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Expression Variations of *MBF*-1, *PKS* and *psbA* Genes in *Umbilicaria decussata* Collected from Horseshoe Island (Antarctic Peninsula) and the Southern Coast of Türkiye

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Abstract: Lichens adapted to extreme conditions through gene expression changes, help monitor environmental and climate change impacts. In this study, *Umbilicaria decussata* (Vill.) Zahlbr. lichen samples were collected with from two different regions: from the Horseshoe Island, located to the west of the Antarctic Peninsula and from the south coast of Türkiye. The *nrITS* gene regions of the samples were studied and the phylogenetic positions of the samples were confirmed as *U. decussata*. After DNA barcoding, comparative determinations of the changes in the expressions of the *multiprotein binding factor-1* (*MBF*-1), *polyketide synthase* (*PKS*) and *photosystem II D1 protein* (*psbA*) genes due to temperature change stress were performed by a qRT-PCR. Specifically, under cold stress conditions such as those found on the Horseshoe Island, the *MBF*-1 gene was highly expressed, while *PKS* and *psbA* gene expression levels were lower compared to the south coast of Türkiye. Specimen collected from different geographical regions exhibited gene expression differences. The results of this study, which is the first in its field, highlight how the adaptation of *Umbilicaria decussata* samples to those habitats and regional climate.

Horseshoe Adası (Antarktika Yarımadası) ve Türkiye'nin Güney Sahilinden Toplanan *Umbilicaria decussata*'da *MBF*-1, *PKS* ve *psbA* Genlerinin İfade Varyasyonları

Anahtar Kelimeler
Antarktika,
İklim değişikliği,
Kutup Biyolojisi,
Gen ekspresyonu,
qRT-PCR

Öz: Likenler gen ifadesi değişiklikleri yoluyla aşırı koşullara adapte olarak, çevresel ve iklim değişikliği etkilerini izlemeye yardımcı olmaktadır. Bu çalışmada *Umbilicaria decussata* (Vill.) Zahlbr. liken örnekleri Antarktika Yarımadası'nın batısında yer alan Horseshoe Adası'ndan ve Türkiye'nin güney kıyısından olmak üzere iki farklı bölgeden toplandı. Örneklerin *nrITS* gen bölgeleri incelenmiş ve örneklerin filogenetik pozisyonlarının *U. decussata* olduğu doğrulanmıştır. DNA barkodlaması sonrasında, *multiprotein bağlama faktörü-1* (*MBF*-1), *poliketid sentaz* (*PKS*) ve *fotosistem II D1 proteini* (*psbA*) genlerinin sıcaklık değişim stresine bağlı olarak ifadelerindeki meydana gelen değişiklikler qPCR ile karşılaştırmalı olarak tespiti yapılmıştır. Spesifik olarak, Horseshoe Adası'nda olduğu gibi soğuk stresi koşullarında *MBF*-1 geni yüksek oranda eksprese edilirken, *PKS* ve *psbA* gen ekspresyonu seviyeleri Türkiye'nin güney kıyılarına göre daha düşüktü. Farklı coğrafi bölgelerden toplanan örnekler gen ekspresyonu farklılıkları sergilemiştir. Alanında bir ilk olan bu çalışmanın sonuçları, *Umbilicaria decussata* örneklerinin söz konusu habitatlara ve bölgesel iklime nasıl uyum sağladığının altını çizmektedir.

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1. Introduction

Antarctica is the southernmost continent in the world and the fifth largest after Asia, Africa, North America and South America. With a surface area of 14.0 million km^2, it is close to twice the size of Australia and 98 % of Antarctica is covered by ice reaching 1.9 km in thickness, which expands to reach the north of the continent. Antarctica is generally not a suitable habitat for plant growth; however, it has an interesting diversity of vegetation [1]. The extreme conditions and isolation from other continents make Antarctica a unique region and the ability to survive in these unusual terrestrial habitats has arisen due to the many exceptional adaptations of organisms living on this continent [2].

Lichens are the most conspicuous and most diverse macro-organisms of Antarctic terrestrial ecosystems. Studies on Antarctica's lichens date back approximately two centuries, but more recently, with the use of DNA-based techniques in taxonomic studies, the determination of the continent's lichen biodiversity has accelerated [3-5]. The number of lichenized fungal species reported from Antarctica now exceeds 500 [6]. In the 20th century, many authors speculated that endemic Antarctic species had a relic origin dating back to pre-Pleistocene times while expanded populations of bipolar and cosmopolitan species originated from more recent colonization [7-9].

This paper focuses on the examination of specimens of *Umbilicaria decussata* (Vill.) Zahlbr. gathered from two distinct geographical regions: Horseshoe Island, situated to the west of the Antarctic Peninsula and the southern coastline of Türkiye. Our primary objective is to compare the variations in gene expression among the *MBF*-1, *PKS* and *psbA* genes which play important roles in response to environmental stressors. *MBF*-1 helps the lichens adapt to stress conditions by regulating gene expression, *PKS* synthesizes protective natural products and *psbA* optimizes the photosynthesis process within this lichen species. *Umbilicaria decussata* is renowned for being a highly adaptable crustose lichen and frequently serves as one of the initial colonizers of recently exposed rock surfaces. Notably, it exhibits a slow growth rate. This particular species thrives in cool temperate and arctic-alpine environments, with a penchant for colonizing siliceous rocks that have been moistened by rain, a characteristic shared in both polar regions [10]. Presence of it is particularly conspicuous in suitable habitats and is notably common on exposed siliceous rocks, particularly in mountainous regions. The distribution of *U. decussata* spans across Europe and North America, with reports of its presence in diverse locations, including the Arctic, Eurasia, Türkiye, South Africa, Central Asia, North and South America and Antarctica. In Antarctica, This is a rather circumpolar common species nearly found everywhere and in Antarctica it is distributed in continental, maritime and subAntarctic parts of the continent [6].

Gene expression analysis is a highly effective molecular technique utilized to assess the impact of various factors on an organism's stress levels. Diverse organisms, including lichens, have contributed to the pool of genes employed in gene expression analyses, as evident in studies by Halici et al., [11], Ying et al. [12], Qin et al. [13] and Bölükbaşı [14]. With the continuous advancements in science and technology, gene expression studies of lichens, among other organisms, have experienced substantial growth. One common thread among all living entities is their capacity to adapt optimally to environmental influences by modulating gene expression within their genetic framework. Specifically, organisms may react to environmental stress conditions by altering the expression levels of a multitude of genes [15, 16].

The *multiprotein binding factor*-1 (*MBF*-1) gene is a transcriptional co-activator, found in the fungal partner of lichens and plays a role in stress tolerance, just as it does in many other organisms. It is primarily involved in the synthesis of MBF1 proteins, which function as transcriptional co-factors, forming a vital link between transcription factors (TFs) and the TATA box-binding protein (TBP). Typically, this gene is activated in response to a range of stressors, including temperature fluctuations, drought, oxidative conditions, salinity, and pathogenic threats [17]. Notably, the overexpression of the *MBF*-1 gene typically leads to heightened stress resistance and an increased capacity to withstand multiple stressors [18-20].

The *polyketide synthase* (*PKS*) gene is responsible for the synthesis of the polyketide synthesis enzyme complex. The *PKS* gene is an important enzyme complex in lichens that plays a role in the synthesis of natural products and secondary metabolites. These products typically originate from the genetic material of the fungal partner. It is known that lichens produce significant numbers of interesting and potentially bioactive polyketide-type metabolites including anthrones, depsides, depsidones, β-orcinol depsidones, and dibenzofurans. Studies have shown that various stress factors (e.g., salt, metal, drought, and oxidative stress) significantly inhibit the transcription and translation of the *PKS* gene encoding the polyketide synthase enzyme [21-24].

Additionally, the *photosystem II D1 protein* (*psbA*) gene is responsible for the synthesis of the D1 protein, which has a key role in structure of PSII core and is important for a protein supercomplex consisting of many photosystem II (PSII) pigment protein complexes. Typically found in the algal (or cyanobacterial) partner of lichens. Photosynthetic processes are heavily influenced by abiotic and biotic stresses [25, 26] causing inhibition of photosynthetic processes in PSII and, as a consequence, reactive oxygen species formation (ROS). Reactive

oxygen derivatives (ROS) formed under stress conditions do not directly cause photodamage to PSII. By oxidative damage, they destroy important components of PSII including D1 protein (acceptor-side photooxidative damage of D1) causing less effective transfer of asorbed light energy through PSII. However, D1 inhibits the repair of damaged PSII subunits by suppressing protein synthesis. Therefore, there is a change in intracellular *psbA* transcript levels [27-29].

In this research, *Umbilicaria decussata* samples were meticulously gathered and identified from two distinct locales: Horseshoe Island, as polar region, and the southern coast of Türkiye, as non-polar region. We performed comparative analyzes of the expression changes in *MBF*-1, *psbA* and *PKS* genes against cold stress in collected samples. This pioneering study stands as the inaugural exploration of its kind within its specialized domain. This study is the first of its kind in its field and it reveals how *U. decussata* lichen samples collected from markedly different geographical regions (polar and non-polar) adapt to their extreme habitats on the level of molecular biology markers.

2. Materials and Methods

2.1 Sample collection

Lichen samples were collected by the second author from Horseshoe Island and the south coast of Türkiye. During the 6th Turkish National Antarctic Scientific Expedition, a total of 60 samples were obtained: 30 from Horseshoe Island and 30 from the south coast of Türkiye. Collected samples were deposited in the Erciyes University Herbarium in Kayseri, Türkiye (ERCH). The samples were marked and coded according to the region where they were collected as ERCH HS 0.013 and ERCH STR 0.046 from the Horseshoe Island and the south coast of Türkiye, respectively. While collecting the samples; Two distinct sets of samples were collected simultaneously. The first set was designated for morphological and molecular phylogenetic analyses, while the second set was allocated for gene expression studies. Additionally, three sets of samples were taken from each sampling point in both regions. Sample Set 1 was kept on its original rock surface for morphological and phylogenetic analyses. Sample Set 2 was collected for gene expression studies, and Sample Set 3 was reserved as a backup for these analyses.

The samples for morphological and phylogenetic analyses were kept on their parent rock surface and transported to the laboratory intact. As *U. decussata* is a crustose species, it was fragmented to maximize penetration of the RNAlater™ Stabilization Solution (Cat. No. V5381, Merck, Germany; Cat. No. AM7021, Thermo Fisher Scientific, USA), which preserves RNA for varying durations: 1 day at 37 °C, 1 week at 25 °C, 1 month at 4 °C, and indefinitely at -20 °C [30-32]. Samples designated for gene expression studies were stored at -20 °C until laboratory analysis. Special cooler bags were used during transportation, ensuring that RNA integrity was preserved in samples from both Horseshoe Island and the west coast of Türkiye.

2.2 Morphological analyses

The specimens were examined by standard microscopic techniques with an Olympus S2X7 dissecting microscope equipped with an Olympus SC30 image capture system (Olympus, Japan). Handmade sections of ascomata were examined with a Leica DM2500 light microscope (Germany) and microphotographs were taken with a Flexacam C1 digital camera (Leica, Germany). Hand-cut sections were studied in water, 10% potassium hydroxide (K), and Lugol's solution (I). Microscope measurements were made in water. Ascospores were measured from five different ascomata. Measurements are given as minimum-maximum values from n = 20 measurements. The thickness of the spore septum was measured at the outer wall.

2.3 DNA extraction, PCR, sequencing and phylogenetic analyses

Total DNA was extracted from apothecia using the DNeasy Plant Mini Kit (Cat. No. 69104, Qiagen, Germany) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was carried out in reaction volumes of 50 µL using 4 µL of 10× reaction buffer, 4 µL of $MgCl_2$ (50 mm), 0.5 µL of each primer (*ITS1F* and *ITS4*), 2 µL of dNTP (10 mm), 0.2 µL of Taq DNA polymerase, 4 µL of template DNA, and 34.8 µL of ddH_2O on a thermal cycler equipped with a heated lid. The primers *ITS1-F* (forward: 5'-CTTGGTCATTTAGAGGAAGTAA-3') [33, 34] and *ITS4-R* (reverse: 5'-TCCTCCGCTTATTGATATGC-3') [34, 35] were used to amplify the *ITS* sequence. PCR amplification was performed under the following conditions: initial denaturation for 7 min at 95 °C; 6 cycles for 1 min at 94 °C, 1 min at 56 °C, and 1 min at 72 °C; and 30 cycles for 1 min at 94 °C, 1 min at 53 °C, and 2 min at 72 °C. A final extension step of 10 min at 72 °C was added, after which the samples were held at 4 °C. The PCR products were visualized on 1.6% agarose gel as bands of approximately 550 bp (*ITS*). Sequence analyses of the lichen samples obtained from the PCR products were performed by Epigen Biotechnology Laboratory (Ankara, Türkiye).

For phylogenetic analyses, the sequences' similarities to available sequences in the National Center for Biotechnology Information (NCBI) GenBank database were analyzed using the Basic Local Alignment Search Tool (BLASTn program: http://blast.ncbi.nlm.nih.gov/Blast.cgi; [33]. Fungal *ITS* reference sequences were selected to carry out phylogenetic analysis. All sequences were aligned with ClustalW in BioEdit V7.2.6.1 [37] using Geneious®6.1.8. The phylogenetic tree was visualized and edited with MEGA XI using the maximum likelihood method with rapid bootstrapping involving 1000 bootstrap replications [38].

2.4 RNA extraction and complementary DNA (cDNA) synthesis assay

Total RNA extraction was done from the lichen samples collected in cryotubes containing RNAlater™. Stabilization Solution was performed with the reagent of the RNeasy Plus Kit (Cat. No./ID 74034, Qiagen, Germany) according to the procedures suggested by the manufacturer. The amount and purity of RNA were subsequently determined using the NanoDrop ND-Spectrometer 1000 device (NanoDrop Technologies, USA) and 1.0% agarose gel electrophoresis. Next, cDNA synthesis was performed using the ProtoScript-II First Strand cDNA Synthesis Kit (BioLabs Inc., USA). The anchored-oligo(dT)18 primer was used because of the long *MBF-1*, *PKS*, and *psbA* gene regions.

2.5 qRT-PCR analyses

The primers for *MBF-1*, *PKS*, *psbA*, and *β-tubulin* as a housekeeping gene used in this study specific to the regions of interest were designed using information from the NCBI GenBank and the literature. Information about these genes and the primers sequences used in this study is given in Table 1.

Table 1. Gene information with sequences and melting temperatures of primers used in qRT-PCR

Genes/ Primer names	Sequence (5'-3')	Melting temperature (°C)	Source
MBF-1	F: 5' ATGGACGACTGGGACACCGT 3'	58-60 °C	[20]
	R: 5' TCACGATTTCGGCGGGAAAAACGGC 3'		
PKS	F: 5' GCTGTTTTTGCGGGCATGGA 3'	58-60 °C	[39]
	R: 5' CATACGGACGGCTTGATGT 3'		
psbA	F: 5' CACTAATCCGTGAAACTACT 3'	58-60 °C	[40]
	R: 5' TAATCGTCCAAAGTAACCGTG 3'		
β-tubulin	F: 5' GGCGTGACCTTACAGATTC 3'	58-60 °C	[24]
	R: 5' CAAGCTCTTGCTCGTAGTC 3'		

Following cDNA synthesis, real-time PCR applications were performed using SYBR Green I Master dye via the Pico Real Time module (Thermo, Germany). The mRNA levels of *MBF-1*, *PKS* and *psbA* were determined by qPCR method to evaluate the expression levels of the genes. PCR conditions consisted of initial denaturation for 10 min at 95 °C; 40 cycles of 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 30 s; and a melting step of 52 to 95 °C with the temperature increasing by 0.5 °C/min. The qRT-PCR analysis entailed three biological replicates, consisting of three technical replicates using the obtained optimal conditions.

2.6 Normalization and statistical analysis of qRT-PCR results

Gene expression results determined as Ct values were normalized considering the *β-tubulin* gene used in this study as a housekeeping gene [41]. The real-time PCR reactions of the *MBF-1*, *PKS*, and *psbA* genes were monitored simultaneously and their peak profiles were recorded. The Ct values of each sample were determined based on these peak profiles. The mRNA levels of the synthesized gene products were determined quantitatively by the obtained Ct values and melting curve analysis [42] and the obtained data were normalized according to the $2^{-\Delta\Delta Ct}$ method of Livak and Schmittgen [41]. The mean, standard deviation, standard error, and statistical significance values were calculated for the obtained data using IBM SPSS Statistics 25.0 for Windows (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Morphological and anatomical analysis

The results of the morphological and anatomical analysis of the *Umbilicaria decussata* (Vill.) Zahlbr. specimens that formed the basis of this study are given below.

Specimens studied: Antarctic Peninsula, Horseshoe Island: Lystad Bay, northeast of the Temporary Turkish Science Base, around Col lake-2, 67°49′40″S 67°13′ 25″W, alt. 4-85 m, 14 February 2022, on rocks, leg. M.G. Halici, ERCH HS 0.013. Türkiye, Muğla, Lake Bafa, southeast of Lake Bafa, siliceous rocks, 37°29′18″N, 27° 25′ 62″ E, alt. 15-150 m, 18 December 2022, leg. M.G. Halici, ERCH STR 0.046.

Description: The thallus is hard and has dense reticulate folds in the center on the upper surface, but decreases towards the edges, there is a pruinose structure in the center, but decreases towards the edges, the thallus color becomes more prominent gray, apothecium is rarely found, the lower surface is black sooty, the thalloconidia are brown unicellular. Talloconidia do not have a septum, but rarely a septum can be found. It is similar to *U. nylanderiana*, but morphologically the most distinctive feature is the hard reticulate folds on the upper surface and rarely it can contain apothetium. Tallus K-, C-, KC-, thallus approx. 300-350 µm, upper cortex approx. 20 µm, colorless layer 50 µm, medulla layer 100 µm, algae layer 40 µm, lower hymenium 25 µm, Paraphyse: Branched, 2.5- 4.5 µm, thallus diameter: 2- 4.5 cm, thallus cross-section: 160- 200 µm, apothetium: 0.9- 1.0 mm, hymenium: 80- 90 µm, ascus: 8 spores, 18-20 x 55- 60 µm, spores 14-18 x 4-5 µm (Figure 1).

Figure 1. General view of *Umbilicaria decussata*

3.2 Sequence alignment and phylogenetic analysis of *U. decussata*

All *ITS* sequences were aligned and edited manually using ClustalW in Bioedit V7.2.6.1 [37]. Ambiguous regions were delimited and excluded from the alignment. The final dataset consisted of newly generated sequences from this study and 56 ITS sequences obtained from GenBank (see Appendix A). All new sequences were deposited in GenBank. Phylogenetic trees with bootstrap values were obtained in MEGA XI using the Maximum Likelihood method with a rapid bootstrap with 1000 bootstrap replications [38]. Kimura two-parameter model was used for the analysis of the ML method. *Mycocalicium albonigrum* (Nyl.) Fink was used as the out-group.

In the BLASTn search, the *ITS* sequences of *Umbilicaria decussata* (480 bp after trimming) was most similar to *Umbilicaria proboscidea* (L.) Schrad. In the phylogenetic analyses, sequences of *U. decussata* (ERCH HS 0.013 and ERCH STR 0.046) appear in clade within the *U. decussata* group, with high support in the *ITS* phylogeny (BS = 87%) (Figure 2).

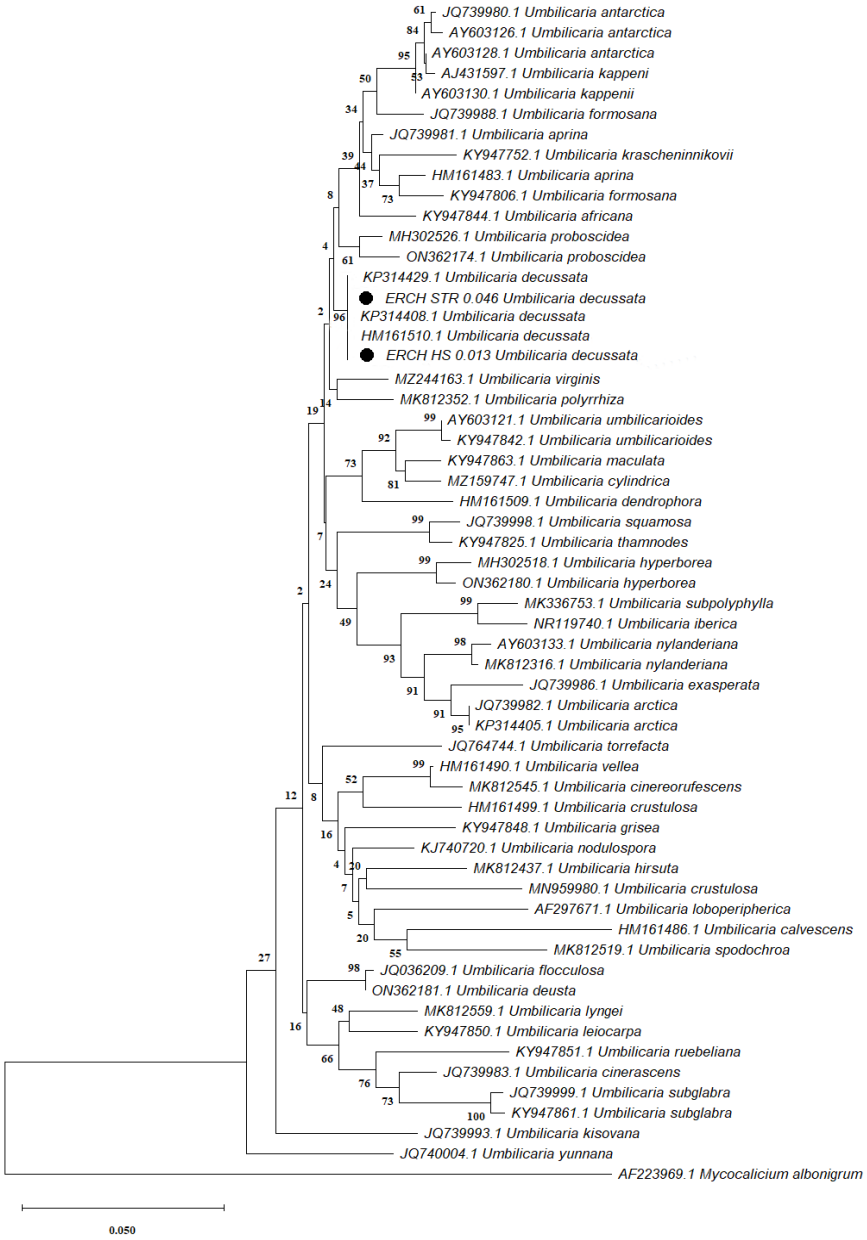

Figure 2. Maximum Likelihood phylogeny of the *ITS* marker. The *U. decussata* species collected from different regions (black) are presented in ERCH HS 0.013 and ERCH STR 0.046 codes.

3.3 qRT-PCR analyses of the *MBF-1*, *PKS*, and *psbA* genes

Based on the normalized gene expression data, the changes in the expression levels of the *MBF-1*, *PKS* and *psbA* genes in *U. decussata* samples collected from different regions were detailed (see Appendix B and C).

From the dataset of the above-specified parameters, it was determined that the expression levels of the *MBF-1* gene were quite different in the *U. decussata* samples collected from the different regions. While the *MBF-1* gene was expressed 10.18-fold in *U. decussata* from the Horseshoe Island, the Antarctic Peninsula (ERCH HS 0.013), it was 3.18-fold in *U. decussata* from the south coast of Türkiye (ERCH STR 0.046). Thus, we observed that the expression change of the *MBF-1* gene in samples of the same species collected from Antarctica and Türkiye varied at a level of approximately 3-fold ($P < 0.05$) (Figure 3).

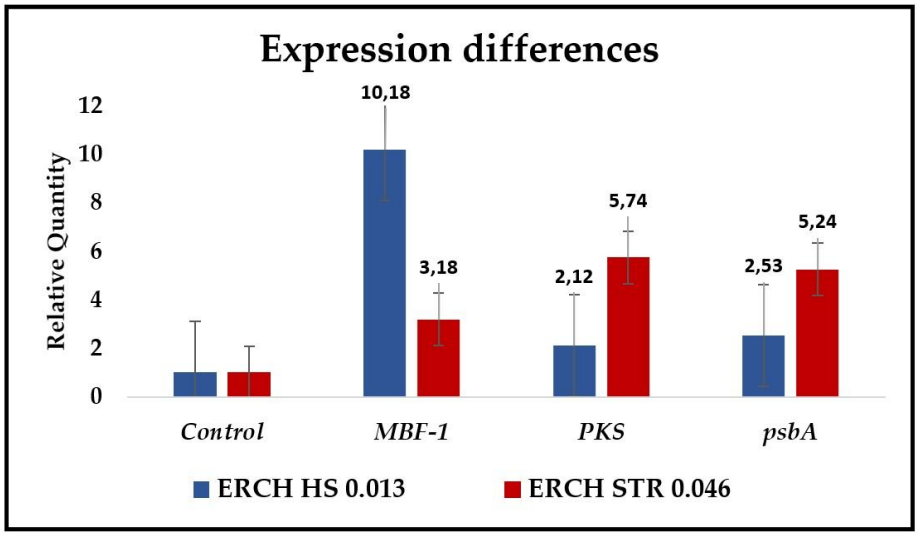

Figure 3. Expression differences of the *MBF-1*, *PKS* and *psbA* genes of the *U. decussata* collected from Horseshoe Island (ERCH HS 0.013) and south coast of Türkiye (ERCH STR 0.046)

Contrary to *MBF-1* gene expression, the level of *PKS* gene expression was lower in the *U. decussata* sample collected from the Horseshoe Island than in the sample collected from the south coast of Türkiye. While this expression level was 2.12-fold in the ERCH HS 0.013 sample, it was approximately 5.74-fold in ERCH STR 0.046 ($P < 0.05$). Similarly, the fold change in *psbA* gene expression was approximately 2.5-fold in the *U. decussata* sample collected from the Horseshoe Island, while it was 5.24-fold in the sample collected from the south coast of Türkiye. Similarly to the *PKS* gene, this constituted a nearly 2-fold decrease in *psbA* gene expression between the regions.

4. Discussion and Conclusion

Lichens stand as remarkable organisms, demonstrating an incredible ability to thrive in the world's most challenging environments. They exhibit survival capabilities in extreme conditions, including deserts, frigid climates, high altitudes and even in simulated outer space conditions. These symbiotic life forms have evolved an array of physiological adaptations, enabling them not only to endure but to thrive in these severe surroundings [43]. Furthermore, they have developed sophisticated mechanisms to shield themselves from the detrimental effects of extreme cold, a particularly vital adaptation for lichens inhabiting high-altitude regions or polar zones. In essence, lichens' capacity to adapt to a diverse range of harsh environments, their resilience, and their survival strategies stand as testament to the remarkable qualities embedded in their genetic makeup [44-47].

Recent studies have focused on conducting phylogenetic analyses of *U. decussata*, a lichen species that inhabits both polar and non-polar regions, shedding light on its evolutionary relationships. By analyzing the genetic relationships between *U. decussata* populations from different geographical locations, researchers have gained insights into the genetic structure of this species [4, 47, 48]. Lichens are often composed of multiple organisms, including both fungal and algal components, which can make DNA extraction and amplification rather difficult. To overcome this challenge, researchers use a variety of molecular techniques, including PCR-based methods and next-generation sequencing, to obtain and analyze DNA from *U. decussata* samples. With these techniques, researchers have been able to generate high-quality genetic data for use in phylogenetic analysis [49]. As a result of the molecular phylogenetic analyses performed in the present study, the diagnosis gene expression differences were found between the *U. decussata* lichen specimens collected from both the Horseshoe Island and those from the southern coast of Türkiye.

When considering the geographical distribution of a single lichen species, lichens encounter a range of location-specific abiotic stresses, including factors like low temperatures, desiccation, intense sunlight and oxidative stress [15, 19]. A limited number of studies have shown that the expression levels of genes such as *MBF-1* [20, 47, 50], *psbA* [26, 28, 47] and *PKS* [22, 23, 47] are significantly altered under various abiotic and biotic stress conditions. Our findings clearly indicate that these genes play important roles in stress tolerance in the lichenized fungal species *U. decussata*.

Some recent research has shed new light on the *MBF-1* gene expression of some plants under cold stress. In these studies, plants samples were subjected to low-temperature conditions for a period of time and the expression of the *MBF-1* gene was analyzed through a variety of techniques. The results indicated that the *MBF-1* gene was significantly upregulated in response to cold stress [47, 51]. Similarly, in our study, significant differences were

detected in the *MBF-1* gene in samples collected from the Horseshoe Island and the southern coast of Türkiye. Compared to the samples collected from the southern coast of Türkiye, the *MFP-1* gene was expressed at levels approximately 3 times higher in the samples collected from Horseshoe Island, providing resistance to extreme cold.

The *PKS* gene encodes the polyketide synthase enzyme, pivotal for generating bioactive polyketide-type metabolites. Its expression is intricately controlled by diverse environmental factors, temperature among them. However, under many stress conditions, including cold stress, the transcription and translation of the *PKS* gene may be significantly inhibited, which could reduce the production of these metabolites. Especially under cold stress, the expression of *PKS* can be upregulated or downregulated depending on the severity and duration. Recent studies have shown that the expression of polyketide synthase genes is altered under cold stress conditions, leading to changes in the production of important natural products [47, 52, 53]. This could be particularly problematic in extreme environments like Antarctica. Our results showed that the *PKS* gene was inhibited in extreme cold. The expression of the *PKS* gene was determined at 5.74-fold in the samples collected from the Horseshoe Island, which is dominated by extreme cold stress. This constituted a comparative decrease in the expression of the *PKS* gene by approximately 2.1-fold, revealing that *U. decussata* could cope with extreme cold stress by reducing the level of *PKS* gene expression.

Another analyzed gene, *psbA* is responsible for synthesizing the D1 protein, which is critical to the functioning of PSII in the biophysical processes of photosynthesis. The D1 protein, encoded by the *psbA* gene, is an essential subunit of the PSII complex. *psbA* gene expression is known to be influenced by various stress conditions, photoinhibition in particular [54] and photoinhibition under chilling conditions [55]. Thus, exposure to environmental stress can increase the production of ROS, which can cause oxidative damage to the PSII complex and result in decreased D1 protein expression. Previous studies have reported that salt stress, oxidative stress [56], metal stress [29] and cold stress [47, 57] prevented the repair of photodamaged PSII subunits by inhibiting the transcription and translation of *psbA*. The *psbA* gene expression levels of the *U. decussata* samples collected from the south coast of Türkiye were approximately 2.1 times higher compared to the samples from Horseshoe Island. Thus, similar to the *PKS* gene, the *psbA* gene was found to be inhibited in extreme cold, as also stated in the literature.

All these findings collectively indicate that the widely distributed lichen species *U. decussata* has evolved intricate mechanisms to thrive in challenging environmental conditions, and the analysis of gene expression can yield valuable insights into these mechanisms. Among these complex adaptations employed by *U. decussata* to confront stress is the regulation of the *MBF*-1, *PKS* and *psbA* genes in response to cold stress. Such adaptive mechanisms hold immense importance for the survival of lichens in the harshest of environments.

In conclusion**,**

This study on gene expression in *Umbilicaria decussata* (Vill.) Zahlbr. offers valuable insights into how lichens adapt to environmental stress. The variations in the expression levels of *MBF*-1, *PKS* and *psbA* genes in samples from different regions suggest that environmental stress may influence gene expression patterns. For example, under cold stress conditions characteristic of Horseshoe Island, the *MBF*-1 gene showed higher expression, while *PKS* and *psbA* expression levels were relatively lower compared to samples from the south coast of Türkiye. These findings indicate that *U. decussata* may regulate its gene expression in response to different stress conditions across geographic locations. The results serve as a preliminary environmental observation, paving the way for more detailed experimental studies in the future.

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Appendices

The data that support the findings of this study are available in the supplementary material of this article. **Appendix A;** ITS sequences used in the phylogenetic analysis. **Appendix B;** Expression data of normalized *MBF*-1, *psbA*, and *PKS* genes of *Umbilicaria decussata* collected from Horseshoe Island and the south coast of Türkiye. **Appendix C;** Mean, standard deviation, and standard error values of expression data of normalized *MBF*-1, *psbA*, and *PKS* genes of *Umbilicaria decussata* collected from Horseshoe Island and the south coast of Türkiye.

Conflicts of interest

Authors have no any financial or personal relationships with other individuals or organizations that might inappropriately influence this work during the submission process.

Statement contribution of the authors

This study's experimentation, analysis and writing, etc. all steps were made by the authors.

Statement of ethics

There is no need for an ethics committee decision for the studies in the article.

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Appendices

Appendix A. *ITS* sequences used in the phylogenetic analysis, with newly generated data in bold

	Genbank Number	**Locality**
ERCH HS 0.013 *Umbilicaria decussata*	**OR506209**	Horseshoe Island, Antarctica
ERCH STR 0.046 *Umbilicaria decussata*	**OR506252**	Lake Bafa, Muğla, Türkiye
Umbilicaria africana	KY947844	Ethiopia
Umbilicaria antarctica	JQ739980	Antarctica
Umbilicaria antarctica	AY603128	Lagoon Island, Antarctica
Umbilicaria antarctica	AY603126	Lagoon Island, Antarctica
Umbilicaria aprina	JQ739981	China
Umbilicaria aprina	HM161483	Bolivia
Umbilicaria arctica	JQ739982	Arctic
Umbilicaria arctica	KP314405	Svalbard
Umbilicaria calvescens	HM161486	Bolivia
Umbilicaria cinerascens	JQ739983	China
Umbilicaria cinereorufescens	MK812545	Norway
Umbilicaria crustulosa	HM161499	Bolivia
Umbilicaria crustulosa	MN959980	-
Umbilicaria cylindrica	MZ159747	United Kingdom
Umbilicaria decussata	HM161510	Bolivia
Umbilicaria decussata	KP314429	Svalbard
Umbilicaria decussata	KP314408	Svalbard
Umbilicaria dendrophora	HM161509	Bolivia
Umbilicaria deusta	ON362181	Canada
Umbilicaria exasperata	JQ739986	Arctic
Umbilicaria flocculosa	JQ036209	-
Umbilicaria formosana	JQ739988	China
Umbilicaria formosana	KY947806	China
Umbilicaria grisea	KY947848	Ukraine
Umbilicaria hirsuta	MK812437	Norway
Umbilicaria hyperborea	MH302518	USA
Umbilicaria hyperborea	ON362180	Canada

Umbilicaria iberica	NR119740	Spain
Umbilicaria kappeni	AJ431597	Lagoon Island, Antarctica
Umbilicaria kappenii	AY603130	Leonie Island, Antarctica
Umbilicaria kisovana	JQ739993	China
Umbilicaria krascheninnikovii	KY947752	Russia
Umbilicaria leiocarpa	KY947850	France
Umbilicaria loboperipherica	AF297671	-
Umbilicaria lyngei	MK812559	Norway
Umbilicaria maculata	KY947863	France
Umbilicaria nodulospora	KJ740720	USA
Umbilicaria nylanderiana	AY603133	Livingston Island, Antarctica
Umbilicaria nylanderiana	MK812316	Norway
Umbilicaria polyrrhiza	MK812352	Norway
Umbilicaria proboscidea	MH302526	USA
Umbilicaria proboscidea	ON362174	Canada
Umbilicaria ruebeliana	KY947851	Norway
Umbilicaria spodochroa	MK812519	Norway
Umbilicaria squamosa	JQ739998	China
Umbilicaria subglabra	JQ739999	China
Umbilicaria subglabra	KY947861	France
Umbilicaria subpolyphylla	MK336753	France
Umbilicaria thamnodes	KY947825	China
Umbilicaria torrefacta	JQ764744	USA
Umbilicaria umbilicarioides	AY603121	Lagoon Island, Antarctica
Umbilicaria umbilicarioides	KY947842	Chile
Umbilicaria vellea	HM161490	Bolivia
Umbilicaria virginis	MZ244163	USA
Umbilicaria yunnana	JQ740004	China
Mycocalicium albonigrum	AF223969	New Zealand

Appendix B. Expression data of normalized *MBF-1*, *psbA*, and *PKS* genes of *Umbilicaria decussata* collected from Horseshoe Island (HS) and the south coast of Türkiye (STR)

	Umbilicaria decussata (Horseshoe Island) (ERCH HS 0.013)											
	MBF-1-a	***MBF-1-b***	***MBF-1-c***	**Mean**	***psbA-1***	***psbA-2***	***psbA-3***	**Mean**	***PKS-1***	***PKS-2***	***PKS-3***	**Mean**
Control	32,12	32,45	32,48	**32,350**	30,12	31,45	31,48	**31,017**	30,12	30,45	30,48	**30,350**
ERCH HS 0.013	28,986	29,36	28,754	**29,033**	30,996	29,891	29,954	**30,280**	29,879	28,125	30,005	**29,336**
	Umbilicaria decussata (south coast of Türkiye) (ERCH STR 0.046)											
	MBF-1-a	***MBF-1-b***	***MBF-1-c***	**Mean**	***psbA-1***	***psbA-2***	***psbA-3***	**Mean**	***PKS-1***	***PKS-2***	***PKS-3***	**Mean**
Control	29,36	29,98	29,44	**29,593**	29,36	29,98	29,44	**29,593**	29,36	29,98	30,44	**29,927**
ERCH STR 0.046	27,698	28,102	28,0025	**27,934**	27,175	27,058	27,091	**27,108**	28,125	28,365	27,074	**27,855**

Appendix C. Mean, standard deviation, and standard error values of expression data of normalized *MBF-1*, *psbA* and *PKS* genes of *Umbilicaria decussata* collected from Horseshoe Island (ERCH HS 0.013) and the south coast of Türkiye (ERCH STR 0.046)

	Mean			**Standard deviation**			**Standard error**		
	MBF-1	***psbA***	***PKS***						
Control	1	1	1	***MBF-1***	***psbA***	***PKS***	***MBF-1***	***psbA***	***PKS***
ERCH HS 0.013	**10,18**	**2,12**	**2,53**	2,651	1,368	2,153	1,530	0,790	1,243
ERCH STR 0.046	**3,18**	**5,74**	**5,24**	0,484	1,616	4,403	0,279	0,933	2,542