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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.

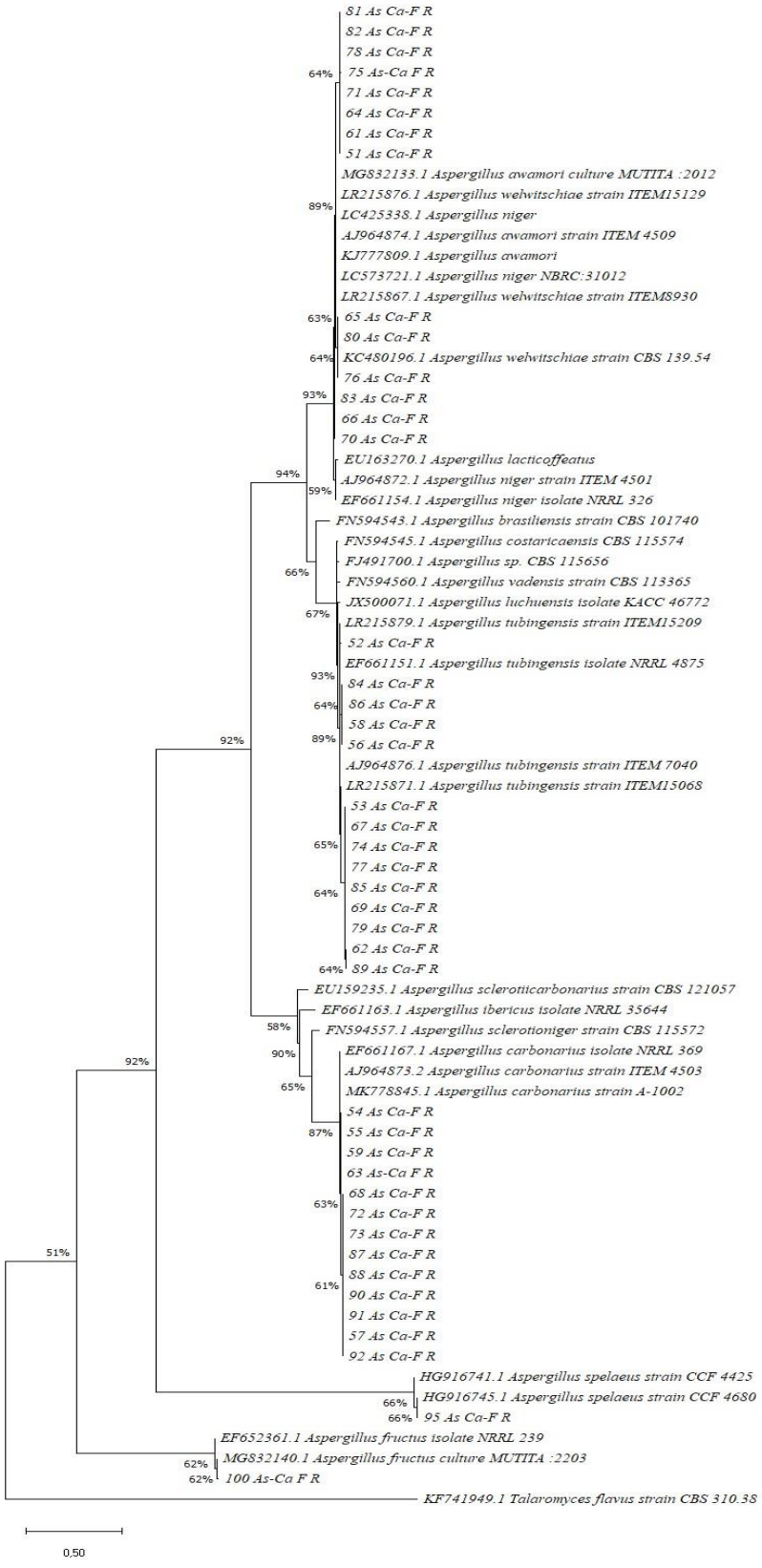

Figure 1. Maximum Likelihood Tree Tamura-Nei model was chosen for maximum likelihood analysis. Bootstrap is set to 1000 replicas. Branch arrangements are selected as TBR. KF741949.1 *Talaromyces flavus* strain CBS 310.38 calmodulin gene, partial cds was used as the outgroup.

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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