Comparative transcriptome analysis of field- and chamber-grown samples of *Colobanthus quitensis* (Kunth) Bartl, an Antarctic flowering plant

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*Colobanthus quitensis* is one of the two vascular plants inhabiting the Antarctic. In natural habitats, it grows in the form of a cushion or mats, commonly observed in high latitudes or alpine vegetation. Although this species has been investigated over many years to study its geographical distribution and physiological adaptations to climate change, very limited genetic information is available. The high-throughput sequencing with a *de novo* assembly analysis yielded 47,070 contigs with blast-hits. Through the functional classification and enrichment analysis, we identified that photosynthesis and phenylpropanoid pathway genes show differential expression depending on the habitat environment. We found that the known ‘plant core environmental stress response (PCESR)’ genes were abundantly expressed in Antarctic samples, and confirmed that their expression is mainly induced by low-temperature. In addition, we suggest that differential expression of thermomorphogenesis-related genes may contribute to phenotypic plasticity of the plant, for instance, displaying a cushion-like phenotype to adapt to harsh environments.

Land plants are susceptible to adverse environmental conditions. Extreme temperatures, drought, high salinity, and high UV radiation are typical environmental stressors that can damage cellular structures and impair physiological function1, leading to inhibited photosynthesis, retarded growth and reduced yields in plants2,3. To cope with these environmental stressors, plants developed stress resistance strategies, including intracellular physiological and metabolic changes such as increasing membrane fluidity and expression of cytoprotective metabolites by regulating stress signal transduction1,3,4. On the other hand, plants are exposed to environmental fluctuations that can drive phenotypic plasticity in plants. Phenotypic plasticity has been defined as the ability of an individual organism to alter its physiology/morphology in response to changes in environmental conditions5. Since the range of phenotypic plasticity varies depending on the species, it can cause different responses to overcome environmental stress and affect crop yields and plant distribution6.

Perennial plants growing in a cushion form commonly found in high latitudes or alpine vegetation are generally round hemispherical or mat shaped7. In extreme ecosystems, these growth forms are thought to play an ecological role in minimizing the loss of heat and moisture which not only helps a plant survive but also creates a microclimate shelter for maintaining species diversity of microbes, insects, or other young plants8,9. In addition, several hundreds of compact cushion plants spanning to various families and genera including *Silene acaulis* (Caryophyllaceae), *Azorella compacta* (Apiaceae), *Androsace helvetica* (Primulaceae), and *Raoulia eximia* (Asteraceae), are considered to be good examples of convergent evolution related to the phenotypic plasticity to cope with extreme environments7.

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**Colobanthus quitensis** (Caryophyllaceae) is a self-fertilizing species that grows in the form of a cushion or mats. It is distributed broadly from Mexico (17°N) to the Antarctic Peninsula (68°S) and at altitudes ranging from 0 to 4200 meters\(^{10-12}\) and is known to exhibit considerable morphological variation depending on their habitats\(^{10-14}\). The morphology analysis of the *C. quitensis* population along an Antarctic-subantarctic latitudinal gradient revealed that populations at higher latitudes have smaller and thicker leaves with higher mesophyll thickness, narrower adaxial surfaces, increased pigments and reduced epidermis\(^{15,16}\).

Structural and physiological characteristics responsible for the high tolerance of *C. quitensis* to extreme conditions were described in terms of the difference in leaf anatomical traits and ultracellular structures and varied photosynthetic responses\(^{16-22}\). In laboratory controlled conditions, the leaves of *C. quitensis* had anatomical and physiological changes when exposed to abiotic stressors such as low-temperature, high light or ultraviolet light\(^{18-21,23}\) and the photosynthetic efficiency was primarily regulated by temperature\(^{21,23}\). In addition, field studies have shown that the OTC (Open Top Chamber) warming effects affect plant growth by influencing plant morphoanatomical traits, cellular chemical composition, and photosynthetic parameters\(^{17,22}\). These suggest that the developmental changes induced by temperature ultimately determine photosynthetic efficiency.

On the other hand, the adaptation of Antarctic *C. quitensis* to the extreme environment has been explained by population-specific genetic evolution\(^{18-20}\). The studies with two geographically separated populations have shown that the Antarctic ecotype of *C. quitensis* undergoes less photoinhibition than an alpine ecotype because it has better recovery rates of PSII than the alpine ecotype\(^{18,20}\). This suggests that the Antarctic ecotype of *C. quitensis* might have developed a unique stress response mechanism operating at the molecular level to survive in a harsh environment.

Despite previous studies on morphological differences and stress resistance of *C. quitensis*, there is no comprehensive report available on genetic regulation using plants growing in a natural habitat. To elucidate the regulatory networks involved in gene expression associated with environmental stress-driven tolerance responses and morphological plasticity of *C. quitensis*, we constructed a de-novo transcriptome assembly and compared the transcriptome profiles on plant samples collected from low-temperature environment of Antarctic natural habitat versus those grown with the mild growth condition in the laboratory. We classified the genes according to functional categories through GO and KEGG analysis, identified genes that differ in their expression level with the habitat, and deduced their potential functions through ortholog analysis with the model plant. Our findings may provide new insights into the genetic control system developed by plants adapted to the extreme environment.

**Results**

**Morphology of ANT and LAB samples of *C. quitensis***. *C. quitensis* plants have shown changes in morphology and physiological responses with respect to geographical distribution and microclimate differences\(^{18,20,24}\). The transcriptome profile of extremophile plants in their natural habitats provides biological information as to how they optimize gene regulation to overcome environmental stresses. Therefore, to investigate the molecular and genetic mechanisms associated with stress tolerance and morphological plasticity due to environmental stress in *C. quitensis*, we compared the transcriptome profiles between plants inhabiting in Antarctic Barton Peninsula and plants cultivated in the mild growth condition in the laboratory. The Antarctic field plants designated ANT were collected in the Baton Peninsula in January 2013 (Fig. 1a). The laboratory cultures designated as LAB were cultivated at 16°C, a temperature within the known optimal leaf temperature range (14–18°C) for photosynthesis of *C. quitensis*\(^{25}\). The morphological differences between the LAB and ANT samples were observed during growth. While the LAB plants have longer leaf blades and elongated hypocotyls, the ANT plants have a denser structure consisting of short and thick leaves (Fig. 1b–d).

**Deep sequencing, de novo assembly, and functional annotation of the *C. quitensis* transcriptome**. Total 160,264,674 reads were generated from Illumina HiSeq. 2500 run. Trimming of raw sequences provided 143,972,486 high-quality reads (Q > 30) comprising 13.7 Gb of sequences. Using CLC Genome Workbench assembly module, the reads were assembled into 101,690 contigs averaging 764 bp and N50 value of 1,058 bp. The assembled contigs were clustered into 95,010 representative contigs using the CD-HIT-EST program (see Supplementary Table S1).

To annotate the assembled unigenes, the sequences were blast searched against GenBank nr or UniProt databases using the BLASTX algorithm. When we blasted them against the nr database, 47,070 contigs had at least one significant alignment to an existing protein data with an E-value of 1. When we blasted them against the nr database, 47,070 contigs had at least one significant alignment to an existing protein data with an E-value of 1. When we blasted them against the nr database, 47,070 contigs had at least one significant alignment to an existing protein data with an E-value of 1. When we blasted them against the nr database, 47,070 contigs had at least one significant alignment to an existing protein data with an E-value of 1. When we blasted them against the nr database, 47,070 contigs had at least one significant alignment to an existing protein data with an E-value of 1.

To classify the functions of the predicted genes of *C. quitensis*, GO terms were assigned to total contigs. Based on classification of GO terms, a total of 26,346 contigs were assigned with at least one GO term and classified into different categories using the PlantGO slim terms (Supplementary Fig. S2, Table S2). In terms of 'biological processes', the top 2 GO terms were 'metabolic process' and 'cellular Process'. In terms of 'molecular function', 'catalytic activity' and 'binding' were top 2 GO terms. To infer the functions and the utilities of the genes in the biological system, total contigs were blast queried against the KEGG databases\(^{27}\) (organism code: ath) with E-value < 10\(^{-10}\). As a result, 11,950 contigs were mapped to KEGG pathways with hierarchy and they are classified into 5 functional categories (Supplementary Fig. S3). The top 5 pathways are 'translation', 'carbohydrate metabolism', 'amino acid metabolism', 'folding, sorting and degradation' and 'energy metabolism'. For the more
specific pathways of the lower hierarchy, the top 3 pathways were ‘ribosome’, ‘protein processing in endoplasmic reticulum’ and ‘oxidative phosphorylation’ (Supplementary Table S3).

SSR Identification. Simple-sequence repeats (SSRs) are well established and have increasingly become the marker of choice for population genetic analyses due to their codominant, highly polymorphic and highly reproducible nature. C. quitensis is an important species for studying plant ecology with geographic parameters. Studies about physiological differences involving genetic diversity have been performed among C. quitensis populations. Given the research interests and the importance of C. quitensis, molecular markers for this species can be valuable resources. We identified EST-SSRs from assembled contigs during the transcriptome analysis of C. quitensis. A total of 8,619 distinct loci containing motifs between one and six nucleotides in size were discovered in 7,749 contigs. Approximately 60.5% of SSRs were mononucleotides, 28.3% were trinucleotides, 9.3% were dinucleotides and the remaining ~2% were tetra-, penta-, and hexanucleotides. The most frequent dinucleotide SSR was AG/CT (4.43%), however, for trinucleotide SSRs, AAC/GTT, AAT/ATT, ACC/GGT, AAG/CTT, and ATC/ATG show similar frequency in the range of 4.2–4.9% (Supplementary Table S4).

Identification of differentially expressed genes in ANT vs. LAB samples. To compare the transcriptome of ANT and LAB samples, we remapped the reads generated from each library to the assembled 95,010 contigs and counted the read numbers that mapped to each contig. Statistical analysis revealed totally 3,902 differentially expressed genes (DEGs) in which 2,127 transcripts were upregulated and 1,775 transcripts were downregulated in ANT samples compared to LAB sample with a cutoff of FDR corrected p-value < 0.05 (beta-binomial test) (Supplementary Tables S5 and S6). Comparative GO enrichment analysis was conducted on the subsets of 3,900 DEGs, 2,127 upregulated DEGs, and 1,775 downregulated DEGs, respectively with the complete set.
of transcripts sets, with Fisher’s exact test (FDR < 0.05). Among the GO terms included in ‘biological process’ category, the ‘response to stress’ (GO: 0006950) and the ‘photosynthesis’ (GO: 0015979) categories were the most enriched GO terms in the differentially expressed gene group (Fig. 2a). To elaborate, GO terms of ‘response to stress’, ‘response to (abiotic, biotic or external) stimulus’ and ‘extracellular region’ were significantly enriched in both upregulated and downregulated gene subsets. In ANT compared to LAB transcriptomes, however, the GO terms of ‘secondary metabolic process’, ‘response to endogenous stimulus’, ‘plasma membrane’, ‘vacuole’, ‘ribosome’ and ‘nucleus’ and the GO terms of ‘photosynthesis’ and ‘carbohydrate metabolic process’, ‘cellular homeostasis’ and ‘plastid’ were enriched only in the upregulated and downregulated gene subsets, respectively (Fig. 2b and Supplementary Tables S7 and S8).

**Differentially altered metabolic pathways in ANT samples.** To determine the altered metabolic pathways in ANT samples, a KEGG enrichment analysis was performed on DEGs in ANT vs. LAB transcriptomes. Among the statistically enriched metabolic pathways (FDR < 0.01, Fisher’s exact test) (Supplementary Tables S9 and S10), the top 10 enriched KEGG pathways of upregulated and downregulated genes are shown in Fig. 3. The ‘ribosome’, ‘phenylpropanoid biosynthesis’, ‘biosynthesis of secondary metabolites’, ‘oxidative phosphorylation’, ‘flavonoid biosynthesis’, ‘glutathione metabolism’, etc. were identified as the enriched pathways in the upregulated genes, whereas ‘biosynthesis of secondary metabolites’, ‘carbon metabolism’, ‘photosynthesis’, etc. were identified as the enriched pathways in the downregulated genes (Fig. 3 and Supplementary Tables S7–S10). KEGG and GO
enrichment analyses on DEGs suggested that C. quitensis exhibits different cellular performance in ANT vs. LAB conditions by regulating genes in various metabolic pathways.

Regarding the ‘photosynthesis’ pathway, it is noteworthy that putative PGR5 (PROTON GRADIENT REGULATION 5, Contig18861) gene, known as an essential protein for photoprotection\textsuperscript{28,29}, was upregulated, while many photosynthesis-related genes were downregulated in ANT samples (Supplementary Tables S7–S10).

We also found that the ‘phenylpropanoid biosynthesis’ (KEGG ID: map00940) pathway genes were considerably enriched in the upregulated gene group in ANT sample ($p$-value: $1.7 \times 10^{-19}$), (Figs 3 and 4 and Supplementary Table S9). In plants, phenylpropanoids are a group of secondary metabolites derived from phenylalanine and have a variety of functions as structural and signaling molecules\textsuperscript{30}. The major modules of ‘phenylpropanoid biosynthesis’ are the ‘monolignol biosynthesis’ (KEGG ID: M0039) and the ‘flavanone biosynthesis’ (KEGG ID: M0039) pathways. We identified 28 contigs in the ‘monolignol biosynthesis’ pathway were found to be increased in ANT samples (Table 1), and they include (1) Phenylalanine ammonia-lyase (PAL; Contig20542), cinnamic acid 4-hydroxylase (C4H; Contig49114, Contig70533) and 4-hydroxycinnamoyl-CoA ligase (4CL; Contig3499), enzymes that play an important role in the formation of the substrate $p$-coumaroyl-CoA in both monolignol and flavanone biosynthesis, (2) hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase (HCT; Contig13431, Contig29449, Contig 40563) enzymes that function in a branched pathway, and 3) hydroxycinnamoyl-CoA reductase (CCR; Contig33804) and cinnamyl alcohol dehydrogenase (CAD; Contig24021) enzymes that form lignin monomers. On the other hand, only two enzymes in the ‘flavanone biosynthesis’ pathway, chalcone synthase (CHS; Contig2685) and chalcone isomerase (CHI; Contig16136) enzymes, showed an increase in expression in ANT samples (Fig. 4 and Table 1).

The increased expression of the ‘plant core stress responsive genes’ in ANT samples. When plants are treated with abiotic stress, a group of stress genes is commonly expressed at the early stage. This gene response is called ‘plant core environmental stress response (PCESR)’, which is well established in Arabidopsis thaliana\textsuperscript{31,32}. PCESR is not limited to Arabidopsis but is also found in other plant species, suggesting that PCESR to various abiotic stimuli is conserved in plants\textsuperscript{31,33–35}. We hypothesized that the plants growing in Antarctic fields would display high expression of abiotic stress tolerance genes to cope with the extreme environmental stress. Among the 8530 reciprocal-best-hit genes between two species (Supplementary Fig. S4), 35 C. quitensis orthologs to the Arabidopsis PCESR genes were found (Table S11), and their expression was compared in LAB and ANT samples. As a result, about 40% (17/35) of the PCESR orthologs showed significantly high levels of transcripts ($p < 0.05$) in ANT samples (Table 2).

We verified their expression levels by quantitative RT-PCR (qPCR) and confirmed if the observed levels are induced by abiotic stimuli including low-temperature, salinity, or drought. Plants were subjected to each abiotic stress for 24 hours and the RNA expression of the genes between treated and non-treated samples was compared. Among the genes tested, 14/17 showed higher expression levels in ANT samples and were induced by abiotic stress.
stresses (Fig. 5). These included the genes with various biological functions such as transcription factors including an APETALATA2 (AP2)/ethylene response factor (ERF) protein (Contig14494), a NAC27-like (Contig38763), a MYB44-like protein Contig16997); C2H2 type zinc finger proteins including ZAT10 (Contig22074) and ZAT12 (Contig28814); a CCR4-associated factor (Contig45609); a calcium-binding protein (Contig38763); a DUF246-containing protein (Contig7995); a sugar transporter 13 (STP13)-like gene (Contig21564); a PUB23 E3 ligase family protein (Contig99760); a major facilitator superfamily gene (Contig15740); ring finger proteins (Contig34911, Contig20878); and a polygalacturonase inhibitor protein (Contig42834). It is noteworthy that these genes respond more specifically to low-temperatures among various abiotic stressors, suggesting that the temperature is the most important factor to induce stress signaling in plants.

Transcription factors associated with morphological changes in different conditions. *C. quitensis* is a representative cushion plant, which forms a dense hemisphere with short leaves, inhabiting the alpine and high latitude. When the field plants were transferred and grown in a 16 °C climate chamber, we noticed that the compact structure of *C. quitensis* loosened and the leaves elongated (Fig. 1c,d). Recent reports have shown that some PHYTOCHROME INTERACTING FACTORS (PIFs), bHLH transcription factors, are associated with thermomorphogenesis36–39. In particular, in the case of Arabidopsis *PIF4* (AT2G43010), gene expression increases with increasing temperature, while gene expression decreases with decreasing temperature36. The length of hypocotyl and leaf petiole was longer than that of WT when these genes were overexpressed, and the opposite when they were knocked out36,38,39. These expression patterns and phenotype of transgenic plants suggest that they participate in leaf elongation caused by change in temperature36,39,40.

In this regard, we hypothesized that expression of *PIF4* and its associated growth-promoting genes would be decreased in ANT samples that have smaller leaves. To prove the hypothesis, first, we tried to determine if the PIF genes of *C. quitensis* were altered in expression under field vs chamber conditions. A TBLASTN search using *Arabidopsis* PIF4 as a query yielded five hits (Contig8088, Contig41295, Contig35032, Contig62363, Contig64547) containing a bHLH DNA-binding domain with an E-box/N-box specificity site were found (E-value < $10^{-10}$) (Fig. 6a). As expected, the expression levels of all orthologs were lower in the ANT sample, and Contig8088 showed a significant decrease ($FDR$ corrected $p$-value < 0.05). In *Arabidopsis*, PIF4 activates the expression of growth-promoting genes such as *HFR*, *IAA29*, *IAA19*, *ATHB*, and *SAUR23*. Therefore, we examined whether the expression of these gene orthologs known to be targets of PIF4 differs between ANT and
tact area between adjacent cell compartments. The phenotypic plasticity is also evident in another Antarctic plant, a Poaceae flowering plant growing in Antarctic regions. Recent studies on Deschampsia antarctica, a vascular plant, have shown that the photosynthesis response to warming is regulated by the plant growth changes due to temperature variations, hyperthermal or hypothermal, has been reported in many plant species. We identified the orthologs of those genes in LAB samples of C. quitensis. We observed when grown at 16 °C or above than at low-temperature conditions (data not shown). Taken together, those grown under control temperature conditions (Fig. 6d). Additionally, proliferation of root and shoot was observed when grown at 16 °C or above than at low-temperature conditions (data not shown). Taken together, the results suggest that the characteristic phenotype of field plants is associated with downregulation of PIF4 and its downstream genes.

**Discussion**

Plant growth changes due to temperature variations, hyperthermal or hypothermal, has been reported in many studies. In the case of Arctic and Antarctic plants, there have been several reports about the phenotypic plasticity. The chilling temperatures have shown to affect the organelle ultrastructure and the organization of palisade cells in C. quitensis mesophyll. The plants naturally growing in the Antarctic field and the cold-acclimated plants, both have deformed chloroplasts with multi-shaped protrusions and invaginations to increase the contact area between adjacent cell compartments. The phenotypic plasticity is also evident in another Antarctic vascular plant, Deschampsia antarctica, a Poaceae flowering plant growing in Antarctic regions. Recent in situ warming tests on C. quitensis have shown that the photosynthesis response to warming is regulated by the anatomical determinants of leaf CO₂ transfer, which enhanced mesophyll conductance and carbon assimilation.

| Contig ID | Arabidopsis ortholog | Genes’ ID | EC | FPKM LAB | FPKM ANT | p-value |
|-----------|-----------------------|-----------|----|-----------|-----------|---------|
| Contig20542 | AT2G37040 | PAL1 | EC:4.3.1.24 | 26.7 | 131.3 | 1.6 × 10⁻³ |
| Contig20541 | AT2G37040 | PAL1 | EC:4.3.1.24 | 8.5 | 31.7 | 4.2 × 10⁻⁴ |
| Contig49174 | AT2G30490 | C4H | EC:1.14.13.11 | 2.4 | 15.8 | 1.7 × 10⁻³ |
| Contig70533 | AT2G30490 | C4H | EC:1.14.13.11 | 1.9 | 18.9 | 2.4 × 10⁻⁴ |
| Contig22559 | AT2G40890 | CYP98A3 | EC:1.14.13.36 | 17.6 | 48.4 | 6.3 × 10⁻⁴ |
| Contig24607 | AT2G40890 | CYP98A3 | EC:1.14.13.36 | 16.6 | 49.2 | 2.4 × 10⁻⁴ |
| Contig33804 | ATIG180820 | CCR2 | EC:1.2.1.44 | 73.4 | 229.9 | 4.4 × 10⁻¹³ |
| Contig7537 | ATIG67980 | CCoAOMT1 | EC:2.1.1.104 | 39.7 | 141.8 | 1.1 × 10⁻¹⁵ |
| Contig16997 | ATIG34050 | CCoAOMT1 | EC:2.1.1.104 | 118.7 | 424.2 | 0 |
| Contig28757 | AT5G54160 | OMT1 | EC:2.1.1.68 | 854.4 | 1773.0 | 0 |
| Contig49114 | AT5G48930 | HCT | EC:2.3.1.133 | 12.2 | 42.2 | 1.4 × 10⁻⁴ |
| Contig33299 | AT5G66390 | PRX | EC:1.11.1.7 | 11.1 | 101.3 | 1.8 |
| Contig24005 | AT1G68850 | PRX | EC:1.11.1.7 | 9.5 | 40.8 | 2.7 |
| Contig3499 | AT1G62940 | 4CL | EC:6.2.1.12 | 3.2 | 49.5 | 9.6 |
| Contig24021 | AT1G72680 | CAD1 | EC:1.1.1.195 | 94.1 | 345.6 | 0 |
| Contig25236 | AT4G37990 | CAD8 | EC:1.1.1.195 | 24.5 | 88.0 | 1.3 × 10⁻⁷ |
| Contig4399 | ATIG62940 | C4H | EC:6.2.1.12 | 3.2 | 49.5 | 9.6 |
| Contig24005 | ATIG68850 | PRX | EC:1.11.1.7 | 9.5 | 40.8 | 2.7 |
| Contig21838 | ATIG71695 | PRX | EC:1.11.1.7 | 46.1 | 179.7 | 4.7 |
| Contig29445 | AT5G48930 | HCT | EC:2.3.1.133 | 22.7 | 80.4 | 3.8 |
| Contig13431 | AT5G48930 | HCT | EC:2.3.1.133 | 15.1 | 76.2 | 3.9 |
| Contig24021 | AT1G72680 | CAD1 | EC:1.1.1.195 | 94.1 | 345.6 | 0 |
| Contig25236 | AT4G37990 | CAD8 | EC:1.1.1.195 | 24.5 | 88.0 | 1.3 × 10⁻⁷ |
| Contig4399 | ATIG62940 | C4H | EC:6.2.1.12 | 3.2 | 49.5 | 9.6 |
| Contig24005 | ATIG68850 | PRX | EC:1.11.1.7 | 9.5 | 40.8 | 2.7 |
| Contig21838 | ATIG71695 | PRX | EC:1.11.1.7 | 46.1 | 179.7 | 4.7 |
| Contig29445 | AT5G48930 | HCT | EC:2.3.1.133 | 22.7 | 80.4 | 3.8 |
| Contig13431 | AT5G48930 | HCT | EC:2.3.1.133 | 15.1 | 76.2 | 3.9 |
| Contig24021 | AT1G72680 | CAD1 | EC:1.1.1.195 | 94.1 | 345.6 | 0 |

Table 1. Expression of C. quitensis genes involved in phenylpropanoid biosynthesis pathway, PAL: phenylalanine ammonia lyase; C4H: cinnamic acid 4-hydroxylase; 4CL: 4-hydroxy cinnamoyl-CoA ligase; CYP98A3: Cytochrome P450 98A3 (Coumaroyl shikimate 3′-monooxygenase); HCT: hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase; CHS: chalcone synthase; CHI: chalcone isomerase.
agreement with Kumar ortholog was dependent on temperature, and the phenotype we observed was in
known as downstream targets, was also found to be lower in the ANT samples than in the LAB samples. In

temperature rises, the density of leaves in a plant decreases with prolonged leaves, thus the CO2 transfer would be
polar habitats form dense cushions with short leaves to minimize heat loss. On the contrary, when the tem-
perature plays a major role in plant growth and adaptation.

wall chemical components (hemicellulose, cellulose and lignin content) than plants grown inside OTC 22.
C. propanoid biosynthesis genes in ANT samples may be the result of cross-talk responses due to various environ-
ments56, suggesting that there may be a common mechanism for how extremophytes adapt to the environment.

Table 2. High expression of PCESR orthologs in ANT sample.

| Contig ID | Arabidopsis ortholog | Description | FPKM LAB | FPKM ANT | p-value |
|-----------|----------------------|-------------|-----------|-----------|---------|
| Contig22074 | AT1G27730 | Zinc finger protein ZAT10-like | 11.7 | 48.5 | 8.8 × 10^{-4} |
| Contig28814 | AT5G59820 | Zinc finger protein ZAT12-like | 2.4 | 20.7 | 1.9 × 10^{-4} |
| Contig8763 | AT1G76650 | Calcium-binding protein CML37 | 0.4 | 44.2 | 2.8 × 10^{-16} |
| Contig14494 | AT4G17500 | AP2 like transcription factor | 8.2 | 54.5 | 2.7 × 10^{-4} |
| Contig35858 | AT1G69490 | NAC transcription factor 027 | 10.0 | 27.0 | 7.0 × 10^{-1} |
| Contig16997 | AT4G37260 | MYB44-like transcription factor | 6.0 | 19.9 | 7.0 × 10^{-1} |
| Contig45609 | AT3G44260 | CCR4-NOT transcription complex family protein | 0.6 | 15.3 | 3.0 × 10^{-4} |
| Contig7995 | AT2G44500 | DUF246 domain- protein | 2.5 | 9.7 | 3.4 × 10^{-2} |
| Contig21564 | AT5G26340 | Hexose transporter | 81 | 138 | 3.0 × 10^{-2} |
| Contig99760 | AT2G35930 | E3 ligase PUB23-like | 0.6 | 6.6 | 2.8 × 10^{-1} |
| Contig15740 | AT5G10190 | Major facilitator superfamily | 12.0 | 32.1 | 4.2 × 10^{-3} |
| Contig34911 | AT3G16720 | Ring finger protein | 2.8 | 13.0 | 9.6 × 10^{-4} |
| Contig20878 | AT3G05200 | Ring finger family protein | 9.7 | 25.9 | 8.3 × 10^{-3} |
| Contig42834 | AT5G08660 | Polygalacturonase inhibiting protein | 22.5 | 125.3 | 2.3 × 10^{-14} |
| Contig21412 | AT5G45340 | ABA8 -hydroxylase CYP707A1 | 2.0 | 39.8 | 1.8 × 10^{-3} |
| Contig_67594 | AT5G64660 | U-box domain protein 27 | 5.8 | 16.9 | 2.0 × 10^{-2} |
| Contig21564 | AT5G26340 | Hexose transporter | 81 | 138 | 3.0 × 10^{-2} |
| Contig38763 | AT1G76650 | Calcium-binding protein CML37 | 0.4 | 44.2 | 2.8 × 10^{-16} |

thereby promoting higher leaf carbon gain and plant growth37,22. This suggests that phenotypic plasticity by tem-
perature plays a major role in plant growth and adaptation.

In this study, we observed that the leaves of C. quitensis elongated when grown in a growth chamber, the
dense cushion shapes of the plants found in nature were loosened. It is expected that plants grown in Alpines/

Phenylpropanoid biosynthesis pathway produces a wide variety of secondary metabolites which contribute to all
aspects of plant responses towards biotic and abiotic stimuli, with lignins and flavonones being representative
products39. In this study, almost all genes involved in the ‘phenylpropanoid biosynthesis’ pathway were strongly
upregulated in ANT samples implying that products and its intermediates could have crucial roles to protect plant
cells from abiotic and biotic stress. In Arabidopsis, Zea mays, Eucalyptus globulus, Lotus japonicus and Miscanthus,
the major enzymes of the phenylpropanoid pathway such as PAL, CCR, CAD, CHS and CHI have been shown to be
strongly induced by cold, drought, UV irradiation or pathogen infection but the gene expression patterns are
temperature-dependent. Although PIFs are well known for the mechanisms involving thermomorphogenesis
associated with high temperature36,48, there is not much discussion of the ecological benefits derived from their
regulatory roles at low-temperature. In this regard, we observed that the expression of all PIF4 orthologs was
found to be lower in the Antarctic samples and the expression of genes such as HFR, IAA19, ATHB, and SAUR23,
known as downstream targets, was also found to be lower in the ANT samples than in the LAB samples. In
addition, the expression of PIF4 ortholog was dependent on temperature, and the phenotype we observed was in
agreement with Kumar et al.36.

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strongly induced by cold, drought, UV irradiation or pathogen infection but the gene expression patterns are
different according to tissue-and species-specific33,49–54. In this regard, transcriptional up-regulation of phenyl-
propanoid biosynthesis genes in ANT samples may be the result of cross-talk responses due to various environ-
mental factors such as cold, drought and pathogenesis, and this result is correlated with the observation that C.
quitensis plants grown in open areas with relatively low ambient temperature exhibited a higher fraction of cell
wall chemical components (hemicellulose, cellulose and lignin content) than plants grown inside OTC22.

Overall DEG analyses revealed that the transcriptome of field plants of C. quitensis has major changes in ‘pho-
tosynthesis’ genes and ‘stress response’ genes to adapt to an extreme environment. These results can be compared with transcriptome results from another extremophyte, Extremna salcusingenom, which has a very clear pheno-
typic plasticity in natural habitats vs. controlled chambers55,56. The transcriptome studies of this species have also shown that stress response genes and photosynthetic genes are correlated with phenotypic plasticity at field con-
ditions56, suggesting that there may be a common mechanism for how extremophytes adapt to the environment.

In Antarctic C. quitensis, about 40% of PCESR orthologs (14 of the 35 genes), which are known to be induced
early in response to abiotic stress in Arabidopsis, were expressed at high levels. In our in-silico study and qPCR
expression assay, we identified 14 PCESR genes with diverse functions that were highly expressed in ANT sam-
pled and induced by abiotic stresses. The stress response is initiated by the intracellular Ca2+ change and the
generation of ROS57. This intracellular signaling induces the expression of transcriptional regulatory elements,
which in turn induce the expression of downstream genes by binding to cis-elements in the regulatory regions55,57.
Among the 14 genes, calmodulin-like proteins and transcription factor genes such as AP2/ERF, NAC, and MYB were included. C2H2 zinc finger proteins ZAT10 and ZAT12 maintained high levels of expression in ANT samples and were specifically induced by low-temperature \(^{58,59}\). ZAT12 is activated by different abiotic stresses and is required for systemic H\(_2\)O\(_2\) signaling \(^{58}\). Another C2H2 zinc finger protein, ZAT10, is known to be systemically regulated and induced by different stresses and essential for priming for systemic acquired acclimation \(^{59}\). We have confirmed that orthologs of ZAT10 and ZAT12 are expressed at high levels in ANT samples of *C. quitensis*.
experiencing various environmental stresses and presume that the expression of these genes will help maintain intracellular homeostasis in response to environmental stress. In addition, an E3-ligase, ring finger proteins, a PGIP protein and various kinds of transporter proteins, which have been reported to be involved in modulation of abiotic stress responses by posttranslational modification, inhibiting of ice-recrystallization or transport of metabolites, were also highly expressed in ANT samples, and expression was induced by cold stress as well. It should be noted that we selected genes that are known to be commonly expressed in the early stages of various stress reactions, and we found that they have higher levels of transcripts in ANT samples that have undergone environmental stress such as low-temperature, high salt, drought, or high UV. However, when we stressed plants individually in the laboratory, many genes were induced by low-temperatures, but not by high salt or dehydration. We cannot completely exclude the possibility that we did not see the earlier reaction by setting a single time point after 24 h in our experiment. However, we would expect that the main cause of gene induction by abiotic stress in field sample is low-temperature.

In this study, we found that multiple adaptations such as modulation of energy metabolism, change in PCESR gene expression pattern, and morphological adaptions are present in *C. quitensis* through a global analysis of field transcriptome of *C. quitensis*. The results suggest that plants adapted to the extreme environment have developed a modified genetic control system as a survival strategy against harsh conditions. Thus, the *C. quitensis* transcriptome profile broadens our understanding of how plants tolerate extreme environments and their adaptive responses to climate change.
Methods

Study area and sampling in the Antarctic field.  *C. quitensis* plants growing under field conditions were collected in the vicinity of the Korean King Sejong Antarctic Station (62°14'/29°S; 58°44'/18°W), on the Barton Peninsula of King George Island in January 2013. The samples were immediately ground in TRizol reagent (Invitrogen, Carlsbad, CA, USA) at the sampling site and transferred in 1 h to the laboratory in King Sejong Antarctic Station for RNA extraction and designated as ANT samples. Photosynthetic active radiati (PAR) and air temperatures at Barton Peninsula were measured during January (2013) with a data logger CR800 (Campbell Scientific, Logan, UT, USA) connected with thermocouples and PAR sensors. Microclimatic conditions on Barton Peninsula during January are shown in Fig. 1A. The air temperature of the site was measured from a temperature sensor installed 5 cm above the soil surface. During the warm months, temperatures dropped below 0 °C for only a few days, and the maximum temperature was 14 °C. The average monthly temperature was ca. 2.7 °C. Maximum PAR intensity varied from ca. 1,845 μmol m⁻² s⁻¹ on a clear day (19th January 2013) to ca. 629 μmol m⁻² s⁻¹ on a cloudy day (15th January 2013). The luminosity was close to zero from midnight to 04:00 AM. We harvested ANT samples between 12:00 ~ 14:00 local time.

Growth conditions of laboratory plants. Some plants from Barton Peninsula were transferred to the laboratory in Korea Polar Research Institute and have been grown hydroponically supplemented with 0.5 × Murashige and Skoog (MS) medium containing 2% sucrose in a climate chamber under a prolonged long day (20:4 h light: dark) cycle with a light intensity of 150 μmol m⁻² s⁻¹ at 16 °C. After growing plants for three weeks in a climate chamber, plants with horizontal diameters of 1.5 ~ 2 cm in similar developmental stages were collected. The old leaves of the lower part of the plant were removed, and the newly developed leaves of the upper part were sampled for RNA extraction and designated as LAB samples. For cold stress treatments, plants grown at 16 °C with 1.5 ~ 2 cm horizontal diameter were transferred to 2 °C and incubated until harvest. For salt stress treatments, plants were transferred onto 0.5 × MS liquid medium, grown for 7 days and then transferred onto the 0.5 × MS liquid medium supplemented with 150 mM NaCl65. For drought stress treatments, plants were transferred onto 3 mm filter paper, exposed to air in a clean bench for 30 min. Subsequently, the plants on filter papers were transferred back to the empty growth boxes and cultivated until harvest65. Plants were harvested for RNA extraction after 0, 1 and 7 days under each condition and prepared in three different biological replicates. All lab–cultured samples were harvested 8 hours after lights-on.

Leaf length measurement. To compare morphological differences depending on temperature, plants grown at 16 °C with 1.5 ~ 2 cm in horizontal diameter were transferred to growth chambers at 2 °C and 8 °C and cultured for 4 weeks. The length of individual leaves was measured by ImageJ programs (https://imagej.nih.gov/ij/). All plants for measurements were prepared with five biological replicates. Statistical analysis was performed by student’s *t*-test (*p* < 0.05).

RNA extraction and RNA-Seq library construction. Total RNAs were extracted using TRizol reagent, treated with DNase I (QIAGEN, Hilden, Germany) to remove contaminant genomic DNA, and were subsequently purified using the RNasy mini kit (QIAGEN) following manufacturer’s protocols. Three biological replicates were prepared. RNA integrity and concentration were determined using a Bioanalyzer (RIN > 6) (Agilent Technologies, Waldbronn, Germany) and a Qubit®RNA Broad-range Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), respectively. To construct RNA-Seq libraries, 1.5 μg of total RNA from each sample was used as input for the TruSeq RNA sample prep kit v2 (Illumina, San Diego, CA, USA). The libraries were quantified using a Bioanalyzer and the library qPCR quantification method following the Illumina guideline. After quantification, they were multiplexed in equal ratios and loaded onto a single flow cell of Illumina HiSeq Rapid SBS kit v2 (2 × 100 runs). Sequencing was performed on a HiSeq. 2500 Sequencer system (Illumina) and a total of 16.2 Gb (160 M paired-end reads) of sequencing data were generated (Q30 > 93%).

*De novo* assembly and annotation. *De novo* assembly was performed using the CLC Genomics Workbench v7.5 software (QIAGEN). The reads were filtered by trimming adapter sequences, excluding low-quality sequences (quality score < 0.001, ambiguity < 2 bps) and removing too short sequences (length > 50 bps) and duplicates. The resulting reads were assembled with following parameters (word size = 20 and bubble size = 50, length > 200 bps), and then the assembled contigs were clustered using CD-HIT63. A total of 95,010 assembled contigs were subjected to BLASTX searches against the non-redundant protein database with an E-value threshold of 1 × 10⁻5. Gene ontology (GO) mapping and annotation were performed with an annotation cutoff of E-value < 1 × 10⁻10 and using Blast2GO platform7. GO enrichment analysis was performed with using Agrigo68 with Fisher’s exact test (FDR < 0.05). Putative full-length cDNAs were predicted by comparison of BLASTX reports from the UniProt databases with a web-based ORF prediction tool, Full-lengther66. To identify orthologs, translated sequences of *C. quitensis* derived from ORF prediction and the *Arabidopsis* protein sequence datasets (TAIR version 10.0) which were obtained from the JGI Phytozome website (https://phytozome.jgi.doe.gov) were compared. The orthologs were identified by reciprocal best-hit analysis for selecting BLASTP parameters with options of soft masking and Smith-Waterman alignment (*-seg yes -soft_masking true -use_sw_back*), lowest e-value, query coverage > 50 and protein identity > 50 as best hits67. To map the contigs to the metabolic pathways, the translated sequences of contigs were blasted to the pathways of Kyoto Encyclopedia of Genes and Genomes (KEGG) databases using internal annotation tool in KEGG website25. KEGG enrichment analysis was performed using a KOBAS web server26.

Differentially expressed gene analysis. The expression values were measured in the reads of exons normalized values69. For statistical analysis, Baggerly’s tests70.
and t-tests were performed on the normalized read counts. In addition, several relevant values for analysis, such as p-values and corrected p-values for multiple corrections, were calculated in the "two-group comparison" option. Through the statistical analysis, the differentially expressed genes were determined using a cut-off value (corrected p-value of false discovery rate <0.05 and difference ≠ 0).

qPCR analysis. Total RNA was extracted from leaves of plant samples and purified using the RNeasy Plant Mini Kit (QIAGEN) as described previously. cDNA was synthesized from 2 ug total RNA extracted from samples using SuperScript III (Invitrogen). Gene-specific primers were designed according to the sequences of the contigs and are listed in Supplementary Table S12. To select internal control genes, we selected 7 candidate genes which have steady expressions at both LAB and ANT transcriptome data as follows: 18S rRNA (contig32901), UBC28, ubiquitin-conjugating enzyme E2 28-like (contig3602), RPB6A, DNA-directed RNA polymerase subunit (contig9755), TIM, triosephosphate chloroplast-like (contig19814), CHC, clathrin heavy chain (contig6535), GLX2-4, lactoylglutathione chloroplast-like (contig18505), RPL3, 50S ribosomal protein L3 (contig9479). To validate the fitness of the reference genes, we performed the amplification efficiency test and the gene stability test by RefFinder (http://leonxie.esy.es/RefFinder/)17. The amplification efficiency between 95–105% (R² > 0.97) was determined as good. And RefFinder analysis carried out using Ct values obtained from all experimental samples. As results of both analyses, ranking for reference gene was followed: TIM > 18S > GLX2-4 > UBC28 > RPL3 > RPB6A > CHC. Also, geNorm algorithm 18 gave a suggestion for a combination of TIM and 18S genes, and thus we used them as references genes. The primer information is listed in Supplementary Table S12. A qPCR was performed with biological triplicates using SYBR® Premix Ex Taq™ DNA polymerase (Takara Bio Inc., Shiga, Japan) and the Mx3000P Real-Time PCR System (Stratagene, La Jolla, CA, USA).

Ethics approval and consent to participate. This study including sample collection and experimental research conducted on these materials was according to the law on activities and environmental protection to Antarctic approved by the Minister of Foreign Affairs and Trade of the Republic of Korea.

Data availability. The Illumina raw sequencing data and the sequence information of assembled contigs are available at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA388703, [NCBI bioproject ID: PRJNA388703, Sequence Read Archive (SRA) ID: SRX2913822 and SRX2913823].

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Author Contributions
J.L., H.L. and H.P. developed the conceptual framework. S.M.C., Y.K., H.J., H.R.L. and J.L. performed the experiment. J.L. and S.M.C. wrote the manuscript. H.L., H.R.L. and H.P. assisted in revising the manuscript. All authors have reviewed the final manuscript.

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