Transcriptome analysis of the common moss *Bryum pseudotriquetrum* grown under Antarctic field condition

Masahiro Otani\(^{1,2,*}\), Haruki Kitamura\(^3\), Sakae Kudoh\(^{4,5}\), Satoshi Imura\(^{4,5}\), Masaru Nakano\(^1\)

\(^1\)Faculty of Agriculture, Niigata University, 2-8050 Ikarashi, Nishi-ku, Niigata 950-2181, Japan

\(^2\)Institute for Research Administration, Niigata University, 2-8050 Ikarashi, Nishi-ku, Niigata 950-2181, Japan

\(^3\)Graduate School of Science and Technology, Niigata University, 2-8050 Ikarashi, Nishi-ku, Niigata 950-2181, Japan

\(^4\)National Institute of Polar Research, Research Organization of Information and Systems, Tokyo, Japan

\(^5\)Polar Science, SOKENDAI (The Graduate University for Advanced Studies), Tokyo, Japan

\(^*\)Masahiro Otani, Ph.D.

Faculty of Agriculture, Niigata University, 2-8050 Ikarashi, Niigata 950-2181, Japan

TEL, FAX: +81-25-262-6680

E-mail: otani@agr.niigata-u.ac.jp

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Abstract

Mosses are distributed all over the world including Antarctica. Although Antarctic mosses show active growth in a short summer season under harsh environments such as low temperature, drought and high levels of UV radiation, survival mechanisms for such multiple environmental stresses of Antarctic mosses have not yet been clarified. In the present study, transcriptome analyses were performed using one of the common mosses *Bryum pseudotriquetrum* grown under Antarctic field and artificial cultivation conditions. Totally 88,205 contigs were generated by de novo assembly, among which 1,377 and 435 genes were significantly up- and down-regulated, respectively, under Antarctic field conditions compared with artificial cultivation condition at 15 °C. Among the up-regulated genes, a number of lipid metabolism-related and oil body formation-related genes were identified. Expression levels of these genes were increased by artificial environmental stress treatments such as low temperature, salt and osmic stress treatments. Consistent with these results, *B. pseudotriquetrum* grown under Antarctic field conditions contained large amounts of fatty acids, especially α-linolenic acid, linolenic acid and arachidonic acid. In addition, proportion of unsaturated fatty acids, which enhance membrane fluidity, to the total fatty acids was also higher in *B. pseudotriquetrum* grown under Antarctic field conditions. Since lipid accumulation and unsaturation of fatty acids are generally important factors for acquisition of various environmental stress tolerance in plants, these intracellular physiological and metabolic changes may be responsible for survival of *B. pseudotriquetrum* under Antarctic harsh environments.

Keywords:

Antarctic moss; environmental stress; lipid metabolism; fatty acid
Introduction

In ice-free areas of Antarctica, limited organisms live under harsh environments such as low temperatures, drought and high UV radiation. Among these organisms, several of moss species grow as dominant terrestrial plants. Therefore, these mosses are assumed to have high adaptability to harsh environments. However, little is known about molecular mechanisms of environmental stress tolerance of mosses grown in Antarctica.

When plants are exposed to severe environments, stress response genes, such as myelocytomatosis (MYC) genes, myeloblastosis (MYB) genes, ethylene-responsive element binding factor (ERF) genes and dehydration-responsive element binding (DREB) genes, are induced, and subsequently various stress responses including expression of molecular chaperon proteins, production of antioxidants such as flavonoids, accumulation of sugars and lipids (He et al. 2018; Hrmova and Hussain 2021; Lata and Prasad 2011). Lipid accumulation and change in fatty acid composition are one of the most important mechanisms, especially for cold stress tolerance in plants (Barrero-Sicilia et al. 2017; Reszczyńska et al. 2020). The fluidity of cell membranes increases with the proportion of unsaturated fatty acids in the membrane phospholipids, and plants maintain cell membrane function by increasing the proportion of unsaturated fatty acids to increase membrane fluidity under cold stress condition. Also, triacylglycerol (TAG), which is biosynthesized from free fatty acids in the endoplasmic reticulum, is accumulated in the oil body for acclimatization to low temperature (Degenkolbe et al. 2012). In some moss species, unsaturated fatty acids are accumulated under low temperature conditions as in seed plants (Aro and Karunen 1988; Beike et al. 2014, 2015; Lu et al. 2022; Resemann et al. 2021). Thus, modification of lipid metabolism for cold acclimatization and acquisition of cold stress tolerance seems to be a common system in plants.
In *Pohlia nutans* (Hedw.) Lindb., one of the mosses grown in Antarctica, changes in gene expression profiles have been investigated in response to artificial stress treatments such as high salinity, low temperature and drought (Fang *et al*. 2022; Liu *et al*. 2021, 2022; Zhang *et al*. 2019). In these studies, expression of transcription factor genes involved in environmental stress response and tolerance (e.g., MYC, MYB, ERF and DREB genes), genes involved in signaling of plant hormones such as abscisic acid (ABA) and jasmonic acid (JA), and antioxidant biosynthesis genes have been shown to be induced by artificial stress treatment. However, no studies using mosses grown under Antarctic field conditions have so far been reported, and gene expression profiles of mosses grown under natural Antarctic conditions is still unclear. The common moss *Bryum pseudotriquetrum* (Hedw.) P. Gaertn., B. Mey. & Scherb. is distributed throughout the world and one of the dominant moss species in Antarctica. Therefore, this moss is expected to have high tolerance to environmental stresses. In the present study, transcriptome analysis was performed to reveal gene expression profiles in *B. pseudotriquetrum* grown in Antarctica. This is the first report on gene expression profiling of mosses grown under Antarctic field conditions and provide important information for understanding adaptation and tolerance mechanisms of mosses to Antarctic harsh environments.

**Materials and methods**

**Sampling of mosses**

Blocks of *Bryum pseudotriquetrum* were cut out and collected from moss colony at four different spots of ice-free regions of Langhovde, Skarvsnes and Skallen in east Antarctica during the summer season (from December 29, 2018 to January 17, 2019) (S1 Fig). GPS location data and environmental conditions of each spot are shown in Supporting information Table S1. After collection, moss blocks were divided into several pieces (approx. 2 cm squares) and some of them were immediately fixed in
Farmer’s solution (acetic acid:ethanol = 1:3, v:v), stored at -20 °C and used for RNA extraction. The rest of moss blocks were stored at -20 °C without fixation and used for artificial cultivation and LC-MS/MS analysis of fatty acids. Mosses, with or without fixation, directly used for analysis were defined as field samples (FSs).

Artificial cultivation of mosses

After six months from collection, stored moss blocks collected from each spot without fixation were slowly thawed at 4 °C. They were then cultivated separately on vermiculite with liquid BCD medium (Nishiyama et al. 2000) under continuous illumination with Light Emitting Diode (LED) lighting (200 μmol m⁻² s⁻¹) at 15 °C. After several weeks of incubation, green gametophores appeared on moss blocks. Such artificially cultivated mosses were defined as cultivated samples (CSs). Before RNA extraction, gametophores from CSs were fixed in Farmer’s solution and stored at -20 °C for two weeks in order to eliminate effects of fixation on gene expression profiles.

RNA extraction and construction of cDNA libraries for RNA-seq analysis

Flow of RNA extraction and RNA-seq analysis is shown in Supporting information Figure S2. Approximately 100 mg fresh weight (FW) of gametophores were used for RNA extraction. Total RNAs were extracted using Trizol regent (Thermo Fisher Scientific, MA, USA), and then purified by RNeasy Mini kit (QIAGEN, Hilden, Germany) and RNase-Free DNase Set (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. Extracted RNAs were further purified by ethanol precipitation. For FSs, RNA extraction was separately performed from gametophores of different blocks derived from same moss colony and these samples were used as biological replicates. Then, total RNAs extracted from mosses
collected at four different spots were mixed by an equal amount and used for cDNA library construction. Likewise, for CS, RNA extraction was performed three times separately from gametophores of cultured mosses derived from the same four spots was used. RIN values of total RNAs from CSs and FSs were 7.5–7.7 and 2.3–2.6, respectively. rRNA was depleted using RiboMinus™ Plant Kit for RNA-Seq (Thermo Fisher Scientific, MA, USA). cDNA libraries were constructed using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs Inc., MA, USA) according to the manufacturer’s instructions. Since total RNAs from FSs were partially fragmented, fragmentation step of library construction was omitted. cDNA libraries were multiplexed at equal proportions (2 nM) and loaded onto a single flow cell of NextSeq500/550 Mid Output Kit v2.5 (150 Cycles) (Illumina, CA, USA). Sequencing was performed on NextSeq 500 (Illumina, CA, USA) with three biological replicates for each sample.

De novo assembly, differential expression analysis and annotation

Low quality reads and contaminated reads derived from rRNAs were filtered from raw transcriptome sequences using FastqPuri version 1.0.7 (Pérez-Rubio et al. 2019) with the parameter -q 30 (minimum quality score: 30) and SortMeRNA version 4.2.0 (Kopylova et al. 2012). Filtered reads were assembled using TransLiG version 1.3 (Liu et al. 2019) with default parameters. Contaminated contigs derived from other organisms such as fungi and bacteria were detected by EnTAP version 0.9.2 (Hart et al. 2020) and removed. Transcripts Per Million (TPM) value of transcripts were quantified by Salmon version 1.3.0 (Patro et al. 2017) and differential expressed genes (DEGs) were generated using tximport version 1.12.3 (Soneson et al. 2015) and DESeq2 version 1.24.0 R package (Love et al. 2014). Gene ontology (GO) annotation of DEGs were performed using EnTAP version 0.9.2.
Cold, salt, and drought stress treatments

Axenic cultures of mosses collected at spot 4 were used for artificial stress treatments. Gametophores cultivated on vermiculite were surface-sterilized with a sodium hypochlorite solution containing 1% active chlorine and cultured on BCD solid medium with 0.8 % agar under continuous illumination at 15 °C. After several months, fully elongated gametophores were isolated from proliferated moss cultures, transferred to fresh solid BCD medium, and cultured for three days in order to avoid an effect of gametophore cutting on gene expression. For cold stress treatment, gametophores were further transferred to fresh BCD solid medium and cultured under a long-day condition (16 h light, 8 h dark) at 4 °C. For salt and drought stress treatments, gametophores were further transferred to BCD solid media containing 200 mM NaCl and 300 mM mannitol, respectively, and cultured under continuous illumination at 15 °C. Each stress treatment was performed three times separately and these samples were used as three biological replicates. For each treatment, gametophores were collected weekly and used for gene expression analysis.

Real-time RT-PCR analysis

Extraction of total RNAs from gametophores and cDNA synthesis were performed using Plant total RNA Purification Kit (BioElegen Technology Co., Ltd., Taichung, Taiwan) and ReverTra Ace® qPCR RT Master Mix (Toyobo Co., Ltd., Osaka, Japan) according to the manufacturer’s instructions. Real-time RT-PCR was performed using KOD SYBR® qPCR Mix (Toyobo Co., Ltd., Osaka, Japan) on the MyGo PCR systems (IT-IS Life Science Ltd., Dublin, Ireland). The specificity of the amplification product was confirmed by melting curve analysis. Real-time RT-PCR analysis was performed in biologically triplicate for each sample. Relative amounts of transcripts were calculated using the comparative cycle threshold method (Livak and Schmittgen 2001), and results were normalized to BTB/POZ domain-containing
protein gene of \textit{B. pseudotriquetrum} (\textit{BpPOB1}; LC767358 from the GenBank/EMBL/DDBJ databases). We have confirmed that there are no significant differences in expression levels of \textit{BpPOB1} between FS and CS. Primer sets used are listed in see Supporting information Table S2.

**LC-MS/MS analysis of fatty acids**

Gametophores from FSs and CSs, which were corrected at spots 1 and 4, were used for fatty acid analysis (see Supporting information Fig. S1). Twenty mg FW of samples were homogenized in liquid nitrogen, and fatty acids were extracted in chloroform/methanol (50:50, v/v). Purified extracts were then analyzed using Prominence XR (Shimadzu, Kyoto, Japan) attached to LTQ Orbitrap XL (Thermo Fisher Scientific, MA, USA) with \textit{L-column}3 ODS (2.1 mm I.D. × 150 mm with 2 μm internal diameter, CERI, Tokyo, Japan). Fatty acid concentrations were calculated from peak areas using MRMPROBS ver. 2.60 software. Sixty-seven fatty acids were initially analyzed using FSs, and the top 15 fatty acids with higher contents were further investigated. Although each analysis was performed in a single replication, data reliability was ensured by examining samples from two different sampling spots.

**Results**

**RNA-seq analysis using FSs and CSs**

In order to examine up- and down-regulated genes in FSs compared with CSs, \textit{de novo} assembly and identification of differentially expressed genes (DEGs) were carried out using transcriptome data. In our transcriptome data, although numbers of total reads were low (3-4.7 million), the mapping rates indicating reliability of the data were good (CSs: approx. 91.7–92.9 %, FSs: approx. 77.6–86.2 %; see Supporting information Table S3). Totally 88,205 contigs were generated by \textit{de novo} assembly (see...
Supporting information Table S4). Among them, 1,377 and 435 genes were significantly up- and down-regulated, respectively, under Antarctic field conditions (Fig 1A). GO enrichment analysis showed that up-regulated DEGs contained many genes involved in various biosynthetic and metabolic processes, such as cellular macromolecule metabolic process, macromolecule metabolic process, cellular biosynthetic process and organic substance biosynthetic process (Fig 1B). A number of genes involved in lipid metabolism and accumulation were also found in the up-regulated DEGs (Table 1). Especially, genes for oleosin Bn-III, which are localized in the oil drop membrane and involved in oil body formation, Δ-15 fatty acid desaturase (Δ15FAD; FAD3), which involved in the conversion from linoleic acid (LA) to α-linolenic acid (ALA) in ERs, and LPTs, which is involved in the transport of wax to cuticular layer; were highly expressed under Antarctic field conditions (Table 1; Fig 3). On the other hand, down-regulated DEGs contained many photosynthesis-related genes (see Supporting information Fig. S3). In particular, expression levels of most genes encoding proteins constituting light-harvesting chlorophyll protein complex (LHC) were down-regulated.

Expression levels of plant core environmental stress response (PCESR) genes, a gene set commonly induced by various environmental stresses in plants (Hahn et al. 2013), were examined. Although 54 out of the 56 PCESR genes were detected in the transcriptome data, only 7 showed elevated expression levels in FSs (see Supporting information Table S4).

Expression analysis of oil body formation-, lipid metabolism- and lipid transfer-related genes

In order to investigate response of oil body formation-, lipid metabolism- and lipid transfer-related genes to various environmental stresses, expression analysis of these genes was carried out in CSs treated with low temperature, salt and drought stresses (Fig 2). Genes for oleosin Bn-III and rubber elongation factor/small rubber particle protein (REF/SRPP) involved in oil body formation responded to
all stress treatments. Expression levels of these genes increased up to 106.6- and 19.6-fold by cold stress treatment, 137.1- and 22.0-fold by salt stress treatment, and 67.5- and 9.7-fold by drought stress treatment within 4 weeks, respectively. Likewise, expression levels of lipid metabolism-related genes, \( \Delta 12FAD \) (FAD2/6), \( \Delta 15FAD \), \( \Delta 8FAD \) and 9-LOX genes were up-regulated in response to all stress treatments. In particular, expression levels of \( \Delta 15FAD \) and \( \Delta 8FAD \) greatly increased up to 127.7- and 583.5-fold by cold stress treatment, 356.8- and 1660.2-fold by salt stress treatment, and 125.8- and 572.6-fold by drought stress treatment within 4 weeks, respectively. Expression levels of LPCAT1, CDS1, cytochrome B5, and NsLTPs increased in response to at least one of the stress treatments.

**Identification and quantification of fatty acids by LC-MS/MS analysis**

Fatty acid composition and content in FSs collected at different two spots, Langhovde (spot 1) and Skallen (spot 4), and CSs collected at the same spots were investigated (Table 2). Both FSs contained mainly \( \alpha \)-linolenic acid and linolenic acid as major fatty acids, followed by arachidonic acid, palmitic acid, oleic acid, \( \gamma \)-linolenic acid, dihomo-\( \gamma \)-linolenic acid, EPA and cis-vaccenic acid. Total amount of fatty acids was greatly higher in FSs compared to CSs. Content of a major saturated fatty acid, palmitic acid (C16:0), was 5.8- to 8.3-fold higher in FSs, while contents of two major unsaturated fatty acids, \( \alpha \)-linolenic acid (C18:3n-3) and linolenic acid (C18:2n-6), were 27.9- to 30.8-fold and 33.3- to 138.5-fold higher in FSs, respectively. Thus, the proportion of unsaturated fatty acid contents among total fatty acid contents were much higher in FSs.
**Discussion**

In the present study, RNA-seq analysis was carried out in the common moss *B. pseudotriquetrum* and genes related to lipid metabolism and oil body formation were found to be highly expressed in FSs (Table 1; Fig 3). In plant cells, lipid accumulation and change in fatty acid composition are important mechanisms for acquiring environmental stress tolerance. Thus, these genes may be involved in multiple stress tolerance in *B. pseudotriquetrum* growing in Antarctica (Fig 2).

Oil body membrane proteins such as oleosin and REF/SRP are involved in regulation of the number and size of oil bodies and accumulation of lipids in cells (Ting *et al.* 1996; Tzen *et al.* 1993). In *Arabidopsis thaliana* and *Oryza sativa*, lipid contents in leaves or seeds were increased by overexpression of the oleosin gene (Fan *et al.* 2013; Liu *et al.* 2013). Likewise, lipid accumulation was promoted by co-overexpression of the oleosin gene and genes involved in TAG-biosynthesis (Bhatla *et al.* 2010; Vanhercke *et al.* 2014). Oil body membrane proteins are also involved in acquisition of environmental stress tolerance through oil body formation. In seeds of *A. thaliana*, oleosin has shown to contribute to freezing tolerance by preventing abnormal fusion of oil bodies (Shimada *et al.* 2008). Overexpression of REF/SRPP gene increased drought stress tolerance, whereas mutation or RNAi suppression of REF/SRPP gene reduced drought tolerance in *A. thaliana* (Kim *et al.* 2016; Laibach *et al.* 2018). In the present study, oleosin and REF/SRP genes were highly expressed and large amounts of fatty acids were accumulated in FSs (Tables 1 and 2). Therefore, oil body membrane proteins may be involved in environmental stress tolerance through promotion of oil body formation and lipid accumulation in cells of *B. pseudotriquetrum* grown under Antarctic field conditions.

Increase in membrane fluidity is essential for reducing damage of environmental stresses such as low temperatures to cell membrane function. Generally, higher proportions of unsaturated fatty acids to the total fatty acids increase the fluidity of cell membrane (Mikami and Murata 2003). Unsaturated fatty
acids are produced through elongation reactions and unsaturation by various elongases and FADs. Cytochrome b5 acts as an electron donor to FADs in this process. In *Solanum lycopersicum*, LeFAD3 (Δ15FAD gene of *S. lycopersicum*) expression was up-regulated by a cold stress treatment, and overexpression of LeFAD3 increased α-linolenic acids (C18:3n-3) contents and induced cold stress tolerance (Yu et al. 2009). FAD2 is essential for proper function of the membrane-attached Na+/H+ antiporter and maintenance of low cytosolic Na⁺ levels, and *A. thaliana* fad2 mutant (mutant for endoplasmic reticulum-localized Δ12FAD) showed high sensitivity to salt stress (Zhang et al. 2009). In addition, the melting point of fatty acids decreases as the degree of their unsaturation increases, e.g. 13.4 °C for oleic acid (18:1n-9, mono-unsaturated fatty acid), -5 °C for linoleic acid (18:2n-6, di-unsaturated fatty acid), -11 °C for α-linolenic acid (18:3n-3, tri-unsaturated fatty acid), and -49.5 °C for arachidonic acid (20:4n-6, tetra-unsaturated fatty acid). In the present study, some FAD genes were highly expressed and unsaturated fatty acids were mainly accumulated in FSs (Tables 1 and 2; Fig 3). Therefore, unsaturation of lipids and change of fatty acid composition may be important adaptation mechanisms to harsh environments in Antarctic mosses.

Very long-chain fatty acids (VLCFAs; C26–C36) are substrates of a cuticle wax mixture covering the plant surface, and therefore, fatty acids are also involved in physical protection against environmental stresses. Lipid transfer proteins (LTPs) are involved in transport of a cuticular wax mixture to the cuticular layer. In many plant species, it has been reported that expression of LPT genes is up-regulated in response to abiotic/biotic stresses and contribute to multiple stress tolerance (Gangadhar et al. 2016; Guo et al. 2013; Martín-Pedraza et al. 2016; Xu et al. 2018). Among LTP family proteins, non-specific LTPs (nsLTPs) are also involved in cuticle formation, wax biosynthesis, plant growth and development (Carvalho et al. 2007; Sterk et al. 1991). In *Nicotiana glauca*, expression level of a nsLTP gene and cuticular wax accumulation increased in response to drought stress, resulting in increased drought resistance (Cameron et al. 2006). In the present study, some nsLPT genes were highly expressed in FSs
(Table 1). Thus, physical protection may also be enhanced by reinforcement of extracellular matrix in mosses growing under Antarctic environments.

Expression of many of genes involved in lipid metabolism and accumulation, such as FADs and oleosin, increased after relatively long periods of various artificial stress treatments (>1 week) (Fig 2). This result indicates that acquisition of environmental stress tolerance in Antarctic mosses by changing fatty acid metabolism requires relatively prolonged exposure to environmental stresses. Although most previous studies using Antarctic mosses have examined changes in gene expression profiles after single artificial stress treatments for short periods (a few hours to several days) (Fang et al. 2022; Liu et al. 2021, 2022; Zhang et al. 2019), longer periods of stress treatments may be required to clarify actual mechanisms of environmental stress tolerance in Antarctic mosses.

In the present study, expression levels of photosynthesis-related genes, especially light-harvesting chlorophyll (LHC) protein genes, decreased in FSs grown under a unique daylength condition in Antarctic summer (midnight sun). It has been reported that LHC proteins are involved both in collecting light energy for driving the primary photochemical reactions of photosynthesis and in photoprotection when absorbed light energy exceeds the capacity of the photosynthetic apparatus (Rochaix and Bassi 2019). In A. thaliana, expression levels of several LHC protein genes were decreased by excessive light treatments (Alboresi et al. 2011). In the present study, ultraviolet-B receptor UVR8-like gene, which has been reported to be involved in UV-protective responses (Rizzini et al. 2011), was also highly expressed in FSs (logFC: 6.294977, padj: 3.18E-06; data not shown). Likewise, artificial UV-B irradiation increased expression of UV-B signaling genes including UVR8-like genes in the Antarctic moss Leptobryum pyriforme (Liu et al. 2021). These gene expression responses might be one of the common mechanisms of tolerance to light stresses and excessive UV exposure under midnight sun in Antarctica.
A number of PCESR genes have been reported to be related to early responses to environmental stress (Hahn et al. 2013). In the present study, however, expression of only a few PCESR genes were up-regulated in FSs (see Supporting information Table S4). In *B. pseudotriquetrum* grown in Antarctica, stress tolerances appear to have already been established by altering their secondary metabolism such as lipid accumulation and alteration of fatty acid composition.

The present study is the first one showing actual genes expression profiles in mosses grown under Antarctic field conditions. The study also showed a possibility that lipid accumulation and change in fatty acid composition are major mechanisms for acquiring environmental stress tolerance in Antarctic mosses. Lu et al. (2022) have reported that amount of storage lipids decreases in *B. pseudotriquetrum* treated with cold stress despite of an increase in the proportion of unsaturated fatty acids. In the present study, since the gene expression of oil body membrane proteins, such as oleosin, was much higher in FSs, we expected that amount of storage lipids would be larger under Antarctic field conditions. These differences might be attributed to differences between the Antarctic environment and artificial stress treatments. In natural fields, plants are exposed to complex environmental factors for a long time. Thus, on-site analysis is essential elucidating actual plant environmental responses and stress tolerance mechanisms.
Data availability

The sequencing data used in this study are openly available in DDBJ Sequence Read Archive (DRA) with the accession number DRA016751.
References


Figure legends

Figure 1. Identification of differentially expressed genes (DEGs) and gene ontology (GO) enrichment analysis of DEGs. (A) MA plot of DEGs. Each dot represents a single gene. Red dots indicate significantly up- or down-regulated genes in FSs compared with CSs (q-value<0.01). (B) GO enrichment analysis of up-regulated DEGs using the GO term Biological process (GO:0008150).

ALT TEXT: Two graphs showing the results of MA plot and GO enrichment analysis of DEGs for cultivated samples vs. field samples.

Figure 2. Real-time RT-PCR analysis of lipid metabolism-related and oil body formation-related genes. Low-temperature (4°C), high-salt (200 mM NaCl), or drought (300 mM mannitol) stress treatment were applied to gametophore of Bryum pseudotriquetrum for 1 to 4 weeks. Relative amounts of transcripts of each gene were normalized to BpPOB1. Values represent the means ± standard error of triplicates.

ALT TEXT: Bar graphs showing expression analysis of 13 genes involved in lipid metabolism and oil body formation after cold, salt and drought stress treatments.

Figure 3. Overview of lipid metabolic pathways in plants (A) and major desaturation and elongation steps of polyunsaturated fatty acids in Endoplasmic reticulum (B). Enzymes whose expression are up-regulated in FSs are indicated by red letters. Abbreviations are as follows: ACP, acyl carrier protein; CoA, coenzyme A; G3P, glycerol-3-phosphate; LPA, lysophosphatidic acid; PA, phosphatidic acid; PC,
phosphatidylcholine; PUFAs, polyunsaturated fatty acids; DAG, diacylglycerol; TAG, triacylglycerol; CDP-DAG, cytidine diphosphate diacylglycerol; PtdGro, phosphatidylglycerol; PtdIns, Phosphatidylinositol; VLCFAs, very long chain fatty acids; 10-OPDA, 10-oxo-11,15-phytodienoic acid; 12-OPDA, 12-oxo-10,15-phytodienoic acid; JA, jasmonic acid; JA-Ile, jasmonic acid-isoleucine; FAS, type II fatty acid synthase complex; KAS, β-ketoacyl-ACP synthase; SAD, Δ9 stearoyl-acyl carrier protein desaturase; FAD, fatty acid desaturase; LOX, lipoxygenase; LPCAT, lysophosphatidylcholine acyltransferase; GPAT, glycerol-3-phosphate acyltransferase; LPAAT, lysophosphatidic acid acyltransferase; PAP, phosphatidic acid phosphatase; DGAT, diacylglycerol acyltransferase; CDS, CDP-diacylglycerol synthase; REF/SRPP, rubber elongation factor/small rubber particle protein; LPTs, lipid transfer proteins; FAD, fatty acid desaturase; OLE, oleic acid; LA, linoleic acid; ALA, α-linolenic acid; EDA, eicosadienoic acid; GLA, γ-linolenic acid; SDA, stearidonic acid; ESA, eicosatrienoic acid; DGLA, dihomo-γ-linolenic acid; ETA, eicosatetraenoic acid; ARA, Arachidonic acid; EPA, eicosapentaenoic acid.

ALT TEXT: Two illustrations showing lipid and fatty acid metabolic pathways with high gene expression under Antarctic condition. A shows overview of lipid metabolic pathways in plant cells. B shows major desaturation and elongation steps of polyunsaturated fatty acids in Endoplasmic reticulum.
Table 1  Up-regulated DEGs involved in lipid metabolism and oil body formation in FSs.

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Table 2  Amount of fatty acids in gametophores of FSs and CSs (μg/g).

<table>
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<tr>
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<th>B. pseudotriquetrum collected in sampling spot 4</th>
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<td></td>
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<td>CS</td>
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<tr>
<td>Linolenic acid</td>
<td>30,000</td>
<td>900</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>9,800</td>
<td>1,300</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>6,900</td>
<td>1,200</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>2,700</td>
<td>61</td>
</tr>
<tr>
<td>γ-Linolenic acid</td>
<td>6,200</td>
<td>140</td>
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<tr>
<td>Dihomo-γ-Linolenic acid</td>
<td>3,700</td>
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<tr>
<td>Eicosapentaenoic acid</td>
<td>1,500</td>
<td>180</td>
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<tr>
<td>cis-Vaccenic acid</td>
<td>1,500</td>
<td>60</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>160</td>
<td>21</td>
</tr>
<tr>
<td>8Z,11Z,14Z,17Z-Eicosatetraenoic acid</td>
<td>490</td>
<td>10</td>
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<tr>
<td>Nervonic acid</td>
<td>550</td>
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<tr>
<td>11Z,14Z-Eicosadienoic acid</td>
<td>360</td>
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<tr>
<td>Stearidonic acid</td>
<td>630</td>
<td>8</td>
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<tr>
<td>11Z,14Z,17Z-Eicosatrienoic acid</td>
<td>160</td>
<td>15</td>
</tr>
</tbody>
</table>
Figure 1

A

Up-regulated genes: 1377
Down-regulated genes: 435

B

GO:0008150 Biological process

- Cellular aromatic compound metabolic process
- Single-organism transport
- Phosphorus metabolic process
- Organic cyclic compound metabolic process
- Oxidation-reduction process
- Transport
- Cellular macromolecule metabolic process
- Macromolecule metabolic process
- Cellular biosynthetic process
- Organic substance biosynthetic process

counts
Figure 2

Oil body formation-related genes

Lipid metabolic enzyme genes

Other genes involved in lipid metabolism and transfer

[Graphs showing relative expression levels of various genes over different time periods]