynthetic pigment amounts

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Tescilli ekmeklik buğday (*Triticum aestivum* L.) genotiplerinin kuraklık stresine fizyo-biyokimyasal yanıtları: Antioksidan parametreler ve fotosentetik pigment miktarlarındaki değişimler

Abstract: In this study, physio-biochemical parameters of 7 registered bread wheat (*Triticum aestivum* L.) genotypes (Gerek 79, Sultan 95, Haymana 79, Grk/Cty, T98-9, Pastor, PM ME1) were investigated under drought stress conditions. Polyphenol oxidase (PPO), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), photosynthetic pigment, total protein, hydrogen peroxide, lipid peroxidation (malonyldialdehyde-MDA) and proline levels were determined in this wheat genotypes exposed to different drought duration (3rd, 6th and 10th day). As a result of this study, among 7 different wheat genotypes, Gerek 79 and Haymana 79 genotypes were the most physiologically sensitive to drought. In comparison, Pastor and Sultan 95 genotypes were the most drought-tolerant varieties. In addition, in parallel with the prolongation of the drought period in wheat varieties in general, it was determined that the content of photosynthetic pigments decreased significantly due to oxidative damage, while proline and MDA content increased.

Key words: Antioxidant enzymes, drought stress, photosynthetic pigments, wheat

Özet: Bu çalışmada, 7 tescilli ekmeklik buğday (*Triticum aestivum* L.) genotipinin (Gerek 79, Sultan 95, Haymana 79, Grk/Cty, T98-9, Pastor, PM ME1) fizyo-biyokimyasal parametreleri kuraklık stresi koşulları altında incelenmiştir. Farklı kuraklık şiddetine (3., 6. ve 10. gün) maruz bırakılan bu buğday genotiplerinde polifenol oksidaz (PPO), peroksidaz (POD), askorbat peroksidaz (APX), katalaz (CAT), fotosentetik pigment, toplam protein, hidrojen peroksit, lipid peroksidasyonu (malonildialdehit-MDA) ve prolin seviyeleri belirlenmiştir. Bu çalışma sonucunda, 7 farklı buğday genotipi arasında Gerek 79 ve Haymana 79 genotipleri fizyolojik olarak kuraklığa en duyarlı genotipler olurken, Pastor ve Sultan 95 genotipleri kuraklığa en toleranslı çeşitler olmuştur. Ayrıca buğday çeşitlerinde genel olarak kuraklık süresinin uzamasına paralel olarak oksidatif hasara bağlı olarak fotosentetik pigment içeriğinin önemli ölçüde azaldığı, prolin ve MDA içeriğinin ise arttığı tespit edilmiştir.

Anahtar Kelimeler: Buğday, kuraklık stresi, antioksidan enzimler, fotosentetik pigmentler

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1. Introduction

Plants are exposed to many biotic (insects and pathogenic fungi, etc.), and abiotic (drought, salinity, UV rays, etc.) stress factors throughout their lives. Drought, one of the abiotic stress factors, causes yield and quality losses, negatively affecting morphological and physiological characteristics in plants (Pulvento et al., 2022). Drought stress due to global climate change seriously affects the physiological functions of plants and appears as a limiting factor, especially in terms of agricultural activities. With the increase in arid areas in recent years, a lower amount of plant production than planned here is a significant loss in terms of economy. (Simelton et al., 2009). The severity of drought appears equivalent to global vegetation loss. It has been the focus of many (ecological, morphological, physiological, etc.) research on the response and adaptation mechanisms of plants to the severity and duration of drought. In this context, growing varieties that are compatible with climatic changes and especially drought-resistant in studies has become essential.

Typically, when plants face drought stress, their initial reaction is to curtail shoot growth, prioritizing water conservation. Simultaneously, they ramp up the production of protective phytochemicals, aiding in osmotic regulation while reducing their overall metabolic demands. Consequently, under water stress conditions, plants undergo a series of transformations in various aspects, including the morphology of leaves, flowers, fruits, and root structures, as well as adjustments in processes like photosynthesis and the activation of antioxidant enzyme systems. Moreover, they activate stress response genes through intricate signal transduction networks, synthesising numerous functional proteins that bolster the plant's resilience against drought stress. This has been corroborated by recent studies (Wahab et al., 2022; Yang et al., 2021). Wheat (*Triticum aestivum* L.), which plays a major role in the nutrition of more than 35% of the world's population, is one of the most important grains affected by drought stress (Zaheer et al., 2019). Deficiency of water causes the detrimental effects at all growth stages of wheat; most prominent effect was observed at the reproductive

stage, particularly at the grain filling stage, which leads to less and reduced grain size in wheat (Yu et al., 2018). In addition, water scarcity reduces nutrient availability, uptake, transport and accumulation in wheat, disrupts nutrient relationships in plants and reduces the rate of photosynthesis, leading to leaf senescence, thus shortening its life cycle (Maghsoudi et al., 2019). Water stress is a growing problem around the world, as well as having a significant impact on grain yield and quality. Global climate changes are making this situation more severe day by day (HongBo et al., 2005). Moreover, all these negativities result in a decrease in wheat yield and quality. The causes of these crop losses include the reduction of net photosynthesis rates due to metabolic limitations, oxidative damage to chloroplasts, and closure of stomata (Bhargava & Sawant, 2013).

Water stress induces oxidative damage due to the overproduction of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and superoxide anion (O_2^-), and that can damage biological membranes through biochemical reactions (Hasanuzzaman et al., 2020). ROS accumulation in plants during stress depends to a large extent on the relationship between ROS production and ROS cleaning system. The main reason for the increase in ROS during stress in plants is the limitation of carbon dioxide fixation in chloroplasts, and in addition, the over-reduction of the electron transport chain (Miller et al., 2010). H_2O_2, one of the free radicals, acts as a signalling molecule that is vital for plants such as photosynthesis processes, growth and development, response to biotic and abiotic stresses, as well as the cell cycle. However, excessive H_2O_2 accumulation causes oxidative stress in cells, and as a result, cell death is inevitable. Antioxidants, which carry out the task of clearing ROS in cells, are divided into two groups non-enzymatic antioxidants (ascorbic acid -AA, vitamins, alkaloids, nonprotein amino acids, carotenoids, glutathione, phenolic compounds) and enzymatic antioxidants (superoxide dismutase-SOD, ascorbate peroxidase-APX, glutathione reductases-GR, glutathione peroxidase-GPX, guaicol peroxidase-GOPX, glutathione-S-GR, polyphenol oxidase-PPO, peroxidase-POD, mono-dehydroasorbate reductase-MSHAR and catalase-CAT). In addition, proline (osmolyte), one of the protective molecules involved in the clearance of ROS, increases in the cell during stress (drought, salinity etc.) and helps to protect the organism. Among these important functions are optimizing mitochondrial functions, influencing cell proliferation and acting as a signal to activate gene expression for plant survival and recovery in stress conditions (Szabados and Savouré, 2010).

In this study, an antioxidant defence system was investigated in 7 different wheat genotypes exposed to drought stress. Comparative analyses were performed by determining the levels of PPO (EC 1.10.3.1), POD (EC 1.11.1.7), APX (E.C. 1.11.1.11), CAT (E.C. 1.11.1.6), photosynthetic pigments, total protein, H_2O_2, lipid peroxidation (malonyldialdehyde-MDA) and proline levels.

2. Materials and Method

2.1. Experiment design

In the research, 7 registered bread wheat genotypes were used (Table 1). Wheat genotypes that were used in the study developed by Republic of Turkey Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute are drought tolerant genotypes related physiological tests results highlights.

Table 1. Bread wheat genotypes and abbreviation used in research.

No	Type Name	Abbreviation
1	GEREK 79	G_{79}
2	SULTAN 95	S_{95}
3	HAYMANA79/ALTAY2000	H_{79}
4	GRK/CTY//MESA/3/RL6043/4*NAC/4 MNCH	GRK
5	T 98-9//VORONA/HD2402	$T_{98\text{-}9}$
6	PASTOR/DEMIR2000//MUFITBEY	Pastor
7	PM ME1 IRR_S-32//TMP64/YY305/3/MUFITBEY	PM ME_1

Seeds of selected wheat genotypes were kept in 70% ethanol for 1 min, then put into 5% NaClO and mixed for 10 min. After this process, the seeds were rinsed 4 times with sterile distilled water. After germination of seeds in petri dishes, 7-10 days old seedlings were taken into pots and grown in pots under the same conditions in the greenhouse environment for 40 days and irrigation was left on the 40th day (Fig. 1). Then plant samples were collected from the application groups on days 3rd, 6th and 10th and stored at -80 °C for analysis (Chakraborty and Pradhan, 2012).

2.2. Determination of total soluble protein content

The total protein amount has been determined according to Bradford's (1976) method. 0.25 g leaf tissue was homogenized in porcelain mortar in 2.5 mL 50 mM KH_2PO_4 (pH:7) buffer with liquid nitrogen and the homogenate was transferred to microcentrifuge tubes. Then, the homogenate was centrifuged at 15.000 g for 20 min at +4 °C. 20 µl of supernatant was taken and 2.5 mL of Coomassie Brilliant Blue G-250 was added and vortexed. After 10 min of incubation, the absorbance of the samples at 595 nm was recorded and the amount of total protein in the leaves was determined by means of a standard curve of bovine serum albumin (BSA) (Öztürk & Demir, 2003).

2.3. Determination of proline content

In order to determine the amount of proline in the application groups, 0.4 g leaf tissue sample was homogenized in 4% $C_7H_6O_6S$ and filtered through filter paper. 0.5 mL of the filtrate 10 times diluted with distilled water and 1 mL of diluted sample, 1 mL of 96% glacial acetic acid and 1 mL of ninhydrin (2,2-dihydroxyindane-1,3-dione) were added and all tubes were incubated in a water bath for 60 min at 100 °C. After incubation, the tubes were simultaneously placed into an ice bath, held for 10 min, 2 mL of toluene were added to each tube and vortexed. Then incubated for a further 5 min and the absorbance of the pink phase formed at the top of each tube was recorded at 520 nm. The results were calculated as the amount of proline per fresh tissue g^{-1} using a standard graph prepared from proline (Öztürk & Demir, 2003).

2.4. Determination of lipid peroxidation

For the determination of lipid peroxidation (MDA); 0.5 g of leaf tissue was homogenized with 5 mL of 0.1% (w/v)

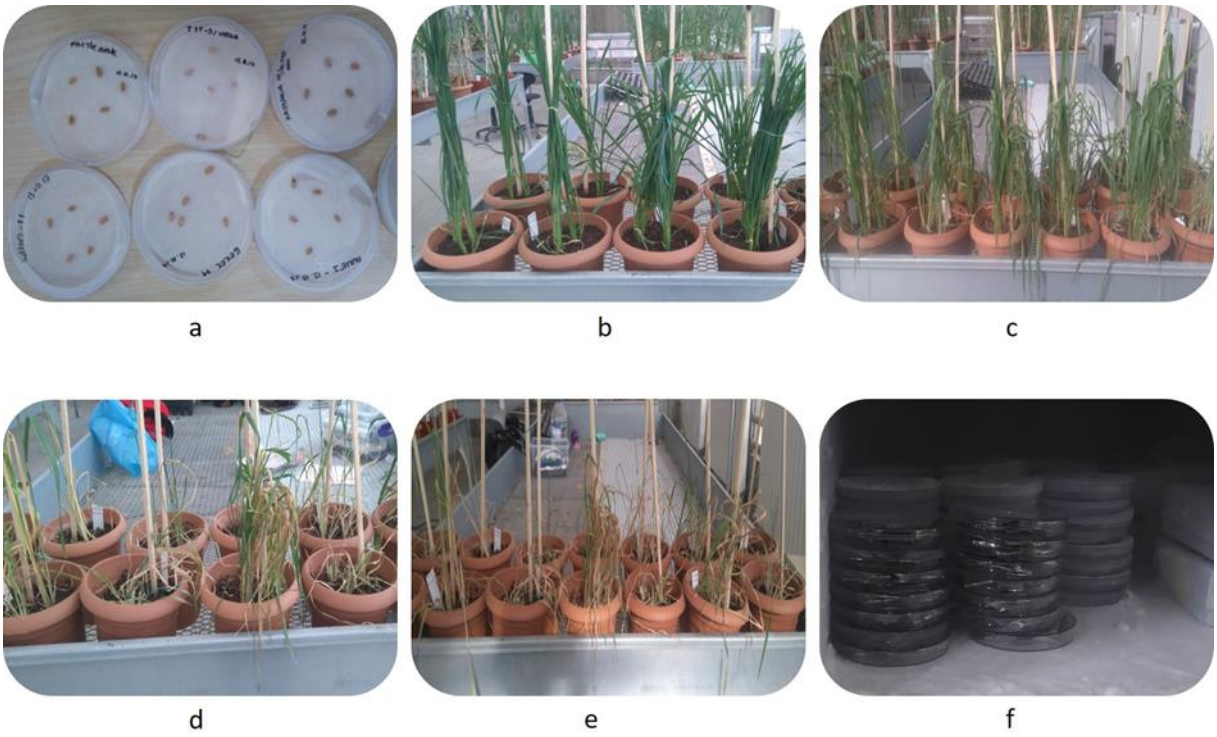

Figure 1. a. Wheat samples germinated in petri dishes **b**. Wheat plants, on the 40th day, grown under greenhouse conditions **c**. 3rd day of drought stress **d**. 6th day of drought stress **e**.10th day of drought stress **f**. Plant samples were stored for analysis at -80°C.

Trichloroacetic acid (TCA) and then centrifuged at 10.000 g for 20 min. On the 0.5 mL supernatant, 1 mL 0.5% (w/v) (Thiobarbituric acid) TBA prepared in 20% TCA was added. The mixture was allowed to stand at 95 °C in a water bath for 30 min and then quickly cooled in an ice bath. After centrifugation at 10.000 g for 5 min, the supernatant absorbance at 532 nm was recorded and corrected for nonspecific turbidity by subtracting the absorbance value at 600 nm wavelength. An extinction coefficient of 1.55×10^5 $M^{-1}cm^{-1}$ was used to quantify lipid peroxides and it was expressed as MDA µmol g^{-1} FW (fresh weight) (Sreenivasulu et al., 1999).

2.5. Determination of hydrogen peroxide (H_2O_2)

For the determination of H_2O_2, leaf tissues (0.5 g) were homogenized with 5 mL of 0.1% (w/v) TCA in an ice bath. The homogenate was centrifuged at 12.000 g for 15 min and 0.5 mL of the supernatant was added to 0.5 mL of 50 mM potassium phosphate (pH: 7) buffer and 1 mL of 1 M KI. Then, the absorbance of the mixture was recorded at 390 nm and the amount of H_2O_2 was determined using the standard curve (Velikova et al., 2000).

2.5. Enzyme activity assays

Fresh leaf tissues (0.2 g) from control and drought treated plants were grounded with liquid nitrogen and homogenized in 3 mL of buffer containing 50 mM KH_2PO_4 buffer (pH 7.0), 0.1 mM EDTA, and 1% PVPP (w/v). The homogenates were centrifuged at 15.000 g for 15 min at 4°C and resulting supernatants were used for the determinations of CAT, POD, APX and PPO activities. Enzyme activity was expressed as enzyme unit (U) per g of fresh tissue.

Catalase (CAT, EC 1.11.1.6) catalyzes the breakdown of hydrogen peroxide (H_2O_2) into H_2O and O_2. Catalase activity measurement is based on the monitoring of the discolouration of H_2O_2 during the reaction of water and oxygen at 240 nm for 3 min by using a spectrophotometer (Schimadzu UV-1800, Japan). In the activity measurement, a 3 mL reaction mixture was prepared with 1450 µl 50 mM KH_2PO_4 buffer (pH: 7), 1500 µl 30% H_2O_2 and 50 µl homogenate. One unit of activity was defined as the amount of enzyme catalyzing the decomposition of 1 µmol H_2O_2 per min, calculated from the extinction coefficient (0.036 cm^2 μmol^{-1}) for H_2O_2 at 240 nm (Ulusu et al., 2017).

Peroxidase (POD, EC 1.11.1.7) activity was defined according to the oxidation of guaiacol previously described (Dursun, 2018). To determine the peroxidase activity spectrophotometrically, a 3 mL reaction mixture was prepared in 970 µl 50 mM KH_2PO_4 buffer (pH: 6), 30% 1000 µl H_2O_2, 1000 µl guaiacol and 30 µl enzyme extract. The reaction was started by finally adding enzyme solution to the activity mixture and the optical density was recorded for 3 min at 470 nm.

In order to determine ascorbate peroxidase (APX, EC 1.11.1.11) activity, a 3 mL reaction mixture was prepared with 1450 µl of 50 mM phosphate buffer (pH: 7), 750 µl of 30% H_2O_2, 750 µl of ascorbic acid and 50 µl of enzyme extract. The reaction was started by adding hydrogen peroxide to the activity mixture and the absorbance at 290 nm was recorded for 3 min. Enzyme activity was calculated using ascorbate Alan's extinction coefficient (2.8 $mM^{-1}cm^{-1}$) (Karabal et al., 2003).

Polyphenol oxidase (PPO, EC 1.10.3.1) activity was determined by measuring the increase in absorbance at 420 nm with a spectrophotometer (UV-1800 Shimadzu JAPAN). 50 µl of crude extract was added to a 3 mL substrate mixture containing 0.20 M sodium phosphate buffer (pH: 6.5), and 25 mM catechol. Enzyme activity was calculated from the linear portion of the curve. One unit of PPO activity was defined as the amount of enzyme that can

cause an increase in absorbance of 0.001/min (Flurkey, 1989).

2.7. Determination of photosynthetic pigment contents

Chlorophyll (Chlorophyll a-Chl a, chlorophyll b-Chl b, total chlorophyll) contents were determined by the methods of Arnon (1949). Leaf tissues (0.2 g) were homogenized with 80% (v/v) acetone and centrifuged at 3.000 g for 5 min. The absorbency of supernatant was measured by spectrophotometer at 450, 645 and 663 nm wavelengths. Photosynthetic pigment amounts were calculated according to the following equations:

Chlorophyll a (mg g^{-1} FW) = 12.7 A_{663} - 2.69 A_{645}

Chlorophyll b (mg g^{-1} FW) = 22.9 A_{645} – 4.68 A_{663}

Total chlorophyll (mg g^{-1} FW) = 20.2 A_{645} + 8.02 A_{663}

2.8. Statistical analysis

According to Duncan's multiple range test, differences in the application groups were analyzed with a significance value of $p \leq 0.05$. Four replicates were performed for each group (n = 4). Statistical analyses were performed with SPSS Standard Version package program and differences between control and application groups were analysed by one-way ANOVA.

3. Results

3.1. Total protein content

The total protein content of 7 different wheat genotypes exposed to drought in the study is shown in Table 2. A result of analysis of H_{79} and G_{79} genotypes showed a relative increase in total protein content, which can be considered statistically significant (Table 2). In H_{79}, a decrease was observed in total protein content in the first days of the drought, while an increase of 3 times was observed in the 10th day compared to the control group. Due to water stress, the total protein content initially decreased compared to the control. It increased again in parallel with the increases in the synthesis of antioxidant enzymes triggered by the continuation of these adverse conditions. In the G_{79} genotype, on the other hand, the total protein content increased periodically during the drought period. This increase may be due to the activities in the synthesis of antioxidant enzymes, which are one of the intracellular antioxidant defence systems. In addition, the increased viscosity due to water loss in the cell cytoplasm under prolonged drought stress can be considered the reason for the increase in protein per tissue compared to the early period. A decrease in total protein content of $T_{98\text{-}9}$, S_{95} and PM ME_1 genotypes was determined during drought. On the other hand, GRK resulted in a serious decrease on the 10th day, although this content increased on the 3rd and 6th days. In many plants, among several cellular responses, variations in protein expression, synthesis, and accumulation have been observed during water deficit, usually mediated by the hormone abscisic acid (Kuromori et al., 2018). Drought stress can induce both qualitative and quantitative changes in plant proteins. For example, Mohammadkhani and Heidari (2008) determined that in *Zea mays* L., in response to progressive drought conditions, about 50 proteins were expressed, while 23 proteins were suppressed and 10 proteins induced synthesis. On the other hand, while there was a significant decrease in the total protein content of the drought-sensitive (Bahar) wheat variety among the 2 different wheat cultivars tested for drought stress, this decrease was slight in the drought-resistant (Kavir) variety (Michaletti et al., 2018).

3.2. Proline content

In all wheat genotypes used in the study, proline content increased in parallel with the prolongation of drought ($p < 0.05$) (Table 2). In G_{79}, the genotype with the highest proline content, on the 3rd, 6th and 10th days of drought, the amount of proline in the plant increased by ~840%, 2089%, and 1988% compared to the control, respectively. All wheat genotypes were able to cope with drought stress in the first days (3rd) of drought, but proline levels increased significantly as the drought continued. Only the proline level of GRK from the investigated wheat genotypes decreased in the first days of drought stress, then increased 13-fold compared to the control.

Proline, which has an important role as an osmolyte, has important functions such as stabilization of membranes and protein conformation, and reduction of photodamage to thylakoid membranes by ROS scavenging when plants are exposed to stress (Ozturk et al., 2021). Similar to our study, it was noted that proline accumulation in pepper increased significantly with increasing drought stress (Kaya et al., 2019). In other experiments, drought has been reported to cause high proline content in peanut (Girija et al., 2002) and wheat genotypes (Keles & Öncel 2004; Sultan et al., 2012).

Proline is known to be able to regulate some drought-resistant genes (Per et al., 2017). Wang et al. (2015) identified the two proline synthesis enzymes pyrroline-5-carboxylate synthetase (P5CS) genes and the pyrroline dehydrogenase PDH gene in *Kosteletzkya virginica* L. It has been reported that although expression of the P5CS1 gene is upregulated in most plant organs during drought, the expression of the PDH1 gene is downregulated (Kiyosue et al., 1996; Strizhov et al., 1997). In this respect, proline accumulation induced by drought stress in plant tissues may occur by upregulating the P5CS gene (Liang et al., 2013).

3.3. Changes in H_2O_2 and MDA content

Toxic ROS production caused by water stress induces lipid peroxidation in plants. Determination of H_2O_2 and MDA content, two important oxidative stress indicators, are commonly used criteria. In general, H_2O_2 and MDA contents of wheat genotypes under drought stress showed a positive correlation with each other. After 10 days of drought, the highest increase in MDA content among genotypes was determined in Pastor with 14-fold, followed by H_{79} with a 9-fold increase. Especially in the G_{79}, H_{79}, GRK, PM ME_1 genotypes, MDA content which reached its highest level as of the 6th day was decreased on the 10th day of drought (Table 2).

In this case, it is thought that it is an indicator that especially membrane lipids begin to disappear due to stress and plant hemostasis is gradually disappearing. Lipid peroxidation in cell membranes is known to be one of the most challenging and most harmful effects on the membranes of all cells exposed to varying degrees of stress. Changes in the amount of malondialdehyde due to the oxidation of membrane lipids provide information about the degree of damage to the cell membranes of plants under stress conditions. Changes in MDA levels in plants vary according

Table 2. Effect of drought treatment on contents of total protein, proline, malondialdehyde and hydrogen peroxide in leaves of wheat types

Type Name	Analysis	Day zero	3rd day	6th day	10th day
H_{79}	Total Protein (mg g^{-1}FW)	0.1595±0.0046c	0.0831±0.0186b	0.0351±0.0022a	0.3109±0.0156d
	Proline (µg g^{-1}FW)	0.9427±0.0245a	1.4244±0.2756a	1.1253±0.1019a	15.3258±0.212b
	MDA(mmol g^{-1}FW)	0.0165±0.0035a	0.051±0.0038b	0.0962±0.0112c	0.0934±0.0105c
	H_2O_2 (µmol g^{-1}FW)	1094.7±162.1b	1975.1±32.3c	185.6±56.9a	1094.7±162.1d
$T_{98\text{-}9}$	Total Protein (mg g^{-1}FW)	0.2168±0.0113c	0.3089±0.0105d	0.0644±0.0027a	0.0988±0.0005b
	Proline (µg g^{-1}FW)	0.9179±0.3656a	5.1177±0.6553b	14.7131±0.4619c	14.6212±0.7838c
	MDA(mmol g^{-1}FW)	0.0287±0.0013a	0.0402±0.0027a	0.0823±0.0082b	0.0991±0.0076b
	H_2O_2 (µmol FW^{-1})	327.6±57.6a	423.5±45.7ab	1072.9±233.9b	2081.6±343.8c
PM ME_1	Total Protein (mg g^{-1}FW)	0.1484±0.0167ab	0.0867±0.0029a	0.1815±0.0009b	0.0664±0.0024a
	Proline (µg g^{-1}FW)	0.7971±0.0475a	7.2482±0.1444b	13.4308±0.4160c	15.8096±0.019d
	MDA(mmol g^{-1}FW)	0.0147±0.0037a	0.0382±0.0076a	0.0657±0.0010b	0.0609±0.0057b
	H_2O_2 (µmol g^{-1}FW)	203.7±43.3a	700.5±45.6b	232.3±34.2a	870.5±50.2b
GRK	Total Protein (mg g^{-1}FW)	0.0632±0.0065a	0.1417±0.0004b	0.1862±0.0222b	0.26±0.01c
	Proline (µg g^{-1}FW)	0.7666±0.1173a	6.4449±1.2665b	15.881±0.0752c	15.8771±0.0728c
	MDA(mmol g^{-1}FW)	0.0518±0.0118a	0.085±0.0122ab	0.1403±0.0162b	0.0814±0.0196ab
	H_2O_2 (µmol g^{-1}FW)	350.5±83.8a	591.1±30.9b	272.2±16.1a	505.7±32.6b
S_{95}	Total Protein (mg g^{-1}FW)	0.2426±0.0063c	0.0965±0.0016a	0.1689±0.0218b	0.175±0.005b
	Proline (µg g^{-1}FW)	0.7854±0.1100a	9.6583±0.6363b	15.7275±0.0786c	15.7559±0.0280c
	MDA(mmol g^{-1}FW)	0.0335±0.0086a	0.1333±0.0060b	0.1709±0.0034b	0.1847±0.0245b
	H_2O_2 (µmol g^{-1}FW)	803.3±173.6a	2149.4±100.8b	495.1±17.4a	574.2±126.1a
Pastor	Total Protein (mg g^{-1}FW)	0.1948±0.0051b	0.0577±0.0028a	0.2457±0.0042c	0.071±0.0077a
	Proline (µg g^{-1}FW)	0.7365±0.09a	9.1423±1.8408c	4.8127±0.8533b	15.9259±0.064d
	MDA(mmol g^{-1}FW)	0.0122±0.0024a	0.0904±0.0215b	0.0585±0.0171b	0.1438±0.0134c
	H_2O_2 (µmol g^{-1}FW)	513.8±107.1a	833.2±71.8ab	318.2±57.9a	1302.6±63.9b
G_{79}	Total Protein (mg FW^{-1})	0.1635±0.0042b	0.2188±0.0019c	0.2227±0.0051c	0.0783±0.0102a
	Proline (µg FW^{-1})	0.9535±0.0652a	0.4588±0.0851a	0.1769±0.0571a	12.5004±1.061b
	MDA(mmol FW^{-1})	0.0032±0.0018a	0.0065±0.0024a	0.0371±0.0024b	0.0127±0.0037a
	H_2O_2 (µmol FW-^{1})	546.1±37.5a	1099.7±55.2b	389.8±15.9a	257.2±47.1a

to the degree of stress and cause an increase in MDA levels in general. If stress conditions cannot be eliminated, MDA levels will continue to increase and deterioration in the endomembrane system will increase, leading to cell damage, similar to our study. Similar to MDA levels, a drought-induced increase was observed in all wheat genotypes. While the levels of H_2O_2 reached their peak on the 3rd day of the drought in the H_{79}, G_{79} and S_{95} genotypes, it was observed that the peak levels were attained on the 10th day of the drought in $T_{98\text{-}9}$, pmme1 and pastor varieties. The results suggest that short-term, gradual temperature increases initially triggered H_2O_2 production in wheat cultivars. Reactive oxygen species (ROS), such as singlet oxygen, superoxide radical, hydrogen peroxide, and hydroxyl radical, responsible for cellular damage, come about due to water stress (Wahid et al., 2007). Damage to plants following exposure to water stress is recognised by impairment to the photosynthetic, mitochondrial and, specifically, cell membranes' fluidity (Gill and Tuteja, 2010; Choudhury et al, 2013). Membrane damage is a stress parameter used to measure the degree of lipid peroxidation (Demirci-Cekic et al., 2022). The elevation in lipid peroxidation and H_2O_2 during biotic and abiotic stress periods is the plant's response to these stress factors. In our study, different H_2O_2 and MDA levels observed in wheat genotypes during different drought periods offer critical information about the tolerance levels of genotypes against drought.

3.4. Antioxidant enzyme activities

Antioxidant enzymes such as CAT, POD, APX, and PPO provide plants with tolerance against stressful conditions and safeguard them from oxidative damage. Plants mitigate the detrimental impacts of reactive oxygen species by activating multiple antioxidant enzymatic systems during water stress exposure. The extent of antioxidant enzyme response in plants exposed to water stress primarily depends on their tolerance and stress levels. To better understand this process in wheat genotypes under gradually increasing drought, we analysed the activities of CAT, POD, APX, PPO antioxidant enzymes. Changes in the activities of four different antioxidant enzymes in wheat plants are shown in Table 3.

CAT activity decreased in all wheat cultivars examined in this study beginning from the initial days of drought. Notably, CAT activity significantly decreased on the sixth day of drought in the H_{79}, PM ME1, G_{79}, Pastor, and GRK genotypes. GRK exhibited the most pronounced decline in CAT (catalase) activity, with a 14-fold reduction compared to its initial levels on the first day of observation. This consistent drop in CAT activity throughout the drought periods suggests that wheat plants may have surpassed their

Table 3. Effect of drought treatment on activity of CAT, POD, APX and PPO enzymes in leaves of wheat types

Type Name	Enzyme Activity (EU FW-1)	Day zero	3rd day	6th day	10th day
H_{79}	CAT	90.375±8.3234b	119.0825±14.2b	22.685±3.8143a	36.5433±6.9284a
	POD	13.2688±0.4434b	12.3891±1.054b	13.4192±0.7466b	8.8891±2.3997a
	APX	2.5071±0.8905a	9.5786±0.7928b	9.45±0.2909b	21.3857±0.5142c
	PPO	2670±785.93a	2560±560a	3420±780a	5700±897.68b
T_{98-9}	CAT	124.6775±10.04b	56.65±5.7907a	82.94±15.2268ab	55.5025±10.63a
	POD	16.7011±0.7586a	18.0376±0.582a	17.0395±0.6328a	17.9605±1.5579a
	APX	3.5679±0.4620a	4.1571±0.3015a	9.5036±0.9604b	5.3893±0.7542a
	PPO	4200±668.13a	6080±335.46b	3080±1247.7a	3800±243.3a
PM ME_1	CAT	38.3756±4.3749b	50.4569±3.147b	5.7868±3.9593a	28.1726±3.961ab
	POD	13.7331±2.5225a	18.6485±0.71ab	16.3872±2.124ab	19.8289±1.3127b
	APX	2.6571±0.0726a	3.4286±0.2726a	2.2071±0.1026a	2.2179±0.2006a
	PPO	2640±202.7a	2520±240a	5100±220ab	5600±211.6b
GRK	CAT	112.0775±16.71c	82.99±15.6442b	14.025±1.505a	*
	POD	14.75±2.0833a	11.4474±1.571a	13.391±2.362a	*
	APX	4.1714±0.3377a	8.775±0.9679b	3.2286±0.3571a	*
	PPO	2640±382.2a	3990±527.5b	2160±600a	*
S_{95}	CAT	67.8173±5.0632b	40.2792±3.136a	44.0102±4.1691a	40.1015±5.9293a
	POD	9.0414±0.7088a	15.6071±1.268b	16.4643±1.762b	18.3008±0.1393b
	APX	2.3679±0.0649b	2.0786±0.0437b	1.7464±0.06671b	0.9571±0.05781a
	PPO	4840±520.7a	3750±453.6a	5910±317.7a	4200±212.9a
Pastor	CAT	96.2437±10.05b	71.4975±10.5ab	54.2132±11.865a	
	POD	10.1767±0.5946a	12.5132±0.156b	13.0226±0.9895b	10.7895±0.81ab
	APX	2.7±0.2693a	4.9071±0.492ab	7.0393±0.8071ab	7.9607±0.0543b
	PPO	4760±262.2b	2910±251.8a	4560±249.7b	4760±204.7b
G_{79}	CAT	112.3075±7.062c	46.5325±6.767b	8.5233±4.02a	26.9475±5.553ab
	POD	18.7782±0.0623a	19.3214±0.042b	19.2223±0.0323b	19.3647±0.0123b
	APX	4.5857±0.2486c	2.2821±0.0915a	2.6143±0.0524a	3.6107±0.5427b
	PPO	2960±693.9a	2220±60a	6720±1440b	2120±312.4a

threshold for drought tolerance, rendering them vulnerable to the adverse consequences of prolonged water scarcity. Furthermore, it's noteworthy that various cultivars display distinct profiles of antioxidant enzyme activities. This divergence underscores the diverse strategies different wheat cultivars employ to combat the effects of water stress. The process of plant adaptation is highly complex and needs fixed guidelines. The process of plant adaptation is complex and needs flexible guidelines. Based on our research, Illescas et al. (2021) also noted a significant decrease in catalase activity (CAT) in wheat plants as drought stress intensified. Similarly, *Festuca arundinacea* Schreb. demonstrated decreased CAT activity when exposed to increasing drought stress.

However, it should be noted that in certain studies, e.g. those investigating *Mentha pulegium* L. (Ulusu et al., 2022) and *Cicer arietinum* L. (Mafakheri et al., 2011) flora, CAT activity has been reported to rise during the initial phases of drought stress before declining over the subsequent days. The decrease in CAT activity is commonly observed as a broad response to various stressors (Abedi & Pakniyat, 2010; Gunes et al., 2008). This decline in CAT activity can be attributed to several factors, including the inhibition of enzyme synthesis, alterations in the composition of enzyme subunits, and potential enzyme degradation mediated by induced peroxisomal proteases, all occurring under stressful conditions.

POD enzymes affect plant growth, development, lignification, suberization, and cross-linking of cell wall compounds (Passardi et al., 2005). In addition, POD decomposes H_2O_2 into H_2O and effectively hinders the accumulation of H_2O_2 in cells, helping to reduce the toxic effect of water stress on plants by protecting them from oxidative damage. In current study, the peroxidase (POD) activity in wheat genotypes subjected to drought conditions was generally higher than in the control. However, only the H_{79} and G_{79} genotypes significantly reduced POD activity on the 10th and 3rd days of drought, respectively. Various research groups have studied the activity of peroxidase enzymes (POD). These studies found that the activity of POD increased in four cultivars of *Carthamus tinctorious* L. (Farooq et al., 2020), in *Brassica napus* L. (Shafiq et al., 2014), in two tomato cultivars (Ghorbanli et al., 2013), and in wheat (Malik & Ashraf, 2012) when subjected to drought stress compared to the controls. These results are in support of the present study.

APX utilises ascorbate as an electron donor to reduce H_2O_2, and its significance in detoxifying H_2O_2 is widely acknowledged (De Gara et al., 2003). The enzyme is activated in the water-water and glutathione-ascorbate cycles. In the Mehler reaction, APX eliminates ROS by reducing them to water (Sui, 2015; Weng et al., 2007). The study found that APX activities varied significantly among wheat genotypes under water stress. H_{79} and Pastor

genotypes showed a significant increase in APX activities, while S_{95} and GRK genotypes exhibited a decrease in APX activities during the later days of drought. PM ME_1 and T_{98-9} genotypes did not present any statistical difference in APX activity under water stress ($p< 0.05$). Another study on antioxidant enzyme activities in three different types of wheat subjected to water stress reported that three varieties showed the highest APX activity at -4 bar water potential level. However, this enzyme activity reduced as the drought level increased (Esfandiari et al., 2007). Enhanced APX activity in plants during water stress augments oxidative stress tolerance (As, 1993). Thus, it minimises the damage caused by drought in plants and protects the plant until a specific period.

PPO activities were directly proportional related to drought stress levels in all the genotypes of wheat studied in this study. In general, PPO activity remained stable in the samples analysed during early drought stress periods, while a relatively increase was observed in H_{79} and PM ME_1 genotypes under long-term drought stress conditions. The results showed that, in general, PPO enzyme activity did not show a clear relationship with stress. Especially in H_{79} and PM ME_1 genotypes, the enzyme activity was significantly lower than the control in the first days of drought, while an increasing trend was observed under severe stress. The diverse reactions of enzymatic antioxidant activities observed across all the tested wheat varieties in this study can be attributed to the genetic distinctions inherent to each of these cultivars (Malik & Ashraf, 2012).

3.5. Chlorophyll (Chl a, Chl b and total Chl) content

In this study, in which Chl a, Chl b, and total Chl analyses were performed to determine how pigment degradation, which reduces the photosynthetic efficiency, changes with drought, it was determined that drought stress significantly reduced these pigment contents (Table 4). Significant differences were identified between the analysed genotypes ($p <0.05$). Especially in the S_{95} on the 10th day of drought, Chl a, Chl b and total Chl contents decreased by 72.17%, 89.74% and 77.25%, respectively and it was the genotype most affected by drought in terms of chlorophyll content. Although Chl a and total Chl content increased on the 3rd and 6th days of drought in the G_{79} genotype, it was greatly affected by the continuation of drought and its vegetative tissues were completely damaged. Among the analyzed genotypes, T_{98-9} was the least affected wheat variety in terms of pigment content from drought. While photosynthetic pigment levels differed according to the genotype difference under stress conditions, the total amount of chlorophyll decreased in general among the cultivars. In Chl a, chlorosis, which occurs due to drought conditions, was less from Chl b. In this case, when Chl a is the dormant type in total Chl, the share of Chl b in the total Chl pool in plants has increased proportionally. These results showed that the water stress experienced had reduced the rate of photosynthesis due to the loss of chlorophyll in plants. Oxidative stress in plants causes chloroplast disruption and can decrease chlorophyll content (Arora et al., 2002). Therefore, the results obtained from the present study can be considered a distinctive oxidative stress symptom (Smirnoff, 1993). Similar to our results, the gradual increase in drought stress resulted in significantly decreased pigment contents in Lilium species (Zhang et al., 2011), *Olea europaea* L. (Khaleghi et al., 2012) and tomato (Zgallaï et al., 2006).

Table 4. Effect of drought treatment on contents of Chlorophyll a, Chlorophyll b, Total Chlorophyll in leaves of wheat types

Type Name	Analysis	Day zero	3rd day	6th day	10th day
H_{79}	Chlorophyll a	23.91±2.49c	15.74±0.78b	7.62±0.91a	6.19±0.67a
	Chlorophyll b	6.72±0.61b	4.44±0.50a	3.18±0.47a	3.20±0.34a
	Total Chlorophyll	23.24±2.30b	16.49±3.11ab	10.80±1.38a	9.39±1.01a
T_{98-9}	Chlorophyll a	17.79±1.14b	21.11±1.34c	12.02±0.45a	15.48±1.11b
	Chlorophyll b	5.25±0.38b	5.33±0.17b	3.63±0.19a	5.18±0.41b
	Total Chlorophyll	23.04±1.53b	23.49±0.83b	15.65±0.56a	20.66±1.52b
PM ME_1	Chlorophyll a	10.20±2.49a	16.21±2.03ab	9.42±1.22a	23.68±3.30b
	Chlorophyll b	3.93±1.08a	4.76±1.02a	3.26±1.08a	12.88±1.05b
	Total Chlorophyll	14.13±3.62a	18.20±4.38a	12.68±1.76a	36.55±6.17b
GRK	Chlorophyll a	24.59±4.59a	24.83±0.91a	27.64±1.15a	*
	Chlorophyll b	16.42±0.41b	8.76±0.72a	17.1±1.18b	*
	Total Chlorophyll	41.01±2.54a	33.59±1.60a	44.87±1.21a	*
S_{95}	Chlorophyll a	25.77±2.33c	29.16±0.55c	16.55±2.75b	7.17±0.48a
	Chlorophyll b	27.1±0.62c	13.63±1.61b	10.12±2.38b	2.68±0.73a
	Total Chlorophyll	42.31±8.28c	42.78±0.15c	26.67±3.53b	9.85±1.15a
Pastor	Chlorophyll a	16.6±2.52c	9.12±0.90b	3.96±0.23a	7.34±0.53ab
	Chlorophyll b	5.61±0.81b	3.03±0.44ab	1.49±0.29a	3.68±0.13ab
	Total Chlorophyll	22.26±2.66c	12.16±1.10b	5.45±0.31a	10.03±0.63b
G_{79}	Chlorophyll a	13.62±1.02c	8.133±0.60b	2.44±0.11a	1.93±0.03a
	Chlorophyll b	4.45±0.04c	2.67±0.43b	0.81±0.09a	0.78±0.02a
	Total Chlorophyll	18.07±0.30c	10.80±0.60b	3.25±0.08a	2.71±0.01a

4. Conclusions

This study provides a significant illustration of how wheat genotypes display diversity in their reaction to drought, covering aspects such as the commencement and severity of drought pressure, water-based mechanisms, capacity for recuperation, and plant biochemical responses. Our findings highlight that G79 and H79 emerge as the most physiologically sensitive to drought among the seven distinct wheat genotypes studied. In contrast, Pastor and S_{95} genotypes display a higher degree of drought tolerance than the other genotypes. The data on antioxidative enzyme activities and proline production in wheat can be instrumental as selection criteria in drought tolerance breeding programs. Ultimately, the selection of drought-tolerant wheat genotypes aims to increase the productivity of the targeted crops.

Conflict of Interest

Authors declare conflict of interest.

Authors' Contributions

The authors contributed equally.

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Sporobolus turcicus (sect. *Crypsis, Poaceae*), a new species from Türkiye

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Sporobolus turcicus (sect. *Crypsis, Poaceae*), Türkiye'den yeni bir tür

Abstract: Some interesting *Sporobolus* (Poaceae) specimens were collected from Eskil, Aksaray, that have narrow inflorescence, long lower glume and caryopsis. In the careful examination made, the specimens resemble *Sporobolus schoenoides* and *S. borszczowii* belonging to the section *Crypsis* (subsect. *Crypsis*), but it was determined that it is different from them due to especially some generative characters. These specimens after compared with the closest taxa, it was decided that, it is a new species for science world and was named *Sporobolus turcicus*. Specimens of the new species grows at altitudes between about 910-920 meters in the dried salt marsh. The size and shape of some organs such as leaf blades (0.6-1.2 mm broad, puberulent to long-pilose inner surface, glabrous outer surface), lower glume (3.2-4.1 mm long), lemma (awn 0.6-0.8 mm long) and caryopsis (ellipsoid, 1.5-2.1 mm long), allow to recognize *Sporobolus turcicus* from its closest taxa. Here, the description of the new species, comparison with the similar taxa, informative photographs, and some ecological preferences have been given.

Key words: Aksaray, new species, *Sporobolus*, taxonomy, Türkiye

Özet: Aksaray, Eskil'den çiçek durumu dar, alt gluması ve karyopsisi uzun bazı ilginç *Sporobolus* (Poaceae) örnekleri toplandı. Yapılan detaylı inceleme sonucunda örneklerin *Crypsis* seksiyonuna (alt seksiyon *Crypsis*) ait *Sporobolus schoenoides* ve *S. borszczowii*'ye benzediği, ancak özellikle bazı generatif karakterler nedeniyle farklı olduğu belirlendi. Bu örneklerin yakın taksonlarla karşılaştırılması sonucunda bilim dünyası için yeni bir tür olduğuna karar verildi ve *Sporobolus turcicus* olarak adlandırıldı. Yeni türün örnekleri kurumuş tuzlu bataklıkta yaklaşık 910-920 metre rakımlarda yetişir. Yaprak ayaları (0,6-1,2 mm genişliğinde, iç yüzey havlı-uzun tüylü, dış yüzey tüysüz), alt gluma (3,2-4,1 mm uzunluğunda), lemma (kılçık 0,6-0,8 mm uzunluğunda) ve karyopsis (elipsoid, 1,5-2,1 mm uzunluğunda) gibi bazı organların boyutu ve şekli, *Sporobolus turcicus*'un en yakın taksonlardan ayrılmasını sağlar. Burada yeni türün tanımı, benzer taksonlarla karşılaştırılması, bilgilendirici fotoğrafları ve bazı ekolojik tercihlerine yer verilmiştir.

Anahtar Kelimeler: Aksaray, *Sporobolus*, taksonomi, Türkiye, yeni tür

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1. Introduction

Poaceae Barnhart is one of the largest families in the world, with 830 genera and 12369 species that are considered valid (WFO, 2023a). According to the latest data, the family is represented with 147 genera and 556 species in Türkiye (Cabi and Doğan, 2012; Cabi et al., 2013; 2015a; 2015b; 2018; Doğan et al., 2015; Cabi and Soreng, 2016; Terzioğlu and Özkan, 2020; Behçet and Yapar, 2021; Aykurt et al., 2022). *Sporobolus* R.Br. s.l. contains about 230 species and is a common genus worldwide (WFO, 2023b). *Sporobolus* is represented by 8 taxa connected to 3 sections in Türkiye (Tan, 1985; Byfield and Pearman, 2000; Cabi and Doğan, 2012; Peterson et al., 2014). None of these species are endemic to Türkiye. Species of the genus mostly prefer edges of terrestrial and coastal salty lakes, marshes or sandy places as habitats (Tan, 1985).

Some interesting *Sporobolus* specimens were collected during the investigation of salt marshes around Tuz Lake (Türkiye, Aksaray, Eskil) in 2018. Due to its spikelets with one floret, annual and rachilla disarticulating below glumes, the specimens were first identified as the genus *Crypsis* Aiton according to "*Flora of Turkey and the East Aegean Islands*" (Davis, 1985). Specimens of the plants with flowers, which has adapted to the edge of salt marshes, were collected around the mid of August. As a result of the investigations carried out on specimens, and the consideration of the most recent revision studies and the Flora of Turkey and the East Aegean Islands, Flora Europaea, Flora USSR, Flora Iranica, Flora of Iraq, and Flora of Syria, Palestine and Sinai, it was decided that the specimens belong to a new species between *Sporobolus schoenoides* (L.) P.M.Peterson and *S. borszczowii* (Regel) P.M.Peterson (sect. *Crypsis* (Aiton) P.M.Peterson, subsect. *Crypsis* (Aiton) P.M.Peterson) (Post, 1933; Rozhevits and Shishkin, 1963; Bor, 1968; 1970; Tutin, 1980; Tan, 1985; Raus and Scholz, 2004; Peterson et al., 2014).

2. Materials and Method

Specimens belonging to the new species were collected in August from the dried salt marshes near Tuz Lake, in Eskil District of Aksaray Province (Central Anatolia, Türkiye). The related literature (Post, 1933; Rozhevits and Shishkin, 1963; Bor, 1968; 1970; Tutin, 1980; Tan, 1985; Raus and Scholz, 2004; Peterson et al., 2014), the high-resolution photographs in the Conservatoire et Jardin Botaniques de la Ville de Genève (G), Royal Botanic Garden Edinburgh (E), Naturhistorisches Museum Wien (W), and Muséum National d'Histoire Naturelle Paris (P) herbaria were utilized in the identification and evaluation of the specimens (Thiers, 2023). A Leica EZ4 stereo microscope, a Samsung A33 5G mobile telephone, and the Canon

EOS60D were used in the examination of the specimens and the taking of photographs, whereas a ruler with a sensitivity of 0.5 mm was used in the measurements.

3. Results

***Sporobolus turcicus* Hamzaoğlu, sp. nova** (Figure 1)

Type. Türkiye. **Aksaray**: Eskil, from Eskil to Tuz Lake, NE of Eskil, road of "Ali Emminin Çardağı", 38°26'56"N - 33°27'09"E, 915 m a.s.l., dried salt marsh, 18 August 2018, *Hamzaoğlu 7519* (**holotype** GAZI!, **isotypes** GAZI!, ANK!, HUB!).

Diagnosis. *Sporobolus turcicus* is related to *S. schoenoides* and *S. borszczowii*. It differs from *S. schoenoides*, mainly by leaf sheaths sparsely pilose hairs between nerves (not glabrous), leaf blades linear-acuminate, 0.6–1.2 mm broad, puberulent to long-pilose inner surface, glabrous outer surface (not acuminate, 2–7 mm broad, glabrous inner surface, villous or sparsely pilose outer surface), lower glume 3.2–4.1 mm long (not 2.2–3 mm long), lemma awn 0.6–0.8 mm long, not ciliate on margin (not unawned), caryopsis ellipsoid, 1.5–2.1 mm long (not oblong-ellipsoid, 1–1.2 mm long). Also, it differs from *S. borszczowii*, mainly by culms erect to ascending (not procumbent to geniculately ascending), leaf blades linear-acuminate, 0.6–1.2 mm broad, puberulent to long-pilose inner surface, glabrous outer surface (not acuminate, 1–3(–5) mm broad, puberulent to long-pilose on both surfaces), inflorescence 4–5 mm broad (not 7–8 mm broad), lemma margin glabrous (not ciliate), caryopsis ellipsoid, 1.5–2.1 mm long (not ovoid, 1–1.5 mm long).

Description. Annual grass. Culms few from base, erect or ascending, 3–6 cm long. Leaves glaucous-green. Sheaths markedly ribbed on outer surface, not scabrid on nerves, sparsely pilose hairs between nerves, with membranaceous, and usually ciliate margins, usually not inflated, conspicuously shorter than internodes, upper 1(–2) usually with reduced blades and bract-like. Ligule a fringe of hairs. Leaf blades distinctly demarcated from sheath, linear-acuminate, involute, 7–25 × 0.6–1.2 mm, conspicuously ribbed on both surfaces, scabrid on margins, puberulent to long-pilose inner surface, glabrous outer surface. Inflorescence a narrowly ovoid or ellipsoid spiciform panicle usually of 10–17 fertile and 1–2 sterile spikelets, 8–15 × 4–5 mm. Rachis glabrous. Spikelets 3–4 mm long including acumens, wedge-shaped, compressed, lower with short- and other one long-pedicellate. Glumes subequal, 3.2–4.1 × 0.7–1.3 mm, 1-nerved, glabrous, scabrid on upper part of keel, narrowly to broadly lanceolate, with membranaceous margin, narrowing into a cuspidate 0.9–1.5 mm acumen, ciliate on upper part either on both margins or at least on one margin. Lemma 3–4 × 0.8–1.1 mm, with membranaceous, margin, 1-nerved, glabrous, scabrid on upper part of keel, narrowing into a cuspidate 0.6–0.8 mm acumen, not ciliate on margin. Palea completely membranaceous,, 4-lobed at apex, 2.3–3.1 × 0.9–1.2 mm, 2-nerved, plicate along nerves. Caryopsis dark brown, ellipsoid, slightly compressed, 1.5–2.1 × 0.9–1.2 mm.

Etymology. The name of the new species was inspired by the name of the country where it was grown and discovered.

Proposed vernacular name. Türk Bakakotu (in Turkish), Turkish Pricklegrass (in English).

Flowering time. July to August.

Figure 1. Habit and flower parts of *Sporobolus turcicus*. A – habit, B – leaves sheaths and hairiness, C – fertile spikelet, D – lemma and palea, E – caryopsis (Scale bars. A: 10 mm; B: 3 mm; C, D and E: 1 mm).

Distribution and habitat. Specimens of *Sporobolus turcicus* were collected from the surroundings of Tuz Lake (Eskil, Aksaray). It is estimated that the species grows in dried salt marsh of northeast of Eskil, approximately between 910 and 920 m a.s.l. The species is also likely to grow in such as Seyfe Lake (Kırşehir Province), Sultansazlığı (Kayseri Province), which are located in the central part of Turkey, but there is no data on this yet. Consequently, at present, the species is an endemic of Türkiye and when the area of distribution is considered, it is an element of the Irano-Turanian phytogeographic region.

IUCN Conservation assessment. According to the existing data, *Sporobolus turcicus* is a species only known from the type locality. Approximately 1.500 individuals were counted in the type locality. The species grows in the dried salt marshes at northeast of Eskil District (Aksaray Province). Since the area where the species grows is a marsh, there are no settlements and agricultural areas in its immediate vicinity. On the other hand, as it is the only pasture area in the vicinity, it is subject to intense grazing by livestock. When the areas where the species could be grown are considered, it is estimated that *Sporobolus turcicus* showed a distribution on an area approximately 3000 km^2. When the existing or envisaged threats are evaluated together, the species being known from only one addresses at present (area of life less than 100 km^2) and the breadth of the area of distribution calculated (less than 3000 km^2), it was decided that it would be suitable to propose the Endangered [EN, B1abiii and B2abii] classification for the extinction risk of the species (IUCN Standards and Petitions Committee, 2019).

Additional specimens examined

Sporobolus schoenoides. **Türkiye**. [Kahramanmaraş]: Maras, Süleymanlı, nr. stream, 04.08.1964, *L.Williams s.n.* (E, E00357226!); prope Smyrnam [İzmir], 22.10.1867, *J.Ball s.n.* (E, E00357226!); prov. Diyarbakır: Diyarbakır-Bitlis, c. 70 km from Diyarbakır, c. 750 m a.s.l., gravel banks in dry river bed, 10.08.1956, *McNeill 508* (E, E00357228!); Elazığ: zwischen Elazığ und Pertek, Südufer des Murat nehri, 500 m östlich der Straßenbrücke über den Fluß, 900 m a.s.l., 21.07.1973, *F.Holtz 00.749b* (E, E00357230!); Hakkari, Yüksekova, 1950 m a.s.l., on path, 07.09.1967, *D.Duncan & S.Tait 218* (E, E00357232!); Çanakkale: Umurbey, Umurbey Çayı, gravel, almost dry riverbed, 22.07.1981, *A.Kurtto 3228* (E, E00357234!). **Greece.** Iles Ioniennes, 0 m a.s.l., 1837, *H.Margot s.n.* (G, G00799112!). **Iran**. Khorasan: Gulestan forest, 1000 m a.s.l., by stream, ground very wet, 24.08.1967, *D.Walton 212* (E, E00357249!); Kazvin: 20 km south of Gazvin (Kazvin), 30.06.1969, J.Andersen & I.Petersen 130 (E, E00357260!); Lorestan: left bank of Kashgan Rud, above Pol-i-Khallor, 60 km W. of Khorramabad, 11.07.1966, J.Archibald 2660 (E, E00357252!). **Iraq**. Baghdad: Tigris Bank, 14.12.1954, *R.Haines s.n.* (E, E00357254!); Amara: cultivated ground by Tigris above Amara, 05.11.1917, *W.Evans s.n.* (E, E00357244!); Ninawa: Sersank, 09.08.1959, *R.Haines 1588* (E, E00357251!).

Sporobolus borszczowii. **Türkiye**. [İzmir]: Station de Boudja, près de Smyrne, vers 150 mètres d'alt. 31.08.1866, *B.Balansa 1541* [as *Crypsis ambigua*], (P, P02520104!; P02904427!; P02904428!; P02904429!; P02520103!; P02520105!; P02520106!; K, K000907264!; K000907266; W, W0027063!; W18890048400!).

4. Discussions

The first comprehensive information in Türkiye about the genus *Sporobolus* (as *Sporobolus* and *Crypsis*), was included in Volume 9 of the work titled Flora of Turkey and the East Aegean Islands (Tan, 1985). Accordingly, *Sporobolus schoenoides* and *S. borszczowii* (incl. all subspecies) are two of the 6 species of the genus known from Türkiye. The validity of these species were also accepted in the later revision studies of the genus *Sporobolus* (Peterson et al., 2014). These species are very similar to each other in habit because their culms erect, ascending or procumbent, leaves linear or linear-acuminate and ligule hairy, inflorescence narrowly ovoid or oblong, spikelets 3–5 mm long and strongly laterally compressed, glumes membranaceous or hyaline; palea 2-nerved and caryopses 1–2.1 mm long. On the other hand, these species differ from each other especially by the dimension of their some vegetative and generative characters (Table 1). According to the latest data, 8 species (incl. *S. turcicus*) of the genus *Sporobolus* grow in Türkiye (Table 2; Tan, 1985; Byfield and Pearman, 2000).

Sporobolus schoenoides and *S. borszczowii* differ slightly from each other with leaf hairiness, lower glume length, and the presence of awn in lemma. However, the differences between *Sporobolus turcicus* and these two species are much more obvious. Current descriptions and the examination of herbaria specimens revealed that *Sporobolus turcicus*, especially for the flowers characters, is different from *S. schoenoides*. For example, the lower

Table 1. Comparison of diagnostic characters of *Sporobolus turcicus* and related species

Diagnostic characters	*S. turcicus*	*S. schoenoides*	*S. borszczowii*
Culms	erect or ascending	procumbent or ascending	procumbent to geniculately ascending
Leaf sheaths	sparsely pilose hairs between nerves	glabrous	glabrous or sparsely pilose
Leaf blades	linear-acuminate, 0.6–1.2 mm broad, puberulent to long-pilose inner surface, glabrous outer surface	acuminate, 2–7 mm broad, glabrous inner surface, villous or sparsely pilose outer surface	acuminate, 1–3(–5) mm broad, puberulent to long-pilose on both surfaces
Inflorescence	4–5 mm broad	4–12 mm broad	7–8 mm broad
Spikelets	3–4 mm long	3–4 mm long	4–5 mm long
Lower glume	3.2–4.1 mm long	2.2–3 mm long	(3–)3.5–4.3 mm long
Lemma	awn 0.6–0.8 mm long, not ciliate on margin	unawned	awn 0.5–1.2 mm long, ciliate on margin
Caryopsis	ellipsoid, 1.5–2.1 mm long	oblong-ellipsoid, 1–1.2 mm long	ovoid, 1–1.5 mm long

Table 2. Taxonomic summary of the genus *Sporobolus* in Türkiye.

Species	According to Peterson et al. (2014)	According to "Flora of Turkey and the East Aegean Islands" (Tan, 1985; Byfield & Pearman, 2000)
	Section *Sporobolus* R.Br.	**Genus *Sporobolus* R.Br.**
1	*Sporobolus fertilis* (Steud.) Clayton	*Sporobolus fertilis* (Steud.) Clayton
	Section *Virginicae* Veldkamp	Genus *Sporobolus* R.Br.
2	*Sporobolus virginicus* (L.) Kunth	*Sporobolus virginicus* (L.) Kunth
	Section *Crypsis* (Aiton) P.M.Peterson	Genus *Crypsis* Aiton
	Subsection *Crypsis* (Aiton) P.M.Peterson	Genus *Crypsis* Aiton
3	*Sporobolus aculeatus* (L.) P.M.Peterson	*Crypsis aculeata* (L.) Aiton
4	*Sporobolus alopecuroides* (Piller & Mitterp.) P.M.Peterson	*Crypsis alopecuroides* (Piller & Mitterp.) Schrader
5a	*Sporobolus borszczowii* Regel subsp. *borszczowii*	*Crypsis acuminata* Trin. subsp. *borszczowii* (Regel) Kit Tan
5b	*Sporobolus borszczowii* Regel subsp. *acuminatus* (Trin.) P.M. Peterson	*Crypsis acuminata* Trin. subsp. *acuminata*
5c	*Sporobolus borszczowii* Regel subsp. *ambiguus* (Boiss. & Balansa ex Boiss.) P.M.Peterson	*Crypsis acuminata* Trin. subsp. *ambigua* (Boiss. & Balansa ex Boiss.) Kit Tan
6	*Sporobolus factorovskyi* (Eig) P.M.Peterson	*Crypsis faktorovskyi* Eig
7	*Sporobolus schoenoides* (L.) P.M.Peterson	*Crypsis schoenoides* (L.) Lam.
8	*Sporobolus turcicus* Hamzaoğlu	In this article.

glume is 3.2–4.1 mm long (not 2.2−3 mm), lemma is awned (not awned), and caryopsis is 1.5–2.1 mm long (not 1−1.2 mm). The new species also has similaries with *S. borszczowii*. But the inflorescence is 4−5 mm broad (not 7−8 mm), lemma is not ciliate on margin (ciliate on margin), caryopsis is ellipsoid and 1.5–2.1 mm long (ovoid and 1–1.5 mm long) (Table 1, Figure 1).

The works titled "A molecular phylogeny and new subgeneric classification of *Sporobolus* (*Poaceae: Chloridoideae: Sporobolinae*)" prepared by Peterson et al. (2014), is a broad-scope study which includes all the species of the genus *Sporobolus*, and includes a total of 177 species belonging to the genus. The moment the *Sporobolus turcicus* was collected for the first time, the most interesting aspects were the narrow leaves, inflorescences, and large caryopsis size. These are still more striking characters differentiating *S. turcicus* from *S. schoenoides* and *S. borszczowii* specimens (Table 1, Figure 1).

Conflict of Interest

Author has declared no conflict of interest.

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A new species from Türkiye: *Bolanthus sertavulus* (*Caryophyllaceae*)

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Türkiye'den yeni bir tür: *Bolanthus sertavulus* (*Caryophyllaceae*)

Abstract: During a field study, performed within the scope of an ongoing revision study on *Bolanthus* (Ser.) Rchb. genus, aiming to contribute to the 'Illustrated Flora of Türkiye', some specimens resembling *B. cherlerioides* (Bornm.) Barkoudah were collected from Sertavul Pass (Mut-Mersin). Detailed investigations on the specimen revealed that some distinct differences exist between *B. cherlerioides* and newly collected specimens. As a result of a carefull comparison with the closest taxon, it was decided that it is new for science, and named as *Bolanthus sertavulus*. It grows at altitudes between 1100-1500 meters, on steppe and rocks. It is flowering in May to June. Cushions 5-10 cm diameter, stems erect, 1-2 cm long, densely upright, and with long glandular hairs; shorter and thicker from *B. cherlerioides*, internodes invisible. Leaves shorter and harder. Calyx surrounded by leaves, rarely visible. Petals protruding from the calyx, pink, with 3 prominent veins. It is known only from type collection, a Mediterranean element, and endemic to Türkiye. A description of the new species, comparison with the closest taxon, photographs and drawings related to its morphology are presented.

Key words: *Bolanthus sertavulus*, endemic, new species, revision, taxonomy

Özet: 'Resimli Türkiye Florası'na katkı sağlamak amacıyla, *Bolanthus* (Ser.) Rchb cinsinin revizyonu konu alan bir çalışma kapsamında gerçekleştirilen bir arazi çalışması esnasında, Sertavul geçidi (Mut-Mersin)'nden *B. cherlerioides* (Bornm.) Barkoudah türüne benzeyen bazı örnekler toplanmıştır. Örnekler üzerinde gerçekleştirilen detaylı çalışma, yeni toplanan örnekler ile *B. cherlerioides* örnekleri arasında belirgin bazı farklılıkların olduğunu ortaya koymuştur. En yakın takson ile dikkatli bir karşılaştırma sonucunda, örneklerin bilim dünyası için yeni olduğuna karar verilmiş ve *Bolanthus sertavulus* olarak isimlendirilmiştir. Bu bitki 1100-1500 metre rakımda, step ve kayalık yerlerde yetişir. Mayıs-Haziran aylarında çiçek açar. Yastıklar 5-10 cm çapında, gövdeleri dik, sık, dik, 1-2 cm boyunda, uzun salgılı tüylerle kaplı *B. cherlerioides*'e göre daha kısa ve kalındır. Boğumlar arası görünmez. Yaprakları daha kısa ve sert, yapraklarla örtülmüş nadiren görülebilen kaliks. Petalleri kaliksten dışarı çıkmış, pembe ve 3 belirgin damarlıdır. Sadece tip lokalitesinden bilinen tür, Akdeniz elementidir ve Türkiye için endemiktir. Yeni türün betimlemesi, en yakın taksonla karşılaştırması, morfolojisine ilişkin fotograf ve çizimleri verilmiştir.

Anahtar Kelimeler: *Bolanthus sertavulus*, endemik, yeni tür, revizyon, taksonomi

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1. Introduction

Although family *Caryophyllaceae*, comprising around 100 genera and 2500 species, is mainly distributed in Northern Hemisphere, the gene center is estimated to be Mediterranean Region (Lawrence, 1951; WFO, 2023). According to our field observations; two differentiation centers have been determined for the genus *Bolanthus* (Ser.) Rchb. within Lakes Region (in Türkiye): First one is the Sultan Mountains (within the boundaries of Isparta, Afyonkarahisar and Konya provinces) and continuation of this mountain, volcanic lands of Bozkır, Hadim, Ermenek districts. The other one is Köpekbeli, around Salda Lake (in Burdur) and Sandras Mountain (in Muğla), serpentine beds. For this reason, distribution area and biodiversity centers of the genus are mainly in Lake Region of Türkiye.

Distribution area of the genus *Bolanthus* (known as Havalotu in Turkish) is Mediterranean countries. There are about 20 species distributed in the region (Türkiye, Greece, Syria, Lebanon and Palestine) (Barkoudah, 1962; Huber-Morath 1967; Phitos, 1997; Koç and Hamzaoğlu 2015). Of these, 11 species currently exist in Türkiye and all of them are endemics. Newly defined species are generally known from type gatherings, 6 in Flora of Europe, one Flora of Palestine (Zohary, 1966; WFO, 2023).

The first revision of the genus for Türkiye was made by Artur Huber-Morath (Huber-Morath, 1967a,b). In this revision, 5 species and 2 varieties were described and recorded for Turkish flora. During the preparation of first supplement volume of the 'Flora of Turkey and the East Aegean Islands', another species, *B. stenopetalus* Hartvig & Strid was added (Davis et al., 1988). Later on, *B. huber-morathii* C.Simon, *B. mevlanaea* Aytaç, *B. turcicus* Koç & Hamzaoğlu and *B. azizsancari* M.Koç & E.Hamzaoğlu were presented, and the number of species in Türkiye increased to 11. In recent publications, existing Turkish *Bolanthus* taxa were revised and an identification key was prepared (Aytaç and Duman, 2004; Özhatay et al., 2009; Koç and Hamzaoğlu, 2015).

Totally 25 members of the genus currently exist in the countries (Syria, Lebanon, Palestine, Greece and Türkiye) localized at Mediterranean coasts. The genus is represented by 8 taxa in European flora, and 6 taxa in Syria, Palestine and Lebanon. All of the taxa distributed in Europe are also known from Greece and East Aegean Islands (Yıldızbaş

Table 1. Existing *Bolanthus* taxa of Türkiye

	Taxon name	Turkish Name
1a	***B. frankenioides*** (Boiss.) Barkoudah var. ***frankenioides***	Tüylü hashavalotu
1b	***B. frankenioides*** (Boiss.) Barkoudah var. ***fasciculatus*** (Boiss. & Heldr.) Barkoudah	Tüysüz hashavalotu
2	***B. spergulifolius*** (Jaub. & Spach) Hub.-Mor.	Yoz havalotu
3	***B. stenopetalus*** Hartvig & Strid	Özge havalotu
4	***B. turcicus*** Koç & Hamzaoğlu	---
5	***B. sandrasicus*** Hamzaoğlu & Koç	Sandras havalotu
6	***B. thymoides*** Hub.-Mor.	Çorak havalotu
7	***B. cherlerioides*** (Bornm.) Barkoudah	Konya havalotu
8	***B. aziz-sancarii*** Koç & Hamzaoğlu	Azizsancar havalotu
9	***B. mevlanaea*** Aytaç	Akseki havalotu
10	***B. minuartioides*** (Jaub. & Spach) Hub.-Mor.	Kaya havalotu
11	***B. huber-morathii*** C.Simon	Bursa havalotu

and Koç, 2018). Twelve of these 26 taxa are distributed only in Türkiye and all of them are endemic to the country.

Existing *Bolanthus* taxa of Türkiye are listed in Table 1 together with their Turkish names (Aytaç and Duman, 2004; Barkoudah and Akeroyd, 1993; Koç and Hamzaoğlu, 2015; Koç et al., 2019; Yıldızbaş and Koç, 2018).

In this study, a new *Bolanthus* species is presented. It was determined within the scope of an ongoing revision study, aiming to contribute to the 'Illustrated Flora of Türkiye'and to solve the problems of small but taxonomically difficult genus *Bolanthus.*

2. Materials and Method

Specimens related to the newly reported *Bolanthus* species were collected in June from Sertavul pass within the boundaries of Mut district of Mersin province, in Türkiye. Photographs related morphology were taken in the herbarium from dry specimens. A stereomicroscope is used for detailed examinations. The determined characteristics of the specimens were compared with Barkoudah (1962), Davis (1967). The herbarium specimens of GAZI, KNYA and especially of GUL which is the richest one in terms *Bolanthus* specimens were also evaluated. The distinguishing features of the specimen are compared, in tabular form, with the closest taxon, *B. cherlerioides*. Diagnosis, description and drawings of holotype specimen were prepared. Based on the investigated specimens, an updated description was also prepared for *B. cherlerioides.* Current identification key for Turkish Bolanthus (Davis, 1967; Koç et al., 2019) were also updated considering the newly identified species and the updates.

3. Results

3.1. Taxonomic treatment

Bolanthus sertavulus Özçelik **sp. nov. (**Figs. 1,2, Table 2)

Affinis ***Bolanthus cherlerioides*** sed partes eius supra humum dense, erectae, pilis longis glandulosis obsita; corpore breviore et crassiore (1-2 cm); foliis brevioribus et durioribus; minora cervicalia magnitudine (5-10 cm) distinguuntur.

Type: İçel (Mersin), Mut, Sertavul pass, steppe and rocks, 1100-1500 m, 26.06.2000, Özçelik 8462. Endemic. Holo: GUL, Iso: GUL, VANF, KNYA.

Diagnosis: Allied to *B. cherlerioides*. But its cushions 5-10 cm, densely upright, and with long glandular hairs; stems erect, 1-2 cm long, shorter and thicker, internodes invisible; leaves shorter and harder,; calyx surrounded by leaves, rarely visible; petals protruding from the calyx, pink, with 3 prominent veins (Table 2).

Description: Glandular perennial herbs. Roots soft, thin, long; young (thin) roots pilose hairy, bright colored. Above-ground parts (3-) 5-10 (-15) cm in diameter, cushion forming, middle of which is like a dome, forming a pillow. Stems short, up to 1 cm tall, clearly shorter than leaves, very branched at base, covered with old and/or dead stem remnants and clumps at base. Internodes very short, slightly visible; stem, leaf, bract and calyx covered with dense long glandular hairs. Leaves in bunches, cylindrical, pointed at ends, 1-veined, up to 4 x 1 mm, often glandular hairy, gradually enlarging with a membranous structure towards base. First leaves oblong-linear, shorter and less hairy than the later leaves. Flowers sessile, usually 1 per fascicle.

Figure 1. Holotype (a), isotype (b,c) specimens, and leaves, bracts, calyx and stamens of *Bolanthus sertavulus* Özçelik (in Hb. GUL)

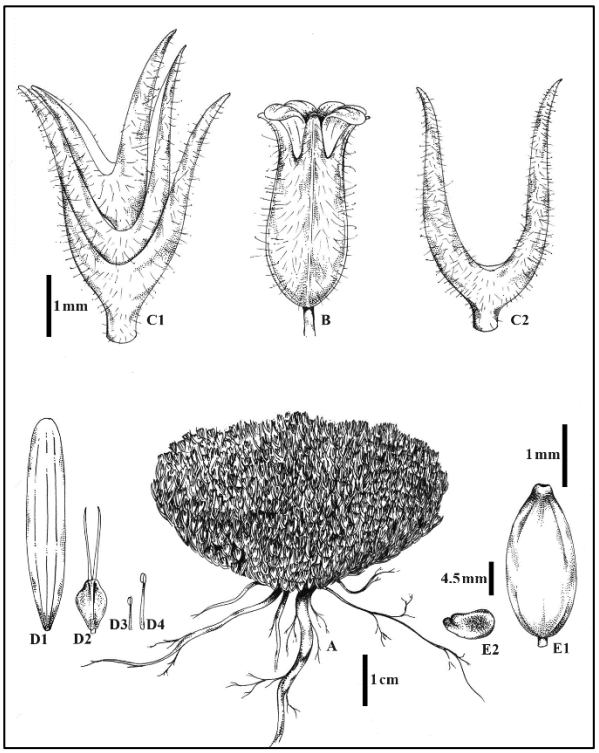

Figure 2. Drawings of holotype specimen of *Bolanthus sertavulus* A- habit, B-flower, C1- leaves and bracts, C2- bracts, D1- petal, D2- pistil, D3,D4- stamens, E1- capsule, E2- seed (GUL Hb., Özçelik 8462) (drawings by G.Aytepe)

Bracts leaf-shaped, smaller. Calyx 5, tubulate, veins prominent, membranous between, 4 × 1.5-2 mm, often erect long glandular hairs; teeth pinkish, linear, pointed tips. Fruity calyx swollen in the middle. Petals 5, pale pink, with 3 prominent veins, long linear, 4-5 × 1.5-2 mm, a little longer than calyx, flush with leaves. Stamens 10, anthers not protruding from calyx, slightly short. Styles 2. Ovary with 8-20 ovules. Fruit capsule, oblong-ovoid, opens with 4 teeth. Seeds tuberculous.

Etymology: This new taxon was detected from Mersin, Sertavul Pass. For this reason, it was named after the place (Sertavul) where it was gathered.

Conservation Status: The habitat of the specimen posses more than 100 square kilometers. But considering the area of occupacy (only one locality) and probable heavy grazing in the area, the IUCN category of the *B. sertavulus* is suggested as Critically Endangered (CR).

Economic Value: No economic use as yet. It can be a ground cover in rock gardens. However, as an endemic, it contributes to biodiversity of Türkiye.

Geography: İçel (Mersin), Mut, Sertavul Pass, steppe and rocks, 1100-1500 m, 26.06.2000, Özçelik 8462. According to Davis (1967), *B. cherlerioides* is also distributed in İçel, Sertavul Pass (Mut, İçel), 29 km south of Karaman, 1610 m, Hub.-Mor. 17098). Since this distribution is thought to belong to *B. sertavulus*, *B. cherlerioides* was redefined in the light of the herbarium specimens given below, and the diagnostic features of it were also stated.

Investigated herbaria specimens for *B. cherlerioides*: Isparta-Konya: Özçelik 7957, 7958, 9161, 9231, 9708, 9229, 9230; 9239, 9233; 9223, 9225, 9228, 8863, 8864, 9227, 9228, 9226, 9224, 9232b! in GUL Herbarium. **Konya:** 9340 (in GAZI!); Özçelik 7264 (GAZI!); H. Ocakverdi 238/804 (KNYA 1213!). **Antalya-Adana:** 805 (KNYA14502!): **Afyonkarahisar:** GAZI 35677!.

Fig. 2. Samples of *Bolanthus cherlerioides* in natural habitat (a), and from GUL Hb.(b), Isparta: Özçelik 9708.

Table 2. A comparison of *Bolanthus cherlerioides* and *B. sertavulus* (new species).

Characters	*B. cherlerioides*	*B. sertavulus*
Plant cushion	(5-)10-15 (-20) cm diameter	5-10 cm diameter
Stems	Young stems 2-3(-8) cm, longer than leaves, internodes visible. Old stems and their remains are black.	Stems 0-1(-2) cm tall, shorter than leaves, internodes very short, invisible. There is no old stems and their remains not black.
Leaves	Leaves linear-subulate, 5-9 mm long	Leaves cylindrical, up to 4 mm long
Calyx	Calyx easily visible, 4-6 mm, short sparsely glandular hairs, rarely glabrous. Calyx teeth linear-subulate to linear-lanceolate, green or pink.	Calyx surrounded by leaves and brackets, rarely visible, up to 4 mm, often erect, with glandular hairs. Calyx teeth linear, with pointed tips, pinkish.
Corolla	Petals linear-lanceolate, white, rarely pale pink, not prominent veins	Petals linear, pale pink, with 3 prominent veins.
Indumentum	Leaves and bracts glabrous or sparsely hairy; stems glandular hairy.	Stems, leaves, bracts and calyx covered with dense, long glandular hairs.
Habitat and geography	Konya, Afyonkarahisar partly to Antalya and Adana provinces, 1600-1900 m altitudes at steppe and subalpinic meadows on slopes.	İçel (Mersin), Mut, Sertavul pass. It grows on steppe and rocks, 1100-1500 m.

3.2. Updated diagnostic characters and description of *B. cherlerioides*

Diagnostic characters: Plant clusters are dense, pillow-shaped; pillows clearly compact (like a dome in the middle), clusters (5-)10-15 (-20 cm in diameter; young stems unbranched, compact, innumerable, upright and short, (1-) 2-3 (-10) cm tall. Internodes visible (0-) 1 (–2) mm; lower part herbaceous or woody. Leaves imbricate, linear-subulate, one-veined, 3-9 mm, base enlarged, cross section approximately circular. Inflorescence dichasium, 1-3(-6). Calyx teeth linear-lanceolate to subulate; petals white or pink, petals 3-pronged with purple veins; central flower sessile.

Description: Cushion-shaped perennial herbs with many-branched and woody rhizomes; cushion compact, 5-15(-20) cm diameter. Young stems much branched; with a mixture of short eglandular and longer glandular hairs. The stems 1-3(-8) cm, longer than leaves. Internodes 1-2 mm, visible, covered by densely imbricated leaves. Old stems and their remains are black. Leaves rather weak, small, (3-)5-7(-9) x 0.2-0.4 mm; linear-subulate, often falcate, densely fasciculate; with ±numerous long spreading glandular and with scarce short eglandular hairs or not. Bracts leaf-like, adpressed to the calyx, enlarged and membranous-margined at base; as long as or longer than flowers. Inflorescence terminal, 1-3(-6) flowered; flowers short

An updated identification key for Turkish *Bolanthus* taxa

1. Petals entirely white, not purple veins
   2. Plants prostrate or decumbent; flowers (5–)10–25 in dense subsessile clusters; calyx 4,0-5,0 mm long ..... *B. minuartioides*
   2. Plants ascending or erect; flowers 1–3 in pedicellate clusters; calyx 5,5-7,0 mm long ..... *B. aziz-sancarii*
1. Petals entirely purple or white with purple veins
   3. Stems glabrous or puberulent hairy; leaves setaceous, glabrous; bracts 1,5 times as long as calyx
      4. Plants prostrate; internodes 5–15 mm long; flowers pedicellate ..... *B. huber-morathii*
      4. Plants ascending or erect; internodes 1–3 mm long; flowers sessile ..... *B. mevlanaea*
   3. Stems glandular-hairy; leaves linear to linear-setaceous, glandular hairy; bracts 1–1.2 times longer than calyx
      5. Cushions compact; leaves falcate; inflorescence 1–3-flowered; calyx teeth 2–2,5 mm long
         6. Cushion diameter (5-)10-15 (-20) cm; stems 2-3(-5) cm long, thin, visible; leaves and bracts glabrous or sparsely glandular hairy ..... *B. cherlerioides*
         6. Cushion diameter 5-10 cm; stems 1-2 cm long, partly thick, invisible, leaves and bracts densely glandular hairy ..... *B. sertavulus*
      5. Cushions lax or prostrate, leaves not falcate; inflorescence 4–15-flowered; calyx teeth 0.5–1,5 mm long
         7. Plants ascending or erect; internodes upto 5 mm long; flowers sessile; petals linear-oblong
            8. Stems (2-) 3-5 cm, internodes 1-2 (-4) mm, bracts as long as calyx; calyx 4-5 mm; petals linear oblong, truncate ..... *B. thymoides*
            8. Stems 1(-2) cm, internodes 0-1(–2) mm; bracts shorter calyx; calyx 2.5–4.0 mm; petals oblanceolate ..... *B. sandrasicus*
         7. Plants prostrate; internodes 5–15 mm long; flowers pedicellate; petals narrowly oblanceolate
            9. Leaves linear-oblanceolate; calyx 3–3.5 mm; inflorescence with lax clusters
               10. Leaves long-ciliate hairy; calyx non-viscid, ovary 8–10-ovulate ..... *B. stenopetalus*
               10. leaves not long-ciliate (pubescent to glabrescent); calyx viscid, ovary 20-ovulate ..... *B. frankenioides*
                  11. Plants ± fasciculate, loosely tufted, prostrate, decumbent; leaves linear-subulated, glabrous; stems puberulent; pedicels 0-2,0 mm ..... var. *fasciculatus*
                  11. Plants not tufted; leaves partially flattened linear, loosely glandular pubescent; stems glandular pubescent or not, pedicels (0-) 2-4 mm ..... var. *frankenioides*
            9. Leaves subulate-setaceous or linear; calyx 3.5–5.5 mm; inflorescence with compact clusters
               12. Leaves linear, 3-nerved; calyx 3.5–4.5 mm long; petals 3.3–4.5 mm, usually as long as calyx ..... *B. turcicus*
               12. Leaves subulate-setaceous, 1-nerved; calyx 4.5–5.5 mm long; petals 6–7 mm, 1.5 times longer than calyx ..... *B. spergulifolius*

pedicellate, only the central flower sessile. Calyx tubular, easily visible, 4-6 mm, with 5 projecting ribs, short sparsely glandular hairs, rarely glabrous. Calyx teeth linear-subulate to linear-lanceolate, 2-2.5 mm, often spreading, green or pink. Petals pure white or rarely bright pink with purple veins; linear-subulate, cylindrical, cuneate, obtuse to truncate, about as long as calyx. Ovary 8-ovulate. Capsule unknown. It is a very distinctive species.

Distribution and Habitat: Endemic to Türkiye. Grows in Konya, Afyonkarahisar partly to Antalya and Adana provinces. Sultan Mountains (north of the pass) and its surroundings, 1600-1900 m, at steppe and subalpinic meadows on sloping slopes and piles of stones. Fl: 6-7. Fr. 7-8. It is an East Medit. element.

4. Discussion

Bolanthus species are taxonomically difficult to study. Perhaps the most difficult species of the genus is *B. sertavulus*. Because the plant is short, flowers small, stuck between the leaves. According to our field observations; in the Lakes region, *Bolanthus* genus becomes more diverse and the population size becomes larger. Most of new species are described from this region. While there were 5 species recorded in the book titled 'Flora of Turkey..' (Davis, 1967), today species number of *Bolanthus* in Türkiye has increased to 12. If the ecological characteristics of the Lakes Region, especially its rock diversity and climate, are examined well, the biology of *Bolanthus* genus will be better understood. These ecological features can also be used as distinguishing features in the revision of the genus

Conflict of Interest

Author has declared no conflict of interest.

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Improving salt stress tolerance in *Zea mays* L. by modulating osmolytes accumulation and antioxidant capacity with Rutin

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Rutin ile osmolit birikimi ve antioksidan kapasite modüle edilerek *Zea mays* L.'de tuz stresi toleransının geliştirilmesi

Abstract: The growth and productivity of maize are severely affected by stress factors**.** Maize seedlings under salt stress were grown hydroponically to study the effect of rutin (Rut), a flavonoid, on changes in the stress parameters (thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2), total chlorophyll), water status (leaf relative water content (RWC), osmolytes; proline, total soluble sugar), and activities of the main antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD)). After 21 days of growth, plants were applied with Rut as foliar spray. After 24 hours, seedlings were exposed to osmotic stress by 100 and 200 mM NaCI in the Hoagland's Solution for 72 hours. Six groups were designed including a control (without NaCl or Rut), 150 mM NaCl, 200 mM NaCl, Rut, Rut+150 mM NaCl, and Rut+200 mM NaCl. Plant leaves were harvested 25 days after treatments. Exogenous significantly decreased TBARS and H_2O_2 contents in leaves of salt-stressed seedlings compared to salt stresses, enhanced the level of osmolytes, leaf RWC, activities of SOD, CAT, APX, and POD, and relative expression levels of *SOD*, *CAT1*, and *APX1*. As a result, findings from the study present reveal the effect of Rut on salt stress tolerance in maize seedlings under different osmotic stress. Here, it was clear that Rut played an active role in stress-alleviating. This application under salt stress can be useful in developing salt stress tolerance in crops for the agriculture sector.

Key words: Antioxidants, gene expression, osmolytes, rutin, salt stress

Özet: Mısırın büyümesi ve verimliliği stres faktörlerinden ciddi şekilde etkilenir. Tuz stresi altındaki mısır fideleri, bir flavonoid olan rutinin (Rut) stres parametrelerindeki (tiyobarbitürik asit reaktif maddeleri (TBARS), hidrojen peroksit (H_2O_2), toplam klorofil), su durumu (yaprak nisbi su içeriği(NSİ), osmolitler; prolin, toplam çözünebilir şeker) ve ana antioksidan enzimlerin (süperoksit dismutaz (SOD), katalaz (CAT), askorbat peroksidaz (APX) ve peroksidaz (POD)) aktiviteleri üzerine etkisini incelemek için hidroponik olarak büyütüldü. 21 günlük büyümenin ardından yapraklara rutin sprey olarak gerçekleştirildi 24 saatin ardından fideler, 72 saat boyunca Hoagland Solüsyonunda 100 ve 200 mM NaCl'nin indüklediği ozmotik strese maruz bırakıldı. Deney grupları, Bir kontrol (NaCl veya Rut içermeyen), 150 mM NaCl, 200 mM NaCI, Rut, Rut+150 mM NaCl ve Rut+200 mM NaCl dahil olmak üzere üç tekrarlı altı grup şeklinde dizayn edildi. Bitki yaprakları uygulamalardan 25 gün sonra hasat edildi. Eksojen Rut, tuz stresine kıyasla tuz stresi altındaki fidelerin yapraklarındaki TBARS ve H_2O_2 içeriğini önemli ölçüde azalttı, osmolit seviyesini, yaprak NSİ'ni, SOD, CAT, APX ve POD aktivitelerini ve *SOD*, *CAT1* ve *APX1*'in nisbi ekspresyon seviyelerini artırdı. Sonuç olarak, mevcut çalışmadan elde edilen bulgular, farklı ozmotik stres altındaki mısır fidelerinde Rut'un tuz stresi toleransı üzerindeki etkisini ortaya koymaktadır. Burada Rut'un stresi azaltmada aktif rol oynadığı açıktı. Tuz stresi altında yapılan bu uygulama, tarım sektörü için bitkilerde tuz stresine toleransın geliştirilmesinde faydalı olabilir.

Anahtar Kelimeler: Antioksidanlar, gen ifadesi, osmolitler, rutin, tuz stresi

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1. Introduction

A significant environmental stressor that limits the production and sustainability of agricultural enterprises in arid and semiarid environments is excessive salinity (Yu et al., 2020; Wei et al., 2020; Mukhopadhyay et al., 2021). The extensive use of improper irrigation techniques that we have recently seen, particularly the excessive use of fertilizers and pesticides, has contributed to a notable deterioration of soil salinization throughout the world in addition to amplifying the consequences of global warming (Farooq et al., 2015; Meng et al., 2016).

One of the most significant food crop species is maize, and maize production has been rising. However, because maize is a somewhat salt-sensitive plant species, its growth is severely constrained by salinity, particularly when it is in the seedling stage (Kang et al., 2004; Zelm et al., 2020). Moreover, salinity negatively affects senescence, transpiration, ion transport, osmotic status, and photosynthetic activity in maize seedlings (Negrão et al., 2017; Zhong et al., 2020; Feng et al., 2021; Ju et al., 2021). In addition, excessive salinity can produce reactive oxygen species (ROS), including hydrogen peroxide, superoxide anions, etc. (Mittova et al., 2004; Huo et al., 2020). Therefore, in order to develop tolerance to salt, maize seedlings have developed various strategies, such as enhanced osmolyte accumulation and activated ROS scavenging systems (Acosta-Motos et al., 2015; Zhu et al., 2016).

Salt-induced osmotic effects change the overall metabolic processes and enzyme activity levels in maize plant cells, which results in excessive ROS accumulation and oxidative stress (AbdElgawad et al., 2016; Ali et al., 2021). Plant cells typically establish a complex antioxidant system that controls redox homeostasis in response to oxidative stress. This system frequently includes the enzymes SOD, CAT, APX, and POD, as well as other free radical scavengers (Liang et al., 2017; Sánchez-McSweeney et al., 2021).

Some compounds, including osmolytes, antioxidants, and signal molecules, have been applied to plants subjected to various stress factors to enhance their stress tolerance. One example is rutin, which is one of the flavonoids and a member of the large group of herbal phenolics. Flavonoids are secondary metabolites and have been reported to provide herbal pigmentation and protection from UV rays as a defense mechanism against herbivores and pathogens (Taiz and Zeiger, 2008). Flavonoids also have antioxidant properties (Yang et al., 2008) and have been reported to inhibit lipid peroxidation under drought stress and act as membrane stabilizers (Singh et al., 2017). Most varieties of flavonoids exist in the form of o-glycosides, a sugar linked by one or more hydroxyl groups, and can be stored in the cell vacuole in this form (Jiang et al., 2007). Rutin also calls quercetin -3-0- rutinoside, consists of the aglycone quercetin and the disaccharide rutinose. It is a yellow, crystalline flavonol glycoside ($C_{27}H_{30}O_{16}$) and is converted to quercetin by being catalyzed by rutinosidase, and quercetin is catalyzed by rutinosidase and synthesized to isoquercitrine, and isoquercitrine is synthesized to rutin by rhamnosyl transferase (Suzuki et al., 2015). It has been reported that because young leaves are more sensitive to UV light, they protect the plants from UV rays by storing rutin more intensively (Suzuki et al., 2015). In addition, with its rutin application to the leaves of tomato plants, it was noted that there was an increase in the number of leaves, leaf area, fruit number, fruit weight, shoot and root dry weight, and elongation in the roots (Gorni et al., 2022). Suzuki et al. (2005) found that the concentration of rutin glucosidase and quercetin increased in buckwheat leaves that were subjected to UV-B, osmotic, and cold stresses. It was observed that the amount of rutin and other phenols increased in *Hypericum brasiliense* Choisy. and *Artemisia* plants exposed to drought stress for a long time (Kumar et al., 2021). Overexpression of the drought stress tolerance gene (*NtCHS*) in the drought-stress-tolerant *Nicotiana tabacum* L. resulted in the accumulation of rutin and other flavonoids. The results of this study showed that rutin increases drought stress tolerance (Chen et al., 2019). As with most flavonoids, rutin also has a reducing effect on lipid peroxidation (Jiang et al., 2007). It has been suggested that this property of rutin may be related to the scavenging of hydroxyl groups. Also by the same researchers, it was found that rutin was synthesized at the highest rate among total phenolics in the leaves of halophyte quinoa species. In this study, the activity of antioxidant enzymes did not increase with rutin application (Ismail et al., 2015).

To the best of our knowledge, it is still unclear whether rutin is useful for protecting plants against stressors. Additionally, there are no studies on the protective role of rutin in salt-stressed maize plants. Therefore, the current study has two hypotheses: (1) Rutin could highly stimulate the accumulation of osmolytes to alleviate the salt stress effect in maize seedlings; and (2) Rutin could modulate the antioxidant defense system to develop salt stress tolerance in maize seedlings. Our study will provide new insights into lighting the physiological and molecular mechanisms of rutin in maize seedlings' responses to salt stress.

2. Materials and Method

2.1. Plant material and applications

Maize seedlings were grown hydroponically in a growth chamber (16 h day/8 h night temperature of 25/22, relative humidity (63±2%) and photon flux density (400 µmol $m^{-2}s^{-1}$) with Hoagland's solution (Hoagland and Arnon 1950). After 21 days of growth, rutin (60 ppm) was applied to the leaves as a spray. After 24 hours, seedlings were exposed to osmotic stress induced by 150 and 200 mM NaCl in the Hoagland's Solution for 72 hours.

The experimental pots were designed as six different treatments: the Nutrient Solution for Control (1), 150 mM NaCl (2), 200 mM NaCl (3), Rut (4), Rut+150 mM NaCl (5), and Rut+200 mM NaCl (6). After treatments, 25 day old seedlings were harvested and stored at -80 °C until analysis.

2.2. Determination of Stress Parameters

2.2.1. Thiobarbituric acid reactive substances (TBARS) Assay

The level of lipid peroxidation was measured in terms of TBARS content. A homogenizer was used to homogenize the leaf samples (0.1 g) in 1.8 mL of 0.1 % trichloroacetic acid (TCA). For 5 min, the homogenate was centrifuged at 16,000 xg. 4 mL of thiobarbituric acid prepared in 20% TCA was added to 1 mL of supernatant. The mixture was heated for 30 min at 95 °C before being quickly cooled. The absorbance of the supernatant was measured at 532 and 600 nm (Heath and Packer, 1968).

2.2.2. Hydrogen peroxide (H_2O_2) assay

The extract produced from crushed with nitrogen leaf samples was centrifuged, 1 mL of supernatant was taken and 10 mM potassium phosphate buffer and 1 M potassium iodide were added. The absorbance was then measured at 390 nm (Velikova et al., 2000).

2.2.3. Total chlorophyll assay

The total amount of chlorophyll was determined according to the method of Arnon (1949). 0.1 g of fresh plant leaf was crushed with liquid nitrogen and homogenized with 1.8 mL of 80% acetone. The obtained samples were centrifuged at 4°C for 10 min. It was diluted by adding 4.5 mL of 80% acetone solution to 0.5 mL supernatants. The absorbances of the obtained supernatants were measured at 645 nm and 663 nm.

2.3. Determination of water status

2.3.1. Leaf relative water content (RWC)

To estimate the water saturated weight (SW), maize leaves plucked from seedlings were immediately weighed on balance (about 0.1g) (fresh weight, FW), and then placed in water and rehydrated for 12 hours. The leaves' dry weight (DW) was obtained by drying them in an oven at 65 °C for 48 hours. To compute the leaf RWC, the formula (RWC(%): (FW-DW)/(SW-DW)*100) was employed.

2.3.2. Osmolyte contents

The proline content of dried samples (0.1 g) was evaluated by filtering them following homogenization with 1.8 mL of 3% sulfosalicylic acid. The filtrate was centrifuged for 5 min at 5,000 xg. 1 mL of supernatant was treated with acetic acid (1 mL) and ninhydrin (1 mL). The mixture was then placed in tubes in a water bath at 100 °C for 1 h. 3 mL of toluene was added to the cooled liquid and vortexed. The mixture was placed in sealed tubes and centrifuged at 5,000 xg for 5 min. After centrifugation, the upper phase was pipetted into the cuvette and measured at 520 nm with a spectrophotometer (Bates et al., 1973).

To calculate the total soluble sugar concentration, after homogenizing the dry leaf sample (0.1 g) with 70% ethanol (5 mL), the homogenate was boiled at 80 °C for 3 min. Homogenate was centrifuged at 10,000 xg for 5 min. 900 µL of filtered water was mixed with 100 µL of supernatant and diluted. After adding 1 mL of 5% phenol, this mixture was stirred using a stirrer. 5 mL of sulfuric acid was completely stirred into the same mixture. After cooling to room temperature, the absorbance values of mixture were measured at 490 nm (Dubois, 1956).

2.4. Determination of changes in antioxidant capacity

The ascorbate (AsA) content was calculated using the method provided by Liso et al. (1984). The leaf samples (0.1 g) were homogenized with 5 mL of 5% metaphosphoric acid and centrifuged at 10,000 xg for 10 min. The supernatant (70 µL) was combined with 3 mL of reaction medium (0.1 M citrate-0.2 M phosphate buffer, pH 6.2). AsA content was evaluated after reading the reduction occuring 5 min after the addition of 2 U ascorbate oxidase to the reaction solution. Ascorbate oxidase was blocked once AsA oxidation was complete by adding 10 mM sodium azide. The combination was then treated with 2.5 mM dithiothreitol. The absorbance was measured at 265 nm again after 3 min of reduction with dithiothreitol.

The samples were produced using the modified method of Sezgin et al. (2018) for enzyme and protein extractions. SOD activity was measured at 25°C using the Beauchamp and Fridovich (1973) technique. CAT activity was measured at 240 nm using the Aebi (1983) technique. The activity of APX was assessed using the Nakano and Asada (1987) technique, which is based on ascorbate oxidation at 290 nm. POD activity was measured according to Urbanek et al. (1991) by following the increase in absorbance at 470 nm.

2.5. The analyis of gene expression

To analyze the relative expression levels of *SOD*, *CAT1*, and *APX1* genes, total RNA was obtained using a Plant Total RNA Purification Kit (FavorPrep). The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used for cDNA synthesis. The cDNAs obtained were used in Real Time PCR tests to determine transcript levels. For RT-PCR analysis, SsoFast EvaGreen Supermix and the RT-PCR System (BioRad) were used. The method stages were modified using Solis BioDyne instructions. The actin (*ACT)* gene was used as a control gene The data were normalized to the level of reference gene expression before being displayed as relative gene expression. Table 1 contains a list of primers.

2.6. Statistical analysis

Every experiment was repeated three times with three biological replicates. Duncan's multiple comparison test (one-way ANOVA) was used for statistical analysis in SPSS (Ver. 23.0). Bio-Rad CFX Manager 3.1 was used to examine relative gene expression during qRT-PCR analysis. The vertical bars reflect the standard deviations of three replicated means. At $P < 0.05$, different letters denote significant differences among all treatments.

3. Results

3.1. Effects of rutin on stress parameters under salinity stress condtions

Salt stress applications (150 and 200 mM) drastically increased the membrane damage (TBARS content) of maize seedlings by 34.3 and 69.8%, respectively, as compared with the control: however, Rut application under 150 and 200 Mm NaCl stress decreased TBARS level by 12.5 and 6.6%, respectively, compared to the same stress level alone. Moreover, Rut statistically significant alleviated the salt-induced membrane damage of maize seedlings under both stress conditions compared to salt stress conditions (Fig. 1A). H_2O_2 content of maize seedlings increased when subjected to salt stresss treatments. However, the application of exogenous Rut under salt stress conditions (150 and 200 mM NaCl) decreased statistically significant H_2O_2 level (31.1 and 16.0%, respectively) compared with salt stress conditions (Fig. 1B). Additionally, decrease in TBARS and H_2O_2 levels in Rut combined 150 mM NaCl were higher than Rut combined 200 mM NaCl treatment (Fig. 1A,B). Salinity

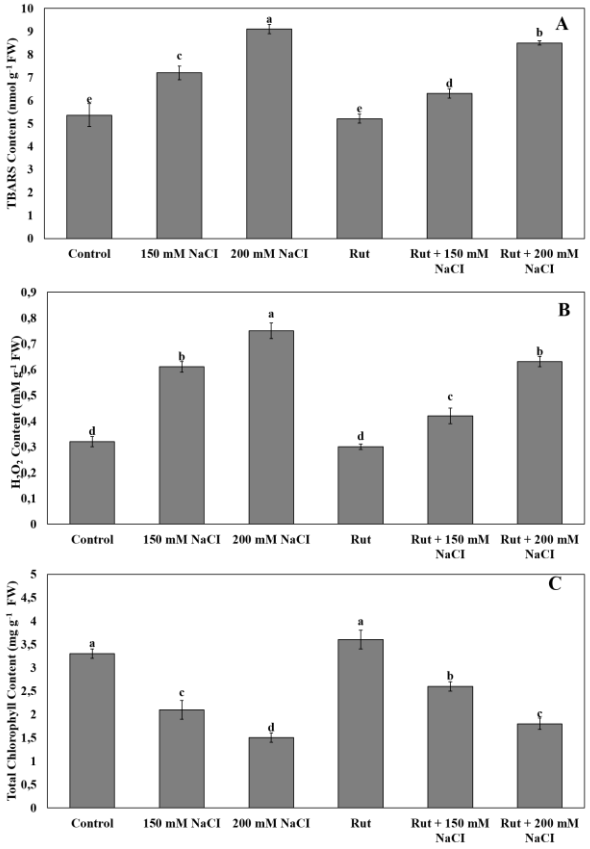

Figure 1. Effects of rutin on stress parameters under salinity stress conditions. TBARS content (**a**), H_2O_2 content (**b**), Total chlorophyll content (**c**).

Table 1. The sequence of gene-specifc primers used for qRT-PCR analysis

Target gene	Oligo name	Sequences 5'-3'
Actin (*ACT*)	ZmACT1_F	ACCAGTTGTTCGCCCACTAG
	ZmACT1_R	GAAGATCACCCTGTGCTGCT
Superoxide dismutase (*SOD)*	ZmSOD_F	TTGTTGCAAATGCTGAGGGC
	ZmSOD_R	AGGCAAGGATGTAACAGCGT
Catalase (*CAT1*)	ZmCAT1_F	TGCTTTCTGCCCAGCGATTA
	ZmCAT1_R	CACTTCTCACGACAGCCTGT
Ascorbate peroxidase (*APX1*)	ZmAPX1_F	GCCTTCTTCAGCTCCCAAGT
	ZmAPX1_R	TGCAAAAGACCACATGCAGC

stress conditions significantly decreased the total chlorophyll content compared with the control but significantly increased in the Rut application under salt stress conditions relative to the salt stress treatments (Fig. 1C).

3.2. Effects of rutin on water status under salinity stress conditions

Both levels of salinity stress decreased Leaf RWC (%), recording as 19.5 and 20.7% higher levels than the control, respectively. When exogenous Rut was applied, there was an increase in the leaf RWC (%) by 14.7 and 8.3% in seedlings subjected to 150 and 200 mM NaCl, respectively, compared to their stress groups. However, leaf RWC (%) was high in Rut combined 150 mM NaCl compared to 150 mM NaCl treatment (Fig. 2A). Total soluble sugar and proline levels markedly increased under both stress conditions compared with the control. Under stress conditions (150 and 200 mM NaCl), Rut application also significantly enhanced total soluble sugar by 14.2 and 30.0%, respectively, compared to the same stress level without Rut. Seedlings treated with rut exhibited an increase proline content by 13.3 and 5.5% under stress conditions (150 and 200 mM NaCl), respectively, compared to the same stress level alone. Additionally, proline content was high in Rut combined 150 mM NaCl in comparison with 150 mM NaCl application, total soluble sugar was high in Rut combined 200 mM NaCl in comparison with 200 mM NaCl application (Fig. 2B,C).

3.3. Effects of rutin on antioxidant capacity under salinity stress conditions

AsA content rose by 25.0 and 55.0 % in the salt applications (150 and 200 mM NaCl compared to the control seedlings. Rut treatment enhanced AsA content by 12.0 and 9.7% in maize seedlings exposed to 150 and 200 mM NaCl in comparison with the salt treatments, respectively. AsA content was high in the Rut combined 200 mM NaCl as compared to 200 mM NaCl treatment (Fig. 3A).

Enzymes activities increased in the both salt treatments as compared to the control seedlings. SOD activities in seedlings treated with Rut under 150 and 200 mM NaCl stress were 75.0 and 14.3% higher, respectively, than the same level of stress without Rut. Exogenous treatment of Rut significantly increased CAT activity by 117.5 and 33.3% under 150 and 200 mM NaCl stress, respectively, compared to the same stress level alone. Additionally, Rut under salt stress conditions (150 and 200 mM NaCl) increased APX activity by 20.0 and 25.0%, respectively, compared to the same stress level. The POD activity in seedlings applied with Rut exposed to 150 and 200 mM NaCl was 50.0 and 4.3%, respectively, higher than the same stress level (Figure 3B,C,D, and E). SOD, CAT, and POD activities also were high in the Rut combined 150 mM NaCl as compared to 150 mM NaCl treatment (Fig. 3B,C, and E)

Relative expression levels of *SOD*, *CAT1*, and *APX1* were significantly up-regulated in the both salt treatments and the Rut treatment in comparison with the control seedlings. Rut+150 mM NaCl and Rut+200 mM NaCl treatments up-regulated the gene expression levels of *SOD* by 30.4 and 30.3%, *CAT1* by 21.2 and 15.2%, and *APX1* by 27.8 and 27.0%, respectively, compared to the same stress levels alone. (Fig. 4A,B, and C).

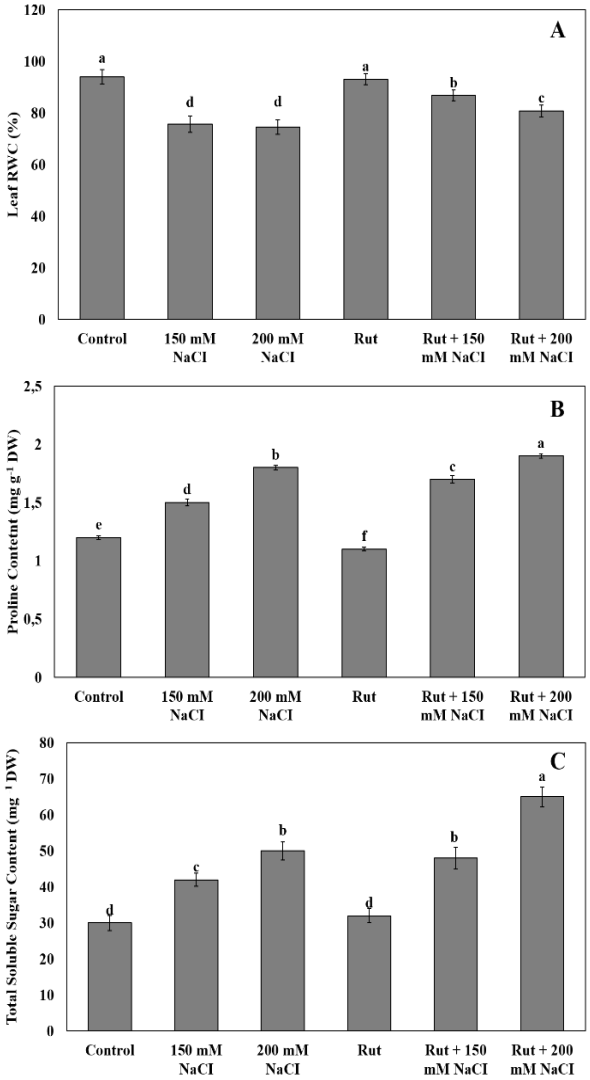

Figure 2. Effects of rutin on water status under salinity stress conditions. Leaf RWC (%) (**a**), Proline content (**b**), Total soluble sugar content (**c**).

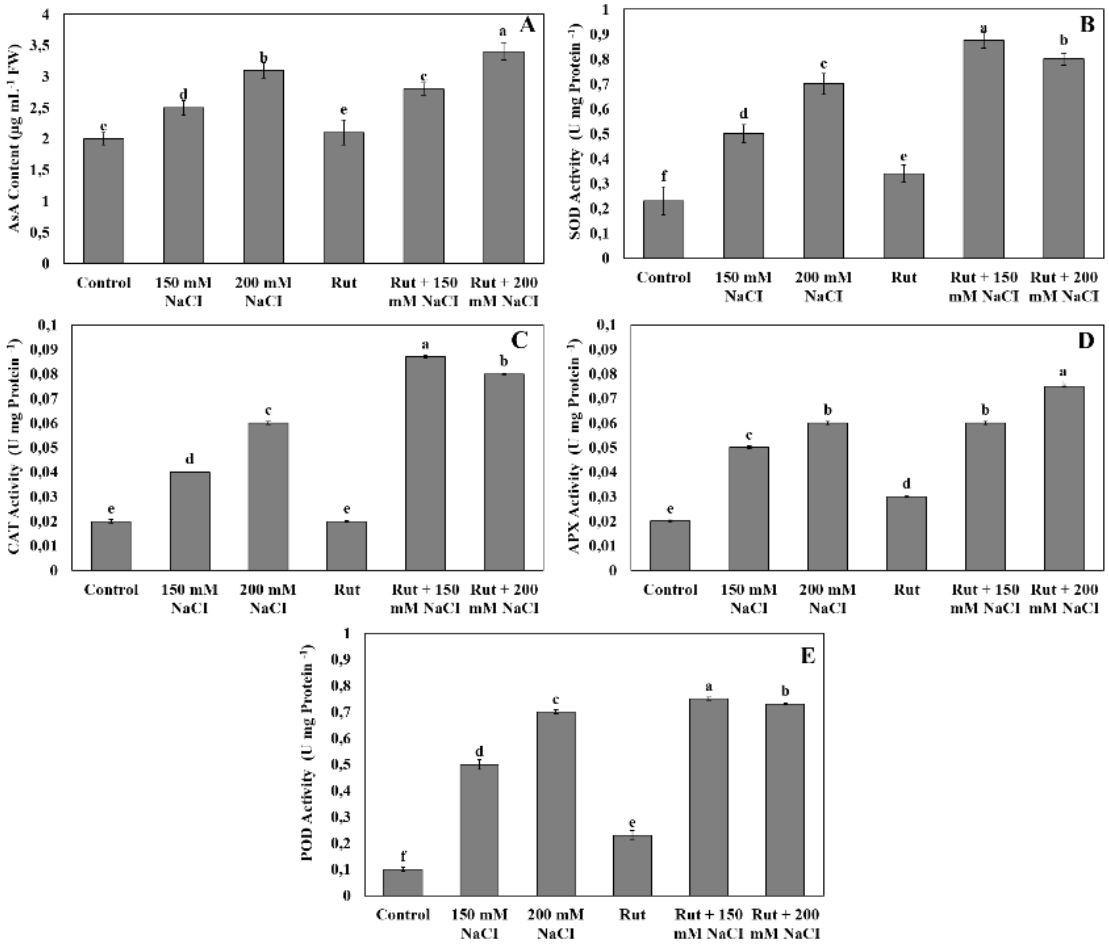

Figure 3. Effects of rutin on antioxidant capacity under salinity stress conditions. AsA content (**a**), SOD activity (**b**), CAT activity (**c**), APX activity (**d**), POD activity (**e**) .

4. Discussions

Our study revealed the effects of foliar Rut application on maize seedlings exposed to various levels of salt stress. We discuss the physiological and molecular mechanisms that seedlings use to tolerate salt stress conditions, as well as how Rut improves salt stress tolerance.

Lipid peroxidation is a process that NaCl, drought, high temperature, etc. cause in cell membranes. In our study, TBARS content increased under salt stress conditions. The use of Rutin reduced the amount of TBARS present as a result of lipid peroxidation brought on by salt stress, demonstrating the role of Rut as a protective and antioxidant molecule against oxidative damage, perhaps as a result of the enhanced activity of antioxidant enzymes. It has been reported that the inhibition of membrane damage increases as the concentration of Rutin increases (Yang et al., 2008). The fact that quercetin, a chemical derivative of Rutin, significantly reduces the amount of TBARS resulting from oxidative stress in salt stress tolerance in tomatoes supports our findings (Parvin et al., 2019). H_2O_2, a ROS produced by cellular metabolism, is a sign of a plant's ability to scavenge ROS in stressful environments. Exogenous Rut application under non-stressed conditions exhibited no obvious effect on H_2O_2 level. According to our findings, salt stress significantly raised the H_2O_2 level, but Rut application (Rut+150 mM NaCl and Rut+200 mM) reduced dramatically the H_2O_2 content under salt stress conditions. Our research further supported the prior papers' results that Rut application reduced H_2O_2 level of tobacco leaves under salt stress (200 mM) (Chen et al., 2019). Similar findings were reported by Parvin et al. (2019) who showed a significant decreased in H_2O_2 content in quercetin applied tomatoes under salt stress. In our current study, we concluded that Rut-treated maize seedlings experienced less cell death when exposed to salt stress, judging from the reduced ROS accumulation (TBARS and H_2O_2). However, the results of the current investigation suggested that Rut application may prevent cell death by lowering the levels of ROS accumulation, TBARS and H_2O_2 that protect against membrane damage under stressful circumstances. These findings suggested that rutin had a potent capacity to scavenge ROS.

Photosynthetic pigments are crucial physiological processes for maintaining plant activity and in solar energy transfer and absorption (Arnao and Hernandez 2010). In our study, salt stress decreased photosynthetic pigment in maize seedlings. However, we observed that pretreatment with rut reduced salt-induced reduction in photosynthesis. Quercetin, a precursor compound of Rutin, has a positive effect by increasing the total amount of chlorophyll in plants under osmotic stress and its antioxidant properties have been supported by studies conducted on wheat (Jańczak-Pieniążek et al., 2021), Arabidopsis (Kurepa et al., 2016) and tomato (Parvin et al., 2019) in which potassium quercetin was applied. Our findings show that exogenous Rut treatment under salinity stress conditions increases chlorophyll content compared to salt stress conditions alone.

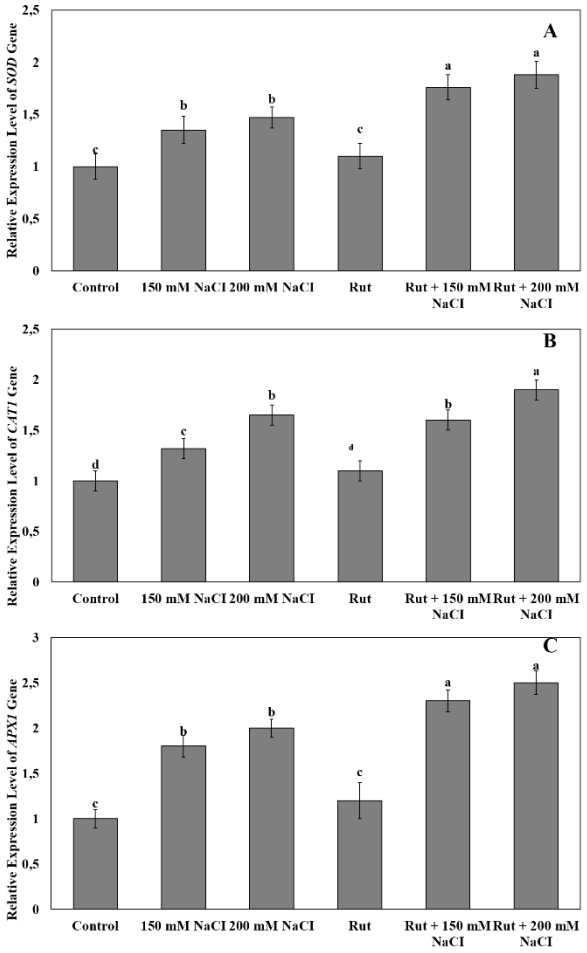

Figure 4. Gene expression analysis. Relative expression levels of *SOD* (**a**), *CAT1* (**b**), *APX1* (**c**).

To adapt to salt stress, plants use a wide range of mechanisms. The activation of the antioxidant defense system, effective ion exclusion, and osmolytes and secondary metabolite accumulation are a few of these tolerance mechanisms (Isayenkov and Maathuis, 2019). Plants have distinct adaptive physio-molecular responses to stress stimuli, such as osmotic adjustment and improved antioxidant capability (Linh et al., 2021). Osmotic adjustment is produced through the assimilation of numerous osmolytes, including proline and glycine betaine, soluble sugar as well as inorganic ions (Safwat and Salam, 2022). Abiotic stress often results in a decrease in leaf water status and an increase in osmotic regulators (Jiang et al., 2016). For evaluating plant tolerance to salt stress, relative water content has been used as an efficient method of water status (Suriya-arunroj et al., 2004). Our findings showed that rut enhanced leaf RWC, proline and total soluble sugar content in the salt stress conditions (150 and 200 mM NaCl). A previous study reported that the total soluble sugar content increased with Rut application to tomato seedlings under drought stress (Gorni, 2022). It was determined that rut application enhanced proline content in quinoa plants under salt stress (400 mM NaCl) (Ismail et al., 2015). In addition, when quercetin, a chemical derivative of Rutin, was applied to tomato seedlings exposed to salt stress, it was found that the amount of proline in the leaf increased as the amount of quercetin increased (Parvin et al., 2019). Our research supported the previous report's results that proline and soluble sugars are soluble compounds that shield plants from environmental challenges through osmoregulation, which scavenges ROS and preserves the integrity of the plasma membrane (Ashraf and Foolad, 2007).

Another important plant tolerance strategy under salt stress is the activation of the antioxidant defense system. The antioxidant system includes SOD, CAT, peroxidases, reductases, AsA, glutathione, polyphenols, etc. (Ren et al., 2020; Joshi et al., 2022). Our results showed that exogenous Rut enhanced the AsA content and the antioxidant enzyme activities such as SOD, CAT, APX, and POD. AsA, a non-enzymatic antioxidant, is used as a reductant in the conversion of H_2O_2 to water by APX enzyme activity (Das and Roychoudhury, 2014). It has been reported that quercetin application increased AsA content in tomato seedlings exposed to salt stress (Parveen et al., 2019). Ismail et al. (2015) in their study, investigated salt stress tolerance of enzymatic and non-enzymatic antioxidants of halophyte and normal genotypes of *Chenopodium quinoa* Willd. species with high flavonoid content. It was found that the flavonoid content, and mostly Rutin, increased when Titicaca plants were exposed to salt stress. The reason for this has been determined that salt stress tolerance is achieved by Rut by regulating K^+ accumulation and Na^+ exclusion in the leaf mesophyll. Superoxide radicals, which increase during osmotic stress, stimulate SOD enzyme activity and try to reduce reactive compounds by converting superoxide into H_2O_2. By converting the H_2O_2 produced into water by APX, CAT, POD enzymes, tolerance is achieved by keeping the amount of ROS below a certain limit (Asada, 1999). APX is an important enzyme of the ascorbate-glutathione cycle. While CAT mostly clears H_2O_2 in peroxisomes, APX serves the same purpose in the cytosol and chloroplasts. APX reduces H_2O_2 to H_2O and dehydroascorbic acid using AsA as the reducing agent. In stressful situations, APX was found to be more effective in scavenging H_2O_2 from CAT (Sharma et al., 2012). Contrary to our findings, a decrease in APX activity was found with quercetin application in tomato seedlings exposed to salt stress (Parvin et al., 2019). When POD is active inside the cell (cytosol, vacuole), cell wall and extracellularly, it is known to play a role as a key enzyme in the scavenging of H_2O_2 (Das and Roychoudhury, 2014). It has been found that excessive increase in *GPX* gene expression increases plant tolerance (Ozyigit et al., 2016). Therefore, Rut treatment might lessen the negative effects of the active oxygen scavenging system of maize seedlings caused by salt and can boost a plant's tolerance to stress. Additionally, in the current study, when we evaluated the gene expressions of *SOD*, *CAT 1*, and *APX* 1 enzymes, it was found to be compatible with their enzyme activities.

According to the findings, exogenous rutin causes changes in physico-biochemical and molecular properties in seedlings and may be a beneficial practice under salt stress conditions. Our findings demonstrated that exogenous rutin treatment in maize seedlings strengthened the antioxidant defense system by reducing ROS accumulation, as indicated by lower TBARS and H_2O_2 levels under salt stress conditions. Rutin supplementation improved seedling chlorophyll content and water status by increasing osmolytes content and leaf RWC.

Conflict of Interest

Author has declared no conflict of interest.

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Elaphomyces anthracinus and *E. septatus,* two new hypogeous ascomycete records for Greek mycota

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Elaphomyces anthracinus and *E. septatus,* Yunanistan mikotası için iki yeni toprakaltı askomiset kaydı

Abstract: *Elaphomyces anthracinus* Vittad. and *E. septatus* Vittad., two seemingly rare hypogeous ascomycete, are reported for the first time in Greece. This paper provides the descriptions of the recorded collections, accompanied by images of their macroscopical and microscopical features.

Key words: *Ascomycota,* mycodiversity, hypogeous macrofungi, new record

Özet: Nadir olduğu değerlendirilen iki toprak altı askomiset olan *Elaphomyces anthracinus* Vittad. ve *E. septatus* Vittad., Yunanistan'da ilk kez rapor edilmiştir. Bu çalışma kaydedilen örneklerin betimlemelerini, makroskopik ve mikroskopik özelliklerine ilişkin görüntüleriyle birlikte vermektedir.

Anahtar Kelimeler: *Ascomycota,* mikoçeşitlilik, toprakaltı mantarları, yeni kayıt

Citation: Kaounas V (2024). *Elaphomyces anthracinus* and *E. septatus,* two new hypogeous ascomycete records for Greek mycota. Anatolian Journal of Botany 8(1): 30-33.

1. Introduction

Elaphomyces species have a cosmopolitan distribution. They grow in forests ranging from tropical to temperate climates, as well as subarctic coniferous forests. They are formed on the roots of many trees and bushes, a symbiotic relationship typical of ectomycorrhizal fungi Castellano et al. (2018). Most of the species of this genus grow below the ground, but some of them are semi-hypogeous. Their ascomata range from spherical to sub-spherical, and can be either fleshy or leathery. They have a single chamber consisting of a powdery spore mass during maturity. Ascospores are primarily spherical or polyhedric such as the form presented in this paper and the asci are spherical or sub-spherical. Only two *Elaphomyces* species, *E. muricatus* Fr., (Diamandis and Perlerou, 2008; Konstantinidis and Kaounas, 2012) and *E. roseolus* Setkos, Kaounas, A. Paz, Lavoise & Fern. Rodr., Paz et al. (2017), were previously known to exist in Greece.

In this paper, a third *Elaphomyces anthracinus* Vittad. and a fourth *E. septatus* Vittad. member of the genus in Greece are introduced, based on collections from Zagori district of Ioannina province and Retine district of Pieria province respectively. According to the existing check-list of Greek ascomycetous macrofungi by Zervakis et al. (1999) and a number of recent studies reporting hypogeous ascomycetes (Diamandis and Perlerou, 2008; Kaounas et al., 2011; Gyosheva et al., 2012; Konstantinidis and Kaounas, 2012, 2014), these two species have not been previously reported in Greece. This study aims to make a contribution to the mycobiota of Greece

2. Materials and Method

Fruit bodies of *E. anthracinus* were collected from Zagori district of Ioannina province while *E. septatus* ascoma was collected from Retine district of Pieria. As they were discovered by truffle hunters, no photograps of them in their natural habitat exist, although notes were taken about the morphological and ecological characteristics. The specimens were later carried to private fungarium, and dried in an air conditioned room. Microscopic studies were performed on dried specimens under a AmScope T120C-E5-3PL trinocular light microscope. The specimens were submerged in water and Melzer reagent. Spore dimensions were obtained from measurement of 30 random, mature spores using Piximetre 5.10 software. The specimens were identified with the help of Gori (2005), Arroyo et al. (2005), Montecchi and Sarasini (2000), Uzun and Kaya (2019), Uzun (2021) and Paz et al. (2017). The nomenclature follows mainly "Index Fungorum" (http://www.indexfungorum.org). Where VK is the initials of the author and the collection code. The samples included in this study are kept in the author's private fungarium.

3. Results

Ascomycota Caval.-Sm.

Elaphomycetaceae Tul. ex Paol.

Elaphomyces anthracinus Vittad., Monogr. Tuberac. (Milano): 66 (1831).

Syn: [*Elaphomyces anthracinus* f. *talosporus* A. Paz & Lavoise; *Lycoperdastrum anthracinum* (Vittad.) Kuntze]

Macroscopic and microscopic features: Ascoma hypogeous, globose to subglobose, at times compressed and broadly umbilicated, with a diameter of 1-2 cm (Fig. 1a). Hard and leathery,but also brittle. It is black with a thick peridium, consisting of two layers visible with naked

eye. The outer layer is carbon-black, hard, brittle, slightly granular or smooth, 0.3-0.7 mm thick. It consists of short hyphae of 20-40 µm long and up to 6 µm thick, with dark brown to black coloured walls of 1-2.5 µm thickness (Fig. 1b). The inner layer is whiteish or pale grey in colour, 1-2 mm thick, consisting of interconnected pale yellow or hyaline, septate, sometimes branched hyphae of 3-6 µm thick (Fig. 1c). Gleba is at first white and cottony, consisting of fine, branched hyphae 1.5-3 µm thick, black at maturity, consisting of spore powder. It gave a weak, indistinct odour. Asci were not observed due to maturity. Ascospores 18.4-24.6 × 17.6-24.3 µm, are initially hyaline, yellow and brown, almost black at maturity, globose to almost polyhedric with up to six faces and rounded corners (Fig. 1d-e). With thick walls, surface decorated with an abundance of striations which are highly variable in morphology but of equal length (0.6-1.2 µm), leaving deep and irregular cracks between them, spore size based on n = 30 spores. Bears fruit in winter and spring.

Elaphomyces anthracinus forma *talosporus* grows in soil and decaying leaves under deciduous or coniferous trees, commonly under *Castanea sativa* Mill., *Fagus sylvatica* L., *Coryllus avellana* L., *Tilia cordata* Mill., *Quercus robur* L., *Betula pendula* Roth and *Pinus sylvestris* L. (Paz et al., 2017).

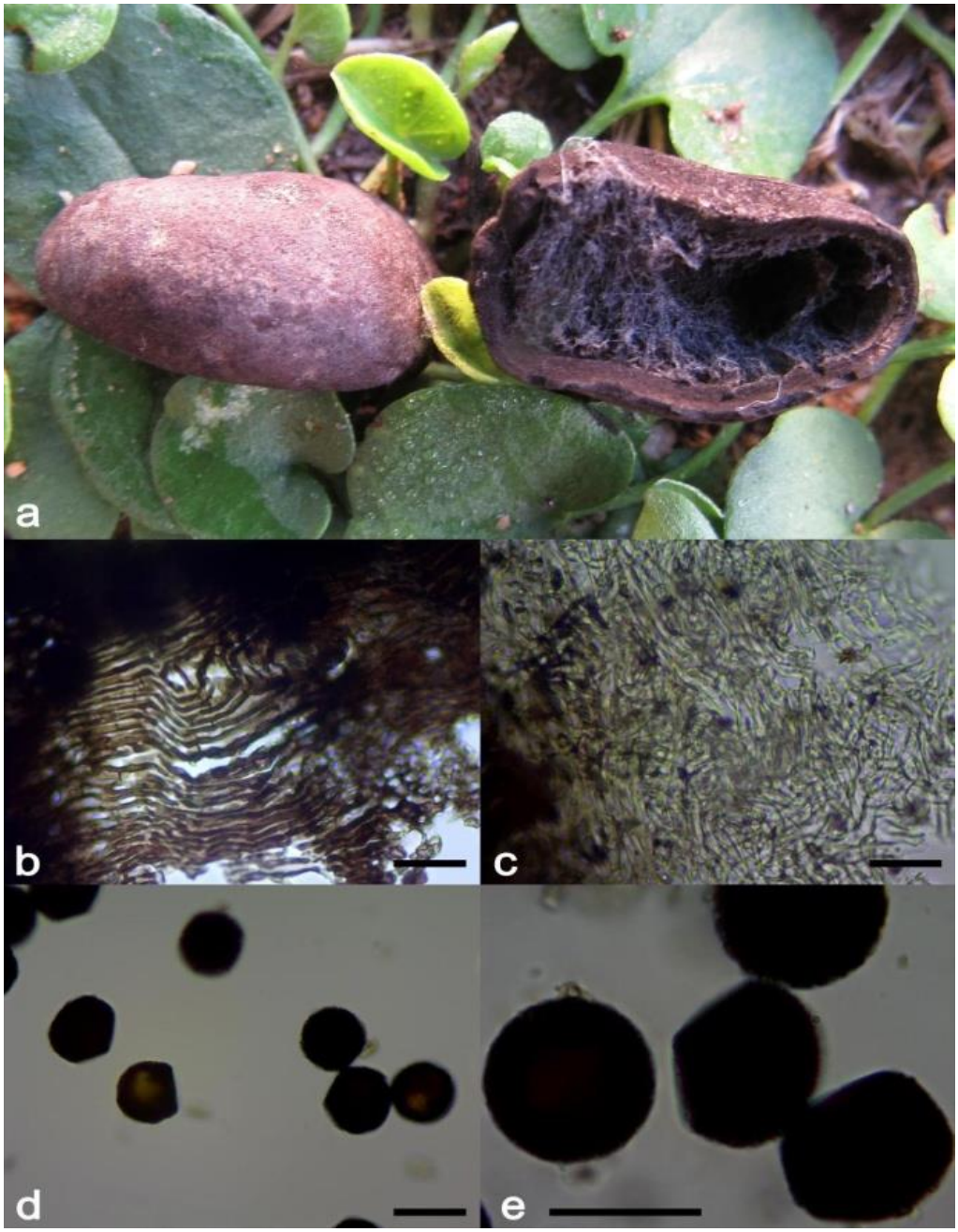

Figure 1. Dried ascocarp (a), outer peridium (b), inner peridium (c), and ascospores (d,e) of *Elaphomyces anthracinus* (bars: 20 µm)

Specimen examined: Greece, Ioannina, Zagori, in soil among decaying leaves under *Quercus sp.*, *Carpinus orientalis* Mill. and *Pinus sp.*, 03.11.2023, Leg: Niaros V, Det: Kaounas V, VK7360.

Elaphomyces septatus Vittad., Monogr. Tuberac. (Milano): 67 (1831).

Syn: [*Lycoperdastrum septatum* (Vittad.) Kuntze]

Macroscopic and microscopic features: Ascoma small, 20 mm in diameter, subglobose, almost smooth to slightly grainy, without basal protrusion, blackish brown. Surface without intertwined mycelial hyphae and with only very few remnants of the substrate. Hard and covered with pale brown septate hyphae of 2.5-7.5 µm thick, and readily severed when handled (Fig. 2a,b). Peridium approximately 2 mm thick, dual-layered. Outer layer 420-480 µm thick, concolorous with the surface, consisting of brown-blackish hyphae of 4-13 µm thick, and walls of 1-2.5 µm thick, arranged in multiple directions (Fig. 2c). Inner layer >800 µm thick, grey-black, consisting of hyaline hyphae of similar dimensions and arrangement (Fig. 2d). Gleba is white when young, cream with pink tones, and pulverized powder at maturity. Odour weak, not distinctive. While asci were not observed, ascospores 29.2-33.7 × 28.4-32.6 µm, including ornamentation, spherical, hyaline, pale grey at first, then greyish to brownish grey at maturity, spore size based on n = 30 spores. Yellow with Melzer (Fig. 2e), covered with quite thin spines of 2-3.5 µm long.

Elaphomyces septatus grows in deciduous montane forests from autumn to spring, under *Quercus* L., *Fagus* L., *Carpinus* Mill. (Vittadini, 1831; Vidal, 2000).

Specimen examined: Greece, Pieria, Retine, in soil among decaying leaves under *Carpinus orientalis* Mill., *Platanus orientalis* L. and *Ruscus aculeatus* L. 20.10.2020, Leg: Gougoulianis D., Det: Kaounas V, VK5940.

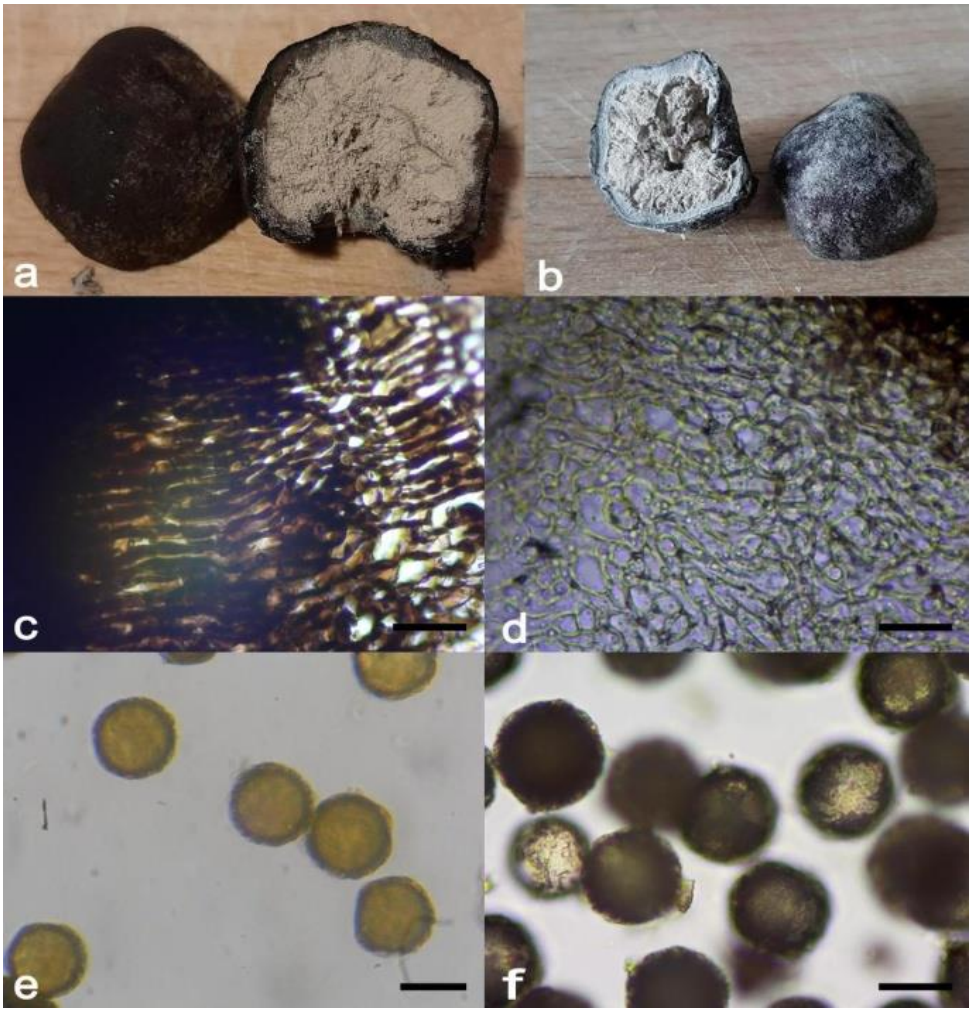

Figure 2. Fresh (a) and dried (b) ascocarp, outer peridium (c), inner peridium (d) and ascospores (e,f) of *Elaphomyces septatus* (bars: 20 µm) foto: Gougoulianis

4. Discussions

The polyhedric shaped ascospores (Fig. 1d-e), leaves no doubt that the specimen is an *E. anthracinus* forma *talosporus*, as described by Paz et al. (2017). The collections, cited as forma *talosporus*, that is quite easily distinguished from typical *E. anthracinus* by several microscopical differences are not distinguishable by either ITS or 28S (Paz et al., 2017). Asci were not observed due to maturity of the specimen, but Paz et al. (2017) report that the asci of forma *talosporus* are spherical, thin-walled and transient with 2-5 ascospores.

Elaphomyces septatus has ascomata similar to *E. maculatus* Vittad. but differs in the smaller size and pale coloration of the ascospores until maturity. Two other characteristics that distinguish it from *E. maculatus* are the absence of green mycelium and of basal protrusion. Another species with pale ascospores is *E. leucosporus* Vittad., however it is smaller than 1 cm, covered with a greenish layer of mycelium and has smaller ascospores of 18-20 µm in diameter.

The macro and micromorphological characters of our samples fit in with the literature. Therefore *Elaphomyces anthracinus* and *Elaphomyces septatus* are new records for

Greek mycobiota. As far as the previous Greek collections of *Elaphomyces* are concerned, *E. muricatus* has a macroscopic difference in the peridium while *E. roseolus* has much smaller ascospores.

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First record of the deadly poisonous *Galerina venenata* (*Hymenogastraceae, Agaricomycotina*) from Türkiye

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Ölümcül zehirli *Galerina venenata* (*Hymenogastraceae, Agaricomycotina*)'nın Türkiye'den ilk kaydı

Abstract: A deadly poisonous fungus, *Galerina venenata* A.H. Sm. collected from the rhizospheric region of *Olea europaea* L., which is distributed in the area with the dominance of Mediterranean climate, is reported for the first time for Türkiye. Molecular phylogenetic analysis based on ITS rDNA sequences from Turkish collections confirmed the position of *G. venenata* in the genus, and being the closest relative of *G. marginata* (Batsch) Kühner. A detailed morphological description of the present species, photographs, and comparison with taxonomically and phylogenetically close species are provided.

Key words: *Agaricales*, macrofungi, phylogeny, ribosomal DNA, taxonomy

Özet: Akdeniz ikliminin hakim olduğu bölgelerde yayılış gösteren *Olea europaea* L.'nın rizosferik bölgesinden toplanan ölümcül zehirli bir mantar olan *Galerina venenata* A.H. Sm., Türkiye için ilk kez rapor edilmektedir. Türkiye koleksiyonlarından elde edilen ITS rDNA dizilerine dayanan moleküler filogenetik analiz, *G. venenata*'nın cins içindeki konumunu ve *G. marginata* (Batsch) Kühner'nın en yakın akrabası olduğunu ortaya koymuştur. Mevcut türün ayrıntılı morfolojik tanımı, fotoğrafları ile taksonomik ve filogenetik olarak yakın türlerle kıyaslanması verilmiştir.

Anahtar Kelimeler: *Agaricales*, makromantar, filogeni, ribozomal DNA, taksonomi

Citation: Çelik A, Türkekul İ, Kaygusuz O (2024). First record of the deadly poisonous *Galerina venenata* (*Hymenogastraceae, Agaricomycotina*) from Türkiye. Anatolian Journal of Botany 8(1): 34-38.

1. Introduction

The taxonomic classification of *Galerina* Earle (Agaricales) can be traced back to Fries (1821), who initially designated it as the tribe *Galera*, comprising "Mycena-like ocher-brown spored fungi". *Galerina* was traditionally classified in the Hymenogastraceae Vittad., and species of the genus are characterized by small to medium-sized basidiomata with yellow-orange or yellow-brown pileus colour, slender and delicate stipe, the distinctive presence of verrucose basidiospores with rusty brown to brown and typically the presence of cheilocystidia (Ammirati et al., 1985; Gulden, 2012; Landry et al., 2021). Originally, it was assigned to the Cortinariaceae Singer due to the ocher brown and tuberculate nature of its basidiospores (Kirk et al., 2001), but subsequent reevaluation placed it within the Strophariaceae Singer & A.H. Sm. (Kirk et al., 2008). A molecular phylogenetic analysis conducted by Matheny et al. (2006) demonstrated that *Galerina* exhibits a closer evolutionary relationship with genera in the Hymenogastraceae as opposed to those in the Strophariaceae.

Members of *Galerine* grow in moss beds or on moss communities, including *Philonotis*, *Polytrichum*, *Sphagnum* and *Tomenthypnum*. However, certain species can be found colonizing decaying wood, stumps, humus, and soil substrates (Wood, 2001; Gulden 2010, 2012; Grzesiak and Wolski, 2015; Liu and Bau, 2021). Several species belonging to the genera *Amanita* Pers., *Conocybe* Fayod, *Galerina*, and *Lepiota* (Pers.) Gray contains amatoxins in amounts that can seriously poison humans (Ammirati et al., 1985). A recent study reported that *Galerina* contains lethal amatoxins at levels comparable to those in *Amanita phalloides* (Vaill. ex Fr.) Link (Landry et al., 2021). *Galerina badipes* (Pers.) Kühner, *G. castaneipes* A.H. Sm. & Singer, *G. marginata* (Batsch) Kühner, and *G. venenata* A.H. Sm. have been found as poisonous species within the genus (Landry et al., 2021). Amatoxins, α- and β-amanitin, which are toxic peptides found in *Amanita*, were also detected in *G. venenata* (Tyler and Smith, 1963).

Galerina comprises more than 300 species worldwide (Landry et al., 2021). Currently, 493 taxa of *Galerina* have been recorded in the IndexFungorum database (www.indexfungorum.org, accessed 10 January 2024), some of which are illegal names or synonyms. In Türkiye, 15 species have previously been reported and their distribution is limited to a few areas of Türkiye such as *Galerina ampullaceocystis* P.D. Orton, *G. atkinsoniana* A.H. Sm., *G. badipes* (Pers.) Kühner, *G. cinctula* P.D. Orton, *G. clavata* (Velen.) Kühner, *G. clavus* Romagn., *G. graminea* (Velen.) Kühner, *G. marginata* (Batsch) Kühner, *G. moelleri* Bas, *G. mycenoides* (Fr.) Kühner, *G. paludosa* (Fr.) Kühner, *G. pumila* (Pers.) Singer, *G. sideroides* (Bull.) Kühner, *G. sphagnorum* (Pers.) Kühner, and *G. stylifera* (G.F. Atk.) A.H. Sm. & Singer (Sesli et al., 2020; Solak and Türkoğlu, 2022). As part of ongoing investigations to

determine the macrofungal biodiversity in Türkiye, this report presents the first record of *Galerina venenata* in Türkiye based on morphological and molecular data.

2. Materials and Method

2.1. Sampling and morphological study

Galerina specimens were collected during the field trips to the Aydın Province in Western Türkiye in 2020 and 2022. By observing the features of fresh materials and morphological characters were described as follow Vellinga (1988). Dried samples mounted with 3% KOH or Melzer's reagent were observed for microscopic characteristics. A minimum of 30 basidiospores had been measured. In the list of abbreviations provided, "L^m" and "W^m" represent the mean dimensions of basidiospore length and width, respectively. "Q" denotes the length-to-width ratios, while "Q^m" signifies the average ratio value derived from the basidiospores measurements. All of the examined samples are deposited in the fungarium at Isparta University of Applied Sciences (ISUF).

2.2. Molecular genetic analysis

The ZR Fungal/Bacterial DNA MiniPrep kit (Zymo research, Irvine, California) was used to extract genomic DNA from tiny fragments of dried materials. For the internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA), the primers ITS1-F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) were utilized. The Polymerase Chain Reaction (PCR) amplification was performed as described by Kaygusuz et al. (2020) and as follows. PCR was conducted in 25 µL reaction volume containing 2 µL DNA template, 8.5 µL ddH2O, 12.5 µL 2 × PCR Master Mix and 1 µL of each primer. For the amplification of the rDNA ITS region, the PCR setup began with an initial denaturation step of 5 minutes at 95°C, followed by 35 cycles that included 1 minute of denaturation at 94°C, annealing for 45 seconds at 54°C, and 1 minute of extension at 72°C. This was concluded with an extension step of 10 minutes at 72°C. PCR products were checked using an ethidium bromide-stained 1.2% agarose electrophoresis gel.

The sequences used for the phylogenetic analysis were selected based on BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) results and relevant publications (Landry et al., 2021). DNA sequences of nrITS were aligned using the multiple sequence alignment program MAFFT v7 (Katoh and Standley, 2013) and were manually adjusted in BioEdit v.7.0 (Hall, 2004) when it is necessary.

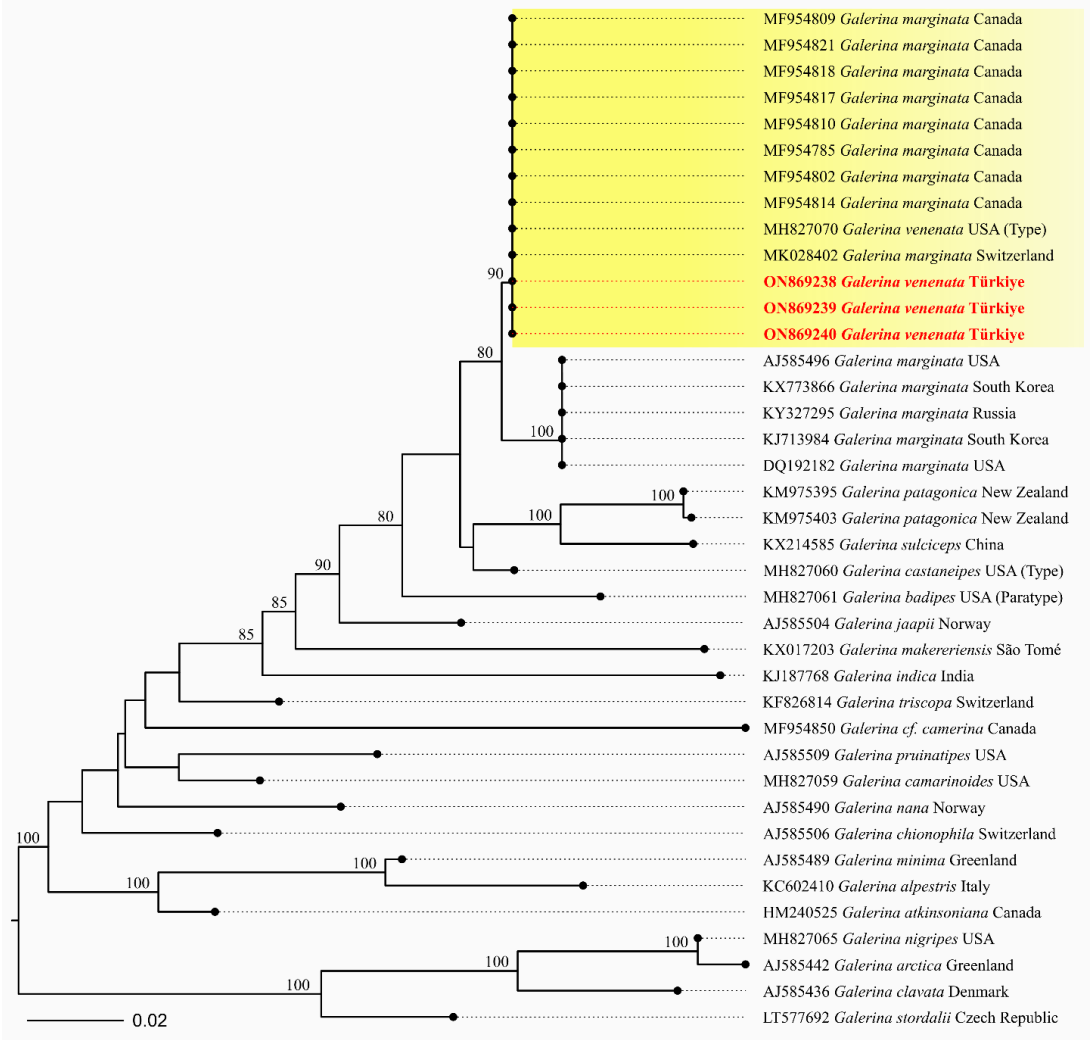

Figure 1. Phylogram generated from Maximum Likelihood (ML) analysis based on nrITS dataset. The tree is rooted with *Galerina arctica* (Singer) Nezdojm., *G. clavata* (Velen.) Kühner, *G. nigripes* A.H. Sm. & Singer and *G. stordalii* A.H. Sm. Newly generated sequences are shown in red

Phylogenetic relationships of taxa were generated through Maximum Likelihood (ML). ML analyses were performed in RAxML v.7.2.6 (Stamatakis et al., 2008) by running 1000 bootstrap (BS) replicates with GTRGAMMA substitution model. Maximum likelihood bootstrap (MLB) values of ≥80% were shown on the branch. The tree was visualized using FigTree v1.4.2 (Rambaut, 2012).

3. Results

3.1. Phylogeny

In this study, three sequences of *Galerina* specimens were generated and deposited in GenBank (ON869238-ON869240). The nrITS data matrix has 39 taxa containing 762 nucleotide sites (including gaps), of which 549 characters were constant and 213 were variable. The sequences from Türkiye were clustered in a separate monophyletic branch with high statistical support with MLB 90% (Fig. 1), with collections including the type sequence of *Galerina venenata* (MH827070).

3.2. Taxonomy

Galerina venenata A.H. Sm., Mycologia 45(6): 922 (1953) (Fig. 2)

Macroscopic and microscopic features:

Pileus 8–20 mm diam., at first convex to broadly convex, finally expanding to applanate, with a broad low umbo or slightly depressed at the centre, surface glabrous, hygrophanous, brightly yellowish white to yellow-brown or cinnamon brown, crenate, and minutely striate margin. Lamellae moderately crowded, subdistant, broadly adnate, yellowish to cinnamon. Stipe 30–65 × 2–6 mm, cylindrical or broadening towards the base, brownish to cinnamon, entirely floccose-fibrillose with white fibrils, without a ring or ring zone. Odour and taste are farinaceous.

Basidiospores (8.0–)9.0–11.8(–12.5) × (4.8–)5.5–6.7(–7.0) µm, $L^m \times W^m = 10.3 \times 6.0$ µm, Q = 1.5–1.9(–2.4), $Q^m = 1.7$, oblong, sometimes subcylindrical, with rugulose-warty in Melzer's reagent, thick-walled. Basidia 20–27 × 7.0–10.0 µm, clavate, 4–spored, thin-walled, hyaline. Pleurocystidia 40–85 × 9.0–15.0 µm, lageniform to fusiform, with a slightly longer neck and acute or subacute apex, thin-walled, hyaline in KOH. Cheilocystidia similar to pleurocystidia 45–80 × 10–18 µm, broadly lageniform to fusiform, with acute or subacute apex, thin-walled, hyaline in KOH. Pileipellis a cutis, terminal hyphae 5.0–18.0 µm wide, intertwined, thin-walled, hyaline in KOH. Caulocystidia 50–70 × 9.0–12.0 µm, lageniform to fusiform, with long tapered apex, mostly at the apex of the stipe, thin-walled, hyaline in KOH. Stipitipellis a cutis, hyphae 5.0–12.0 µm wide, cylindrical, thin-walled, hyaline in KOH. Clamp connections present in all tissues.

Habit and habitat: Gregarious or sometimes in small groups, terrestrial, near rhizosphere of *Olea europaea* L., growing on calcareous sandy soils.

Additional collections examined: Türkiye, Aydın Province, Kuşadası District, around Güzelçamlı, on calcareous soil, near rhizosphere of *Olea europaea*, alt. 10 m, 5 March 2020, leg. O. Kaygusuz, OKA-TR0124, GenBank: ON869238 for nrITS; *ibid.,* on calcareous soil, under *O. europaea*, alt. 5 m, 17 March 2021, leg. O. Kaygusuz, OKA-TR0125, GenBank: ON869239 for nrITS; *ibid.,* on soil, near rhizosphere of *O. europaea*, alt. 8 m, 23 March 2022, leg. O. Kaygusuz, OKA-TR0126, GenBank: ON869240 for nrITS.

4. Discussions

Galerina belongs to the family Hymenogastraceae and is a polyphyletic genus (Gulden et al., 2005). The main colour range of the fruit bodies of members of the genus varies from ochre to reddish brown, and the pileus is hygrophanous, in most species, it is translucently striate (Gulden, 2012). Among them, *Galerina venenata* was originally described by Smith (Smith, 1953) from the USA. Three collections from Türkiye grouped into a monophyletic branch, which includes the holotype sequence of *G. venenata* (Fig. 1). The sequences previously labelled as *G. marginata* from Canada (MF954809, MF954821, MF954818, MF954817, MF954810, MF954785, MF954802, and MF954814) and Switzerland (MK028402) clustered in the same clade as *G. venenata* (Fig. 1). This study is the first report of *Galerina venenata* from Türkiye.

Members of *Galerina* were reported to grow saprotrophic in mosses, on wood or soil, or rarely parasitic (Gulden, 2012). The type specimen of *G. venenata* was recorded from the lawn (Smith, 1953). Türkiye collections of *G. venenata* have been reported from calcareous soil under *Olea europaea*, which is a new possible host or mycorrhizal partner for this species.

Molecular phylogenetic analysis based on ITS rDNA sequences from Türkiye collections revealed the position of *Galerina venenata* within the genus *Galerina*, indicating it as a sister to *G. marginata*. However, *Galerina marginata* differs from *G. venenata* in having a pale to dark ochraceous to yellow pileus colour, and it usually grows in lignicolous habitats (Smith, 1953; Landry et al., 2021). In addition, α-amanitin has been isolated from *G. marginata*, while β-amanitin was detected in *G. venenata* (Landry et al., 2021). Morphologically, *Galerina autumnalis* (Peck) A.H. Sm. & Singer resembles *G. venenata*, but it is distinguished by having a membranous annulus and pleurocystidia that mostly have enlarged apices (Smith, 1953).

For many species of *Galerina*, habitat is a very important characteristic. *G. venenata*, *G. brunneimarginata* (Murrill) A.H. Sm. & Singer, and *G. semilanceata* (Peck) A.H. Sm. & Singer prefers open grassy areas. However, *G. brunneimarginata* and *G. semilanceata* differ from *G. venenata* in the colour and characteristics of the pileus, the small size of the basidiospores, and the absence of pleurocystidia (Smith and Singer, 1964).

Conflict of Interest

The authors have declared no conflict of interest.

Authors' Contribution

The authors contributed equally.

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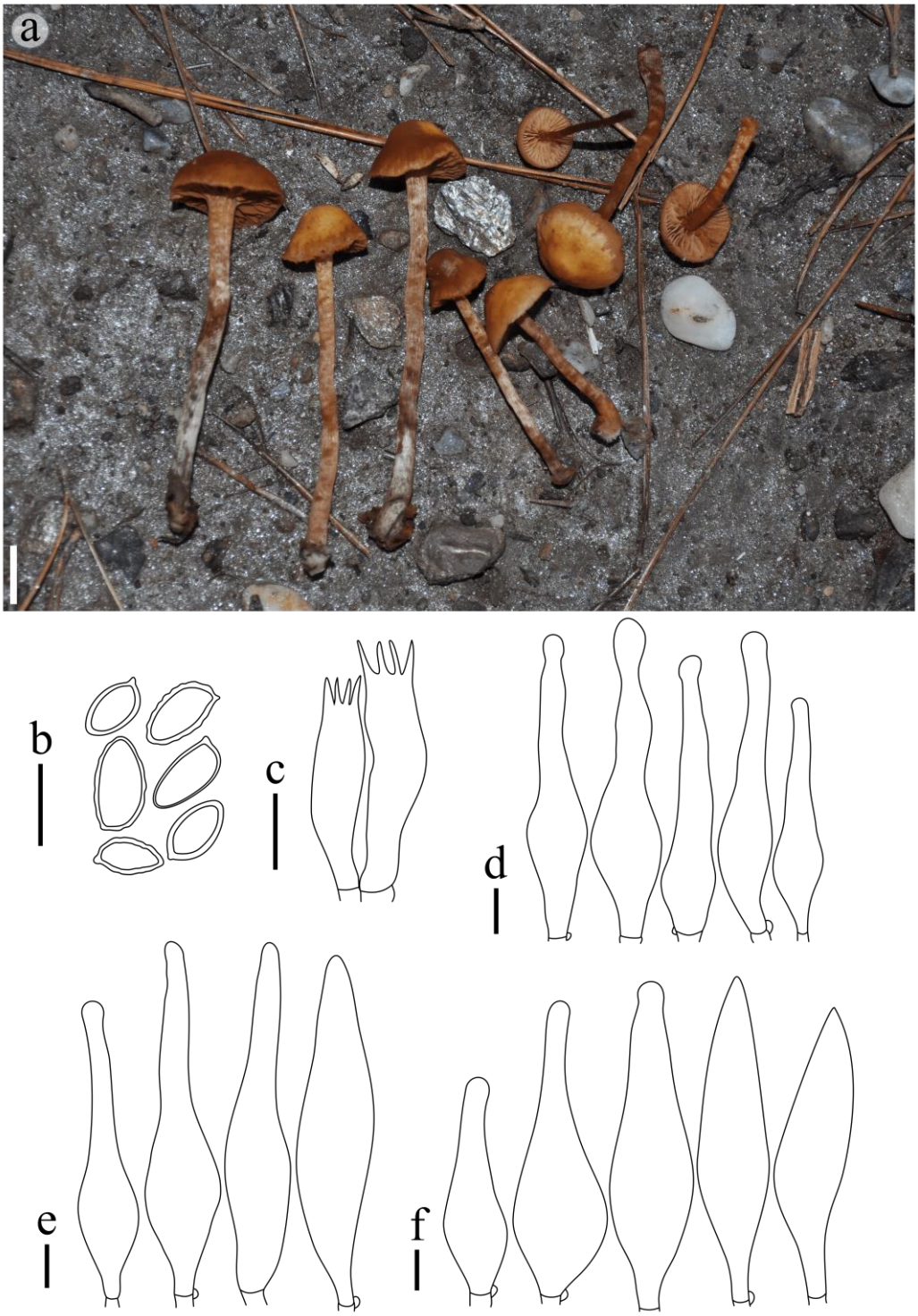

Figure 2. Basidiomata (a), basidiospores (b), basidia (c), caulocystidia (d), pleurocystidia (e) and cheilocystidia (f) of *Galerina venenata* (Scale bars: a- 10 mm, b-f- 10 µm)

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Biodiversity of ochratoxigenic *Aspergillus* species isolated from çavuş and karalahna grapes in Bozcaada, Türkiye

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Bozcaada çavuş ve karalahna üzümlerinden izole edilen okratoksijenik *Aspergillus* türlerinin biyoçeşitliliği

Abstract: This study reported the presence and OTA production of ochratoxigenic *Aspergillus* species isolated from Bozcaada Çavuş and Karalahna grapes. The study was conducted with *Aspergillus* isolates isolated from grape samples collected during ripening in 2015 and 2016. Thin-layer chromatography was used to determine the secondary metabolite profiles of 290 *Aspergillus* isolates. Out of these, 122 isolates were found to be possible OTA producers. 43 isolates, selected based on their colony morphology of 122 isolates in different culture media, were identified using calmodulin gene sequencing analysis. The identified isolates were determined to be *A. tubingensis*, *A. carbonarius*, *A. niger/welwitschia/awamori*, *A. welwitschia*, *A. spelaeus*, and *A. fructus*. OTA production was investigated in six isolates. Using HPLC-FLD, these isolates were found to produce OTA in quantities ranging from 3.550 ± 0.240 to 92.346 ± 0.818 ppb. Consequently, OTA-producing *Aspergillus* species were isolated from grapes. The presence of *A. spelaeus* and *A. fructus* was reported for the first time in grapes. *A. fructus* has been found to be a new record for Türkiye.

Key words: *Aspergillus*, calmodulin, HPLC, molecular identification, TLC

Özet: Bu çalışmada, Bozcaada Çavuş ve Karalahna üzümlerinden izole edilen okratoksijenik *Aspergillus* türlerinin varlığı ve OTA üretimi rapor edilmiştir. Çalışma, 2015 ve 2016 yıllarında olgunlaşma sırasında toplanan üzüm örneklerinden izole edilen *Aspergillus* izolatları ile gerçekleştirilmiştir. İlk olarak 290 *Aspergillus* izolatının ikincil metabolit profilleri İnce Tabaka Kromatografisi kullanılarak belirlenmiştir. İzolatlardan 122 tanesinin ise muhtemel OTA üreticisi olduğu tespit edilmiştir. 122 izolatın farklı kültür ortamlarındaki koloni morfolojilerine göre seçilen 43 izolat, calmodulin gen dizileme analizi kullanılarak tanımlanmıştır. Tanımlanan izolatların *A. tubingensis*, *A. carbonarius*, *A. niger/welwitschia/awamori*, *A. welwitschia*, *A. spelaeus* ve *A. fructus* olduğu belirlenmiştir. OTA üretimi ise altı izolatta araştırılmıştır. HPLC-FLD kullanılarak bu izolatların 3,550 ± 0,240 ile 92,346 ± 0,818 ppb arasında değişen miktarlarda OTA ürettiği bulunmuştur. Sonuç olarak üzümlerden OTA üreten *Aspergillus* türleri izole edildi. *A. spelaeus* ve *A. fructus* türlerinin varlığı üzümlerde ilk kez rapor edilmiştir. A. fructus Türkiye için yenit kayıt olduğu tespit edilmiştir.

Anahtar Kelimeler: *Aspergillus*, calmodulin, HPLC, moleküler identifikasyon, TLC

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1. Introduction

Bozcaada (Tenedos), located south of the Dardanelles Strait and northeast of the Aegean Sea, is connected to Çanakkale and is the only district of Türkiye that does not have a village. With its low and flat geographical structure at the exit of the Bosphorus, the north and south winds are quite effective, and the Mediterranean climate type is observed. Most of Bozcaada's vegetation consists of vineyards. Climate and winds allow various types of grapes to be grown. For this reason, viticulture is one of the most important livelihoods of the island people. Both edible and wine grape varieties are grown on the island. These grapes are processed in wine factories on the island and presented to the market (Dardeniz et al., 2007; Anonymous, 2023).

Aspergillus is a genus with high economic and social effects. The *Aspergillus* genus is a very important species that affects food, indoor air, and human health and is used in biotechnology. The genus *Aspergillus* includes many species based on their morphological, physiological, and phylogenetic structures. *Aspergillus* is an anamorph genus containing approximately 840 species. These species have been classified into about ten different teleomorph genera. It has been determined that the genus *Aspergillus* is related to nine teleomorph genera by conventional identification, but with the phylogenetic data, it forms a monophyletic branch closely related to the genera such as *Polypaecilum*, *Phialosimplex*, *Dichotomomyces*, *and Cristaspora* with genus *Penicillium* together. Classification and identification of *Aspergillus* species are based on phenotypic characteristics, but there have been many advances in molecular and chemotaxonomic characterisation in recent years (Krijgsheld et al., 2013; Samson et al., 2014).

Recent studies have determined that the morphological methods used alone are insufficient for identifying *Aspergillus* species, which are especially important from a medical point of view. It is increasingly recognised that

comparative sequence-based methods combined with traditional phenotype-based methods can provide better species resolution within this genus. They stated that molecular identification requires the use of ITS regions for interdepartmental level identification and β-tubulin and Calmodulin gene regions for identification of individual species within various *Aspergillus* divisions (Balajee et al., 2007; Samson et al., 2014).

From the past to the present, there have been many studies on the determination of fungal loads and mycotoxin contents of grapes and their products grown in Türkiye (Eltem et al., 2004; Askun et al., 2007; Taskin et al., 2008; Eltem et al., 2009; Gulsunoglu et al., 2019). Studies have mostly been on determining mycotoxins in grape and grape products. Yield in the vineyards where grapes are grown in Bozcaada is variable due to fungal and other diseases. However, the literature shows no published research on the microbiota of these grapes grown on the island except for Özcan Ateş & Zorba (2021). In this study, the mycobiota of Çavuş (table) and Karalahna (table and wine) grapes native to Bozcaada were determined (Özcan Ateş & Zorba, 2021). Therefore, this study aimed to perform molecular identification of *Aspergillus* species isolated from grape berries obtained from Çavuş and Karalahna vineyards and to determine the OTA production potential of the characterised isolates.

2. Materials and Method

2.1. Cultures

Aspergillus isolates isolated from Karalahna and Çavuş grapes grown in Bozcaada in 2015 and 2016 were used. First, the stock cultures (containing 0.2% agar + 0.05% Tween 80 spore solution in slanted Potato Dextrose Agar (PDA)) were inoculated by the three-point method on PDA, and culture purity controls were made. Analyses were carried out with 290 *Aspergillus* isolates that were revived and checked for purity.

2.2. Identification of possible ochratoxin a producer ısolates and selection of ısolates

Secondary metabolite profiles and ochratoxin production of *Aspergillus* isolates were examined by the Thin Layer Chromatography method, according to Samson et al. (2010). Besides, colony morphology of isolates on Coconut cream agar (CCA) (Dyer & McCammon, 1994), Czapek Yeast Extract Agar, Malt Extract Agar, and Creatine Sucrose Agar (Samson et al., 2010; Samson et al., 2014). According to TLC results, 122 isolates were determined to be possible OTA producers. Then, the colony morphologies of these isolates were examined. As a result of comparing TLC results with colony morphologies, 43 possible OTA producer isolates for molecular identification were selected in 122 isolates.

2.3. Molecular identification

DNA extraction from cultures was done with QuickGene DNA tissue kit S protocol DF-13 (Kurabo, South Korea). Cultures grown on Malt Extract Agar (MEA) medium at 25°C for seven days were used. Cultures were scraped from Petri dishes, and 25-50 mg fungal pellets were transferred to 1.5 mL Eppendorf tubes containing zirconium beads. Then, the protocol given in the kit was applied. Nucleic acid measurements of the DNAs of the samples were made with a fluorescent spectrophotometry device (Colibri Microvolume Spectrometer, Berthold Titertek Instruments, Inc., Germany) at the wavelength of OD260/OD280. The concentrations of the isolates, whose quality and quantity of gDNA were controlled, were prepared at 20 ng/ µL and used in molecular studies.

Calmodulin (CaM) (AS_CA_F; GCKWAAYAGGACAAGGATGG and AS_CA_R; CTGGTCVGCCTCACRAAT) (Ayan et al., 2018) gene region was selected for fungal molecular diagnosis of *Aspergillus* isolates. Before sequence sequencing, the PCR reactions of the samples were prepared with 50ng gDNA, 10 µL Sensifast 2X Probe Mastermix, 0.4 nM forward and reverse primers and ddH2O in a total volume of 20 µL. PCR reaction conditions as the first denaturation at 95°C for 10 min followed by 40 cycles at 95°C-15 sec, at 61°C for 30 sec, at 72°C for 15 sec, and the final extension phase as one cycle 72°C-7 min. has been made. Electrophoresis of PCR products was carried out on a 1% agarose gel containing 1 µg of nucleic acid fluorescent marker ethidium bromide at 200 V for 15 min. 100 bp Plus DNA Ladder Thermo was used as a marker in the electrophoresis of PCR products.

Sequence analyses were performed to investigate the phylogenetic relationships of 43 isolates identified within the scope of the study over the CaM gene region. The PCR products were first purified with Rapid Alkaline Phosphatase and Exonuclease I, and sequencing was performed forwards and backwards with the ABI 3500xL Genetic Analyzer (Applied Biosystems) device. The corrupted readings were caused by the primer attachment points of the samples whose sequencing process was completed and cut with the program Bioedit v7.0.53 (Hall, 1999). After editing the sequence data of isolates, sequences multiple alignment process MEGA v6. (Tamura et al., 2013). To check whether the polymorphisms are correct from the sequence peaks. Reference isolates were used to evaluate all isolates within the scope of the study, and the results were also checked over NCBI-Blast-n.

2.4. Determination of ochratoxin-a production amounts of ısolates by HPLC

OTA production amounts of six of the molecularly identified *Aspergillus* strains were determined according to the HLPC method given by Özcan Ateş and Zorba (2021). The study determined HPLC performance parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability and reproducibility. The linearity of the method was determined by drawing a 5-point calibration curve. OTA 0.05-20.00 µg/kg ranges were used to create calibration curves. The detection limit was calculated based on the signal-to-noise ratio being S/N=3/1, and the measurement limit was calculated based on the signal-to-noise ratio being S/N=10/1 (British Standard, 2000). The calculated LOD and LOQ are below the legal limit. To determine method precision, relative standard deviation percentages were calculated regarding repeatability and reproducibility, as six replications on the same day at one standard concentration and three replications on three separate days.

3. Results

The CaM gene regions of 43 *Aspergillus* isolates selected by considering TLC and colony morphologies in 290 *Aspergillus* isolates isolated from Karalahna and Çavuş grapes grown in Bozcaada in 2015 and 2016 were amplified by PCR. Sequencing was performed for 43 isolates of CaM

gene regions. The resulting sequences were checked on NCBI-Blast-n. The gene bank numbers and nomenclature of the isolates are given in Table 1. Maximum Likelihood Tree Tamura-Nei model is given in Figure 1.

Table 1. Identification of the *Aspergillus* isolates determined by amplifying CaM gene and nucleotide sequences

Isolate no	NCBI Gene Bank Number	Closest relative	Closest Accession Number	Identity (%)	Collection date	Isolation source	Location
51	-*	*A. welwitschiae*	LR215876.1	99.00%	20-Jul-2016	Karalahna Grape	Bozcaada Çayır
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
52	PP264190	*A. carbonarius*	MK778845.1	99.00%	11-Aug-2016	Çavuş Grape	Bozcaada Çayır
53	PP264189	*A. tubingensis*	LR215871.1	99.00%	30-Jun-2016	Çavuş Grape	Bozcaada Sulubahçe
54	PP264188	*A. carbonarius*	MK778845.1	100.00%	04-Sep-2015	Çavuş Grape	Bozcaada Çayır
55	PP264187	*A. carbonarius*	MK778845.1	100.00%	24-Aug-2015	Karalahna Grape	Bozcaada Çayır
56	PP264186	*A. tubingensis*	LR215879.1	99.00%	24-Aug-2015	Karalahna Grape	Bozcaada Çayır
57	PP264185	*A. carbonarius*	MK778845.1	99.00%	24-Aug-2015	Çavuş Grape	Bozcaada Çayır
58	PP264184	*A. tubingensis*	LR215879.1	99.00%	14-Aug-2015	Çavuş Grape	Bozcaada Sulubahçe
59	PP264183	*A. carbonarius*	MK778845.1	99.00%	14-Aug-2015	Çavuş Grape	Bozcaada Sulubahçe
61	-	*A. welwitschiae*	LR215876.1	99.00%	20-Jul-2016	Çavuş Grape	Bozcaada Sulubahçe
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
62	PP264182	*A. tubingensis*	LR215871.1	99.00%	22-Aug-2016	Karalahna Grape	Bozcaada Sulubahçe
63	PP264181	*A. carbonarius*	MK778845.1	99.00%	01-Aug-2016	Karalahna Grape	Bozcaada Sulubahçe
64	-	*A. welwitschiae*	LR215876.1	99.00%	20-Jul-2016	Karalahna Grape	Bozcaada Sulubahçe
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
65	-	*A. welwitschiae*	LR215867.1	99.00%	10-Jul-2016	Çavuş Grape	Bozcaada Çayır
		A. awamori	KJ777809.1	99.00%			
		A. niger	LC573721.1	99.00%			
66	-	*A. welwitschiae*	LR215876.1	99.00%	10-Jul-2016	Çavuş Grape	Bozcaada Çayır
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
67	PP264180	*A. tubingensis*	LR215871.1	99.00%	20-Jun-2016	Çavuş Grape	Bozcaada Çayır
68	PP264179	*A. carbonarius*	MK778845.1	100.00%	04-Sep-2015	Çavuş Grape	Bozcaada Sulubahçe
69	PP264178	*A. tubingensis*	LR215871.1	99.00%	04-Sep-2015	Karalahna Grape	Bozcaada Çayır
70	-	*A. welwitschiae*	LR215876.1	99.00%	20-Jun-2016	Çavuş Grape	Bozcaada Çayır
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
71	-	*A. welwitschiae*	LR215876.1	99.00%	20-Jul-2016	Karalahna Grape	Bozcaada Çayır
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
72	PP264177	*A. carbonarius*	MK778845.1	99.00%	01-Aug-2016	Çavuş Grape	Bozcaada Çayır
73	PP264176	*A. carbonarius*	MK778845.1	99.00%	22-Aug-2016	Karalahna Grape	Bozcaada Sulubahçe
74	PP264175	*A. tubingensis*	LR215871.1	99.00%	01-Aug-2016	Çavuş Grape	Bozcaada Çayır
75	-	*A. welwitschiae*	LR215876.1	99.00%	14-Aug-2015	Karalahna Grape	Bozcaada Çayır
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
76	PP264174	*A. welwitschiae*	LR215867.1	99.00%	04-Aug-2015	Çavuş Grape	Bozcaada Sulubahçe
77	PP264173	*A. tubingensis*	LR215871.1	99.00%	04-Aug-2015	Karalahna Grape	Bozcaada Sulubahçe
78	-	*A. welwitschiae*	LR215876.1	99.00%	14-Aug-2015	Karalahna Grape	Bozcaada Çayır
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
79	PP264172	*A. tubingensis*	LR215871.1	99.00%	04-Aug-2015	Karalahna Grape	Bozcaada Çayır
80	PP264171	*A. welwitschiae*	LR215867.1	99.00%	04-Aug-2015	Çavuş Grape	Bozcaada Çayır
81	-	*A. welwitschiae*	LR215876.1	99.00%	20-Jul-2016	Karalahna Grape	Bozcaada Çayır
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
82	-	*A. welwitschiae*	LR215876.1	99.00%	20-Jul-2016	Çavuş Grape	Bozcaada Sulubahçe
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
83	-	*A. welwitschiae*	LR215876.1	99.00%	20-Jul-2016	Çavuş Grape	Bozcaada Sulubahçe
		A. niger	LC425338.1	99.00%			
		A. awamori	MG832133.1	99.00%			
84	PP264170	*A. tubingensis*	LR215871.1	99.00%	10-July-2016	Karalahna Grape	Bozcaada Sulubahçe
85	PP264169	*A. tubingensis*	LR215871.1	99.00%	04-Sep-2015	Karalahna Grape	Bozcaada Çayır
86	PP264168	*A. tubingensis*	LR215871.1	98.00%	24-Aug-2015	Karalahna Grape	Bozcaada Çayır
87	PP264167	*A. carbonarius*	MK778845.1	99.00%	04-Sep-2015	Çavuş Grape	Bozcaada Çayır
88	PP264166	*A. carbonarius*	MK778845.1	99.00%	04-Sep-2015	Çavuş Grape	Bozcaada Çayır
89	PP264165	*A. tubingensis*	LR215871.1	99.00%	24-Aug-2015	Çavuş Grape	Bozcaada Sulubahçe
90	PP264164	*A. carbonarius*	MK778845.1	99.00%	24-Aug-2015	Çavuş Grape	Bozcaada Sulubahçe

91	PP264163	*A. carbonarius*	MK778845.1	99.00%	14-Aug-2015	Çavuş Grape	Bozcaada Çayır
92	PP264162	*A. carbonarius*	MK778845.1	99.00%	04-Sep-2015	Çavuş Grape	Bozcaada Sulubahçe
95	PP264161	*A. spelaeus*	HG916745.1	99.00%	04-Aug-2015	Çavuş Grape	Bozcaada Sulubahçe
100	PP264160	*A. fructus*	MG832140.1	99.00%	20-Jul-2016	Karalahna Grape	Bozcaada Sulubahçe

-* Since exact species discrimination could not be made, gene bank data was not entered.

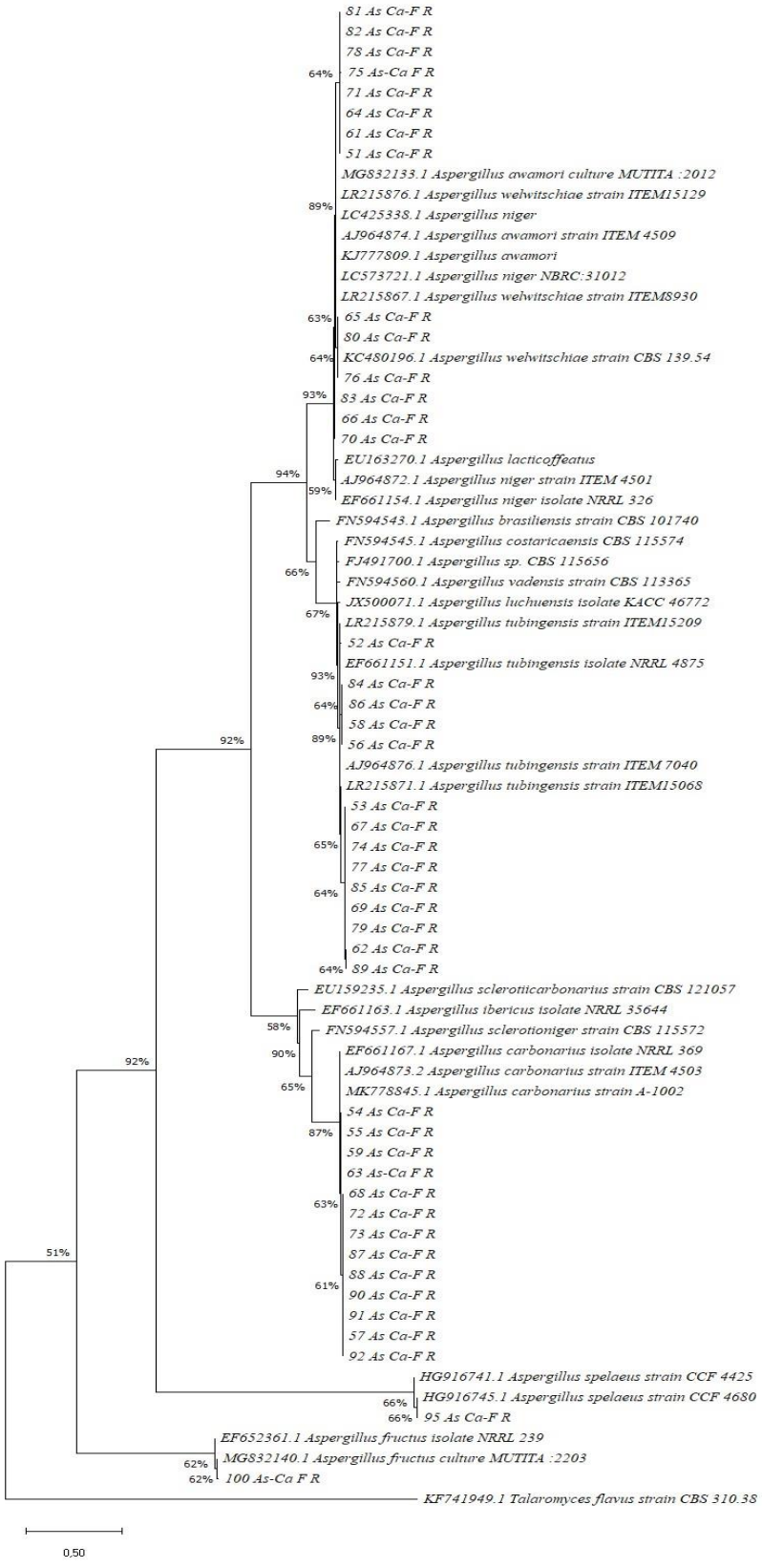

Figure 1. Maximum Likelihood Tree Tamura-Nei model was chosen for maximum likelihood analysis. Bootstrap is set to 1000 replicas. Branch arrangements are selected as TBR. KF741949.1 *Talaromyces flavus* strain CBS 310.38 calmodulin gene, partial cds was used as the outgroup.

Table 2. Haplotype information of isolates

Haplotype	Number of isolates	Isolates		
Hap_1	1	1,011	28,397	1,616
Hap_2	14	51_As_Ca_F_R	70_As_Ca_F_R	80_As_Ca_F_R
		61_As_Ca_F_R	71_As_Ca_F_R	81_As_Ca_F_R
		64_As_Ca_F_R	75_As_Ca_F_R	82_As_Ca_F_R
		65_As_Ca_F_R	76_As_Ca_F_R	83_As_Ca_F_R
		66_As_Ca_F_R	78_As_Ca_F_R	
Hap_3	13	52_As_Ca_F_R	69_As_Ca_F_R	85_As_Ca_F_R
		53_As_Ca_F_R	74_As_Ca_F_R	86_As_Ca_F_R
		56_As_Ca_F_R	77_As_Ca_F_R	89_As_Ca_F_R
		62_As_Ca_F_R	79_As_Ca_F_R	
		67_As_Ca_F_R	84_As_Ca_F_R	
Hap_4	1	58_As_Ca_F_R		
Hap_5	1	100_As_Ca_F_R		
Hap_6	13	54_As_Ca_F_R	68_As_Ca_F_R	90_As_Ca_F_R
		55_As_Ca_F_R	72_As_Ca_F_R	91_As_Ca_F_R
		57_As_Ca_F_R	73_As_Ca_F_R	92_As_Ca_F_R
		59_As_Ca_F_R	87_As_Ca_F_R	
		63_As_Ca_F_R	88_As_Ca_F_R	
		63_As_Ca_F_R	88_As_Ca_F_R	

It was determined that 13 of the identified isolates were *A. tubingensis*, 14 of them *A. carbanorius*, 12 of them *A. niger/welwitschia/awamori*, 2 of them *A. welwitschia*, 1 of them *A. spelaeus*, and 1 of them *A. fructus*. In the identification of 43 *Aspergillus* isolates with the CaM gene region, six different haplotypes were detected in the polymorphism data of 43 isolates, haplotype information of the isolates was given in Table 2.

Information on the OTA production potential of six isolates out of 43 isolates that we determined to be possible mycotoxin producers was given in Table 3.

OTA production amounts of 6 isolates selected according to the molecular identification results were determined by HPLC: 12.483 ± 0.187 and 49.448 ± 0.354 ppb of *A. carbanorius* isolates, 32.884 ± 0.554 ppb of *A. tubingensis* isolate, 3.550 ± 0.240, 7.519 ± 0.134, and 92.346 ± 0.818 ppb of 3 *A. niger/welwitschia/awamori* isolates.

4. Discussions

In mycological studies on grapes and grape products, especially black *Aspergillus* species were identified, and OTA production amounts were determined (Battilani et al., 2003; Magnoli et al., 2003; Serra et al., 2003; Guzev et al., 2006; Khoury et al., 2008; Chiotta et al., 2009; Lasram et al., 2012; García-Cela et al., 2015; Garmendia & Vero, 2016; Oliveri et al., 2017). The ecological distribution of mycotoxigenic mould populations is very important, as many agricultural products, including grapes, are at risk of contamination by mycotoxins. Therefore, there is a need to develop tools for the distribution of the mould population and to identify the moulds (Palumbo & O'Keeffe, 2015). There is a previous study by Özcan Ateş and Zorba (2021) on the mould biodiversity of Bozcaada grapes. Although diversity has been determined with *Aspergillus* species isolated from grapes, the time-consuming isolation of mould and the need to identify individual strains by morphological or molecular techniques limit the scope of studies.

Table 3. Ochratoxin A production amounts (in ppb) of selected isolates.

Isolate No	Closest relative	OTA
51	*A. welwitschiae*	7.519 ± 0.134*
	A. niger	
	A. awamori	
52	*A. tubingensis*	32.884 ± 0.554
57	*A. carbonarius*	49.448 ± 0.354
75	*A. welwitschiae*	92.346 ± 0.818
	A. niger	
	A. awamori	
82	*A. welwitschiae*	3.550 ± 0.240
	A. niger	
	A. awamori	
91	*A. carbonarius*	12.483 ± 0.187

*Results are expressed as means ± standard deviations.

Among the *Aspergillus* species isolated and identified from grapes, species such as *A. niger, A. awamori, A. japonicus, A. aculeatus, A. foetidus, A. carbonarius, A. candidus*, and *A. flavus* were determined by traditional methods (Magnoli et al., 2003; Ponsone et al., 2007) and when molecular techniques were used, in addition to these species, *A. nidulans, A. ochraceus, A. tamarii, A. terreus, A. wenti, A. welwitschiae, A. sclerotioniger, A. sclerotiocarbonarius*, and *A. ibericus* species were also detected (Martinez-Culebras & Ramon, 2007; Pantelides et al., 2017). It was determined that 41 isolates detected in the present study were *Aspergillus* Nigri and similar species to those in the literature. However, it was not possible to distinguish whether 12 isolates were *A. niger/welwitschia/awamori* in the study. When the phylogenetic analysis of the sequences produced from three gene fragments encoding β-tubulin (benA), calmodulin (CaM) and translation elongation factor-1 alpha (TEF-1α) proteins of the strains isolated from grapes in Europe and defined as *A. niger* were evaluated, it was stated that the species could be *A. awamori* and *A. niger*. However, the researchers noted that these species could not be distinguished. The cultural traits of these species are also very similar and differ only on five bases in their identification with the calmodulin gene region. Therefore, additional analyses such as AFLP are needed to differentiate these species (Perrone et al., 2011). While one

isolate obtained in the study was defined *A. spelaeus* (Flavipedes group), one isolate was determined as *A. fructus* (Versicolores group). *A. spelaeus* was previously reported by Ayan et al. (2018) isolated from the forest lands of Edirne province and reported as a new record from Türkiye. This species was also isolated from the land where *Serapias vomeracea* is distributed in Samsun, Turkey (Özdener Kömpe et al., 2022). However, no studies were found on isolating the 2 species (*A. spelaeus* and *A. fructus*) mentioned in the literature from grapes. In addition, it was determined that the *A. fructus* (there is no synonymy according to Index Fungorum) was a new record for Türkiye (Sesli et al., 2020; Asan et al., 2022; Index Fungorum, 2023).

Magnoli et al. (2003) found that 41.3% of *Aspergillus* section Nigri isolates produced OTA in 2 to 24.5 ng/ml medium in their study on OTA production of *Aspergillus* genus isolated from grapes. Martinez-Culebras and Ramon (2007) found that *A. carbonarius* and *A. tubingensis* isolates produced OTA at a maximum level of 2.85 µg/g. There are also different studies on *A. niger* aggregate, *A. niger* and *A. carbonarius* species producing OTA (Ponsone et al., 2007; Pantelides et al., 2017). OTA production from the same species was also detected in our study.

As a result of this study, it was determined that Bozcaada Çavuş and Karalahna grapes were contaminated with ochratoxigenic *Aspergillus* species. Phylogenetic analysis of the isolated moulds revealed that *A. tubingensis, A. carbanorius* and *A. niger* species were dominant. Most importantly, *A. spelaeus* and *A. fructus* were isolated from grapes for the first time.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

G.Ö.A, N.N.Z and B.Ş. designed the experiments, G.Ö.A. performed the experiments, analyzed the data, and wrote and edited the article. GÖA, N.N.Z, and B.Ş. reviewed and edited the article. All authors have read and agreed to the published version of the manuscript.

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Simocybe centunculus, a new record for the mycobiota of Türkiye

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Simocybe centunculus, Türkiye mikobiyotası için yeni bir kayıt

Abstract: *Simocybe centunculus* is reported for the first time from Türkiye, based on the identification of the specimens collected from the Beykoz (İstanbul) district. This species is the first member of the genus *Simocybe* reported from Türkiye. A brief description of the presented species is provided, together with the photographs illustrating its macro- and micromorphologies.

Key words: Biodiversity, *Crepidotaceae,* new record, Türkiye

Özet: *Simocybe centunculus* Beykoz (İstanbul)'dan toplanan örneklerin teşhis edilmesine bağlı olarak Türkiye'den ilk kez rapor edilmiştir. Bu tür, *Simocybe* cinsinin Türkiye'den rapor edilen ilk üyesidir. Belirlenen türün kısa bir betimlemesi, türün makro ve mikromorfolojilerine ilişkin fotoğrafları ile birlikte verilmiştir.

Anahtar Kelimeler: Biyoçeşitlilik, *Crepidotaceae,* yeni kayıt, Türkiye

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1. Introduction

Simocybe P. Karst. is a genus in the family *Crepidotaceae* (*Agaricales*). Species of the genus are characterized by small, brown basidiomes with minutely pruinose or micaceous pileus lacking veil remnants, emarginate or subdecurrent brown lamellae, central or eccentric pruinose stipe without veil remnants, conspicuous cheilocystidia and caulocystidia and smooth, brown, ovoid, amygdaliform or phaseoliform spores usually without a germ pore (Pegler and Young, 1975; Horak and Ronikier, 2011).

Early classifications placed *Simocybe* and its allies within the *Cortinariaceae* (Breitenbach and Kränzlin, 2000), but phylogenetic analyses indicate that *Simocybe* is monophyletic and closely related to *Crepidotus* and is thus much better placed in the *Crepidotaceae* (Moncalvo et al. 2002; Aime et al. 2005; Petersen et al. 2010). Although confirmed records of *Simocybe* are not so frequent, the geographical range of locality records spans from tropical-subtropical regions to arctic-alpine habitats (Horak and Ronikier, 2011).

IndexFungorum (2023) lists 71 confirmed species names within the genus *Simocybe,* but the current checklists (Sesli et al., 2020; Solak and Türkoğlu, 2022) and the latest contributions (Keleş et al., 2022; Işık et al., 2023; Kaygusuz et al., 2023; Sesli, 2023; Yeşilyurt et al., 2023) indicate that any member of the genus *Simocybe* has yet been reported from Türkiye.

The study aims to contribute to the knowledge of the mycobiota of Türkiye.

2. Materials and Method

The fresh basidiomata of *Simocybe* were collected from the Beykoz district in İstanbul province during a field survey in 2023. In the field, the specimens were photographed and documented. Then they were transferred to the fungarium in the paper bags, and dried in an air conditioned room. Microscopic investigations were conducted using a Leica DM 2500 trinocular compound microscope on dry specimens mounted on slides in pure water. Congo Red, Melzer's reagent and 3% KOH were used for additional observations. Measurements of microscopic structures were performed at least 20 times, on slides prepared in pure water. The samples were identified by comparing the obtained data with literature (Breitenbach and Kränzlin, 2000; Kuo, 2007; Senn-Irlet, 2008; Desjardin et al., 2014; Desjardin and Perry, 2016; Siegel and Schwarz, 2016). Voucher specimens are preserved at Karamanoğlu Mehmetbey University, Science Faculty, Department of Biology.

3. Results

Basidiomycota R.T. Moore

Agaricales Underw.

Crepidotaceae (S. Imai) Singer

Simocybe centunculus (Fr.) P. Karst., Bidr. Känn. Finl. Nat. Folk 32: 420 (1879)

Syn: [*Agaricus centunculus* Fr., *A. centunculus* var. *concolor* Fr., *A. centunculus* var. *enchymosus* Fr., *A. enchymosus* (Fr.) Fr., *Agrocybe centunculus* (Fr.) Romagn., *A. centunculus* var. *luxurians* (Romagn.) Romagn., *A. Laevigata* (J. Favre) Romagn., *Hylophila centunculus* (Fr.) Quél., *Naucoria centunculus* (Fr.) P. Kumm., *N. centunculus* f. *luxurians* Romagn., *N. centunculus* var. *laevigata* J. Favre, *N. centunculus* var. *obscura* Romagn.,

N. enchymosa (Fr.) Sacc., *N. laevigata* (J. Favre) Kühner & Romagn., *N. laevigata* var. *maritima* Bon, *Ramicola centunculus* (Fr.) Watling, *R. Laevigata* (J. Favre) Watling, *R. Maritima* (Bon) Bon, *R. obscura* Romagn. ex Watling, *Simocybe centunculus* var. *laevigata* (J. Favre) Senn-Irlet, *S. centunculus* var. *maritima* (Bon) Senn-Irlet, *S. centunculus* var. *obscura* Romagn. ex Senn-Irlet, *S. Laevigata* (J. Favre) P.D. Orton, *S. laevigata* var. *maritima* (Bon) Courtec., *S.laevigata* var. *maritima* (Bon) Courtec., *S. obscura* Romagn. ex D.A. Reid].

Macroscopic and microscopic features: Pileus 10-25 mm in diameter, initially convex then becoming plano-convex to almost plane, with a somewhat depressed or slightly umbonate center, surface minutely pruinose to velvety when young, dark yellowish brown to olive-brown at maturity, paler, somewhat striate or cracked at the marginal zone. Flesh thin, almost concolorous with the surface. Taste and odor not distinct. Lamellae adnate to adnexed, subdistant up to three-seried, pale brown to olive-brown with paler or white pruinose edges. Stipe 10-35 × 2.2-4.2 mm, cylindrical, somewhat slightly tapering upwards, hollow, almost concolorous with the cap, pruinose to striate pubescent, partially glabrescent in age, with a conspicuous tuft of white mycelium at the base (Fig. 1).

Basidia 19-23 × 7–8.6 µm, clavate, 4-sterigmate (Figs. 2a,b). Basidioles clavate. Basidiospores (5.7) 5.73 - 6.6 (6.8) × (3.7) 3.9 - 4.6 (4.7) µm, Q = 1.4 - 1.6 (1.7), ellipsoid

Figure 1. Basidiocarps of *Simocybe centunculus*

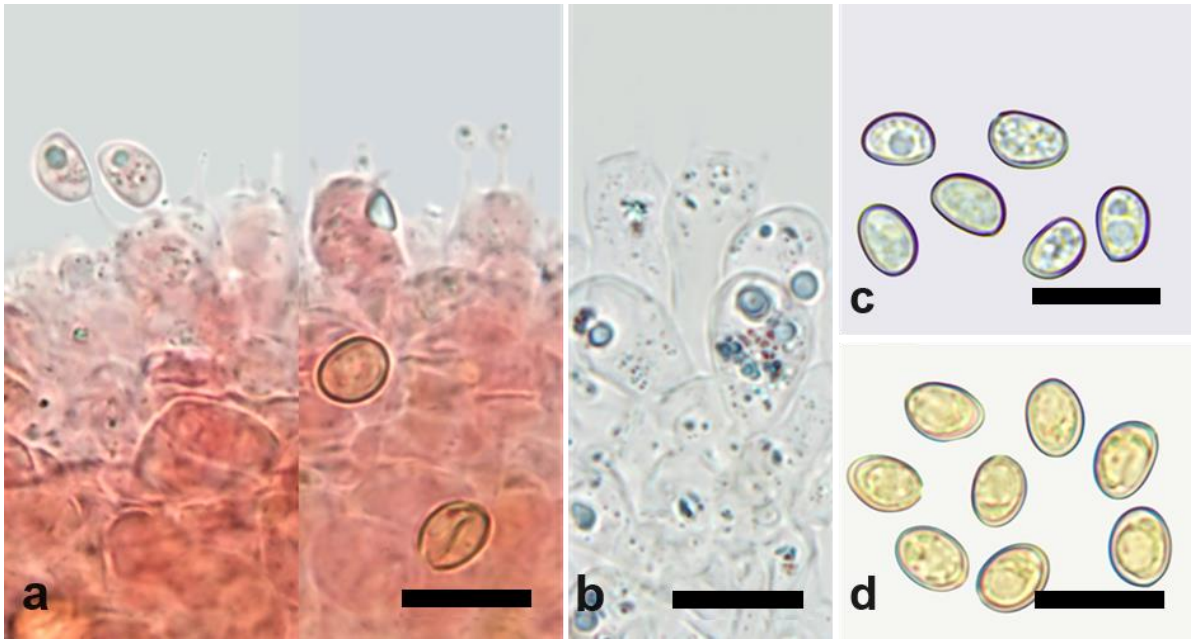

Figure 2. Basidia and basidioles (a,b) and basidiospores (c,d) of *S.centunculus* (bars 10 µm) (a in Congo Red, b in %3 KOH, c in water, d in Melzer)

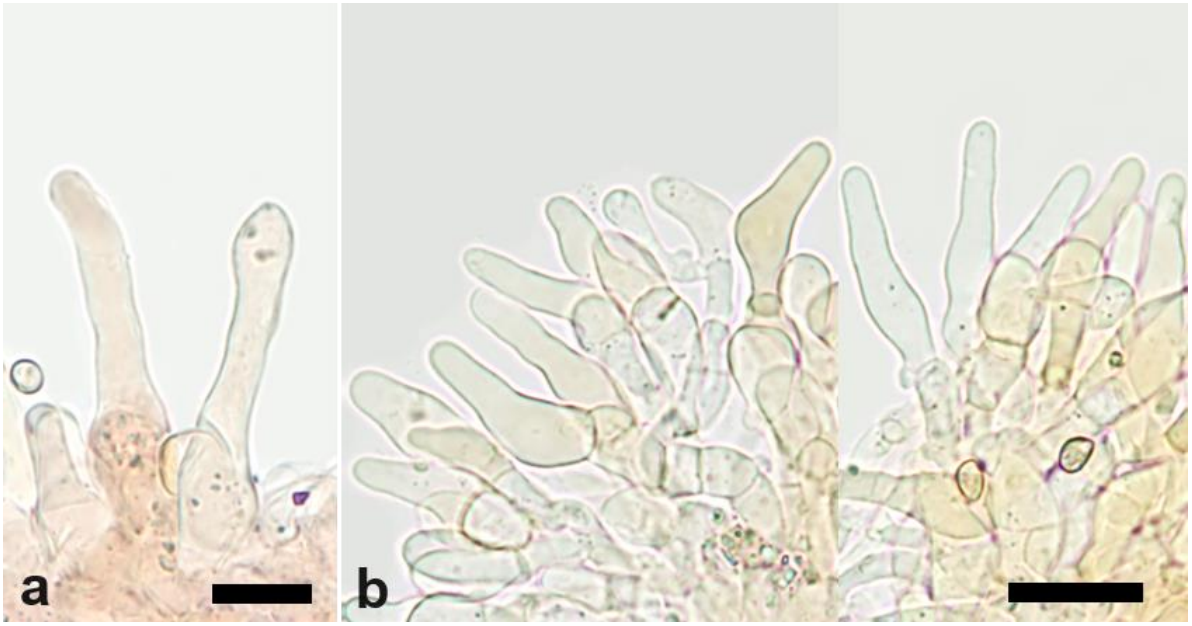

Figure 3. Cheilocystidia (a) and pileipellis (b) of *S. centunculus* (bars a- 10 µm, b- 20 µm) (a in Congo Red, b in %3 KOH)

to mostly phaseoliform or reniform without a germ pore, smooth (Figs. 2c,d). Spore print brown. Cheilocystidia abundant, generally cylindrical to subventricose, hyaline (Fig. 3a). Pleurocystidia were not observed. Pileipellis nearly a palisade with numerous subcylindrical to fusoid-ventricose or lageniform, obtuse, hyaline and erect pileocystidia arising from inflated to vesiculose cells (Fig. 3b). Clamp connections are present at some septa.

Simocybe centunculus is reported to grow as solitary or scattered in small troops on stumps and decaying hardwood branches and logs, especially of *Quercus* L. and *Lithocarpus* Blume members, from fall to late spring (Kuo, 2007; Desjardin et al., 2014; Desjardin and Perry, 2016; Siegel and Schwarz, 2016).

Specimen examined: İstanbul, Beykoz, Polonezköy, on decaying *Quercus* sp. stump in mixed forest composed of *Carpinus*, *Fagus*, *Castanea* and *Pinus* L. spp., 41.118580N - 29.190857E, 250 m, 14.10.2023, YKaraduman 012.

4. Discussions

Simocybe centunculus has been presented as a new addition to the mycobiota of Türkiye, marking the first presence of the genus *Simocybe* in the country. General characteristics of the Turkish collection are in agreement with Breitenbach and Kränzlin (2000), Kuo (2007), Desjardin et al. (2014), Desjardin and Perry (2016) and Siegel and Schwarz (2016).

This species is easily distinguished by its minutely pruinose to velvety, dark yellowish brown to olive-brown pileus; concolorous and cylindrical stipe with a conspicuous tuft of white mycelium at the base; adnate to adnexed, subdistant, pale brown to olive-brown lamellae with paler or white pruinose edges; ellipsoid to phaseoliform or reniform, yellowish brown basidiospores, a palisade-type pileipellis with subcylindrical to fusoid-ventricose pileocystidia, cylindrical to subventricose cheilocystidia, and its growth on rotten wood. *Simocybe haustellaris* (Fr.) Watling and *S. sumptuosa* (P.D. Orton) Singer may share similar habitat with *S. centunculus.* But *S. haustellaris* has larger spores (7-10 x 4.5-6.5 µm), and *S. sumptuosa* has larger fruit bodies with cheilocystidia having broad round heads and tapered bases (Siegel and Schwarz, 2016; Marchadier, 2023).

Simocybe centunculus is somewhat similar to *Naucoria fusco-olivacea* Bres. & Roum. but the latter species have longer (60-70 mm) and reddish brown stipe (Desjardin and Perry, 2016). With a similar macromorphology, *Callistosporium luteo-olivaceum* also has olive colors. However this species differs from *S. centunculus* by having larger basidiomata, smooth pileus, white spores, and growth on conifer wood (Siegel and Schwarz, 2016). In terms of general macromorphology, *S. centunculus* may resemble some species of *Mycena* (Pers.) Roussel and small species of *Pluteus* Fr. But they are easily distinguished from *S centunculus* by the colour of their spores. *Mycena* species have white spores, and *Pluteus* species have pinkish brown spores (Desjardin et al., 2014).

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contribution

Authors contributed equally.

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Glu-A3b allele has a significant effect on gluten quality of bread wheat in a recombinant inbred line population

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Glu-A3b allelinin rekombinant kendilenmiş hat popülasyonunda ekmeklik buğdayın gluten kalitesi üzerinde önemli bir etkisi vardır

Abstract: In this study, a total of 147 wheat lines with variying low molecular weight glutenin subunits (LMW-GS), obtained by crossing Tosunbey and Tahirova2000 bread wheats, were included. Milling, protein, dough-mixing properties of the genotypes were measured and their relations with LMW-GS were investigated in eight different environments. As the LMW-GS of the parents were quite different; milling, protein and dough-mixing properties of the lines were significantly influenced. In this regard, presence of rye translocation (*Glu-B3j*) reduced flour yield and increased damaged starch and protein contents. In terms of protein quality, *Glu-A3b+Glu-B3b* allellic combinations were better than *GluA3b+Glu-B3j* or *Glu-A3e +Glu-B3j* allellic combinations. It was observed that negative effects of rye translocation could be minimized by selecting proper *Glu-3* alleles, such as *Glu-A3b* instead of *Glu-A3e*. LMW-GS combinations of the lines influenced mixolab mixing and thermorheological properties. In this respect, the lines with *Glu-A3b* or *Glu-B3b* allelles showed increased mixing time and stability as compared to the lines with *Glu-A3e* or *Glu-B3j* allelles. The effect of LMW-GS alleles on gluten quality and dough strength was statistically bb > eb > bj > ej. In terms of myxolab stability value related to bread volume; 1 = 2*, 7 + 9 > 17 +18, b > e and b > j; in terms of mixing time; 1 > 2*, 7 + 9 < 17 +18, b > e and b > j. As a result, the *Glu-A3b* allele can be used to increase gluten quality, and the *Glu-B3j* allele can be used to increase protein content. Proper allellic combinations of LMW-GS in wheat can be developed for a given bakery product.

Key words: Bread wheat, technological quality, low molecular weight glutenin subunits, wheat-rye translocation

Özet: Bu çalışmaya, Tosunbey ve Tahirova2000 ekmeklik buğdaylarının melezlenmesiyle elde edilen, farklı düşük molekül ağırlıklı glutenin alt birimlerine (LMW-GS) sahip toplam 147 buğday hattı dahil edilmiştir. Genotiplerin öğütme, protein, hamur karıştırma özellikleri ölçülmüş ve LMW-GS ile ilişkileri sekiz farklı ortamda incelenmiştir. Ebeveynlerin LMW-GS'leri oldukça farklı olduğundan; hatların öğütme, protein ve hamur karıştırma özellikleri önemli ölçüde etkilenmiştir. Çavdar translokasyonunun (*Glu-B3j*) varlığı un verimini azaltmış, hasarlı nişasta ve protein içeriğini arttırmıştır. *Glu-A3b+Glu-B3b* allelik kombinasyonları protein kalitesi açısından *GluA3b+Glu-B3j* veya *Glu-A3e +Glu-B3j* allelik kombinasyonlarına göre daha iyi sonuç vermiştir. *Glu-A3e* yerine *Glu-A3b* gibi uygun *Glu-3* allellerinin seçilmesiyle çavdar translokasyonunun olumsuz etkilerinin en aza indirilebileceği gözlenmiştir. Hatların LMW-GS kombinasyonları, mixolab karışımını ve termoreolojik özellikleri etkilemiştir. Bu bakımdan *Glu-A3b* veya *Glu-B3b* allellerine sahip hatlar, *Glu-A3e* veya *Glu-B3j* allellerine sahip hatlara kıyasla daha yüksek karıştırma süresi ve stabilite göstermiştir. LMW-GS allellerinin gluten kalitesi ve hamur sertliği üzerine etkisi istatistiksel olarak bb > eb > bj > ej şeklindedir. Ekmek hacmine bağlı myxolab stabilite değeri açısından; 1 = 2*, 7 + 9 > 17 +18, b > e ve b > j; karıştırma süresi açısından; 1 > 2*, 7 + 9 < 17 +18, b > e ve b > j'dir. Sonuç olarak, *Glu-A3b* alleli gluten kalitesini arttırmak için, *Glu-B3j* alleli ise protein içeriğini arttırmak için kullanılabilir. Belirli bir unlu mamul için buğdaydaki LMW-GS'nin uygun allelik kombinasyonları geliştirilebilir.

Anahtar Kelimeler: Ekmeklik buğday, ekmeklik kalitesi, düşük moleküler ağırlıklı glutenin alt üniteleri, buğday-çavdar translokasyonu

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1. Introduction

The quality of wheat, which has a wide adaptability and rich biodiversity, varies depending on many criteria and has a very broad meaning depending on its intended use in industry. Wheat contains 60-70% starch, 10-15% protein, 5-10% non-starch carbohydrates, 1-2% lipids and 1-2% minerals (Lineback and Rasper, 1988; Pomeranz, 1988).

The amounts, properties, ratios and interactions of these components determine the suitability and intended use of wheat for different end products (Pomeranz, 1988; Hoseney, 1994).

Protein quality is an important trait that determines the intended use. The effect of cultivation on protein quality is less and more genetically controlled (Graybosch et al., 1996). An important storage protein that influences the quality of wheat is gluten. Gluten proteins are composed of prolamin (gliadin) and glutelin (glutenin) fractions (MacRitchie, 2016). Gluten proteins, which have viscous, elastic and cohesive characteristics, are functional proteins unique to wheat due to their ability to form dough and retain gas, and have an irreplaceable role in the production of many bakery products. The viscous, elastic and cohesive stability of gluten proteins is due to the protein content of wheat and the structural properties of gluten proteins (Hoseney, 1994; Barak et al., 2015).

The gliadins that make up gluten proteins are monomeric proteins and are prominent in the viscous and cohesive properties of dough, while glutenines, which are polymeric proteins, are more determinant in dough elasticity (MacRitchie, 2016). The glutenin fraction is divided into high molecular weight (>80 kDa) glutenin (HMW-G) and low molecular weight (<80 kDa) glutenin (LMW-G) proteins according to electrophoretic mobility. (Rasheed et al., 1988). HMW-G on dough rheology and bread quality proteins have been studied in detail and largely clarified. However, the effects of LMW-G proteins on dough rheology and bread quality are still under investigation (Gianibelli et al., 2001, Dupont et al., 2007, Rasheed et al, 2014).

LMW-G protein alleles are encoded by the *Glu-A3*, *Glu-B3* and *GluD3* loci localized on the short arms of the group 1 chromosomes of wheat (Weegels et al., 1996). A total of 15 to 20 *Glu-3* alleles have been identified in bread wheat (Liu et al., 2001; Dangi et al., 2019; Cho et al., 2017). *Glu-A3* and *Glu-B3* alleles were reported to be more effective than *Glu-D3* alleles on dough and bread quality (Zhang et al., 2012). Studies have shown that the c or d allele on *Glu-A3*, the d, b, c or g allele on *Glu-B3* and the a or b allele on *Glu-D3* are associated with higher wheat quality, while the *Glu-A3e* allele is negatively associated with quality (Gupta et al., 1994; Bonafede et al., 2015; Yasmeen et al., 2015; Aktas and Baloch, 2017).

Glu-B3 alleles have a determining effect on dough stability (He et al., 2005; Ahn et al., 2014). In terms of sedimentation volume, the *Glu-A3b* allele, a simple but robust quality test for wheat, was found to be superior to other *Glu-B3* alleles (Ito et al., 2015), while the *Glu-A3e* allele was inversely related to wheat protein content (Gupta et al., 1989) and decreased the gluten index of wheat flour (Zhen et al., 2014). In particular, studies using mixographs have proven that the *Glu-A3e* null allele causes weakening of gluten (Flaete and Uhlen, 2003; Bonefede et al., 2015; Ito et al., 2015).

In addition to dough and bread quality, wheat has been reported to affect the milling characteristics of LMW-GS combinations. Ahn et al. (2014) and Bonafede et al. (2015) found that *Glu-A3* alleles affected flour yield, but *Glu-B3* alleles had no effect on flour yield and particle size distribution. Shin et al. (2012) compared the *Glu-B3h* allele with the *Glu-B3d* allele and concluded that the differences in flour yield and particle size distribution were due to puroindolines (*Pina-D1borPinb-D1b*). Similarly, Ahn et al. (2014) found that flour yield and grain physical properties are influenced by puroindolines rather than allelic combinations of gluten proteins.

Many wheat genotypes in the world carry the *1BL.1RS* wheat-rye translocation. The rye translocation, commonly referred to as the *Glu-B3j* allele, is known to increase grain yield and protein content but reduces the gluten quality of wheat (Gobaa et al., 2008; Moiraghi et al., 2013). Some studies (Fenn et al., 1994; Burnett et al., 1995; Kim et al., 2005) did not identify any effect of rye translocation on flour yield; however, Moiraghi et al (2013) found that rye translocation caused an increase in starch damage, pentosans and water absorption of flours.

Literature shows that LMW-GS or *Glu-3* allelic combinations affect milling characteristics, protein content and gluten quality of wheats, dough mixing characteristics and final product quality. Therefore, it is important to further expand our knowledge on the effects of various *Glu-3* allelic combinations on wheat quality. In this study, the effects of different *Glu-3* combinations (*Glu-A3b - Glu-A3e, Glu-B3b - Glu-B3j etc.)* in 147 recombinant selfed lines obtained by crossbreeding of Tosunbey and Tahirova2000 bread wheat varieties on milling, protein and dough mixing characteristics were investigated in eight different environments.

2. Materials and Method

A recombinant inbred line (RIL) population was developed by crossing Tosunbey and Tahirova2000 cv, was used in the study. Tahirova2000 variety carries the combination of 2*, 7+9, 5+10; *GluA3e* and *GluB3j*; Tosunbey variety carries the combination 1, 17+18, 5+10; *GluA3b* and *GluB3b* alleles. The RILs, composed of 147 genotypes including parents, were grown in eight environments to produce the data for flour yield, protein content, sedimentation volume, damaged starch content, 1000-kernel weight and test weight. The environments were Bafra/Samsun and Eskişehir locations for 2012-2013 growing season and Bafra/Samsun, Eskişehir and Pamukova/Sakarya locations for 2013-2014 and 2014-2015 growing seasons. Therefore, the study contained a grand total of 2352 wheat samples for these quality traits. For Mixolab dough-mixing studies, we used 524 samples from four environments that Bafra/Samsun and Eskişehir for 2012-2013 and 2013-2014 growing seasons.

2.1 Molecular analysis

HMW-GS and LMW-GS of wheats were extracted by Singh et al. (1991) and separated on SDS-PAGE electrophoresis by Masci et al. (2000) and Gianibelli et al. (2001). HMW-GS and LMW-GS were identified following the nomenclature of Bekes et al. (2006). SDS-PAGE electrophoresis was used in the determination of *1BL.1RS* wheat-rye translocation and Glu-B3b allel, which were further confirmed by PCR technique (Wang et al., 2009). DNA was isolated from the leaves of plants that reached the 4-5 leaf stage. For this, the Plant/Seed DNA miniPrep™ Kit was used. Analysis of the *Glu-B3b* allele in the genotypes was also detected using the specified primers and as specified by Wang et al. (2009). PCR gels were imaged using the BioRad gel imaging system.

2.2 Quality analysis

Hectoliter weights and moisture contents of wheat kernels were measured on Dickey-John GAC Plus (Auburn, IL, USA) by the AACCI method 55-10 (AACCI, 2010). Thousand-kernel weights of RILs were determined using Numigral-I (Chopin, Villeneuve-la-Garenne, France) and converted to 14% moisture basis. Wheat samples (200 g) were milled on a Quadrumat Jr. type laboratory mill (Bastak, Ankara, Turkey) upon tempering at 15% moisture content for 24 hours, as elucidated by the AACCI method 26-50 (AACCI, 2000). Moisture contents of flours were measured using Ohaus MB-45 moisture tester (Ohaus, Greifensee, Swetzerland). Damaged starch contents of flours (14% moisture basis) were measured on SD-Matic device (Chopin, Villeneuve-la-Garenne, France) by the ICC method 172 (ICC, 2011). Protein contents of flours were quantified using Perten 9500 NIR spectroscopy (Perten, Hagersten, Sweden) and expressed as 14% moisture basis. SDS sedimentation volumes of flours (14% moisture basis) were measured according to the method of Maghirang et al. (2006), which was modified from the AACCI method 56-70 (AACCI, 2000). Dough-mixing properties of flours were measured on Mixolab system (Chopin, Villeneuve-la-Garenne, France) employing the "Chopin+" protocol by the ICC method 173 (ICC, 2011).

2.3 Statistical analysis

The experiments were conducted according to a balanced-lattice experimental design with two replications. The data collected within the scope of the study were analyzed by this experimental design using the JMP software (Patterson and Hunter, 1983) We investigated the effects of HMW-GS and LMW-GS and, their interactions on quality traits.

3. Results

3.1. Molecular analysis

DNA markers developed by Wang et al. (2009) were used to determine the *Glu-B3b* allele carrying status of the lines in the recombinant selfed line population and the lines carrying and not carrying the *Glu-B3b* allele were determined (Fig. 1). As a result of molecular screening using *Glu-B3b* primers, 1570 bc (base pair) long bands were obtained in lines carrying the relevant allele. Lines not carrying the relevant allele did not produce bands. In addition, lines carrying the *Glu-B3b* allele do not carry rye translocation. The results obtained are in agreement with the results determined by SDS-PAGE method (Fig. 2).

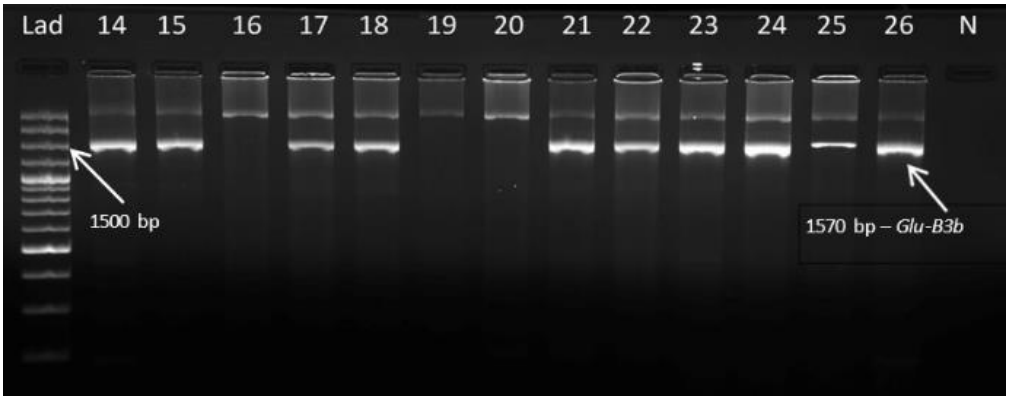

Figure 1. Gel photograph of some lines for *Glu-B3b* allele (Lad: Ladder, N: Control)

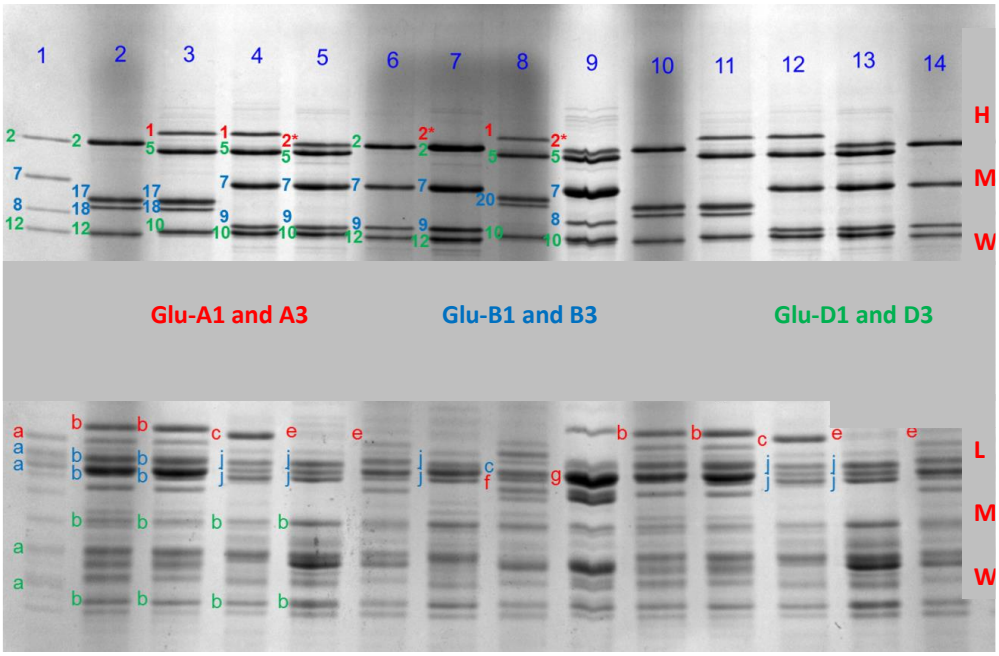

Figure 2. SDS-PAGE screening results of some genotypes (11: Tosunbey, 13: Tahirova2000)

SDS-PAGE analysis was performed for HMW and LMW subunits in all lines and alleles carried by some lines are shown in Figure 2. As can be seen in the Figure 1, *GluA3e* and *GluB3j* alleles are null alleles for wheat, while the presence of *GluB3j* allele indicates the presence of rye translocation.

3.2. Quality analysis

The mean values of some technological traits of *Glu-A1* alleles 1 and 2*, *Glu-B1* alleles 17+18 and 7+9 are given in Table 1. Variance analysis results of some technological traits of the genotypes in the study are given in Table 2. Year, location, *Glu-A1, Glu-B1, Glu-B1, Glu-B3* and some interactions were found significant at 1% or 5% level. In other words, besides the environment, both HMW-G and LMW-G alleles seem to have an effect on technological quality traits.

There was no statistical difference between *Glu-A1* alleles (1 and 2*) in terms of damaged starch content, hectoliter weight and protein ratio. In *Glu-A1* 1 allele, hectoliter weight and flour yield of 7+9 were higher than 17+18, while kernel weight and damaged starch content were lower. It was noteworthy that the SDS sedimentation values indicating gluten quality were higher (28.2 ml) in the 17+18 carriers of these two subunits with the same protein ratio average (11.2%) in 1 of the *Glu-A1* alleles. When the same two alleles were evaluated in 2* *Glu-A1* allele, 7+9 again had slightly higher flour yield, but lower hectoliter weight and kernel weight and higher damaged starch content. Although the protein ratio was slightly lower (11.0%), the SDS sedimentation values of the two subunits were close. The 17+18 subunit had a significantly higher SDS sedimentation value and damaged starch value at a protein ratio close to the 1 allele compared to the *Glu-A1* 2* allele. In addition, grain physical properties and flour yield were lower. The 7+9 subunit also gave higher gluten quality and protein content with the 1 allele. Although kernel weight and damaged starch content were lower, hectoliter weight and flour yield were similar in both alleles.

In the *Glu-B1* 17+18 subunit, the effects of *Glu-A3/B3* LMW-GS alleles on gluten quality (sedimentation value) were statistically *bb* > *eb* > *bj* > *ej*. Statistically, *eb* = *bj* for the *Glu-A1* 1 allele and *bj* = *ej* for the *Glu-A1* 2 allele. Clearly, the presence of the *-bb* allele was associated with high and the presence of the rye translocation *(j)* with low gluten quality. For protein ratio there is an inverse effect *bj* = *ej* > *bb* = *eb*. The effects of *Glu-A3/B3* alleles on gluten quality are similar in the 7+9 allele. *Glu-A3/B3 -bb* was highly effective and *-ej* was low while the other two alleles were close to each other. In general, in terms of protein ratio, *-ej* and *-bj* gave high values in both *Glu-A1* alleles, while the other two alleles had low values.

In the study, it was noteworthy that in the *Glu-A1* 1 allele, the *-bj* and *-ej* alleles had higher amounts of damaged starch than the other two alleles. Kernel weight in *Glu-A1* 1 allele of *-ej* allele is lower than the other alleles, while it is good in 2* allele. Among alleles, *-bj* allele did not decrease the kernel weight in general. In 2*, 7+9 alleles, high kernel weight of *-eb* allele (43.4 g) and high hectoliter weight of *-bb* and *-eb* were remarkable. Apart from these alleles, *-bj* allele had low hectoliter weight. Flour yield was found to be high in all combinations of *-bb* allele and *-eb* allele which are effective in terms of protein quality. The presence of wheat rye translocation was therefore found to be unfavorable in terms of both bread making and milling quality. Especially in combination 1, 17+18, low kernel weight in *-bj* allele is remarkable (Table 1).

The mean values of alleles at *Glu-A1, B1, Glu-A3* and *Glu-B3* loci are statistically compared in Table 3. While sedimentation value (gluten quality) was higher in *Glu-A1* 1 allele, flour yield and kernel weight were higher in 2* allele. No significant difference was observed among other parameters. *In Glu-B1*, 7+9 gave higher values in terms of flour yield and 17+18 gave higher values in terms of damaged starch content, while no statistically significant difference was observed between the other parameters. At the *Glu-A3* and *B3* loci, the b allele showed superiority over

Table 1. The mean results of grain yield and based on the averaged data from eight environments

HMW-GS		LMW-GS		Line number	Flour yield (%)	Protein content (14% mb)	SDS (ml)	Damaged starch content (%)	1000-kernel weight (g)	Test weight (kg)
Glu-A1	*Glu-B1*	*Glu-A3*	*Glu-B3*							
1	17+18	b	b	18	55.5 cd	10.9 ef	33.3 b	3.72	40.8	77.1
1	17+18	e	b	16	55.6 bc	10.8 fg	27.4 ef	3.71	40.6	77.2
1	17+18	b	j	1	50.9 j	11.9 a	28.9 de	3.91	42.6	75.7
1	17+18	e	j	7	53.4 hi	11.0 d-f	23.0 j	3.86	39.0	76.6
					53.9	**11.2**	**28.2**	**3.80**	**40.8**	**76.7**
2*	17+18	b	b	21	55,3 cd	10.9 ef	30.9 c	3.63	40.3	77.4
2*	17+18	e	b	17	55.3 cd	11.0 de	26.1 h	3.74	41.3	77.2
2*	17+18	b	j	3	55.0 c-f	11.6 ab	24.2 i	3.49	42.9	76.9
2*	17+18	e	j	5	54.5 d-g	11.5 a-c	20.5 i	3.67	43.1	77.2
					55,0	**11.3**	**25.4**	**3.63**	**41.9**	**77.2**
1	7+9	b	b	1	56.2 a-e	11.4 a-e	36.8 a	3.27	41.8	77.3
1	7+9	e	b	4	57.2 a	10.5 g	23.6 i	3.59	39.4	77.6
1	7+9	b	j	15	53.4 i	11.2 cd	26.8 fg	3.73	41.7	76.7
1	7+9	e	j	6	53.5 g-i	11.6 a	20.2 j	3.67	38.4	76.6
					55.1	**11.2**	**26.9**	**3,57**	**40.3**	**77.1**
2*	7+9	b	b	5	55.7 bc	10.6 fg	28.4 d	3.65	41.0	76.6
2*	7+9	e	b	5	56.2 ab	10.8 fg	25.6 h	3.66	43.4	76.8
2*	7+9	b	j	9	54.1 f-h	11.3 bc	26.5 gh	3.68	40.7	77.2
2*	7+9	e	j	14	54.7 ef	11.3 bc	20.6 j	3.71	41.0	77.1
					55.2	**11.0**	**25.3**	**3.68**	**41.5**	**76.9**

Table 2. ANOVA of grain yield properties by *Glu-1* and *Glu-3* alleles based on averaged data from four environments

		Flour yield (%)	Protein content (14% mb)	SDS (ml)	Damaged starch content AACC-7631	1000-kernel weight (g)	Test weight (kg)
Locus	*DF*			MS			
Lokasyon (Yıl)	2	433.49	44.47	1299,27	2.76	87.61	10.3
Tekekkür (Yıl, Lokasyon)	4	13.25	19.82	89.08	0.36	23.72	4.8
Glu-A1	1	113,9**	0,65	1194,7**	0,19	336,7**	8,88
Glu-B1	1	129,9**	2,87	94,7	2,39**	39,7	1,97
Glu-A1* Glu-B1	1	62,8*	5,60*	20,1	4,89**	0,18	28,2
Glu-A3	1	86,6**	7,80*	9482,9**	1,13**	122,2	8,20
Glu-A1*Glu-A3	1	32,8	12,8**	777,5**	0,05	726,2**	3,86
Glu-B1*Glu-A3	1	0,21	1,53	256,1**	0,01	0,49	2,53
Glu-A1* Glu-B1*Glu-A3	1	37,9	0,66	290,5**	1,27**	44,5	1,73
Glu-B3	1	1260,3**	74,0**	6847,7**	2,29**	3,80	35,6
Glu-A1*Glu-B3	1	321,6**	0,41	70,2	4,05**	31,5	68,8
Glu-B1*Glu-B3	1	17,3	0,48	0,01	0,99**	273,9**	15,9
Glu-A1*Glu-B1*Glu-B3	1	24,5	0,66	355,2**	0,04	137,6	2,55
Glu-A3* Glu-B3	1	1,78	0,08	107,7*	0,40	205,7*	1,95
Glu-A1*Glu-A3* Glu-B3	1	17,3	2,14	383,3**	1,02**	9,37	0,00
Glu-B1*Glu-A3* Glu-B3	1	33,7	22,4**	29,4	0,61*	5,07	17,6
Glu-A1* Glu-B1* Glu-A3* Glu-B3	1	50,4*	18,4**	492,5**	0,33	57,1	0,65
Error	2336	12,3**	1,40**	27,0**	0,12**	38,1**	24,9

* P <0.05, ** P <0.01 level of significance, DF: degrees of freedom, MS: mean square

the *e* and *j* alleles in both. This is the opposite for the amount of starch damaged. In terms of protein ratio, *b* > *e* in *Glu-A3* and *j* > *b* in *Glu-B3*. In terms of flour yield, *e* > *b* and *b* > *j*.

In the study, the averages of some myxolab traits of HMW-Gs *Glu-A1* and *Glu-B1*, LMW-Gs *Glu-A3* and *Glu-B3* alleles were statistically evaluated and given in Table 4. The analysis of variance results of allele combinations on myxolab traits are also given in Table 6. Year, location, *Glu-A1, Glu-B1, Glu-B1, Glu-B3* and some interactions were found significant at 1% or 5% level. Besides environment, both HMW-G and LMW-G alleles seem to have an impact on myxolab traits. In *Glu-A1* 1, when looking at 17+18 and 7+9, the water absorption of 17+18 was slightly higher, while the stability and kneading time of 7+9 were found to be higher. In *Glu-A1* 2*, the water absorption and kneading time of 17+18 were higher, while the stability value was slightly lower. The 17+18 subunit gave similar values in *Glu-A1* 1 and 2* alleles The water absorption and kneading time of 17+18 were slightly higher in 1 and the stability value was slightly lower. When the 7+9 subunit was compared in *Glu-A1* 1 and 2* alleles, better results were obtained with 1 in terms of all parameters.

In *Glu-B1* 17+18 subunit, the effects of myxolab stability value, which is the most important parameter related to dough strength, are statistically *bb* > *eb* > *bj* > *bj* > *ej*. Water absorption is the opposite. Kneading time similarly indicates dough strength. In both *Glu-A1* alleles, *-bb* stood out in dough kneading time. While *-ej* gave the lowest value; *-eb* and *-bj* gave close values. In the *Glu-B1* 7+9 subunit, the effect on stability at 1 and 2* is *bb* > *eb* > *bj* > *ej*. 1, this effect is more pronounced. *-ej* gave the lowest stability value in both alleles. In the 2* allele *-ej* did not differ from the other alleles in terms of stability value. In terms of water absorption, *-ej* gives the highest value in both alleles (1 and 2*) and *-bb* gives the lowest value, while the other two alleles have similar values. In terms of kneading time, *-bb* is clearly separated in *Glu-A1* 1, while *-bj* is high in the allele. Interestingly, *-bb* and *-ej* had the same value in the 2* allele.

The mean values of alleles at *Glu-A1, Glu-B1, Glu-A3* and *Glu-B3* loci are statistically compared in Table 5. In terms of myxolab stability value, which is related to dough durability and bread volume, 1 = 2*; 7+9 > 17+18 and *b* > *e* and *b* > *j*; in terms of kneading time, 1 > 2*; 7+9 < 17+18 and *b* > *e and b* > *j*. Especially the *b* allele was found to be

Table 3. Mean values of alleles at *Glu-A1, B1, Glu-A3* and *Glu-B3* loci

Locus	Subunit	Line number	Flour yield (%)	Protein content (14%)	SDS (ml)	Damaged starch content AACC-7631	1000-kernel weight (g)	Test weight (kg)
Glu-A1	1	68	54.5 b	11.2	27.6 a	3.68	40.6 b	76.9
	2*	79	55.1 a	11.1	25.4 b	3.65	41.7 a	77.0
Glu-B1	7+9	60	55.2 a	11.1	26.2	3.62 b	40.9	77.0
	17+18	87	54.4 b	11.2	26.8	3.72 a	41.3	77.0
Glu-A3	b	73	54.5 b	11.2 a	29.5 a	3.63 b	41.5	76.9
	e	74	55.1 a	11.1 b	23.4 b	3.70 a	40.8	77.0
Glu-B3	b	87	55.9 a	10.9 b	29.1 a	3.62 b	41.1	77.1
	j	60	53.7 b	11.4 a	23.9 b	3.71 a	41.2	76.8

Table 4. Mixolab dough mixing properties of wheat lines by *Glu-1* and *Glu-3* allelic combinations based on averaged data from four environments

HMW-GS	LMW-GS			Line number	Optimum water absorption(%)	Optimum mixing time (min)	Stability (min)
Glu-A1	*Glu-B1*	*Glu-A3*	*Glu-B3*				
1	17+18	b	b	7	57.0 cd	5.04 abc	9.66 a
1	17+18	e	b	4	56.3 def	4.48 c-f	8.50 c
1	17+18	b	j	1	57.9 a-d	4.95 a-f	7.14 efg
1	17+18	e	j	5	58.2 a	4.25 c-f	6.05 gh
					57.4	**4.68**	**7.84**
2*	17+18	b	b	5	56.8 cde	5.36 ab	9.54 ab
2*	17+18	e	b	5	56.9 cde	4.21 def	8.98 c
2*	17+18	b	j	3	57.0 b-e	4.56 a-f	6.91 f
2*	17+18	e	j	4	57.6 abc	3.90 def	6.13 fgh
					57.1	**4.51**	**7.89**
1	7+9	b	b	1	56.3 c-f	5.83 a	10.01 a
1	7+9	e	b	4	57.0 ef	4.17 def	8.63 c
1	7+9	b	j	6	57.1 bcd	4.87 a-d	7.84 de
1	7+9	e	j	5	57.9 ab	4.15 def	5.69 h
					57.1	**4.76**	**8,04**
2*	7+9	b	b	4	55.7 f	3.71 ef	8.95 bc
2*	7+9	e	b	3	56.7 cde	4.57 b-e	8.78 c
2*	7+9	b	j	5	56.1 ef	4.70 a-e	8.46 cd
2*	7+9	e	j	4	58.1 a	3.71 f	5.62 h
					56.7	**4.17**	**7.95**

associated with high dough strength. In terms of water absorption, *Glu-A3* and *Glu-B3 b* allele gave lower values than other alleles. Again, in terms of water absorption, 1 = 2* and 17+18 > 7+9. As a result, lines with the *Glu-A3b* or *Glu-B3b* allele showed longer kneading time and stability compared to lines with the *Glu-A3e* or *Glu-B3j* allele.

4. Discussions

Within the scope of the study, milling, protein and dough kneading characteristics of the genotypes were measured and the relationships between these quality parameters and HMW-GS and especially LMW-GS allele combinations were investigated. Recent studies have shown that the effects of small regions formed by translocations or a locus should be investigated in the investigation of protein quality and that determining the variation to be generated for this purpose is more important for genomic studies on quality (Lukaszewski et al., 1987; Wang et al., 2016).

Among the parents used in the study, Tosunbey cultivar 1, 17+18, 5+10 and Tahirova2000 cultivar 2*, 7+9, 5+10 carry HMW-GS alleles and their quality scores were 10 and 9, respectively (Payne et al., 1987). In terms of LMW-GS alleles, Tosunbey has *Glu-A3b*, *Glu-B3b* and *Glu-D3b* alleles and Tahirova2000 has *Glu-A3e* (null/empty), *Glu-B3j* (null/empty, rye translocation) and *Glu-D3b* alleles. Since the Tahirova2000 variety carries a rye translocation (*Glu-B3j*), the *Glu-1* score corrected for rye decreases from 9 to 6. Among the parents, Tosunbey variety is considered as first class bread wheat in terms of protein quality, while Tahirova2000 variety has low protein quality (Aydın et al., 2016). Differences in the LMW-GS alleles of the cultivars, especially the fact that Tahirova2000 carries *Glu-A3e* (null/empty) and *Glu-B3j* alleles are effective in this difference, since the cultivars have similar quality score in terms of HMW-GS. As a result of this, the variation resulting from the crossing of Tosunbey variety, which has high protein content and quality, and Tahirova2000 variety, which has relatively high protein content but poor protein quality, is important and constitutes the essence of this study.

Flour yield and damaged starch content are important milling quality parameters of bread wheats. Commercial flour yields of bread wheats are desired to be high (>75%) and damaged starch contents within a certain range (5-8%) (Hoseney, 1994; Bushuk, 1998; Elgün et al., 2002). Since a laboratory type mill was used to grind the wheat in this study, it is expected that the flour yields and damaged starch contents of the genotypes (Table 1,3) would be lower than the values in commercial flour production.

Tables 1 and 3 show that *Glu-A1*, *Glu-B1* and *Glu-B3* alleles have an effect on flour yield, while *Glu-A3* allele has no effect. Ahn et al. (2014) reported that *Glu-A3* allele had a significant effect on flour yield. The reason for this difference may be the difference in *Glu-1* and *Glu-3* allele combinations of the material used in the studies. When the tables are analysed, the average flour yields of the combinations carrying rye translocation (*Glu-B3j*) are lower than the combinations not carrying rye translocation (*Glu-B3b*). While *Glu-A3* allelic difference (*Glu-A3b* and *Glu-A3e*) had no effect on flour yield, *Glu-B3* allelic difference (*Glu-B3b* and *Glu-B3j*) caused significant difference in flour yield. However, in previous studies (Fenn et al., 1994; Burnett et al., 1995; Kim et al., 2005), the effect of rye translocation on flour yield was found statistically insignificant.

It is known that rye translocation not only improves the breeding characteristics of wheat but also increases its protein content, but decreases its quality (Dhaliwal et al., 1987; Fenn et al., 1994; Burnett et al., 1995; Graybosch, 2001; Lelley et al., 2004; Kim et al., 2005; Liu et al., 2005; Gobaa et al., 2008; Moiraghi et al., 2013). However, the data in this study indicate that protein quality may vary depending on the *Glu-A3* allele despite carrying the rye translocation. Indeed, it was observed that the protein quality of the combinations carrying the *Glu-A3b* allele was

Table 5. Mixolab dough mixing properties of wheat lines by specific *Glu-1* and *Glu-3* alleles based on averaged data from four environments

Locus	Subunit	Line number	Optimum water absorption(%)	Optimum mixing time (min)	Stability (min)
Glu-A1	1	33	57.1	4.72	7.94
	2*	33	56.9	4.34	7.92
Glu-B1	7+9	32	56.7 b	4.46	8.00
	17+18	34	57.2 a	4.59	7.86
Glu-A3	b	32	56.7 b	4.88 a	8.54 a
	e	34	57.2 a	4.18 b	7.30 b
Glu-B3	b	33	56.5 b	4.67	9.13 a
	j	33	57.5 a	4.39	6.73 b

higher than those carrying the *Glu-A3e* (null/null) allele, although they carried the rye translocation (Table 3).

Gupta et al. (1989) found that *Glu-A3e* (null/empty) allele was negatively correlated with protein content in wheat, while Zhen et al. (2014) found that it decreased gluten index. Wang et al. (2016) reported that deficiency of the *Glu-B3* allele decreased protein content and dough kneading properties. Ito et al. (2015) found that *Glu-B3b* allele was better than other *Glu-B3* alleles in terms of sedimentation volume. The effects of wheat HMW-GS alleles on dough and bread quality have been largely clarified and are listed as follows: 1>2*>null/empty for *Glu-A1* locus, 7+8≥13+16>17+18=7+9 for *Glu-B1* locus and 5+10>2+12>4+12 for *Glu-D1* locus (He et al., 2005). The effects of wheat LMW-GS alleles on dough and bread quality are still being intensively studied. The ranking of wheat in terms of dough strength and bread quality according to LMW-GS alleles was *Glu-A3d>Glu-A3b>Glu-A3c>Glu-A3f>Glu-A3a>Glu-A3e* for *Glu-A3* locus, *Glu-B-3b=Glu-B3d=Glu-B3g>Glu-B3h>Glu-B3a>Glu-B3c>Glu-B3j* for the *Glu-B3* locus and *Glu-D3d=Glu-D3f>Glu-D3e>Glu-D3a=Glu-A3c=Glu-D3b* for the *Glu-D3* locus (Zhang et al., 2012). Jin et al. (2013) and Bonafede et al. (2015) also reported that *Glu-A3e* allele weakened the dough. The results of these studies are compatible with the results found in this study. HMW-GS alleles of the lines were similar in terms of bread quality, but LMW-GS alleles were different. These differences were significantly reflected on the protein contents and sedimentation values of the wheats (Tables 1-3).

As shown in Tables 4 and 5, the optimum water holding capacities of the lines were higher in combinations carrying *Glu-A3e* and *Glu-B3j* (rye translocation) alleles. This result is closely related and compatible with the higher protein and damaged starch contents of the same combinations (Tables 1 and 3). This is because the water holding capacity of flours increases depending on their protein and damaged starch contents (Hoseney, 1994). In addition, since the pentosan content of the lines carrying rye translocation may also be high (Moiraghi et al., 2013), it may have contributed to the water retention capacity of the flours.

Table 6. ANOVA of Mixolab dough mixing properties by *Glu-1* and *Glu-3* alleles based on averaged data from four environments

Locus	DF	Optimum water absorption (%)	Optimum mixing time (min)	Stability (min)
			MS	
Yıl	1	117.41	30.93	66.95
Lokasyon (Yıl)	2	110.12	11.35	50.03
Tekekkür (Yıl, Lokasyon)	4	4.94	2.01	1.18
Glu-A1	1	4.12	8.81	0.02
Glu-B1	1	14.7*	1.04	1.10
Glu-A1* Glu-B1	1	0.09	2.55	0.31
Glu-A3	1	15.5*	29.89**	98.60**
Glu-A1*Glu-A3	1	12.11*	2.77	1.89
Glu-B1*Glu-A3	1	10.13*	0.31	8.21**
Glu-A1* Glu-B1*Glu-A3	1	1.97	7.29	0.13
Glu-B3	1	57.13**	4.64	329.34**
Glu-A1*Glu-B3	1	8.71	0.11	0.85
Glu-B1*Glu-B3	1	0.41	0.33	2.68
Glu-A1*Glu-B1*Glu-B3	1	1.10	3.46	3.63
Glu-A3* Glu-B3	1	11.38*	0.30	12.08**
Glu-A1*Glu-A3* Glu-B3	1	0.07	4.40	4.61*
Glu-B1*Glu-A3* Glu-B3	1	0.25	1.52	10.37**
Glu-A1* Glu-B1* Glu-A3* Glu-B3	1	0.31	11.22	2.48
Error	2336	2.43	2.04	1,12

When the optimum kneading times and stability of the genotypes (Tables 4 and 5) are analysed, the effect of LMW-GS alleles is clearly seen. In general, the kneading times and stability of the genotypes carrying *Glu-A3b* and *Glu-B3b* alleles were higher than the other combinations (*Glu-A3e* and especially *Glu-B3j*). Long kneading times and stability of bread flours indicate strong dough and quality bread production. This result coincides with the previously discussed high sedimentation values of the same combinations (Table 6). In previous studies using a mixograph (Flaete and Uhlen, 2003; Bonafede et al., 2015; Ito et al., 2015), the *Glu-A3e* (null/empty) allele was found to cause gluten weakening. Both *Glu-A3* (Zhen et al., 2014) and *Glu-B3* alleles were found to be significant in dough stability measured using Farinograph (He et al., 2005; Ahn et al., 2014). Ito et al. (2015) associated *Glu-A3b* and *Glu-A3d* alleles with long dough stability and good bread quality.

Within the scope of the study, milling, protein and dough kneading properties of the genotypes were measured and the relationships between these quality parameters and HMW-GS and especially LMW-GS allele combinations were investigated in eight different environments. The effects of LMW-GS alleles on gluten quality and dough strength were statistically as *bb* > *eb* > *bj* > *ej*. In terms of myxolab stability value, which is related to dough durability and bread volume, 1 = 2*; 7 + 9 > 17 + 18 and *b* > *e* and *b* > *j*; in terms of kneading time, 1 > 2*; 7 + 9 < 17 + 18 and *b* > *e* and *b* > *j*. The milling, protein and dough kneading data of the RIL lines, especially those obtained due to the LMW-GS allele combination, are internally compatible and the related ones support each other. Rye translocation *(Glu-B3j*) reduced flour yield although it increased damaged starch and protein content in genotypes. It was also observed that the negative effects of rye translocation could be minimized by selecting appropriate alleles such as *Glu-A3b* instead of *Glu-A3e.* In conclusion, this study shows that it is important to work on mapping populations containing different HMW-GS and LMW-GS alleles; with this approach, the most suitable HMW-GS and LMW-GS allele combinations can be developed for any bakery product.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Authors' Contribution

All authors contributed to the study conception and design. All authors read and approved the final manuscript.

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Determination of sugar contents in *Hyacinthella* Schur bulbs and identification of sugars by cluster analysis method

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Hyacinthella Schur soğanlarında şeker içeriğinin belirlenmesi ve şekerlerin kümeleme analizi yöntemiyle tanımlanması

Abstract: Türkiye has a rich floristic diversity and is accepted as the gene center of the *Hyacinthella* genus. Nineteen species of the *Hyacinthella* genus have been described in the World, and eleven of these species are distributed in Türkiye. Additionally, ten of the eleven *Hyacinthella* species are endemic to our country. Therefore, any study on these species will contribute to the protection, promotion and sustainability of our country's genetic resources. Plants may have different metabolite contents depending on their genetic structure and environmental conditions. Sugar content in bulbous plants depends on sucrose metabolism and varies with species and environmental effects. In this study, the sugar contents of 11 different *Hyacinthella* species distributed in our country were analyzed. The presence of 9 different sugar types in the species was analyzed qualitatively and quantitatively. In addition, depending on the sugar content, identification and grouping techniques were used with the Cluster Analysis Method. As a result of sugar analysis, while glucose, sucrose and fructose are found in all species, differences were detected between species in other sugar contents In cluster analysis, *Hyacinthella* species were divided into 3 different groups in terms of sugar content. The study both identifies the sugar contents found in the bulbs of *Hyacinthella* species and suggests a different identification method By combining morphological, molecular and metabolic data, a complete and accurate identification of species will be achieved.

Key words: Classification, hyacint, endemic

Özet: Türkiye floristik açıdan zengin bir çeşitliliğe sahiptir ve *Hyacinthella* cinsinin gen merkezi olarak Türkiye kabul edilmektedir. Dünya'da *Hyacinthella* cinsine ait on dokuz tür tanımlanmakla, bu türlerin on bir'i Türkiye'de yayılış göstermektedir. Ayrıca *Hyacinthella* cinsinin on bir türü'nün on'u ülkemiz için endemiktir. Bu nedenle, bu türler ile ilgili yapılacak her türlü çalışma, ülkemiz gen kaynaklarının korunması, tanıtılması ve sürdürülebilirliğine katkı sağlayacaktır. Bitkiler genetik yapıları ve çevresel şartlara göre farklı metabolit içeriklerine sahip olabilirler. Soğanlı bitkilerde şeker içeriği sukroz metabolizmasına bağlı olup, tür ve çevresel etkiler ile değişkenlik göstermektedir. Bu çalışmada ülkemizde yayılış gösteren 11 farklı *Hyacinthella* türüne ait şeker içerikleri analiz edilmiştir. Dokuz farklı şeker grubunun tür içinde varlığı kalitatif ve kantitatif olarak analiz edilmiştir. Ayrıca şeker içeriklerine bağlı olarak, küme analizi yöntemiyle tanımlama ve gruplama teknikleri kullanılmıştır. Sonuç olarak yapılan şeker analizlerinde glikoz, sukroz ve fruktoz tüm türlerde rastlanır iken, diğer şeker içeriklerinde türler arasında farklılıklar tespit edilmiştir. Kümeleme analizinde *Hyacinthella* türleri şeker içerikleri bakımından 3 farklı gruba ayrılmıştır. Yapılan çalışma hem *Hyacinthella* türlerinin soğanlarında bulunan şeker içeriklerini tanımlamakta hem de farklı bir tanımlama yöntemi önermektedir. Morfolojik, moleküler ve metabolik verilerin birleştirilmesi ile türlerin tam ve doğru bir şekilde teşhis edilmesi sağlanacaktır.

Anahtar Kelimeler: Sınıflandırma, sümbül, endemik

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1. Introduction

Türkiye is one of the most important centers of the world in terms of plant genetic resources also a gene center for many genera and species. *Hyacinthella* Schur is a genus of bulbous ornamental plants belonging to the Asparagaceae family. *Hyacinthella* species emerge from bulbs whose tunics are often covered in powdery white crystals. Nineteen taxa belonging to the *Hyacinthella* species have been identified in the world and 11 of them are distributed in Türkiye. The genus *Hyacinthella* consists of 11 taxa [10 species and 1 hybrid species [*H. micrantha* (Boiss.) Chouard × *H. heldreichii* (Boiss.) Chouard] (Davis, 1965; Persson and Wendelbo, 1982; Persson, 2000).

Leaves of plants are the primary photosynthetic organs that fix atmospheric carbon to make sucrose, which is then transported great distances to non-photosynthetic tissues via phloem. Sugars and sugar derivatives were originated from fixed carbon during the photosynthesis. Triose phosphates is the first sugar derivates that released from chloroplasts by carbon fixation and triose transformed to sugars in the cytosol of photosynthetic (source) cells (Malkin and Niyogi, 2000). This sugar reserve is ready for export to heterotrophic (sink) organs like bulb, corm, tuber, etc., The variety of different sugar concentrations throughout leaf development is related with the significant requirement of decreasing sugars for energy and carbon skeletons (Buchanan et al., 2000). Researchers reported a relationship between sucrose metabolizing enzymes and transcript levels

in four different developmental stage in *Hevea* Aubl. leaves.

Sugar contents interact with other signaling molecules, including phytohormones therefore effect plant growth and development because of their position as energy and carbon sources, as well as their regulatory activities (Rolland et al., 2006; Smeekens et al., 2010). Environmental conditions (crop management) and genetic variety of speices can have an impact effect on the sugar content of tubers and bulbs (Thompson et al., 2008). Many environmental factors and climate change can influence a variety of biochemical processes; balance of partitioning of sugars within plant cells and their transportation from source to sink organs.

Sugar levels, transportation, consumption, and storage are regulated and dependent on physiological activity, plant organs, extreme conditions, circadian rhythms, and physilogic age. (Lemoine et al., 2013). Sucrose can be degraded by a variety of enzymes (invertases and sucrose synthase) or regenerated from degradation products. Furthermore, sucrose, glucose, fructose, and trehalose operate as metabolic signaling molecules in host plant cells and stimulating the activation of gene sets, including defense genes.

Bulb growth requires starch accumulation and storage of carbohydrates (Xu et al., 2019; Wu et al., 2020; Li et al., 2021). In this context, growth of the bulb are tightly linked to starch and sucrose metabolism. Starch and sucrose metabolism is a complex biochemical process involving multiple enzymes (Liu et al., 2022). Soluble sugars are degradation product of starch and they can provide energy for morphogenesis, carbon fixation, emergence of leaves and development of flower buds (Liu et al., 2022).

The objective of this study was to determine the sugar content of bulbs of all *Hyacinthella* species grown in Türkiye. According to our research, no previous study has been found on this subject. Additionally, the data obtained was evaluated with the Cluster analysis method, which is a useful statistical technique used in the fields of recognition, classification and machine learning.

2. Materials and Method

2.1. Material

The materials of this study consists of all *Hyacinthella* taxa distributed in Türkiye. Between March and May of 2019 - 2021, samples of *Hyacinthella* genus taxa were collected both in flowering and fruiting form from their localities specified in the records in the Flora of Turkey. The collected samples were dried in accordance with herbarium methods, identified and preserved in the Research Laboratory of Yüzüncü Yıl University, Faculty of Science.

Eleven different *Hyacinthella* species distributed in Turkey were collected and bulbs of them were dried in shadow. Species names are given in Table 1.

2.2. Methods

2.2.1. Analysis of Sugars

Five grams of bulb samples of 11 different *Hyacinthella* species crushed with liquid nitrogen thoroughly by mortar and pestle. Fourty ml of methanol was added and the final mixture was homogenized with a magnetic stirrer for 20 minutes at 50 °C. After centrifugation at 3000 rpm for 10 minutes at the appropriate temperature, the volume of the supernatants was made up to 50 ml with methanol and mixed thoroughly. The methanol phase was then evaporated

Table 1. *Hyacinthella* species used in the study

No	Species name
1	*H. campanulata* K. Perss. & Wendelbo (End.)
2	*H. acutiloba* K. Perss. & Wendelbo (End.)
3	*H. glabrescens* (Boiss.) K.Perss. & Wendelbo (End.)
4	*H. heldreichii* (Boiss.) Chouard (End.)
5	*H. hispida* (J.Gay) Chouard (End.)
6	*H. lazulina* K.Perss.& Jim.Perss. (End.)
7	*H. lineata* (Steud. ex Schult. & Schult.f.) Chouard (End)
8	*H. micrantha* (Boiss.) Chouard (End.)
9	*H. nervosa* (Bertol.) Chouard
10	*H. siirtensis* B.Mathew (End.)
11	*H. venusta* K.Perss (End.)

evaporated in a rotary evaporator. The remaining solution was made up to 5 ml with water.

The extracts were load to Sep-Pak C18 cartridges and 7.5 ml of acetonitrile was added to 2.5 ml of the filtrate. Extractions were loaded to 0.45 µm membrane filter. Samples were injected into the HPLC device. Analyzes of free sugars were performed by Torija et al. (1998), and Karkacier et al. (2003).

2.2.1.2. HPLC conditions and calibration

Regression curves for linearity of the HPLC method were tested by injecting 9 different sugar standarts at 0.2, 0.5, 1.0, 2.5, and 5 mg/mL concentrations. Acetonitrile/water (80:20) was used as the mobile phase. Analyze sugars at 30 °C with an HPLC refractive index detector at a flow rate of 2 mL/min. NH_2 (Amino) column was used as the column. Sugar content and amounts were expressed as mg/ml. Sugar analyzes were completed in three replicates. HPLC was calibrated using standard sugars such as glucose, fructose, sucrose, maltose, galactose, ribose, xylose, triose, mannose, arabinose.

Nineteen different sugars standarts were tested to detect the sugars content in the bulbs of *Hyacinthella*, however 9 of them could be detected.

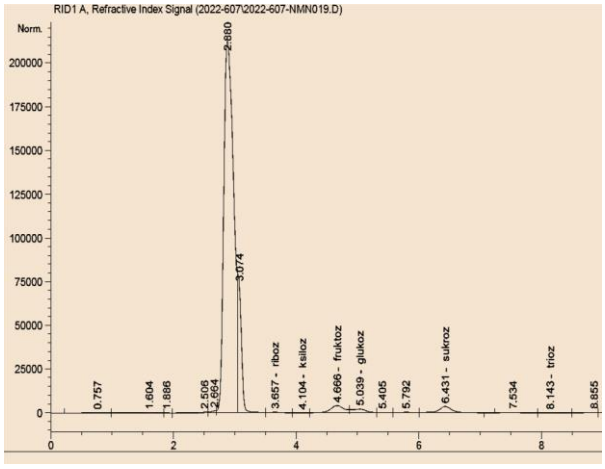

Figure 1. Chromatogram of sugar standards

2.2.2. Cluster Analysis

K-means is a clustering algorithm used to cluster a dataset into a certain number (K) of clusters or groups. This algorithm clusters data points according to a specific centroid, determining a set of centroids that represent the center of each cluster. Data points are assigned to the center closest to them, and this process is repeated until the clustering result of data points becomes stable.

The step-by-step working principle of the algorithm is as follows:

1. Initially, K random centers are selected. These centers may be randomly selected from the data set or determined according to a specific rule.
2. Each data point is assigned to the closest center. In this step, it is determined which cluster each data point is in.
3. A new center is calculated for each cluster. This is done by averaging all the data points of the cluster.
4. Steps 2 and 3 are repeated until a convergence criterion is reached where the centers change positions. The convergence criterion may be that the locations of the centers no longer change or that the amount of change does not exceed a certain threshold.

These steps are repeated until the dataset finds the optimal centers for clustering. The algorithm works by updating the position of the centroids and reclustering the data points at each iteration. As a result, each data point is associated with a cluster and the center of each cluster is determined.

3. Results

3.1. Sugar analysis

Although 19 different sugar standards were used for 11 different *Hyacinthella* bulbs in the study, 9 of them could be detected by HPLC analysis. Different sugar groups were obtained in different *Hyacinthella* species (Table 2.).

When the sugars in bulbs were evaluated qualitatively, glucose, fructose and sucrose sugars were found in all species naturally however the presence of other sugar types varied according to species (Table 2). It was determined that the differences between the species were mostly arabinose, triose and xylose sugars.

In the sugar analysis, the values differed in quantity levels. Ribose is the highest amount in *H. acutiloba*. Xylose, Glucose and Arabinose were found at higher level in *H. Campanulata*, Fructose at the highest amount founded in *H. Heldreichii*, Galactose at the highest levels in *H. Nervosa*. Sucrose and triose at the highest levels in *H. Venusta*.

Significant differences were detected between species, especially in terms of xylose and triose sugar amounts (Table 3).

Table 2. Qualitative analysis of sugars in *Hyacinthella* bulbs

Species	Ribose	Xylose	Arabinose	Fructose	Glucose	Galactose	Sucrose	Maltose	Triose
H. campanulata	-	-	+	+	+	+	+	+	+
H. acutiloba	+	+	+	+	+	+	+	+	+
H. glabrescens	-	+	-	+	+	+	+	+	+
H. heldreichii	-	-	-	+	+	+	+	-	+
H. hispida	-	+	-	+	+	+	+	-	+
H. lazulina	+	+	+	+	+	-	+	-	-
H. lineata	+	+	-	+	+	-	+	-	-
H. micrantha	-	+	-	+	+	-	+	-	+
H. nervosa	-	+	+	+	+	+	+	-	+
H. siirtensis	+	+	+	+	+	-	+	+	-
H. venusta	-	-	+	+	+	+	+	+	+

3.2. Cluster analysis

In this study, sugar data sets of 11 different *Hyacinthella* bulbs were used for cluster analysis. Clustering was done according to whether they were present or not. Clustering was performed by applying the K-means method to the data set. Plant species were clustered using the attributes of 9 different sugar types. In the K-means method, K=3 was chosen.

When the uure of the cluster analysis is examined, 3 different clusters are obtained. In the 1st group, *H. siirtensis*, *H. acutiluba*, *H. lazuilana* and *H. lineta* species (green), in the 2nd group, *H. campanulata* and *H. venusta* species (red), in the 3rd group, *H. glabrescens*, *H. nervosa*, *H. micrantha*, *H. hispida* and *H. helderichii* (blue) were defined. Same groups appear to be similar to each other.

4. Discussions

It was determined that there were significant differences between the species and their sugar contents. Naturally, glucose, fructose and sucrose sugars are found in all bulbs. These sugars are natural forms found in all plants. Differences were observed between species in sugar contents such as arabinose, triose and xylose. These sugar

Table 3. Quantitative analysis of sugars in *Hyacinthella* bulbs

EO	Ribose	Xylose	Arabinose	Fructose	Glucose	Galactose	Sucrose	Maltose	Triose
H. campanulata	1.09	9,.7	8.72	4.68	5.39	4.30	2.55	1.74	4.61
H. acutiloba	4.93	6.80	1.90	4.11	2.28	3.34	3.84	2.58	2.44
H. glabrescens	-	1.18	-	2.55	4.84	1.76	2.69	1.00	5.76
H. heldreichii	1.32	-	1.51	8.65	5.63	-	2.57	-	1.32
H. hispida	-	4.04	-	4.7	2.03	3.76	2.20	-	4.23
H. lazulina	-	3.85	6.16	1.60	1.24	-	3.08	-	-
H. lineata	-	3.85	6.16	1.60	1.24	-	3.08	-	-
H. micrantha	-	1.72	5.25	2.74	4.08	-	1.30	-	7.31
H. nervosa	2.55	4.86	-	3.01	5.24	-	3.16	3.29	5.00

H. siirtensis	-	3.98	1.98	3.02	3.20	5.64	2.58	-	4.60
H. venusta	2.33	2.38	2.38	6.25	3.69	-	2.84	1.89	7.67

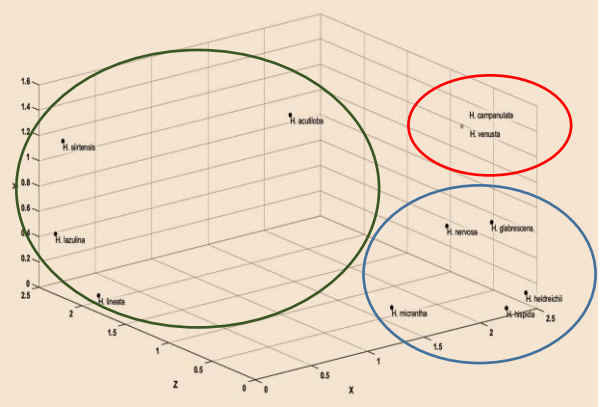

Figure 2. Cluster analysis of *Hyacinthella* species

were defined as specific sugars. Different sugar types play an active role in many metabolic events in plants; cell wall content to signaling, stress response to root tip elongation.

Responses to environmental changes; enzymatic and sugar content changes occurring in plant tissues are act as important indicators of the adaptive capacity of a species. This metbaolic process was defined as a necessity for the survival of the plant (López-Millán et al., 2000). In fact, the ability to maintain carbohydrate production and consumption, the stabilization of its levels, are the other responses, have been identified as a crucial adaptation for cold tolerance (Allen and Ort, 2001).

In the sugar analysis, it was seen that not only the sugar contents were different, but also their concentrations were variable. This may be due to the location and physiological age of the species, or due to their different adaptations. Locations of the *Hyacinthella* species are quite variable and their habitats also vary. Moreover, the environmental effects they exposed to are different from each other. In this context, sugar contents in *Hyacinthella* bulb may provide information about their habitat and adaptations.

There are quite different literature regarding sugar contents and diversity. These studies stated that sugar contents and concentrations may vary depending on metabolic and genetic differences, as well as ecological and stress situations. High sugar levels in plant tissues boost the plant's immunological response to fungal infections. Sugars most likely serve as priming chemicals that cause immunity in plants activated by pathogen-associated compounds (Morkunas and Ratajczak, 2014). Sugar content, qualitative composition, and transport in plant tissues change continually at day and night period also during all developmental phases. Plants have evolved an effective system by decreasing or increasing of sugar content related with metabolic process. Cell division, germination, vegetative development, flowering, and senescence are all impacted by changes in their concentrations. (Patric et al., 2013).

As a result, the reasons why sugar content varies in different *Hyacinthella* species studied are; It is thought to arise from the environment they are in and the conditions they are exposed to (biotic and abiotic), physiological conditions such as growth, development and flowering, and ultimately genetic differences.

In recent studies, anatomical and palynological features on *Hyacinthella* species were published (Eroğlu et. al, 2022; Sahin et al, 2022) However, the sugar content of bulbs belonging to *Hyacinthella* species were analyzed for the first time in current study. Moreover, the similarity and closeness between the species were tried to be evaluated by cluster analysis. More comprehensive and effective studies on *Hyacinthella* will contribute to the evaluation of this genus as a pharmaceutical or ornamental plant.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

Mehmet Acar: Analysis- Investigation-writing. Mehmet Emre Erez: Project administration – Investigation- review and editing. Hüseyin EROĞLU: Field investigation, anaylsis- writing.

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Predicting the effect of climate change on the geographic distribution of the endemic *Fritillaria aurea* in Türkiye

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İklim değişikliğinin Türkiye'deki endemik *Fritillaria aurea*'nın coğrafi dağılımına etkisinin tahmin edilmesi

Abstract: *Fritillaria aurea* is a rare, high altitude, endemic, and bulbous plant species in Türkiye. Although it is classified as least concern according to IUCN criteria, the species has a narrow distribution. This study utilized ensemble modeling to forecast potential future changes in suitable habitats for *F. aurea* by two Shared Socio-Economic Pathways (SSPs: SSP 1-2.6 and 5-8.5). These pathways were constructed using two General Circulation Models (GCMs) and covered the years 2035, 2055, and 2085. The results showed that the minimum temperature of the coldest month (bio6), mean temperature of the wettest quarter (bio8), and precipitation of the warmest quarter (bio18) have the largest influence on the potential species distribution. The ensemble model predicted that the highly suitable habitats of *F. aurea* would contract under all future SSP scenarios and it would lose almost all of its potential highly suitable distribution areas by the end of the century. The remained population of *F. aurea* could possibly harbour in only minor areas of the North Anatolian Mountains in the north and Taurus Mountains in the south. The results of the study could contribute to establishing conservation strategies and natural resource management policies for *F. aurea* against the potential impacts of climate change. The highly suitable habitats under pessimistic scenarios at the end of this century projected by the present study can be determined as protected areas for the species.

Key words: CMIP6, global warming, habitat prediction, ensemble model, plant species distribution

Özet: *Fritillaria aurea*, Türkiye'de nadir bulunan, yüksek rakımlı, endemik ve soğanlı bir bitki türüdür. IUCN kriterlerine göre az endişe kategorisinde sınıflandırılmasına rağmen, dar yayılışlı bir türdür. Bu çalışma, *F. aurea* için uygun habitatlarda gelecekteki potansiyel değişiklikleri iki Paylaşılan Sosyo-Ekonomik Yol (SSP'ler: SSP 1-2.6 ve 5-8.5) aracılığıyla tahmin etmek için topluluk modellemeyi kullanmıştır. Bu yollar 2035, 2055 ve 2085 yıllarını kapsayan iki Genel Dolaşım Modeli (GCM) kullanılarak oluşturulmuştur. Sonuçlar, en soğuk ayın minimum sıcaklığının (bio6), en yağışlı çeyreğin ortalama sıcaklığının (bio8) ve en sıcak çeyreğin yağışının (bio18) potansiyel tür dağılımı üzerinde en büyük etkiye sahip olduğunu gösterdi. Topluluk modeli, *F. aurea*'nın son derece uygun habitatlarının gelecekteki tüm SSP senaryoları altında daralacağını ve yüzyılın sonuna kadar potansiyel olarak son derece uygun dağıtım alanlarının neredeyse tamamını kaybedeceğini öngördü. *F. aurea'nın* geriye kalan popülasyonunun kuzeyde Kuzey Anadolu Dağları'nın ve güneyde Toros Dağları'nın yalnızca çok küçük bir kısmında barınması muhtemeldir. Çalışmanın sonuçları, *F. aurea*'nın iklim değişikliğinin olası etkilerine karşı koruma stratejileri ve doğal kaynak yönetimi politikalarının oluşturulmasına katkı sağlayabilir. Bu çalışmanın öngördüğü, bu yüzyılın sonundaki kötümser senaryolar altında son derece uygun habitatlar, tür için korunan alanlar olarak belirlenebilir.

Anahtar Kelimeler: CMIP6, küresel ısınma, habitat tahmini, topluluk modeli, bitki türlerinin dağılımı

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1. Introduction

Climatic drivers are the major determinants of plant species distribution along with geographical gradients (Woodward and Williams, 1987). Therefore, any alterations of climatic conditions highly overpressure plant species and diversity. According to the IPCC's 2021 report, it is projected that the average global temperature will rise by at least 1.5 °C by the end of 2100 (Allen et al., 2018). The human-induced warming triggers other impacts on plant species such as fragmentation, invasive species, overexploitation, and habitat loss (Mantyka-Pringle et al., 2012). Plant species are predicted to respond differently to climate change, and its effects are assumed to differ from region to region (Thuiller et al., 2005). Particularly, most studies reported that mountain regions will be most affected by climate change (Guisans and Theurillat, 2000; Liu and Chen, 2000; Diaz and Eischeid, 2007). The most serious and irreversible impact of climate change on species distributions will be loss of species. Due to both changes and vanish in the distribution limits of the species changes in their taxonomic and functional diversity are expected to be observed.

Fritillaria aurea Schott which is distributed in the Central Anatolian region in Türkiye is a rare, alpine, endemic, and bulbous plant species. According to IUCN (The International Union for Conservation of Nature 2024), it is assessed in the least concern threatened category. The species colonizes limestone substrates on north-facing rock outcrops and snow beds at an altitude of 1650-3000 m, particularly in Juniper openings (Rix, 1984; Tekşen, 2018). The current distribution is restricted to eastern and south-eastern Central Anatolia which includes middle and upper Kızılırmak (Halys) basin, the northern parts of the Euphrates, Konya and Adana provinces (Tekşen and Aytaç, 2011; Tekşen, 2018). *F. aurea* is a typical Irano-Turanian element, and it has campanulate-shaped and reddish-brown tessellated yellow flowers. The main threats to the

populations of the species are overgrazing, habitat loss because of agricultural activities, and reduced water availability due to human activities. The ever-increasing anthropogenic disturbance and influence of climate change are likely to have disproportionately negative impacts on populations of the species more recently.

To acquire a deeper understanding of the potential effects of global climate change, various worldwide climate model simulations have been generated within the Coupled Model Intercomparison Project (CMIP) under the World Climate Research Program (WCRP). IPCC releases climatic conditions for the past, present, and future using different models under various emission scenarios based on greenhouse gases with the help of the Coupled Model Intercomparison Project (CMIP) as reports, and these are occasionally updated. The most recent publication of the Intergovernmental Panel on Climate Change sixth assessment report (IPCC, 2021) contains the findings of the Coupled Model Intercomparison Project Phase 6 (CMIP6). Species distribution models (SDMs) predict the potential impacts of climate change on the extent of a species' range and biodiversity in the past or the future with the help of these climatic conditions based on various emission scenarios of IPCC reports and using different algorithms (Pearson, 2007; Elith and Leathwick, 2009).

Endemic species have a high potential to disappear owing to narrow geographical range, small population size, low genetic variability, and the requirement for specialized ecological niches (Işık, 2011). These species are most sensitive to future climate changes (Da Silva et al., 2019). Accordingly, we deemed it necessary to investigate the potential spatial distribution of the species and the impact of climate changes in the future on *F. aurea* in Türkiye. We applied the ensemble technique to establish the potentially current suitable habitats for *F. aurea* under the current climate and understand the alteration of these potentially suitable habitats in the future due to climate change. Considering the survival of endemic species totally relies on national policies, the results of this study will supply useful information for academic research and aid to design effective sustainable ecological assessments for the conservation of the species.

2. Materials and Method

2.1. The study area and occurrence data

The region of Central Anatolia including the study area is located between latitude 39°N to 41°N and longitude 30°E to 38°E. Central Anatolia is a vast plateau that is limited between the North Anatolian Mountain ranges in the north and the Taurus Mountain ranges in the south. The topography is generally flat, with the elevation varying between 700 to 1100 m. There are a few volcanic mountains that can rise up to above 3000 m on this vast area. Regional precipitation varies between about 330 and 700 mm and average maximum temperatures are between 23°C and 29°C in this region, thus, the climate of the region is classified under an arid and semi-arid Mediterranean climate (Kenar and Kikvidze, 2019).

Species presence records were obtained from the field works by M. Tekşen and the verified occurrence points in different herbaria (AEF, AIBU, AKSU, ANK, E, EBKA, EGE, GAZI, HUB, ISTE, ISTF, ISTO, NGBB, and RSA [the acronyms follow Thiers, 2024]) listed in 33 locations in Türkiye (Tekşen, 2018) seen below.

***Fritillaria aurea*:** TÜRKİYE. Adana: Seyhan, Pozantı, Armutoluk, E of Hondu, 12 May 1952, *İ. Akkaş* 11873 (ISTF); Aksaray: Hasan Mt., 05 May 2021, *M. Tekşen* 5975 (AKSU); Kahramanmaraş: Göksun, between Püren pass and Değirmendere village, Kartallık, 1700-1800 m, 21 May 2000, *M. Tekşen* 1994 (fr.) (GAZI); ibid, 22 April 2001, *M. Tekşen* 2049 (fl.) (GAZI); ibid., 9 July 2001, *M. Tekşen* 2104 (fr.) (GAZI); ibidem, 24 May 1993, *Ekici* 1275 (GAZI); Göksun, Berit Mt., 19 June 1981, *B. Yıldız* 3005 (AIBU; HUB); Göksun, Binboğa Mt., above Karlı plateau, *P.H. Davis* 20024 (E); Kayseri: Sarız, Binboğa Mt., Yalak, 1700-1900 m, 7 May 1991, *Z. Aytaç* 3702 & *H. Duman* (AEF, HUB, GAZI); Pınarbaşı, Eğrisöğüt village, Şirvan Mt., 1900-2000 m, 25 July 2003, *M. Tekşen* 2204 (fr.) (GAZI); 24 km S. of Pınarbaşı, 1800-1900 m, 24 May 1965, *Coode & Jones* 1423 (ISTO, E, ISTF); Pınarbaşı, Eğrisöğüt-Beyçayır villages, around Kumuk Adil, c. 1700 m, 17 April 2001, *A. M. Özkan* s.n. (AEF); Pınarbaşı to Gürün, Ziyaret hill, 2000 m, 23 May 1965, *Coode & Jones* 19810 (ISTF); Pınarbaşı, Solaklar Köyü, Şirvan Mt., 4 June 2006, *A. Güner* 14239 et al. (NGBB); Yahyalı, Maden, Aladağlar, 25 May 2008, *A. Güner* 14857 et al. (NGBB); Malatya: Akçadağ, Bayramuşağı village, İskender Hayması, 26 April 1992, *B. Yıldız* 9087 (ISTE 94755); Darende, Ağılbaşı district, Ağılbaşı, Akbabaçalı Mt., 30 April 2008, *H. Yıldırım* 1372 (EGE); Doğanşehir, 29 April 2008, *M. Aslay* F44043 & *M. Tekşen* (EBKA); Doğanşehir, Eskiköy, Bey Mt., 3 May 1992, *B. Yıldız* 9102 (ISTE 94761); Doğanşehir, Eskiköy, Koçdere village road, 28 April 2008, *H. Yıldırım* 1294 (EGE); Doğanşehir, Söğüt, Kudılı village, Boğaboynu, 25 April 2009, *M. Aslay* F44063 & *M. Tekşen* (EBKA); Sürgü, Eski Kurucaova village, 24 May 1992, *B. Yıldız* 9308 (ISTE 94801); Mersin: Aslanköy, Ballık Mt., 11 May 1976, *T. Baytop* (ISTE 34852); Toros, Siehe 216 (ANK); Niğde: Aladağ, SW (south-west) Flank of Demir Kasık, 2400-2800 m, *Parry* 170 (E); Aladağ, 2700 m, 24 June 1964, *Wood & Gibson* 106 (E); Aladağ, Emli Valley, Parmaktaş, 2 May 2010, *M. Tekşen* 2359 (AKSU!); Aladağ, Tekneli plateau, 2700 m, 15 July 1979, *R. Carle & H. Kürschner* 79-433 (RSA); Sivas: Pınarbaşı to Gürün, 2000 m, 26 May 1960, *Stainton & Henderson* 5179 (E, RSA).

2.2. Current and future climatic data

We used the 19 bioclimatic layers of the CHELSA version 2.1 (Climatologies at High Resolution for the Earth's Land Surface Areas) dataset (Karger et al., 2021) as climatic environmental variables at a 30-arc sec spatial resolution (~1km) for four temporal ranges (1981-2010 "current times", 2011-2040 "2035s", 2041-2070 "2055s", and 2071-2100 "2085s"). We extracted the bioclimatic variable values of the grid cell using QGIS 3.18.2 (QGIS Development Team, 2021). We utilized Max Planck Institute Earth System Model (MPI-ESM1-2-HR; Gutjahr et al., 2019) and the Meteorological Research Institute Earth System Model Version 2.0 (MRI-ESM2.0; Yukimoto et al., 2019) data of the Global Circulation Model (GCM) produced by the Coupled Model Intercomparison Project Phase 6 database (CMIP6) (IPCC, 2021).

We used two Shared Socio-Economic Pathways (SSPs) having different scenarios based on the amount of greenhouse gas emissions and radiative forcing level

considering the population and economic growth. The SSP 1-2.6 is a scenario that predicts a low level of greenhouse gas emissions, as indicated by its radiative forcing route. This scenario forecasts a global warming of less than 2°C by the year 2100, with a radiative forcing level of 2.6 W/m² by the same year. The SSP5-8.5 represents a scenario characterized by significant emissions, where radiative forcing is stabilized at 8.5 W/m^2 by the year 2100. This scenario is based on the assumption of unrestricted and rapid increases in both economic output and energy consumption resulting in the greatest levels of greenhouse gas emissions (Meinshausen et al., 2020).

2.3. Data analysis

We computed Variance Inflation Factors (VIF) for climatic variables using the "usdm" package in R to mitigate the issue of multi-collinearity. Environmental variables with a VIF exceeding 5 were subsequently eliminated (Naimi et al., 2014).

Species Distribution Models (SDMs) attempt to estimate the spatial patterns of species occurrences based on correlations between available presence records and the environmental conditions (Beery et al., 2021). We utilized the R package 'biomod2' version 3.5.1 which supports an ensemble model that combines different modeling techniques to generate potential distribution maps of *F. aurea* (Thuiller et al., 2009; R Core Team, 2013). We performed ten modeling algorithms: Multivariate Adaptive Regression Splines (MARS), Generalized Linear Model (GLM), Random Forest (RF), Generalized Boosted Model (GBM), Functional Data Analysis (FDA), Artificial Neural Network (ANN), Generalized Additive Model (GAM), Surface Range Envelope (SRE), Maximum Entropy (Maxent), and Classification Tree Analysis (CTA). We constructed 350 pseudo-absence records (PAs) using random generation. These records were then calibrated using an 80% occurrence dataset and assessed using a 30% occurrence dataset performing using 3-fold cross-validation. We chose to utilize the Receiver Operating Characteristic Curve (specifically the Area Under the Curve, AUC) and true skill statistics (TSS) to assess the correctness of the model. The AUC values range from 0 to 1. Models with AUC values closer to "1" exhibit greater accuracy. The formula for TSS is calculated by adding the sensitivity and specificity values and then subtracting 1. A higher number for TSS indicates a higher level of accuracy for the model (Allouche et al., 2006).

We generated the final potential species distribution map including habitat suitability levels ranging of values from 0 to 1 based on the ensemble model. The levels of suitability are categorized as follows: unsuitability (0-0.2), low suitability (0.2-.0.4), medium suitability (0.4-0.6), suitability (0.6-0.8), and high suitability (0.8-1).

3. Results

3.1. Model performance and the importance of environmental variables

We used only those bioclimatic variables (bio3, bio4, bio6, bio8, bio18, and bio19) after calculation of VIF values for the models (Table 1).

AUC value is an important metric used for evaluating the performance of the models. In our study, these values varied between 0.986 and 0.807, which shows that the models were highly accurate (Table 2). According to the results, the most accurate algorithm was MARS, whilst the least accurate was CTA. The performance of the ensemble model had an AUC value of 0.998 and a TSS value of 0.972, indicating that the ensemble model best performed in this study and could be used in the following analysis.

Table 1. The climatic variables utilized in constructing the models obtained from CHELSA Version 2.1 (Karger et al., 2021), along with their corresponding Variance Inflation Factor (VIF) values employed in model construction

Code	Bioclimatik variable	VIF value
BIO3	Isothermality (BIO2/BIO7) (* 100)	2.33
BIO4	Temperature Seasonality (standard deviation *100)	1.81
BIO6	Minimum Temperature of Coldest Month	1.14
BIO8	Mean Temperature of Wettest Quarter	4.47
BIO18	Precipitation of Warmest Quarter	3.39
BIO19	Precipitation of Coldest Quarter	2.14

Table 2. Receiver operating characteristics (ROC) and True Skill Statistics (TSS) values (± SD) of all the algorithms, ensemble model, multivariate adaptive regression splines (MARS), generalized linear model (GLM), random forest (RF), generalized boosted model (GBM), functional data analysis (FDA), artificial neural network (ANN), generalized additive model (GAM), surface range envelope (SRE), maximum entropy (MaxEnt), and classification tree analysis (CTA) performed with present climate conditions (1981–2010).

Algorithm	ROC ± SD	TSS ± SD
Ensemble model	0.998 ± 0.001	0.972 ± 0.002
MARS	0.986 ± 0.020	0.952 ± 0.050
GLM	0.979 ± 0.028	0.938 ± 0.073
RF	0.958 ± 0.050	0.862 ± 0.114
GBM	0.958 ± 0.045	0.847 ± 0.129
FDA	0.955 ± 0.046	0.828 ± 0.115
ANN	0.927 ± 0.008	0.804 ± 0.036
GAM	0.843 ± 0.089	0.690 ± 0.172
SRE	0.828 ± 0.050	0.657 ± 0.099
MAXENT	0.826 ± 0.036	0.652 ± 0.073
CTA	0.807 ± 0.068	0.614 ± 0.137
Algorithm	ROC ± SD	TSS ± SD

The environmental variables which were used in the model and the percent predictive contribution of each variable were calculated and converted to percentages (Fig. 2). Each environmental variable had different importance for the models in the study. The minimum temperature of the coldest month (bio6) had the maximum contribution to most algorithms such as MARS, GLM, and GBM, followed by precipitation of the warmest quarter (bio18; Fig. 1). The mean temperature of the wettest quarter (bio8) could be the third variable with an average contribution except for RF, FDA, and MaxEnt. Compared to precipitation variables, temperature variables (bio3, bio4, and bio6) contributed more to the GLM, which was among the first four algorithms with the highest AUC value.

3.2. Potential suitable habitat under current and future changes

The extent of potential distribution areas in the current and future climate conditions predicted by the ensemble model

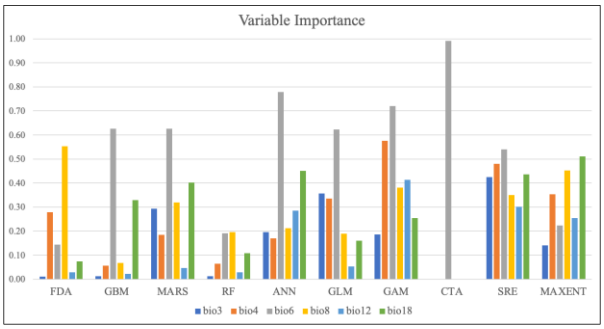

Figure 1. The importance of the selected environmental variables to FDA, GBM, MARS, RF, ANN, GLM, GAM, CTA, SRE (BIOCLIM), and MaxEnt models

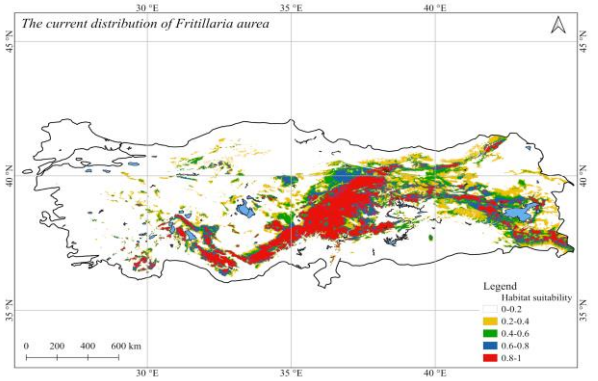

Figure 2. The ensemble model was used to forecast the suitable habitats for *Fritillaria aurea* from 1981 to 2010. The color white (0-0.2) indicates unsuitability, while yellow (0.2-0.4) indicates low suitability. Green (0.4-0.6) represents medium suitability, blue (0.6-0.8) signifies suitability and red (0.8-1) indicates high suitability.

are shown in Table 3. The current potential distribution area of *F. aurea* covers a large region at the intersection of eastern and south-eastern Central Anatolia, and further, the possible distribution of the species continues towards the south of Anatolia in the Mediterranean coastal region (Fig. 2). We established that 69.201 km^2 of these areas are highly suitable areas for *F. aurea.* Considering the projections for the current distribution of the species, both scenarios (SSP1-2.6 and SSP5-8.5) remarked that there would be a decline in the distribution of the species in both climate change scenarios and global climate models (Figs. 3-4).Although there were some small increases in the middle of the century, the MPI-ESM1-2-HR GCM model projected that the species' potential range would drop by 27% (equivalent to a loss of 18.776 km^2) under the SSP1-2.6. However, under the SSP5-8.5, the species' range would be reduced by 62% (equivalent to a loss of 42.821 km^2) by the end of the century. The MRI-ESM2.0 offers us more pessimistic scenarios. Accordingly, highly suitable habitats exhibit a strong downward trend; the possible habitat loss would be 42% (29.014 km^2) under SSP1-2.6, whilst the loss would be 92% (63.806 km^2) under SSP5-8.5 by the end of the century.

Furthermore, future models predicted that the species could have very narrow potential refuge areas in the transition zone between the Irano-Turanian and the Euxine region, in mountainous habitats in the west of the Black Sea region, and in the subalpine zone of central and western Taurus Mountains.

4. Discussions

Fritillaria aurea is one of the nearly 52 *Fritillaria* species recorded in Türkiye (Tekşen, 2018; Tekşen et al., 2024). The population size of *F. aurea* is progressively decreasing in its natural habitats and thus, the species may be at risk of extinction due to human disturbance and environmental change in the near future (Tekşen and Aytaç, 2011; Tekşen, 2018). The model under current climate conditions accurately predicted a large part of the suitable habitat of *F. aurea* in the southeast of Central Anatolia and central Taurus mountains which were consistent with our field

Table 3. Percentage and the predicted suitable area of the presence of *F. aurea* for the present day (1981-2010), 2035s (2011-2040), 2055s (2041-2070), 2085 (2071-2100) and under two different climate scenarios. Mean predicted results are from two global climate models [MPI-ESM1-2-HR and MRI-ESM2-0] which were modelled under 2035s-SSP126; 2035s-SSP585; 2055s-SSP126; 2055s-SSP585; 2085s-SSP126; and 2085s-SSP585. SSP5-8.5 refers to a Shared Socio-economic Pathway (SSP) scenario from the IPCC sixth assessment report (AR6) for a scenario with very high greenhouse gas emissions; SSP1-2.6 refers to a second SSP scenario with stringent mitigation of greenhouse gas emissions

Ensemble model	**MPI-ESM1-2-HR**				
Suitability Class Code	0	1	2	3	4
Unit of measure	km2	km2	km2	km2	km2
Current	541.931	75.980	51.823	40.995	69.201
2035/SSP126	519.006	95.720	59.747	53.830	51.628
2055/SSP126	528.669	91.864	59.959	50.407	49.030
2085/SSP126	529.417	88.102	56.803	55.183	50.425
2035/SSP585	530.440	89.367	51.339	47.647	61.137
2055/SSP585	528.669	91.864	59.959	50.407	49.030
2085/SSP585	570.710	90.028	53.238	39.575	26.380
Ensemble model			**MRI-ESM2-0**		
Suitability Class Code	0	1	2	3	4
2035/SSP126	541.931	75.980	51.823	42.198	37.893
2055/SSP126	566.086	83.490	43.055	41.170	46.115
2085/SSP126	580.578	77.902	44.607	36.656	40.187
2035/SSP585	552.292	91.606	54.877	42.460	38.692
2055/SSP585	595.871	76.174	40.994	40.029	26.863
2085/SSP585	662.493	56.426	36.934	18.684	5395

Total area: 779.932 km^2

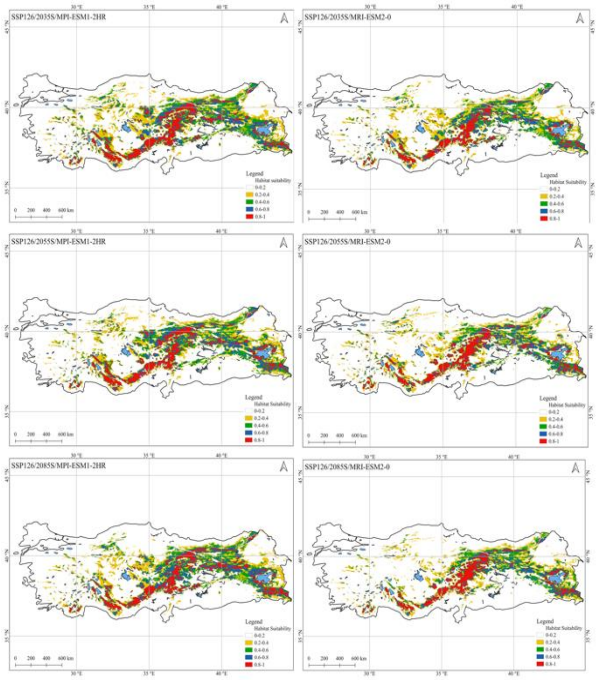

Figure 3. The predicted suitable habitats of *F. aurea* in 2035s, 2055s, and 2085s under the SSP5-8.5 scenario using the MPI-ESM1-2-HR and MRI-ESM2-0 GCMs derived from the ensemble model. White (0-0.2) represents unsuitability, yellow (0.2-.0.4) represents low suitability, green (0.4-0.6) represents medium suitability, blue (0.6-0.8) represents suitability, and red (0.8-1) represents high suitability.

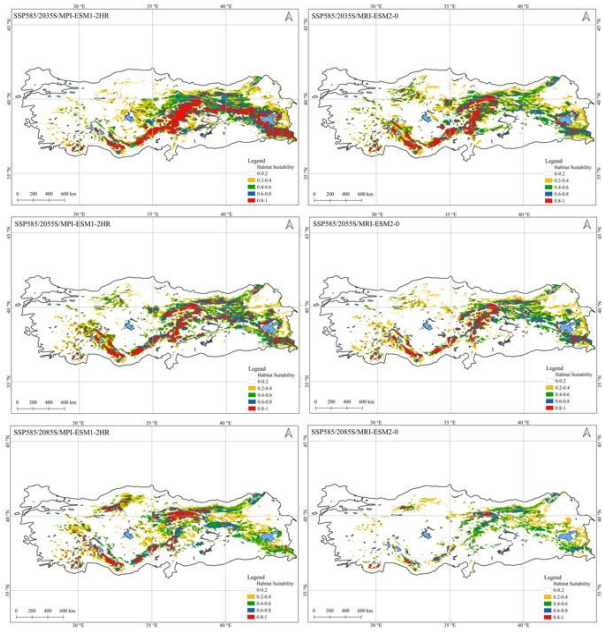

Figure 4. The predicted suitable habitats of *F. aurea* in 2035s, 2055s, and 2085s under the SSP5-8.5 scenario using the MPI-ESM1-2-HR and MRI-ESM2-0 GCMs derived from the ensemble model. White (0-0.2) represents unsuitability, yellow (0.2-.0.4) represents low suitability, green (0.4-0.6) represents medium suitability, blue (0.6-0.8) represents suitability, and red (0.8-1) represents high suitability.

studies. Besides this region, the ensemble model also predicted the south and east of Sivas province, the western Taurus Mountains, East of Van Lake, and high mountains in Hakkari province in southeasternmost of Anatolia as suitable habitats. Since the species grows at high altitudes (1000-3000 m a.s.l.), it is considered an alpine geophyte. The altitudes of the regions which are shown as potential distribution areas in the model are similar to the elevation of the current distribution areas. Therefore, we can state that similar climatic conditions and elevations prevail in these regions.

Seasonality and continentality are important factors in high altitude environments (Testolin et al., 2020). While seasonality comprises both temperature and precipitation (Lisovski et al., 2017), continentality is directly dependent upon annual monthly temperature-precipitation extremes (Deniz et al. 2011). The plants in alpine habitats adapted to extreme conditions such as cold temperatures and a short growing season. Therefore, the plants in these habitats are easily affected by seasonal changes in precipitation and temperature caused by global warming (Hamid et al., 2020). Low temperatures and precipitation are usual conditions during the whole year for the temperate high-altitude zone and the intensity of reduction in both these low temperatures and in precipitation tends to increase considerably with elevation (Billings and Mooney, 1968). The distribution range of our species is mostly determined by the lowest temperature during the coldest month (bio6), the amount of precipitation during the warmest quarter (bio18), and the average temperature during the wettest quarter (bio8).

Due to their adaptation to lower temperature regimes, the plants in alpine habitats are considered to be highly sensitive to global warming (Singh, 2008). In particular, it is estimated that endemic species of perennial plant, geophyte, and tree life forms will be adversely affected by climate change (Kobiv, 2017; Inouye, 2020). The plant species could respond to climate change usually contracting or shifting and expanding at best their distributions (Chen et al., 2011). Our study revealed the possible spatial changes in the suitable habitat of *F. aurea*, which is an alpine geophyte, under different future climate change scenarios. Accordingly, the potential highly suitable habitats of the species showed a downward tendency based on both two global climate model simulations, and further the potential distribution of the species were severe differences considering greenhouse gas emissions and radiative forcing level scenarios (SSPs). *Fritillaria aurea* would lose from one third to half of its potential distribution area under SSP1-2.6, whilst this ratio would be two out of three and even almost all under SSP5-8.5 until end of the century.

Narrow-ranging species usually grow in idiocratical habitats, and they are vulnerable to climate change (Da Silva et al., 2019). They will presumably encounter distribution shifts, and range restrictions or they will vanish due to limited ecological adaptability as a response to global warming (Dubos et al., 2022). Hereunder, *F. aurea* would contract a major part of its distribution to certain points that would mostly be mountainous habitats. These could be the transition zone between the Irano-Turanian and the Euxine regions and the subalpine zone of the central and western Taurus Mountains.

In conclusion, we predicted the potential distribution of *F. aurea* in the future by using various modeling algorithms and two global climate models under two different scenarios in our study. The minimum temperature of the coldest month (bio6), precipitation of the warmest quarter (bio18), and mean temperature of the wettest quarter (bio8) had the largest influence on *F. aurea* distribution. The large parts of the habitat of *F. aurea* were estimated to be lost by 2100 according to both climate scenarios. The distribution

modeling of our species was created using only climatic parameters without including anthropogenic effects such as overgrazing, agriculture, and urbanization. It is a known fact that human activities have already generated negative effects on the distribution of species. When the negative impact of climate change joins with the pressure of human activities, the threat to the distribution of the species will increase further. Our results provide useful information to establish conservation strategies and determine the options for suitable areas in the future against the expected changes in the distribution of *F. aurea* which is an endemic species under changing climatic conditions.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Nihal Kenar and Mehtap Tekşen conceived and designed research. The corresponding author analysed the data, wrote and edited the manuscript. Mehtap Tekşen provided data collection. Both authors read and approved the manuscript.

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Morphology and phylogeny of *Cortinarius strenuipes* (*Basidiomycota, Agaricales*) reported for the first time from Türkiye

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Türkiye'den ilk kez kaydedilen *Cortinarius strenuipes* (*Basidiomycota, Agaricales*)'in morfolojisi ve filogenisi

Abstract: *Cortinarius strenuipes* Rob. Henry is reported for the first time from Türkiye based on morphological features and molecular analysis. It is found in mixed forest and distinguished by a gray or reddish-brown pileus with blackish spots, dark brownish ochre to chocolate-brown lamellae, brown or brownish-gray, cylindrical stipe slightly bulbous at base. Internal transcribed spacer region (ITS) and the large subunit (LSU) of nuclear ribosomal RNA region sequences of the specimen are determined and compared with similar taxa.

Key words: *Basidiomycota*, *Cortinariaceae*, new record, phylogenetic analysis, Türkiye

Özet: *Cortinarius strenuipes* Rob. Henry morfolojik özellikleri ve moleküler analizlere dayalı olarak Türkiye'den ilk kez rapor edilmiştir. Karışık ormanlarda bulunur ve siyahımsı benekli, kahverengimsi veya kırmızımsı-kahverengi şapka, koyu okra-kahverengi ile çikolata-kahverengi lamelleri ve önce beyazımsı, sonra kahverengi veya kahverengimsi-gri, tabanda hafif şişkin silindirik sap ile ayırt edilir. Örneğin, ribozomal RNA bölgesine ait transkribe edilen aralayıcı bölge (ITS) ve büyük altbirim (LSU) sekansları belirlenip benzer taksonlarla karşılaştırılmıştır.

Anahtar Kelimeler: *Basidiyomycota, Cortinariaceae,* yeni kayıt, filogenetik analiz, Türkiye

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1. Introduction

Cortinarius (Pers.) Gray is the largest genus of Agaricales with an estimated number of more than 5,000 scientific names according to the IndexFungorum (Kirk et al., 2008; Liimatainen et al., 2014) and about 150 species in Turkey (Sesli et al., 2020; Şengül Demirak et al., 2022; Sesli, 2023). Members of the genus distributed in temperate and subtropical forests and they form mycorrhizal associations with a wide range of tree and plant families including, Caesalpiniaceae, Cistaceae, Dipterocarpaceae, Fagaceae, Malvaceae, Myrtaceae, Nothofagaceae, Pinaceae, Rhamnaceae, Rosaceae and Salicaceae (Frøslev et al., 2006; Garnica et al., 2011; Liimatainen et al., 2014; Soop et al., 2019).

Classification of species within the genus *Cortinarius* is very problematic due to convergence of macroscopic and microscopic features which causes description of the same species under different names. These problems are partly solved by the use of molecular techniques where the nuclear ribosomal internal transcribed spacer (ITS) is widely used as a universal barcode marker for fungal barcoding (Schoch et al., 2012). More specifically, it is suggested that ITS is suitable for species delimitation in *Cortinarius* (Frøslev et al., 2005, 2007; Garnica et al., 2011; Liimatainen et al., 2014). Thus, molecular studies are necessary for an accurate identification of a *Cortinarius* species.

The present study identifies a *Cortinarius* species, *Cortinarius strenuipes* Rob. Henry, a new record for the Turkish mycota, based on molecular and morphological analyses.

2. Materials and Method

2.1. Morphological studies

Fresh specimens of *Cortinarius* were collected from Avlunlar village (Tokat) on November 2018. Color photographs were taken in the field and macromorphological and ecological features were noted. The samples were transported to the laboratory wrapped in aluminum foil, then dried using a fan heater and placed in zip lock bags for further studies. The dried samples are kept in the Fungarium of the Biology Department, Tokat Gaziosmanpasa University (GOPUF). The measurements of micromorphological structures were determined using dried samples and chemicals such as 5% KOH, Melzer reagent, 1% Congo Red under a Nikon Research Microscope (100x). Micromorphological features were examined and descriptions were made following Bidaud (1992), Muñoz (2018), Gane (2016) and Maletti (2021).

2.2. Molecular studies

Genomic DNA was extracted from lamella using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo research, Irvine, California) as described by the manufacturer's protocol. The ITS1-5.8S-ITS2 region of the rDNA gene was amplified using the primer pair ITS4-ITS5 (White et al., 1990) and the 28S LSU gene region was amplified using the primer pair LROR-LR5 (Vilgalys and Hester, 1990).

Polymerase chain reactions (PCR) were prepared in a 30 µl final volume mixture containing 3 µl 10X buffer, 3 µl dNTP mix, 3 µl primer pair (final concentration of 1 µM each), 0.3 µl Dream Taq DNA polymerase (Thermo), 10 µl gDNA and 7.7 µl sterile double distilled H_2O. PCR amplifications for ITS and LSU gene regions included 5 min initial denaturation at 95ºC followed by 40 cycles of denaturation at 95ºC for 30 sec, annealing at 53ºC for ITS and 48ºC for LSU for 30 sec, and extension at 72ºC for 1 min and a final extension for 10 min. PCR products were verified by using 1 % agarose gel electrophoresis and sent for sequencing (Aquatayf Biotechnology Laboratories, Istanbul, Türkiye).

For both ITS and LSU genes, sequences were generated from both ends and then assembled to produce a final gene sequence. These sequences were BLASTed for homology based searches using Basic Local Alignment Search Tool (BLAST) program. Best matches were retrieved from GenBank for phylogenetic analysis. Sequence alignment is performed using ClustalW and phylogenetic tree is constructed using MEGA 6.0 (Tamura et al., 2013). Phylogenetic trees were constructed using the maximum likelihood (ML) method where Tamura-Nei model (Tamura and Nei, 1993) was used to construct the ML tree with bootstrap support of 1000 replicates and default settings. The bootstrap support values ≥ 50% were marked on the branches of the phylogenetic tree.

3. Results

3.1. Taxonomy

Cortinarius strenuipes Rob. Henry, Bulletin de la Société Mycologique de France 71 (3): 230 (1956) (Fig. 1,2)

Mycobank MB# 295972

Macroscopic and microscopic features: Pileus 60-100(130) mm across, firstly convex, later plane-convex, finally flat-convex and even slightly depressed; brown-gray, brown-cream or reddish-brown; margin regular, smooth, firstly inrolled and then incurved; surface dirty in appearance, dry, hygrophanous, initially covered with a grayish veil, with abundant whitish fibrils cuticle and dark blackish spots. Lamellae dark ocher-brown to chocolate-brown; not very tight, thick, broad, several lengths, crowded, convoluted indentation, slightly decurrent. Stipe 60-70(100) x 15-20(25) mm, dry, solid, fleshy, hard, rusty brown fibrillose, cylindrical, slightly bulbous at base;

Figure 1. *Cortinarius strenuipes* (Collection HIS-47): a- basidiomata in situ, b- basidiospores, (Scale bars: a = 40 mm; b = 10 µm).

whitish, then slightly brown, covered with a grayish silky veil; cortina short-lived, whitish, rarely leaves a slight ring-shaped scar on the upper. Flesh thick, whitish grayish on stem, pale reddish brown on pileus; unpleasant weak odor. Basidiospores 8.5–11.5(13.0) x 6.0–7.0(8.0) µm, ellipsoidal to amygdaliform, fine to medium-sized warts. Basidia 33-35(45) x 10-12(14) µm, clavate, 4-spored. Marginal cells 5-7 µm in diameter, clavate. Pileipellis filamentous, cylindrical hyphae, septate with clamps. Clamp connections present. Potassium hydroxide (KOH) on the cuticle black, on the flesh dark gray.

Ecology and distribution: Rare, in hygrophilous (moisture-loving) deciduous forests in summer and autumn, under trees (beech, hornbeam and oak), on calcareous soils, in groups of 6-8 specimens. Reported from Mediterranean regions.

Specimen examined: Türkiye. Tokat province, Avlunlar village, in a mixed forest on calcareous soil. 24.11.2018, 40°32ˈ35"N, 36°45ˈ41"E, 1146 m, HIS-47.

3.2. Phylogeny

Approximately a 580 bp ITS and 960 bp LSU gene sequences for *C. strenuipes* are generated in this study with accession numbers PP425965 and PP425973, respectively. The nrITS data set included 865 sites, of which 582 were conserved, 105 were variable, and 61 were parsimony informative. According to ITS sequence analyses, the Turkish specimen was identified as belonging to *C. strenuipes*, where each species formed a monophyletic clade with high bootstrap support (Fig. 3). Within the clade, sequence analyses between our collection and the remaining species indicated an intraspecific genetic

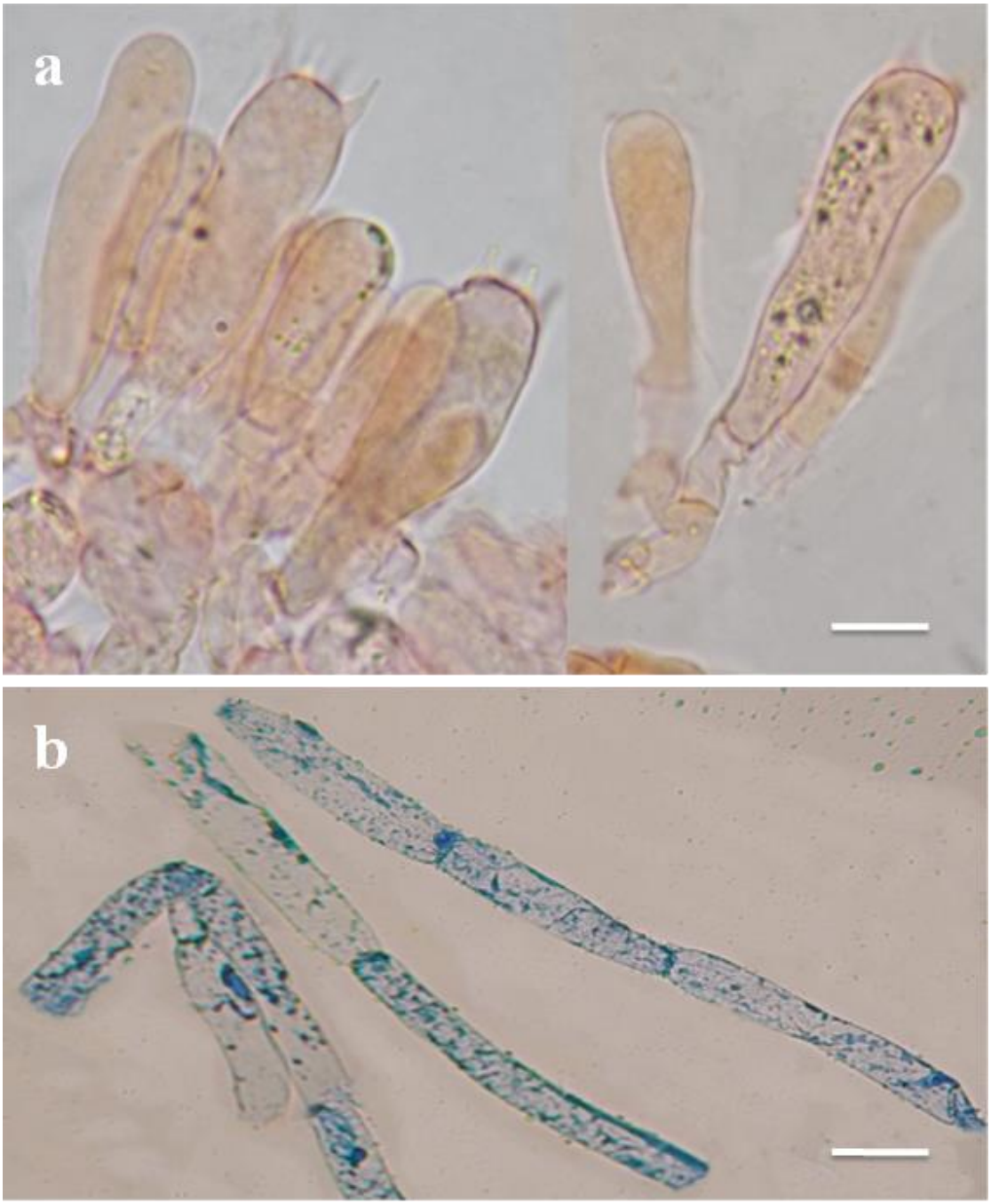

Figure 2. *Cortinarius strenuipes* (Collection HIS-47): a- basidia and basidiole, b- pileipellis (Scale bars: a = 10 µm, b = 30 µm).

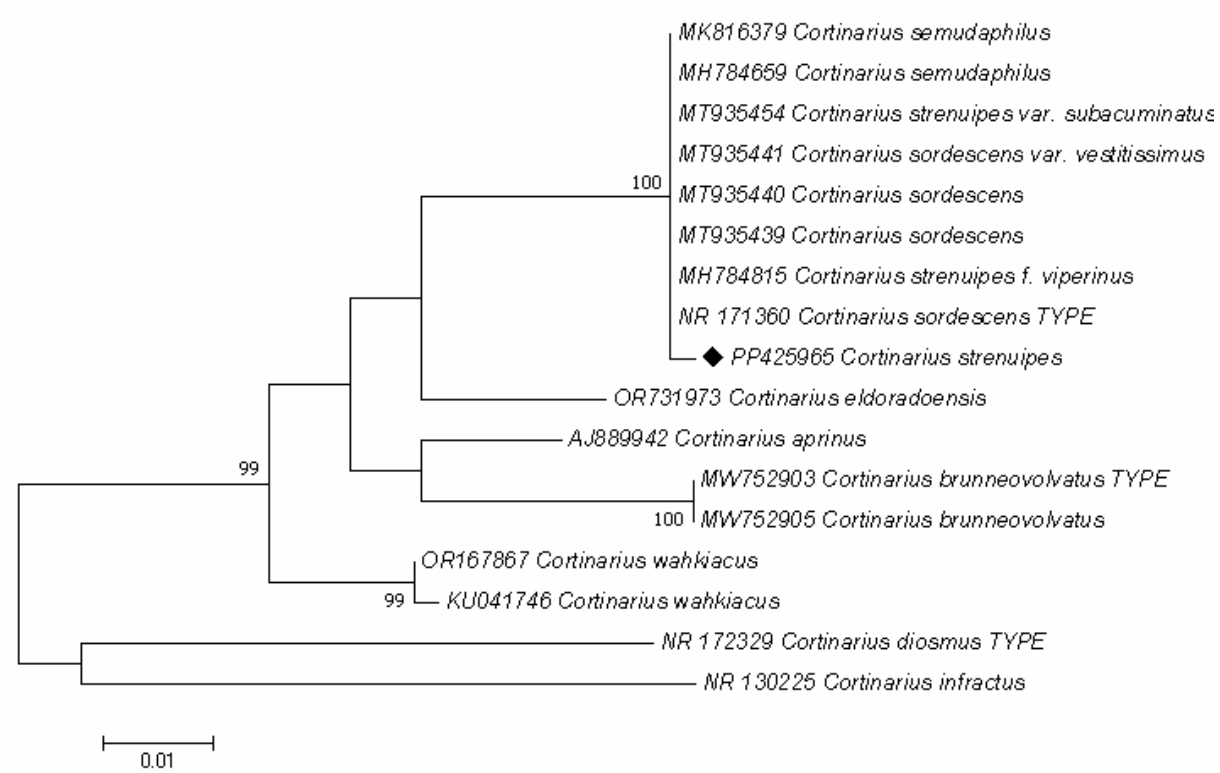

Figure 3. Maximum likelihood tree generated from nrITS sequences belonging to Turkish collection (labelled in diamond) in this study and closely related taxa. Bootstrap support values ≥ 50% from ML analysis were shown on the branches. Bar indicates 0.01 expected change per site per branch.

variation of 3 to 6 bp. Analysis with LSU sequence did not show any significant results since no sequence from this gene region existed for the studied specimen and closely related species for an accurate comparison. LSU based tree is uninformative, thus is not presented here.

4. Discussions

This study reports *Cortinarius strenuipes* Rob. Henry for the first time from Türkiye with molecular data and morphological description. This species belongs to C. subg. Dermocybe (Fr.) Trog, C. sect. Sericeocybe (P.D. Orton) Melot., and C. subsect. Strenuipedes (Moser and Horak, 1975). Its characteristic features include a gray or reddish-brown, hygrophanous, fibrous pileus; cylindrical, slightly bulbous at the base, brownish fibrillose stipe; loosely packed, thick, ocher brown to chocolate brown lamella. It is a rare species reported only from Mediterranean regions including Spain and France in *Quercus ilex* L. forests on calcareous soil (Henry, 1956; Mahiques, 2010; Muñoz, 2018).

According to IndexFungorum (2024), this taxon appears in two forms and two varieties, which are *Cortinarius strenuipes* f. *strenuipes*, *Cortinarius strenuipes* f. *viperinus* Reumaux, *Cortinarius strenuipes* var. *strenuipes* and *Cortinarius strenuipes* var. *subacuminatus* Rob. Henry ex Reumaux. The latter species has shorter pileus and stipe, cream-colored and rusty brown lamella, slightly smaller basidiospores when compared to *C. strenuipes* Rob. Henry, and differs from *C. strenuipes* var. *strenuipes* based on negative reaction to phenol anilin and positive for guaiac tincture (Bidaud et al., 2002; Mahiques et al. 2013). More recently, Liimatainen et al. (2020) used the name of *C. sordescens* Rob. Henry as the current name of *C. strenuipes* var. *subacuminatus* based on molecular analyses.

In the literature, there is no molecular sequence data exists for *C. strenuipes* Rob. Henry. This study provides the first molecular data from this species. While *C. strenuipes* and its varieties macroscopically differ from each other, sequence analysis revealed high sequence identity among them. Our phylogenetic analysis showed that *C. strenuipes* is closely related to *C. strenuipes* var. *strenuipes*, *C. strenuipes* f. *viperinus*, and *C. sordescens* with high support and clustered them together. The observation of little intraspecific genetic variation among them could be due to no enough time to for ITS sequence divergence. It is clear that multiple collections of this taxon from different geographical locations should be analzyed for a better explanation of morphological and genetic variations.

Cortinarius is an extremely difficult genus for species identification due to overlapping characters among its species. In the last decade, studies including DNA sequence analyses have helped delimitation problems. It is evident that both molecular and morphological investgation should be conducted for accurate nomenclature of *Cortinarius* species.

Conflict of nterest

Authors have declared no conflict of interest.

Authors' Contribution

Dr. Şengül Demirak conducted the molecular studies, wrote original draft, revised and edited the manuscript. Drs. Türkekul and Işık conducted morphological studies.

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Macromycetes determined in Baykan (Siirt) district

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Baykan (Siirt) ilçesinde belirlenen makromantarlar

Abstract: The current study was based on the macrofungi collected from Baykan (Siirt) district. As a result of field and laboratory studies, 88 species belonging to 56 families were identified. Nine taxa belong to *Ascomycota* and 79 to *Basidiomycota*. Considering the taxonomic categories of the species identified in the research area, it is seen that the order, family and genus represented by the most members are *Agaricales*, *Agaricaceae* and *Agaricus* L., respectively. The identified species are listed along with their localities, habitats/substrates, geographical positions, collection dates, and voucher numbers. All of the determined species are new records for the study area.

Key words: Biodiversity, macrofungi, Siirt, Baykan, Türkiye

Özet: Mevcut çalışma Baykan (Siirt) ilçesinden toplanan makromantarlar üzerinde yapılmıştır. Arazi ve laboratuvar çalışmaları sonucunda 56 familya'ya mensup 88 tür teşhis edilmiştir. Belirlenen taksonlardan dokuzu *Ascomycota* ve 79'u *Basidiomycota* bölümlerinde yer almaktadır. Araştırma alanında tespit edilen türlerin taksonomik kategorileri dikkate alındığında, en çok üye ile temsil edilen takım, familya ve cinsin sırasıyla; *Agaricales*, *Agaricaceae* ve *Agaricus* L. olduğu görülmektedir. Teşhis edilen türler, lokalitesi, habitatı/substratı, toplandığı coğrafi koordinat, toplanma tarihi ve toplayıcı numaraları ile birlikte listelenmiştir. Tespit edilen türlerin tamamı araştırma alanı için yeni kayıttır.

Anahtar Kelimeler: Biyoçeşitlilik, makromantarlar, Siirt, Baykan, Türkiye

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1. Introduction

Baykan (Siirt) district is 47 km away from cenral district of Siirt Province. It is surrounded by Şirvan to the east, Kurtalan and Kozluk (Batman) to the west, Siirt (central district) to the south, and Bitlis to the north. Continental climate prevails in the district. Summers are hot and dry, winters are cold and rainy. The district center is situated in a narrow valley and surrounded by high hills (Figure 1).

Macrofungi, are a diverse group of organisms that form fruiting bodies which can be seen by the naked eye, and handled by hand (Berber et al., 2022). They are an important natural source of food and medicine and have great importance in the ecosystem, especially in ecology, pathology (Kamble et al. 2021). Members of macrofungi may be edible, unedible, or poisonous, and some species are used in traditional medicine for prevention and treatment of different diseases (Badalyan & Rapior, 2020).

According to the latest checklist studies (Sesli et al., 2020; Solak and Türkoğlu, 2022), around 2750 macrofungi species have been identified in Türkiye. However, considering the current plant richness of our country, this number is expected to be much higher. By the presentation of contributory studies (Kesici et al., 2022; Acar and Dizkırıcı, 2023; Akata et al., 2023; Akçay et al., 2023; Kaplan et al., 2023; Sesli, 2023a,b; Uzun and Kaya, 2023a,b; Karaduman et al., 2024) on macrofungi of Türkiye, the existing number of species is continiously being updated.

The aim of this study is to determine the macrofungal diversity of Baykan (Siirt) district, and to make a contribution to Turkish mycobiota.

2. Materials and Method

The macrofungi specimens were collected periodically from Baykan (Siirt) district between 2011 and 2012. Relevant morphological and ecological characteristics of the samples were noted and they were photographed in their natural habitats. Then the samples were taken to the fungarium, and dried in an air conditioned room. The dried samples were put in zip lock polyethylene bags, and kept as fungarium materials. Microscopic investigations were carried out on dried samples. After obtaining necessary macroscopic and microscopic data, they were identified with the help of relevant literature (Phillips, 1981; Buczacki, 1989; Breitenbach and Krânzlin, 1984-2000; Bresinsky and Besl, 1990; Hansen and Knudsen, 1992, 2000; Jordan, 2004; Dähncke, 2004; Bessette et al., 1997, 2007; Krânzlin, 2005; Bessette and Bessette, 2006; Antonin and Noordeloos, 2010; Beug et al., 2014; Siegel and Schwarz, 2016). The specimens are kept at Yüzüncü Yıl University.

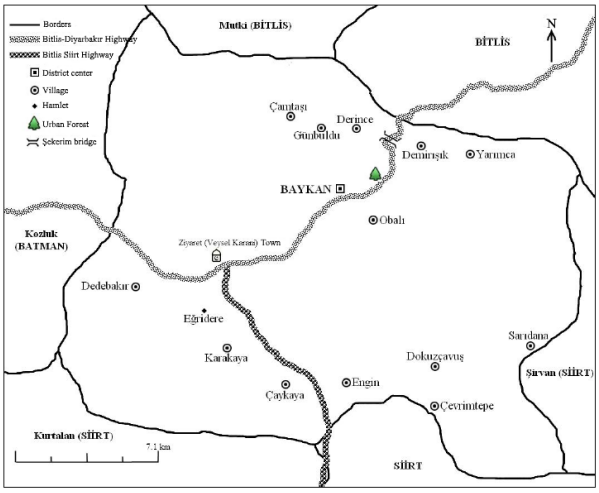

Figure 1. Map of study area.

3. Results

The determined taxa are listed in alphabethical order. Current names of the species are in accordance with IndexFungorum (2024). Each taxa are presented together with their localities, habitats, geogharphical position, collecting dates and voucher numbers.

Ascomycota Whittaker

Pezizomycetes O.E. Erikss. & Winka

Pezizales J. Schröt.

Ascobolaceae Boud. ex Sacc.

1. ***Ascobolus furfuraceus*** Pers.

Near the Şekerim Bridge, on dung, 38º11.809′N, 41º49.015′E, 775 m, 03.11.2012, BYK. 1624.

Helvellaceae Fr.

2. ***Helvella acetabulum*** (L.) Quél.

Derince village, under *Quercus* sp. trees, 38º12.123′N, 41º48.151′E, 821 m, 27.04.2011, BYK. 1373.

3. ***Helvella lacunosa*** Afzel.

Near the Şekerim Bridge, under *Populus* sp. trees, 38º11.708′N, 41º49.026′E, 790 m, 27.04.2011

4. ***Helvella leucopus*** Pers.

Derince village, in mixed forest, 38º12.247′N, 41º47.966′E, 809 m, 27.04.2011, BYK. 1360.

Morchellaceae Rchb.

5. ***Morchella crassipes*** (Vent.) Pers.

Near the Şekerim Bridge, in mixed forest, 38º11.809′N, 41º49.015′E, 775 m, 27.04.2011, BYK. 1343.

6. ***Morchella elata*** Fr.

Gülbuldu village, near the mosque, in mixed forest, 38º12.341′N, 41º47.534′E, 968 m, 27.04.2011, BYK. 1379.

7. ***Morchella esculenta*** (L.) Pers.

Sarıdana village, near the fountain, under *Prunus* sp. trees, 38º05.348′N, 41º53.861′E, 1154 m, 28.04.2011, BYK. 1431.

Pezizaceae Dumort.

8. ***Peziza granularis*** Donadini

Demirışık village, in mixed forest, 38°11.406′N, 41°50.145′E, 880 m, 30.11.2012, BYK. 1906.

9. ***Peziza praetervisa*** Bres.

Baykan Urban Forest, under Conifer trees, burnt area, 38º10.712′N, 41º48.460′E, 727 m, 01.12.2012, BYK. 1928.

Basidiomycota R.T. Moore

Agaricomycetes Doweld

Agaricales Underw.

Agaricaceae Chevall.

10. ***Agaricus bisporus*** (J.E. Lange) Imbach

Dedebakır village, near the *Quercus* sp. forest, meadow, 38°07.123′N, 41°39.517′E, 828 m, 05.11.2012, BYK. 1678.

11. ***Agaricus campestris*** L.

District center, near the Baykan State Hospital, conifer forest clearance, meadow, 38°09.628′N, 41°47.001′E, 720 m, 17.11.2012, BYK. 1863.

12. ***Agaricus comtulus*** Fr.

Near the Şekerim Bridge, conifer forest clearance, meadow, 38º11.806′N, 41º49.018′E, 754 m, 15.11.2012, BYK. 1711.

13. ***Agaricus langei*** (F.H. Møller) F.H. Møller

Baykan Urban Forest, under conifer trees, 38°10.712′N, 41°48.460′E, 727 m, 01.12.2012, BYK. 1947.

14. ***Agaricus pseudopratensis*** (Bohus) Wasser

Near the Şekerim Bridge, edge of agricultural land, meadow, 38º11.806′N, 41º49.018′E, 754 m, 12.05.2011, BYK. 1712.

15. ***Agaricus sylvicola*** (Vittad.) Pec

Baykan Urban Forest, under conifer trees, 38°10.712′N, 41°48.460′E, 727 m, 01.12.2012, BYK. 1946.

16. ***Coprinus comatus*** (O.F. Müll.) Pers.

Karakaya village, Eğridere hamlet, under *Quercus* sp. trees, 38º03.875′N, 41º46.565′E, 559 m, 12.05.2011, BYK. 1555

17. ***Cyathus olla*** (Batsch) Pers.

Near the Şekerim Bridge, on woody remains, 38º11.809′N, 41º49.015′E, 775 m, 30.11.2012, BYK. 1901.

18. ***Lepiota cristatoides*** Einhell.

Derince village, near the *Juglans* sp. trees, meadow, 38°12.267′N, 41°47.977′E, 821 m, 12.05.2011, BYK. 1607.

19. ***Lepiota ignivolvata*** Bousset & Joss. ex Joss.

District center, near the Baykan State Hospital, under conifer trees, 38°09.628′N, 41°47.001′E, 720 m, 17.11.2012, BYK. 1866.

20. ***Leucoagaricus barssii*** (Zeller) (Zeller) Vellinga

Demirışık village, in mixed forest, 38°11.406′N, 41°50.145′E, 880 m, 17.11.2012, BYK. 1913.

21. ***Leucoagaricus leucothites*** (Vittad.) Wasser

District center, Gümüşhane neighbourhood, meadow, 38º10.093′N, 41º47.161′N, 815 m, 02.12.2012, BYK. 2024.

22. ***Leucoagaricus serenus*** (Fr.) Bon & Boiffard

Derince village, in mixed forest, 38º12.267′N, 41º47.977′E, 821 m, 12.05.2011, BYK. 1604.

23. ***Lycoperdon decipiens*** Durieu & Mont.

Batman provincial border, under conifer trees, 38º08.759′N, 41º38.928′E, 861 m, 28.04.2011, BYK. 1975.

24. ***Lycoperdon excipuliforme*** (Scop.) Pers.

District center, near the Baykan State Hospital, under conifer trees, 38°09.628′N, 41°47.001′E, 720 m, 02.12.2012, BYK. 1864.

25. ***Macrolepiota excoriata*** (Schaeff.) Wasser

Demirışık village, under *Quercus* sp. trees, 38°11.406′N, 41°50.145′E, 880 m, 30.11.2012, BYK. 1914.

26. ***Macrolepiota mastoidea*** (Fr.) Singer

Gümüşkaş village, under conifer trees, 38º08.905′N, 41º38.941′E, 830 m, 04.11.2012, BYK. 1654.

Amanitaceae R. Heim ex Pouzar

27. ***Amanita argentea*** Huijsman

Demirışık village, under *Quercus* sp. trees, 38°11.406′N, 41°50.145′E, 880 m, 15.11.2012, BYK. 1743.

28. ***Amanita magniverrucata*** Thiers & Ammirati

Karakaya village, Eğridere hamlet, under *Quercus* sp. trees, 38º03.875′N, 41º46.565′E, 559 m, 13.05.2011, BYK. 1562.

29. ***Amanita phalloides*** (Vaill. ex Fr.) Link

Demirışık village, under *Quercus* sp. trees, 38°11.406′N, 41°50.145′E, 880 m, 15.11.2012, BYK. 1744.

30. ***Amanita proxima*** Dumée

Eğridere hamlet entrance, under *Quercus* sp. trees, 38º06.374′N, 41º42.734′E, 745 m, 02.12.2012, BYK. 1994.

Bolbitiaceae Singer

31. ***Bolbitius titubans*** (Bull.) Fr.

Dedebakır Village Cemetery, meadow, 38°07.123′N, 41°39.517′E, 828 m, 16.11.2012, BYK. 1791.

32. ***Conocybe pulchella*** (Velen.) Hauskn. & Svrček

Opposite Baykan district center, under conifer trees, 38º09.725′N, 41º47.473′E, 716 m, 17.11.2012, BYK. 1875.

Hymenogastraceae Vittad.

33. ***Psilocybe coronilla*** (Bull.) Noordel.

Çevrimtepe village, around Şeyh Ali tomb, meadow, 38º04.630′N, 41º50.757′E, 1243 m, 17.11.2012, BYK. 1849.

Inocybaceae Jülich

34. ***Inocybe nitidiuscula*** (Britzelm.) Lapl.

Derince village, under *Quercus* sp. trees, 38º12.123′N, 41º48.151′E, 821 m, 27.04.2011, BYK. 1477.

35. ***Inocybe pyriodora*** (Pers.) P. Kumm.

Gümüşkaş village, under conifer trees, 38º08.759′N, 41º38.928′E, 861 m, 28.04.2011, BYK. 1467.

36. ***Pseudosperma rimosum*** (Bull.) Matheny & Esteve-Rav.

Near the Şekerim Bridge, in mixed forest, 38º11.806′N, 41º49.018′E, 754 m, 15.11.2012, BYK. 1707.

Incertae sedis

37. ***Clitocybe rivulosa*** (Pers.) P. Kumm.

Eğridere hamlet entrance, under *Quercus* sp. trees, 38º06.374′N, 41º42.734′E, 745 m, 02.12.2012, BYK. 2005.

38. ***Lepista irina*** (Fr.) H.E. Bigelow

Baykan Urban Forest, under conifer trees, 38º10.712′N, 41º48.460′E, 727 m, 01.12.2012, BYK. 1927.

39. ***Lepista nuda*** (Bull.) Cooke

Baykan district entrance, under conifer trees, 38º10.396′N, 41º48.880′E, 719 m, 01.12.2012, BYK. 1923.

40. ***Lepista personata*** (Fr.) Cooke

Near the Şekerim Bridge, under *Populus* sp. trees, 38º11.708′N, 41º49.026′E, 790 m, 02.12.2012, BYK. 2042.

41. ***Melanoleuca exscissa*** (Fr.) Singer

Obalı village, meadow, 38º09.566′N, 41º48.651′E, 798 m, 01.12.2012, BYK. 1954.

42. ***Melanoleuca graminicola*** Kühner & Maire

Baykan district entrance, under conifer trees, 38°10.396′N, 41°48.880′E, 719 m, 01.12.2012, BYK. 1918.

Marasmiaceae Roze ex Kühner

43. ***Marasmius rotula*** (Scop.) Fr.

Demirışık village, on herb remains, 38°11.406′N, 41°50.145′E, 880 m, 30.11.2012, BYK. 1909.

44. ***Omphalotus olearius*** (DC) Singer

Gümüşkaş village, under conifer trees, 38°08.905′N, 41°38.941′E, 830 m, 16.11.2012, BYK. 1761.

Mycenaceae Overeem

45. ***Hemimycena angustispora*** (P.D. Orton) Singer

District center, under conifer trees, on pine cone remains, 38º00.725′N, 41º47.473′E, 716 m, 02.12.2012, BYK. 2016.

46. ***Mycena filopes*** (Bull.) P. Kumm.

Opposite Baykan district center, under conider trees, 38º09.725′N, 41º47.473′E, 716 m, 17.11.2012, BYK. 1880.

47. ***Mycena pura*** (Pers.) P. Kumm.

District center, near the Baykan State Hospital, under conifer trees, 38°09.628′N, 41°47.001′E, 720 m, 17.11.2012, BYK. 1870.

Physalacriaceae Corner

48. ***Armillaria ostoyae*** (Romagn.) Herink

Dedebakır village, on log, 38°07.123′N, 41°39.517′E, 828 m, 16.11.2012, BYK. 1796.

49. ***Armillaria solidipes*** Peck

Dedebakır village, on log, 38°07.123′N, 41°39.517′E, 828 m, 16.11.2012, BYK. 1793.

Pleurotaceae Kühner

50. ***Hohenbuehelia petaloides*** (Bull.) Schulzer

Gümüşkaş village, on conifer log, 38º08.759′N, 41º38.928′E, 861 m, 28.04.2011, BYK. 1468.

51. ***Hohenbuehelia tremula*** (Schaeff.) Thorn & G.L. Barron

Baykan Urban Forest, under conifer trees, 38º10.712′N, 41º48.460′E, 727 m, 01.12.2012, BYK. 1926.

52. ***Pleurotus eryngii*** (DC.) Quél.

Çevrimtepe village, on *Ferulae* sp. remnants, 38º03.741′N, 41º50.836′E, 1012 m, 12.05.2012, BYK. 2043.

53. ***Pleurotus ostreatus*** (Jacq.) P. Kumm.

Engin village, around TPO water tank, on *Salix* sp. trees, 38º03.609′N, 41º46.835′E, 569 m, 02.12.2012, BYK. 2013.

Pluteaceae Kotl. & Pouzar

54. ***Pluteus salicinus*** (Pers.) P. Kumm.

Near the Şekerim Bridge, in mixed forest, 38º11.809′N, 41º49.015′E, 775 m, 15.11.2012, BYK. 1696.

55. ***Volvariella caesiotincta*** P.D. Orton

Gümüşkaş village, under conifer trees, 38º08.759′N, 41º38.928′E, 861 m, 12.05.2011, BYK. 1527.

56. ***Volvopluteus gloiocephalus*** (DC.) Justo

Demirışık village, meadow, 38°11.406′N, 41°50.145′E, 880 m, 30.11.2012, BYK. 1911.

Psathyrellaceae Vilgalys, Moncalvo & Redhead

57. ***Candolleomyces candolleanus*** (Fr.) D. Wächt. & A. Melzer

Near the Şekerim Bridge, in mixed forest, 38º11.809′N, 41º49.015′E, 775 m, 30.11.2012, BYK. 1896.

58. ***Coprinellus disseminatus*** (Pers.) J.E. Lange

Dokuzçavuş village, near woody remains, 38º04.630′N, 41º50.757′E, 1243 m, 17.11.2012, BYK. 1848.

59. ***Coprinellus micaceus*** (Bull.) Vilgalys, Hopple & Jacq. Johnson

Dokuzçavuş village, meadow, 38º04.630′N, 41º50.757′E, 1243 m, 17.11.2012, BYK. 1851.

60. ***Coprinopsis picacea*** (Bull.) Redhead, Vilgalys & Moncalvo

Dokuzçavuş village, cemetery, in mixed forest, 38°04.584′N, 41°49.961′E, 1233 m, 17.11.2012, BYK. 1859.

61. ***Panaeolus papilionaceus*** (Bull.) Quél.

Çevrimtepe village, around Şeyh Ali tomb, on dung, 38º03.465′N, 41º50.369′E, 954 m, 02.12. 2012, BYK. 1992.

62. ***Parasola auricoma*** (Pat.) Redhead, Vilgalys & Hopple.

Baykan Urban Forest, under conifer trees, 38°06.580′N, 41°41.481′E, 788 m, 17.11.2012, BYK. 1890.

63. ***Psathyrella olympiana*** A.H. Sm.

Near the Şekerim Bridge, streamside, in mixed forest, 38º11.806′N, 41º49.018′E, 754 m, 15.11.2012, BYK. 1727.

64. ***Psathyrella phegophila*** Romagn.

Near the Şekerim Bridge, field edge, 38º11.809′N, 41º49.015′E, 775 m, 30.11.2012, BYK. 1890.

65. ***Psathyrella pseudogracilis*** (Romagn.) M.M. Moser

Near the Şekerim Bridge, streamside, under *Platanus* sp. tree, 38º11.806′N, 41º49.018′E, 754 m, 15.11.2012, BYK. 1721.

Pseudoclitocybaceae Vizzini, Consiglio, P.-A. Moreau & P. Alvarado

66. ***Pseudoclitocybe expallens*** (Pers.) M.M. Moser

Obalı village, meadow, 38º09.566′N, 41º48.651′E, 798 m, 01.12.2012, BYK. 1955.

Strophariaceae Singer & A.H. Sm.

67. ***Agrocybe paludosa*** (J.E. Lange) Kühner & Romagn. ex Bon

Opposite Baykan district center, under conifer trees, on moss, 38º09.725′N, 41º47.473′E, 716 m, 12.05.2012, BYK. 1520.

68. ***Cyclocybe cylindracea*** (DC.) Vizzini & Angelini

Yarımca village, on log, 38º11.244′N, 41º51.929′E, 1064 m, 5.11.2012, BYK. 1754

69. ***Deconica coprophila*** (Bull.) P. Karst.

Çevrimtepe village, around Şeyh Ali tomb, on dung, 38º03.456′N, 41º50.406′E, 937 m, 28.04. 20112, BYK. 1483.

70. ***Pholiota decussata*** (Fr.) M.M. Moser

Demirışık village, under *Quercus* sp. trees, 38°11.406′N, 41°50.145′E, 880 m, 30.11.2012, BYK. 1917.

71. ***Protostropharia semiglobata*** (Batsch) Redhead, Moncalvo, Vilgalys

Baykan Urban Forest, on dung, 38º10.712′N, 41º48.460′E, 729 m, 01.12.2012, BYK. 1936.

Tricholomataceae R. Heim ex Pouzar

72. ***Tricholoma virgatum*** (Fr.) P. Kumm.

Baykan district entrance, under conifer trees, 38°10.396′N, 41°48.880′E, 719 m, 01.12.2012, BYK. 1919.

Tubariaceae Vizzini

73. ***Tubaria furfuracea*** (Pers.) Gillet

Near the Şekerim Bridge, under *Populus* sp. trees, 38º11.806′N, 41º49.018′E, 754 m, 03.11.2012, BYK. 1594.

Boletales E.-J. Gilbert

Boletaceae Chevall.

74. ***Butyriboletus fechtneri*** (Velen.) D. Arora & J.L. Fr

Demirışık village cemetery, under *Quercus* sp. trees, 38°11.406′N, 41°50.145′E, 880 m, 17.11.2012, BYK. 1902.

75. ***Rubroboletus lupinus*** (Fr.) Costanzo, Gelardi, Simonini & Vizzini

Demirışık village cemetery, under *Quercus* sp. trees, 38°11.406′N, 41°50.145′E, 880 m, 15.11.2012, BYK. 1746.

76. ***Rubroboletus satanas*** (Lenz) Kuan Zhao & Zhu L. Yang

Eğridere hamlet entrance, under *Quercus* sp. trees, 38º06.362′N, 41º42.740′E, 743 m, 13.05.2011, BYK. 1565.

Gomphidiaceae Maire ex Jülich

77. ***Chroogomphus rutilus*** (Schaeff.) O.BYK. Mill.

Baykan Urban Forest, under conifer trees, 38º10.289′N, 41º48.678′E, 758 m, 12.05.2011, BYK. 1508.

Rhizopogonaceae Gäum. & C.W. Dodge

78. ***Rhizopogon luteolus*** Fr.

Baykan district entrance, under conifer trees, 38º10.396′N, 41º48.880′E, 719 m, 01.12.2012, BYK. 1925.

Suillaceae Besl & Bresinsky

79. ***Suillus collinitus*** (Fr.) Kuntze

Gümüşkaş village, under conifer trees, 38º08.905′N, 41º38.941′E, 830 m, 16.11.2012, BYK. 1766.

80. ***Suillus granulatus*** (L.) Roussel

Baykan district entrance, under conifer trees, 38º10.396′N, 41º48.880′E, 719 m, 01.12.2012, BYK. 1921.

Gomphales Jülich

Gomphaceae Donk

81. ***Ramaria gracilis*** (Pers.) Quél

Baykan Urban Forest, under conifer trees, 38º10.712′N, 41º48.460′E, 727 m, 01.12.2012, BYK. 1938.

Polyporales Gäum.

Fomitopsidaceae Jülich

82. Laetiporus sulphureus (Bull.) Murrill

Günbuldu village, on log, 38º11.945′N, 41º47.982′E, 898 m, 27.04.2011, BYK. 1387.

Polyporaceae Fr. ex Corda

83. ***Cerioporus squamosus*** (Huds.) Quél.

Obalı village, on *Salix* sp. trees, 38º09.566′N, 41º48.651′E, 798 m, 08.05.2010. BYK. 1318.

84. ***Lentinus tigrinus*** (Bull.) Fr.

Dedebakır village, on log, 38°07.123′N, 41°39.517′E, 828 m, 04.11.2012, BYK. 1660.

Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David

Russulaceae Lotsy

85. ***Lactarius deliciosus*** (L.) Gray

Baykan district entrance, under conifer trees, 38º09.725′N, 41º47.473′E, 716 m, 02.12.2012, BYK. 2017.

86. ***Russula delica*** Fr.

Çaykara village, under *Quercus* sp. trees, 38º03.895′N, 41º46.558′E, 566 m, 12.05.2011, BYK. 1535

87. ***Russula pallidospora*** J. Blum ex Romagn.

Karakaya village, Eğridere hamlet, under *Quercus* sp. trees, 38º03.875′N, 41º46.565′E, 559 m, 13.05.2011, BYK. 1544.

Stereaceae Pilát

88. ***Stereum hirsutum*** (Willd.) Pers.

Gümüşkaş village, on conifer log, 38º08.905′N, 41º38.941′E, 830 m, 01.12.2012, BYK. 1973.

4. Discussions

Eighty-eight macromycete species were determined from the research area. Nine of them (%10.23) belong to *Ascomycota* and seventy nine (%89.77) to *Basidiomycota*. The taxa are distributed in 2 classes (*Agaricomycetes* 79, *Pezizomycetes* 9) and 6 orders. The order-wise distribution of the determined taxa is presented in Figure 2.

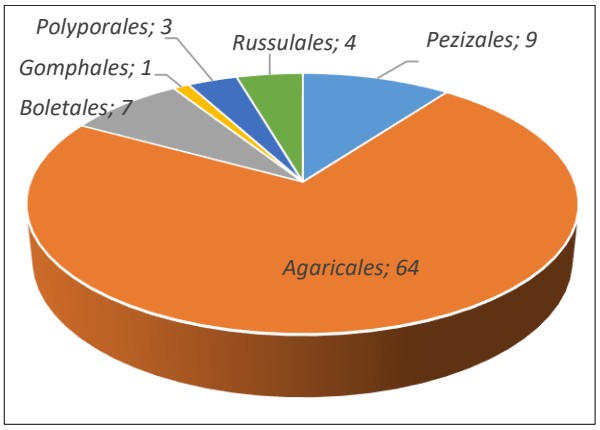

Figure 2. Distribution of identified taxa into orders

The taxa are distributed in 29 families and 56 genera. Six of the determined 88 species are currently in Incertae sedis position at the family level.

Agaricaceae is the most crowded family with 17 taxa. *Psathyrellaceae* is the second with 9 taxa. *Strophariaceae* comprise 5 taxa. *Amanitaceae* and *Pleurotaceae* comprise 4 taxa. In the region seven families (*Helvellaceae*, *Morchellaceae*, *Inocybaceae*, *Pluteaceae*, *Mycenaceae*, *Boletaceae*, *Russulaceae*) are represented with three taxa, and six families (*Pezizaceae*, *Bolbitiaceae*, *Marasmiaceae*, *Physalacriaceae*, *Suillaceae*, *Polyporaceae*) are represented with two taxa, while the rest of ten families are represented with only one taxon.

The genus *Agaricus* L. was found to be the most crowded genus in the area with 6 taxa. It is followed by *Amanita* Pers. with 4 taxa. *Helvella* L., *Morchella* Dill. ex Pers., *Lepista* (Fr.) W.G. Sm., *Leucoagaricus* Locq. ex Singer and *Psathyrella* (Fr.) Quél. are represented with 3 taxa, while *Armillaria* (Fr.) Staude, *Coprinellus* P. Karst., *Hohenbuehelia* Schulzer, *Inocybe* (Fr.) Fr., *Lepiota* (Pers.) Gray, *Lycoperdon* Pers., *Macrolepiota* Singer, *Melanoleuca* Pat., *Mycena* (Pers.) Roussel, *Peziza* Dill. ex Fr., *Pleurotus* (Fr.) P. Kumm., *Rubroboletus* Kuan Zhao & Zhu L. Yang, *Russula* Pers. and *Suillus* Gray are represented with 2 taxa, other 35 genera are represented in the region with only one taxon.

Forty seven of the taxa are edible, 30 are inedible while 11 are more or less poisonous. Although the research area is very rich in terms of edible species, local people do not consume many unfamiliar mushrooms for fear of being poisonous. Tough %53.41 of the determined taxa are edible, only two of them (*Agaricus campestris* and *Pleurotus eryngii*) are collected and consumed by the locals.

The species identified in the district show some similarities with those determined by nearby investigations. The details of these investigations and the corresponding similarity percentages are provided in Table 1. The likely reason for this similarity and dissimilarity could be the shared vegetation and climate.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

Table 1. The percentage of similarity of the findings to the investigations conducted in neighboring districts

Study	Study area	Total taxa	Number of identical taxa	Similarity percentage (%)
Kaya (2001)	Bitlis	60	18	30.00
Demir et al. (2007)	Batman	50	17	34.00
Acar et al. (2015)	Hani (Ağrı)	101	23	22.77
Demirel et al. (2015)	Van	122	27	22.13
Demirel and Dengiz (2016)	Şirvan (Siirt)	53	15	28.31
Demirel et al. (2016)	Lice (Diyarbakır)	55	18	32.72
Sadullahoğlu and Uzun (2020)	Karz Dağı (Tatvan-Bitlis)	95	17	17.89

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