



Identification of Molecular Markers for Population Diagnosis of Korean Fir (*Abies koreana*) Vulnerable to Climate Change

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ABSTRACT

Korean fir (*Abies koreana*) is an evergreen coniferous tree species that is unique to South Korea. *A. koreana* is found in a limited sub-alpine habitat and is considered particularly vulnerable to climate change. Identification of populations vulnerable to climate change is an important component of conservation programs. In this study, a heat stress-induced transcriptome RNA-seq dataset was used to identify a subset of six genes for assessment as candidate marker genes for ecologically vulnerable populations. Samples of *A. koreana* were isolated from ecologically stable and vulnerable regions of the Halla and Jiri mountains, and the expression levels of the six candidate markers were assessed using quantitative real-time polymerase chain reaction. All six of the candidate genes exhibited higher expression levels in samples from vulnerable regions compared with stable regions. These results confirm that the six high temperature-induced genes can be used as diagnostic markers for the identification of populations of *A. koreana* that are experiencing stress due to the effects of climate change.

Keywords: *Abies koreana*, Climate change, Heat stress, Vulnerable ecosystem


Introduction

Global climate change is causing environmental shifts such as sea level rise, drought, and flood. The resulting ecosystem changes are leading to changes in the habitats of plants and animals, and a decrease in biodiversity (van der Geest & Warner, 2020). Efforts are ongoing to identify and protect organisms that are threatened by the reduction in biodiversity. Korean fir (*Abies koreana*) is a native

evergreen coniferous species found in sub-alpine regions of South Korea, including the Halla and Jiri mountains. *A. koreana* is considered vulnerable to climate change and is a protected endangered species (Hong *et al.*, 2011; Lee, 1982; Lee *et al.*, 2010).

Plant responses to environmental stress are important in the mitigation of climate change effects. In recent years, leaf dryness has been observed in large areas of *A. koreana* woodland. This collective leaf drying phenomenon is thought to be due to complex interrelationships among environmental factors such as high temperature and dry environment, which have emerged as a result of global warming. Although *A. koreana* has been recognized as a species sensitive to climate change by the South Korean government, only limited research has been conducted into its resilience to environmental stresses (Woo, 2009).

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It is therefore essential to identify the molecular response mechanisms that underlie the responses to environmental stress and thus gauge the resilience to such stresses in *A. koreana* (Fig. 1).

To support conservation efforts, our previous research used *de novo* RNA sequencing to obtain a base transcriptome and examine environmental stress responses in *A. koreana*. *A. koreana* was heat-treated to identify genes whose expression changed specifically under high-temperature conditions (Hwang *et al.*, 2018). In this study,

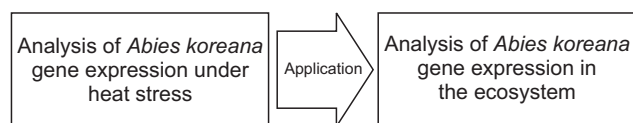


Fig. 1. Schematic representation of identification of heat-induced genes in *Abies koreana* and their assessment as marker genes for ecologically vulnerable populations.

a subset of genes that responded to high temperature in the previous analysis were tested as candidate marker genes for diagnosis of natural *A. koreana* populations vulnerable to high temperature stress.

The results of this analysis will improve the diagnostic aspects of measures to counteract damage to vulnerable ecosystems due to climate change (Park *et al.*, 2018).

Materials and Methods

Identification of candidate genes for diagnosis of vulnerable ecosystems in *Abies koreana*

Raw read sequences from a previously published *A. koreana* high-temperature-stress transcriptome (Hwang *et al.*, 2018) were filtered using Trimmomatic (version 0.32; <http://www.usadellab.org/cms/index.php?page=trimmomatic>) and assembled using Trinity software (version R20140717; [https://github.com/trinityrnaseq/wiki](https://github.com/trinityrnaseq/trinityrnaseq/wiki)). Cluster database at high identity with tolerance (CD-HIT) was used to identify the longest

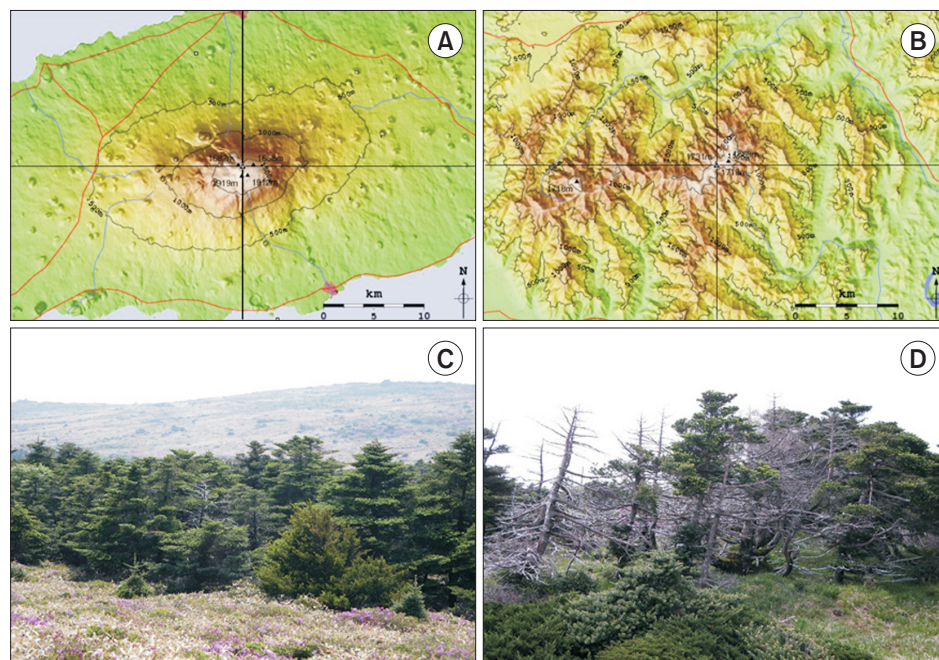


Fig. 2. *Abies koreana* sampling locations. (A) Topography of Halla mountain. (B) Topography of Jiri mountain. (C) Image of ecologically stable *A. koreana* population. (D) Image of ecologically vulnerable *A. koreana* population. Topography images from <https://www.mountain-forecast.com>.

Table 1. Primers for quantitative real-time polymerase chain reaction analysis

Contig	Forward primer sequence (5' → 3')	Reverse primer sequence (5' → 3')
c93462_g1_i1	AAGCGCTGGACAAAGATCAC	TCTTTGCTGATCCCGTCACT
c142609_g1_i1	CGGTTGGATGACTGGGTA	AGGCAGATTCAGGAGCAACT
c156586_g1_i1	GCGCAGATACGTTTCGAGAAA	ACCTTCACCTCCGGATTCAG
c173884_g1_i1	CCAGTGCATTCCGGCTCATA	TTGGCAGCCTGACTTAACT
c205642_g1_i1	GCATTATCCCGCGTCTCT	GTATGCTCCGCGTCCAATAG
c207159_g1_i1	GGCGTCCCTTCTGACTCTAA	TGGCACCATGATTGGAGGT

Table 2. High temperature-inducible genes used as candidate diagnostic markers for ecologically vulnerable *Abies koreana* populations

Contig	Fold change	BLAST	
		Arabidopsis	Poplar
c93462_g1_i1	44.1	AT4G38810.2 Calcium-binding EF-hand family protein	Potri.009G127800.1
c142609_g1_i1	84.7	AT1G69490.1 NAP, ANAC029, ATNAP	Potri.001G404400.1
c156586_g1_i1	13.9	AT5G12030.1 AT-HSP17.6A, HSP17.6, HSP17.6A	Potri.006G223900.1
c173884_g1_i1	7.1	AT5G56960.1 bHLH DNA-binding family protein	Potri.006G148800.1
c205642_g1_i1	2.9	AT2G43790.1 ATMPK6, MPK6, MAPK6, ATMAPK6	Potri.007G139800.1
c207159_g1_i1	40.4	AT5G35550.1 TT2, ATMYB123, MYB123, ATTT2	Potri.006G221800.1

NAP, nucleosome assembly protein; HSP, heat shock protein, bHLH:basic helix-loop-helix; MPK, mitogen-activated protein kinase; MYB, myoblastosis; AT, arabidopsis thaliana.

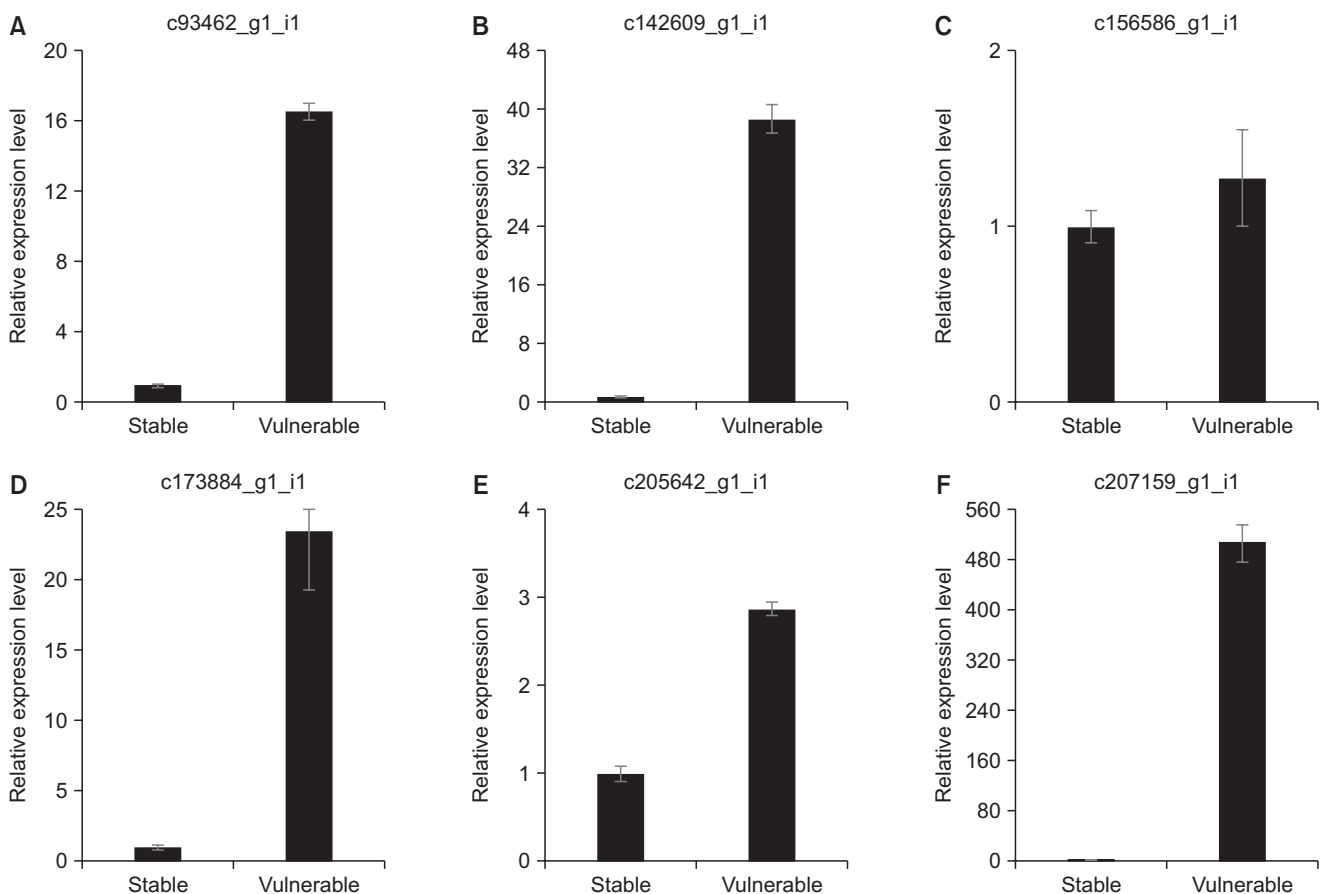


Fig. 3. Analysis of candidate diagnostic markers in ecologically stable and vulnerable populations of *Abies koreana* from Halla mountain. Gene expression in vulnerable samples is shown relative to expression in stable samples, which is set to 1. (A) c93462_g1_i1. (B) c142609_g1_i1. (C) c156586_g1_i1. (D) c173884_g1_i1. (E) c205642_g1_i1. (F) c207159_g1_i1.

sequences from overlapping fragments. Transcripts were annotated by NCBI-nr BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and grouped by predicted gene functions using WEGO (<http://wego.genomics.org.cn/>). High-temperature specific genes were classified as those with more than 2-fold increased expression and P -values ≤ 0.05 with differential expression sequence analysis using RNA-seq by expectation-maximization.

Abies koreana sample collection

A. koreana leaf samples were collected from ecologically stable and vulnerable areas in the Halla and Jiri mountains (Fig. 2). Samples were lyophilized immediately and stored at -80°C for RNA extraction. RNA extraction was performed using a Ribospin Seed/Fruit Kit (GeneAll, Seoul, Korea) according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (qRT-PCR)

A. koreana RNA was reverse transcribed to cDNA using

a ReverTra Ace- α kit (Toyobo, Osaka, Japan). For qRT-PCR, $1\ \mu\text{L}$ diluted cDNA was added to $10\ \mu\text{L}$ of $2\times\text{IQ SYBR Green Supermix}$, $1\ \mu\text{L}$ of $10\ \mu\text{M}$ forward primer, $1\ \mu\text{L}$ reverse primer, and $7\ \mu\text{L}$ H_2O . Reactions were denatured at 95°C for 10 minutes, followed by 55 cycles of 95°C for 15 seconds, 55°C for 15 seconds, and 75°C for 30 seconds. Reactions were performed using a CFX 96 real-time system (Bio-Rad, Hercules, CA, USA), and data were analyzed using the Bio-Rad CFX Manager program. Primers used are listed in Table 1.

Results and Discussion

Identification of high temperature-responsive markers in *Abies koreana*

To identify high-temperature responsive transcription factors in *A. koreana*, known transcription factor domains were used to search our previously published *A. koreana* RNA-seq database (Hwang *et al.*, 2018). Six transcriptional regulators whose expression increased more than 2-fold

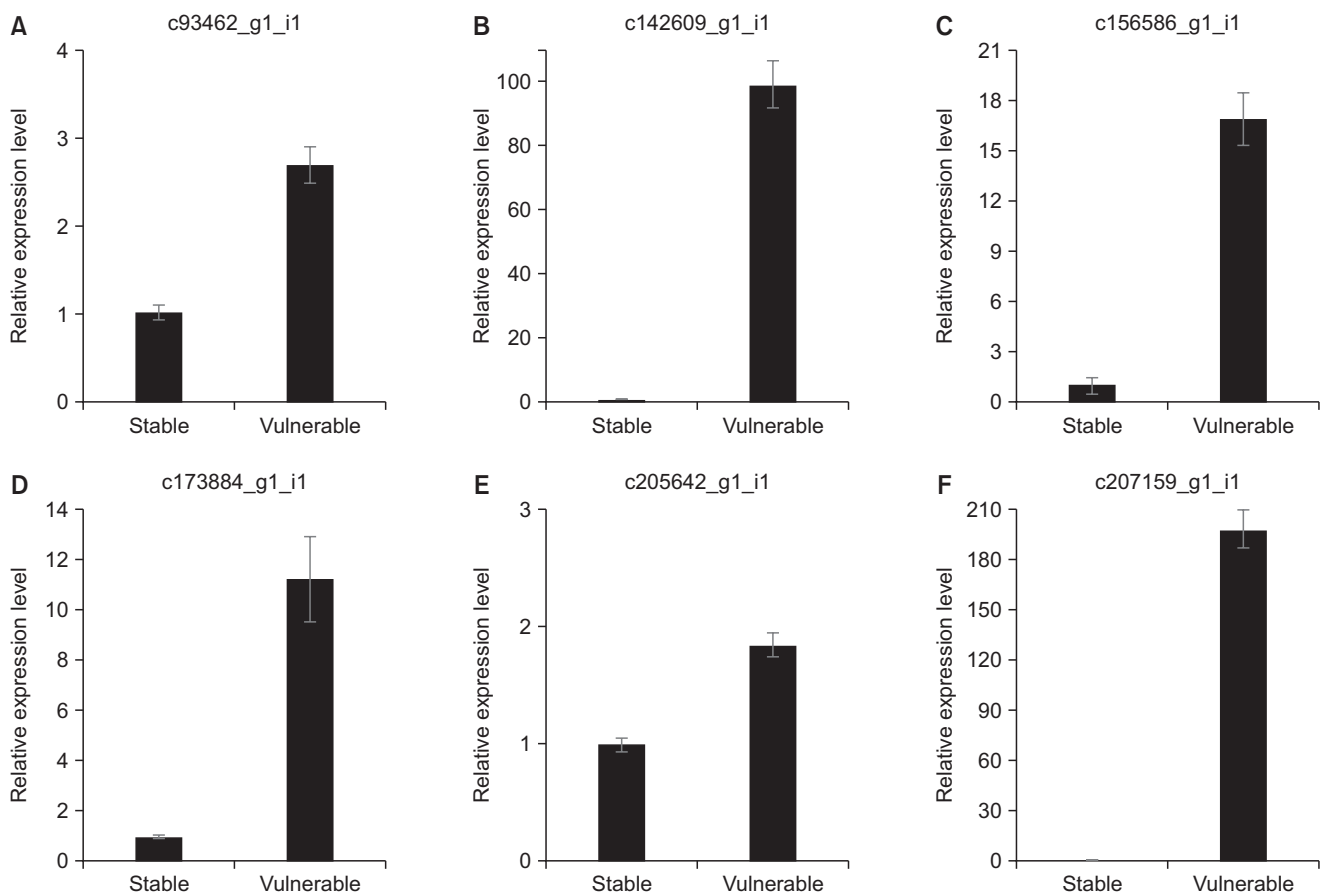


Fig. 4. Analysis of candidate diagnostic markers in ecologically stable and vulnerable populations of *Abies koreana* from the Banya region of Jiri mountain. Gene expression in vulnerable samples is shown relative to expression in stable samples, which is set to 1. (A) c93462_g1_i1. (B) c142609_g1_i1. (C) c156586_g1_i1. (D) c173884_g1_i1. (E) c205642_g1_i1. (F) c207159_g1_i1.

upon heat exposure were identified, as follows: c93462_g1_i1, c142609_g1_i1, c156586_g1_i1, c173884_g1_i1, c205642_g1_i1, c207159_g1_i1 (Table 2). These six high temperature-induced genes in *A. koreana* were then assessed for their utility as diagnostic markers for ecologically vulnerable populations using samples from the Halla and Jiri mountains in South Korea.

Evaluation of six high temperature-responsive genes as markers for ecologically vulnerable *Abies koreana* populations using samples from Halla mountain

Expression of the six candidate marker genes was assessed in samples from ecologically stable (Youngsil region) and vulnerable (Nambuk region) areas of Halla mountain, by qRT-PCR. All six markers exhibited higher expression levels in samples from vulnerable regions compared with samples from stable regions (Fig. 3). Expression was 1.3-fold higher for c156586_g1_i1, 38.6-fold higher for c142609_g1_i1, 507.7-fold higher for c207159_g1_i1, 26.3-fold higher for c173884_g1_i1,

16.5-fold higher for c93462_g1_i1, and 2.9-fold higher for c205642_g1_i1. These results were consistent with qRT-PCR analysis of *A. koreana* samples collected in 2017 (Park et al., 2017).

Evaluation of six high temperature-responsive genes as markers for ecologically vulnerable *Abies koreana* populations using samples from Jiri mountain

Expression of the six candidate marker genes was assessed in samples from ecologically stable and vulnerable areas of the Banya and Seseok regions of Jiri mountain, by qRT-PCR. In samples from the Banya region, all six markers exhibited higher expression levels in samples from vulnerable regions compared with samples from stable regions (Fig. 4). Expression was 16.8-fold higher for c156586_g1_i1, 98.6-fold higher for c142609_g1_i1, 198-fold higher for c207159_g1_i1, 11.2-fold higher for c173884_g1_i1, 2.7-fold higher for c93462_g1_i1, and 1.8-fold higher for c205642_g1_i1. In samples from the Seseok region, all six markers also exhibited higher

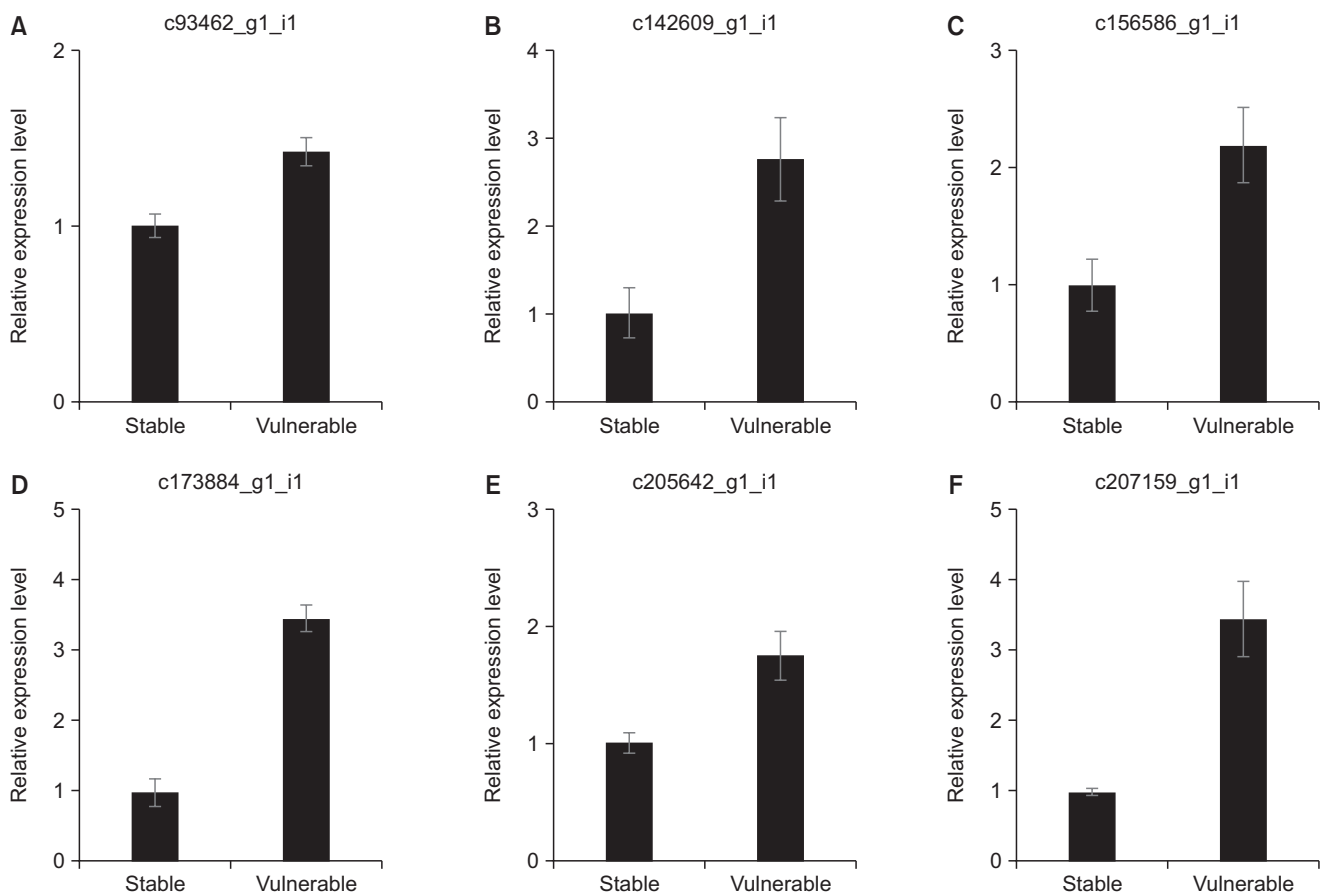


Fig. 5. Analysis of candidate diagnostic markers in ecologically stable and vulnerable populations of *Abies koreana* from the Seseok region of Jiri mountain. Gene expression in vulnerable samples is shown relative to expression in stable samples, which is set to 1. (A) c93462_g1_i1. (B) c142609_g1_i1. (C) c156586_g1_i1. (D) c173884_g1_i1. (E) c205642_g1_i1. (F) c207159_g1_i1.

expression levels in samples from vulnerable regions compared with samples from stable regions (Fig. 5). Expression was 2.2-fold higher for c156586_g1_i1, 2.8-fold higher for c142609_g1_i1, 3.4-fold higher for c207159_g1_i1, 3.4-fold higher for c173884_g1_i1, 1.4-fold higher for c93462_g1_i1, and 1.7-fold higher for c205642_g1_i1.

Overall, expression of diagnostic marker genes in ecologically vulnerable and stable regions showed the same trends in *A. koreana* samples from the Jiri and Halla mountains.

Recent declines in *A. koreana* have been attributed to heat stress caused by climate change. The purpose of this study was to identify genes with increased expression upon exposure to heat stress, and to assess their utility as diagnostic markers for ecologically vulnerable natural populations of *A. koreana*. Six candidate marker genes were identified from a heat-stress RNA-seq dataset, and their expression was assessed in leaf samples from ecologically vulnerable and stable natural populations using qRT-PCR. All six markers exhibited elevated expression in samples from vulnerable populations, confirming their value as diagnostic markers.

Conflict of Interest

The authors declare that they have no competing interests.

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