

Assessing Genetic Plasticity in Response to New Environmental Conditions in Coniferous Tree Seeds from Multiple Provenances

Peng-Kai Zhu

College of Landscape Architecture and Art, Fujian Agriculture and Forestry University

<https://orcid.org/0009-0007-5598-1300>

Yan-Ping Xu

College of Forestry, Fujian Agriculture and Forestry University

Ju-Dong Rong

Fujian Agriculture and Forestry University

Kai Liu

Fujian Agriculture and Forestry University

Tian-You He

Fujian Agriculture and Forestry University

Li-Guang Chen

Fujian Agriculture and Forestry University

Yu-Shan Zheng

Fujian Agriculture and Forestry University

Ling-Yan Chen

fafucly@fafu.edu.cn

Fujian Agriculture and Forestry University

Research Article

Keywords: Coniferous tree, Environmental changes, Gene expression, *Chamaecyparis hodginsii*, Adaptability, Seed experiments

Posted Date: March 8th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4022821/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Abstract

In the context of climate change, assessing the adaptive potential of species and populations is crucial for developing effective conservation strategies. Changes in plant gene expression play a significant role in the adaptation process to climate change. This study aims to explore the adaptive responses of the near-threatened conifer species *Chamaecyparis hodginsii* to climate change and analyze the molecular-level reactions of these long-lived trees to climatic shifts. It seeks to understand their phenotypic responses to climate change, identify key environmental factors driving adaptive gene expression, and provide information for transplantation conservation strategies based on genetic adaptability. By conducting mixed-tissue RNA sequencing on seeds from multiple provenances and employing redundancy analysis (RDA), weighted gene co-expression network analysis (WGCNA), and partial least squares path modeling (PLS-PM), the study assesses the impact of climatic variables on gene expression and phenotype. It identifies key gene groups associated with environmental responses and elucidates the complex relationships between environmental factors, functional gene groups, and phenotypic traits. The findings reveal that *C. hodginsii* adapts to environmental stresses by regulating specific gene activities related to morphological trait adjustments. Moreover, environmental factors such as the impact on tree architecture emphasize the importance of Precipitation Seasonality, Isothermality, and Precipitation of Driest Quarter for adapting to climate stresses. This research not only unveils the complex adaptive responses of *C. hodginsii* to climate change but also provides critical insights for the management and conservation of long-lived tree species facing climate change threats.

1 Introduction

Climate change is one of the major threats to biodiversity, with studies showing it has led to localized extinctions of animal and plant species (Walther et al. 2002). Assessing the adaptive potential of species and populations is crucial for developing effective conservation strategies (Eizaguirre and Baltazar-Soares 2014; Razgour et al. 2019). Species with higher adaptive potentials are more likely to survive under the threat of climate change (Aitken et al. 2008; Yang et al. 2022). For long-lived species, such as forest trees, the pace of mutation and migration may not keep up with environmental changes, limiting their ability to adapt (Holliday et al. 2017; Bisbing et al. 2021; Dauphin et al. 2021). Therefore, changes in plant gene expression play a crucial role in the adaptation of species to environmental changes. Rapid environmental shifts can induce changes in gene expression programs, thereby regulating plant growth and metabolism to adapt to new conditions and increasing phenotypic variation at the population level (López-Maury, Marguerat, and Bähler 2008; Berger et al. 2011). Changes in plant gene transcription levels not only directly reflect the species' adaptive potential to current environments but are also essential for assessing the viability of natural species under climate change and for developing effective ecological and forest management strategies. During the process of adapting to environmental stress, plants undergo extensive changes in gene expression (Z.-H. Chen and Soltis 2020; Kan et al. 2023), providing a basis for natural selection on short evolutionary timescales (Xu et al. 2016; Innes et al. 2023).

Differences in gene expression patterns can explain the varying abilities of different populations to adapt to environmental changes (Xu et al. 2016; Poncet et al. 2010). While traditional methods such as reciprocal transplants or common garden experiments have provided valuable insights into plant local adaptation, they face challenges of experimental difficulty and long generation times, especially for long-lived species like trees (Anderson, Willis, and Mitchell-Olds 2011; Fournier-Level et al. 2011; Sang et al. 2022). However, translocating organisms from their native habitats to new environments remains an effective means of conserving genetic diversity, especially for threatened and rare species (Hufford and Mazer 2003), and offers opportunities for studying adaptability (Weeks et al. 2011). Notably, seedlings, being in the early stages of growth, may be more sensitive to environmental changes (Hummel et al. 2004; Huang and Wang 2016). RNA sequencing technology plays a key role in revealing the function of gene expression in plant adaptation to environmental changes (Hess et al. 2016) and has been successfully applied to the study of adaptability in conifer species (Ma et al. 2019; Yang et al. 2022). This technique, by precisely quantifying gene activity, provides us with a powerful tool to understand how plants respond to environmental stress at the molecular level. Furthermore, Weighted Gene Co-expression Network Analysis (WGCNA) allows us to identify gene networks closely associated with climate change adaptability, revealing how populations adapt to rapidly changing climates through gene expression regulation. With WGCNA, we can explore key gene groups that show significant changes under different environmental conditions (Rezaie, Reese, and Mortazavi 2023), deepening our understanding of species adaptation mechanisms. This analytical approach not only reveals potential mechanisms for coping with climate change but also offers new perspectives for predicting species' responses to future environmental changes.

Trees act as effective carbon sinks and are crucial in fighting climate change (Isabel, Holliday, and Aitken 2020; Zhu et al. 2023). Their long generation times and frequent extreme climate events make them vulnerable to climate change (Nepstad et al. 2007; Dauphin et al. 2021). This study focuses on *Chamaecyparis hodginsii*, a near-threatened species. We aim to understand its adaptability to climate change using mixed-tissue RNA sequencing. Our goals include revealing tree phenotypes' response to climate change at the gene expression level, identifying key environmental factors affecting adaptability, and analyzing gene expression regulation. We also aim to support conservation translocation strategies based on gene adaptability. This work seeks to provide insights and strategies for protecting long-lived tree species against climate change.

2 Materials and Methods

2.1 Plant Materials

All plant samples in this study were sourced from the Bailai State-owned Forest Farm in Hutou Town, Anxi County, Quanzhou City, Fujian Province, China (118°3' E, 25°49' N, 590-600 m above sea level). The plant experiments and field studies, including the collection of plant materials, complied with relevant institutional, national, and international guidelines and regulations. The germination and sprouting experiments of cypress seeds were conducted in the greenhouse of Fujian Agriculture and Forestry

University, with seeds disinfected before sowing. At the end of 2015, seed germination began under controlled environmental conditions, maintaining a temperature of 22°C to 25°C and a relative humidity of 70%-80%. Successfully germinated seeds were then transferred to seedling trays filled with standard nutrient soil to promote seedling growth. Seedlings received at least 8 hours of natural light, supplemented by grow lights to ensure adequate lighting, and were watered and fertilized according to standard care procedures. In April 2016, when the seedlings reached about 15cm in height, they were transplanted to outdoor plots with similar soil texture, drainage conditions, and light exposure. The experiment employed a completely randomized block design (CRD) with 8 plots, each containing representative seedlings from 14 different provenances (Table S1; Figure S1). Five seedlings from each provenance were randomly assigned to each plot to ensure even distribution across the experimental area, with a planting distance of 2m to minimize competition. Appropriate measures were taken to alleviate transplant shock, and the growth and adaptability of the seedlings were regularly monitored post-transplantation. In April 2020, four years after transplantation, key growth parameters of the surviving seedlings were measured, including Height, Diameter at Breast Height (DBH), Crown Width (CW), and the Average Angle of Dips, the latter obtained by calculating the average angle of the three lowest secondary branches of each plant.

2.2 Transcriptome Sequencing

In April 2020, we randomly selected seven individuals from each seed provenance and collected their stem-differentiating xylem, leaves, branches, and roots. These tissues were mixed by category and disinfected uniformly with alcohol for 30 seconds to one minute, then rinsed in deionized water for 2 minutes to remove any alcohol residue and dead microbes. One gram of mixed tissue sample from each type was immediately weighed, flash-frozen in liquid nitrogen, and stored at -80°C for later use. Total RNA was extracted using the RNeasy Pure Plant Kit (Qiagen, Beijing, China) and the quality of RNA was assessed by 2% agarose gel electrophoresis. The concentration of RNA was determined by the NanoPhotometer[®] spectrophotometer (IMPLEN, California, USA) and the Qubit[®] 2.0 Fluorometer in conjunction with the Qubit[®] RNA Assay Kit (Life Technologies, California, USA). RNA integrity was evaluated using the RNA Nano 6000 Assay Kit on the Agilent[®] 2100 Bioanalyzer (Agilent Technologies, California, USA), with an RNA integrity number (RIN) greater than 7 considered satisfactory. Subsequently, libraries were constructed using the NEB-Next[®] Ultra[™] RNA Library Prep Kit for Illumina[®] (NEB, USA) and sequenced on the Illumina[®] NovaSeq 6000 platform to produce 150 bp paired-end reads.

2.3 RNA-seq read mapping and quantification

We performed initial processing of the raw RNA-Seq reads using fastp v0.23.2 (S. Chen et al. 2018) with parameters set to ensure quality (-qualified_quality_phred 15 -unqualified_percent_limit 50 -n_base_limit 15). The resulting clean reads, along with those from other *C. hodginsii* RNA-Seq datasets (NGDC: CRA013478), were aligned to the genome using the Two-pass alignment mode of STAR v2.7.8a (Dobin et

al. 2013). The alignment parameters (`–alignIntronMin 20 –alignIntronMax 50000 –sjdbOverhang 149 –outFilterMismatchNmax 2 –outSJfilterReads Unique –outSAMmultNmax 1 –outSAMmapqUnique 60`) were chosen to enhance the accuracy and reliability of the alignment. After alignment, structural annotation files based on the new alignment results were generated using `gffcompare v0.12.6` (Pertea and Pertea 2020). The clean reads were then realigned to the *C. hodginsii* reference genome, which included new annotations, to ensure the accuracy of the alignment. Finally, gene expression levels for each gene were quantified using `featureCounts v2.0.1` (Liao, Smyth, and Shi 2014) with default parameters.

2.4 Static analysis

First, we obtained information on 19 environmental factors from various seed provenance locations (Table S1), sourced from the WorldClim database (Fick and Hijmans 2017). Spearman correlation analysis was employed to assess the interrelationships among these environmental factors. Subsequently, we conducted gradient forest model analysis using the R package `gradientForest v0.1-37` (Ellis, Smith, and Pitcher 2012), setting the tree count to 500, to evaluate the impact of each environmental factor on gene expression levels and determine their significance. Based on the results of the gradient forest analysis and the correlations between environmental factors, we selected factors that were both significant and had low intercorrelations to reduce potential collinearity issues. These selected environmental factors were then used as explanatory variables in subsequent Redundancy Analysis (RDA) to delve deeper into their relationship with gene expression data. RDA, known for its low false-positive rate (Capblancq and Forester 2021), was conducted using the R package `vegan v2.6-4` (Jari Oksanen et al. 2017) to further reveal how environmental factors influence gene expression patterns. Significant environment-associated expressed genes were defined by their loadings on the tails of the distribution, using a standard deviation cutoff of 3 along one or more RDA axes.

To gain a deeper understanding of the modular characteristics of gene expression, this study employed WGCNA. We utilized the Python package `PyWGCNA v2.0.1` (Rezaie, Reese, and Mortazavi 2023) with default parameter settings. This analysis clusters genes into modules based on similar expression patterns, aiding in exploring the correlations among these modules, environmental factors, and morphological traits of each seed provenance. It helps identify groups of genes that may be influenced by specific environmental factors. Within each module significantly related to environmental and phenotypic factors, we further filtered to select genes with a KME value greater than 0.6 as key genes. These key genes are considered to play central roles within the modules and may be sensitive to environmental changes. Finally, we selected these key genes representing modules with high correlations to environmental factors to construct a Partial Least Squares Path Modeling (PLS-PM) using the R package `plspm v0.5.1` (Tenenhaus et al. 2005), with the maximum iteration count set to 300. This model integrates the relationships among environmental factors, functional genes, and phenotypic changes. The PLS-PM model reveals the direct and indirect effects among these variables, offering profound insights into how

gene expression is regulated by environmental factors at the seed provenance location, impacting the phenotype.

3 Results

3.1 Assessing and Addressing Collinearity in Environmental Factors Affecting Gene Expression

In this study, we initially conducted a correlation analysis for all considered environmental factors to identify potential collinearity issues (Figure 1A). The results revealed strong positive correlations among some temperature indices such as Annual Mean Temperature, Mean Temperature of Driest Quarter, and Mean Temperature of Warmest Quarter. Additionally, precipitation indices like Annual Precipitation, Precipitation of Coldest Quarter, and Precipitation of Wettest Quarter also showed very strong positive correlations. These highly correlated factors could affect the accuracy and interpretability of the RDA. Therefore, we assessed the impact of each environmental factor on gene expression using the gradient forest model. The results indicated significant differences in the importance of various environmental variables for model prediction. Precipitation of Driest Quarter and Precipitation of Driest Month had the highest importance scores, highlighting the critical impact of drought conditions on the prediction model. Mean Temperature of Driest Quarter and Precipitation of Wettest Month also showed high importance, emphasizing the effects of temperature and extreme wet conditions.

Furthermore, we grouped highly correlated environmental factors (correlation coefficient $|r| > 0.65$) together. During processing, we considered the importance scores of collinear factors within each group and selected the factor with the highest importance score as the representative for each group (Figure 1B). Based on the analysis, we ultimately chose five relatively independent and important environmental factors to avoid collinearity issues while retaining the most explanatory factors. These factors include Precipitation of Driest Quarter, Mean Temperature of Driest Quarter, Temperature Annual Range, Isothermality, and Precipitation Seasonality. This selection process ensured our model accounted for the importance of environmental factors while avoiding potential problems caused by collinearity.

3.2 Linking Environmental Variability to Gene Expression Patterns

The RDA results revealed a strong connection between gene expression and environmental factors, with the first axis explaining 38.6% of the environmental variation, while the second and third axes accounted for an additional 21.7% and 16.5% of variation, respectively, highlighting the profound impact of environmental factors on gene expression (Figure 2 A&B; Table S2). Notably, the significant orientation of Precipitation Seasonality on the first axis indicated it as a key environmental factor driving major variations in gene expression. The similar direction of arrows for Isothermality and Precipitation of Driest Quarter suggested some similarity in their effects on gene expression. In contrast, the unique direction of

Mean Temperature of Driest Quarter pointed to a different pattern of influence compared to other factors. Furthermore, the directions of Temperature Annual Range and Precipitation of Driest Quarter nearly overlapped on the second and third axes (Figure 2B), suggesting similarities in their effects on gene expression patterns in these specific dimensions. However, this does not imply a lack of differences between these two factors; rather, such differences may not be evident on these two axes.

Further analysis revealed that 2941 genes had significant loadings on at least one RDA axis, indicating high sensitivity to specific environmental factors and significant expression variation. Through 999 permutation tests, our model was statistically significant ($p < 0.05$), confirming the substantial impact of Precipitation of Driest Quarter and Mean Temperature of Driest Quarter on gene expression (Table S3). Overall, environmental factors accounted for 25.06% of the total variance, with the remaining variance likely due to other unconsidered variables. These findings highlight the complexity of plant gene regulatory networks and suggest that plants may respond to diverse environmental conditions through transcriptional regulatory mechanisms.

3.3 Identifying Key Gene Modules Linked to Environmental and Phenotypic Variations

Through WGCNA, we categorized the gene expression data into 14 modules, each containing a cluster of genes with similar expression patterns (Figure S2). The analysis indicated significant correlations between specific modules and environmental factors or phenotypic traits, suggesting that certain genes may respond to environmental stresses or phenotypic changes. For instance, the Rosybrown module showed a positive correlation with Precipitation of Driest Quarter ($r = 0.65$, $P < 0.05$), while the Brown module was negatively correlated with DBH ($r = -0.63$, $P < 0.05$) (Figure 3). To identify genes most sensitive to environmental changes, we selected those with module membership (KME) values greater than 0.6 as key genes, highly correlated with the representative gene expression patterns of their respective modules. Integrating these findings with RDA results, we further pinpointed key genes within the Dimgrey, Firebrick, Rosybrown, and Snow modules. These modules demonstrated strong correlations with environmental factors or phenotypic traits, reinforcing the credibility of the RDA findings. Notably, the Dimgrey module contributed the majority of key genes, with fewer key genes from the other three modules (Figure S3), suggesting that while multiple modules are associated with environmental or phenotypic characteristics, the Dimgrey module may play a leading role in responding to environmental changes. Functional enrichment analysis of these key genes revealed their involvement in the synthesis of cyclic and aromatic compounds and various enzymatic activities (Figure 4). These functions are crucial for cellular metabolic activities, the transmission of genetic information, and genome integrity, highlighting the pivotal role of these genes in plant adaptive responses and phenotypic shaping.

3.4 Modeling the Influence of Environmental Factors on Phenotypic Traits via Key Gene Pathways

To further understand how environmental factors influence plant phenotypic traits through key genes, we employed PLS-PM. This analysis revealed a network of relationships among environmental variables, functional gene groups within the key genes, and phenotypic traits ($p < 0.05$). The environmental variables included only Precipitation Seasonality, Isothermality, and Precipitation of Driest Quarter. These factors significantly impacted two groups of genes: one involved in the biosynthesis of cyclic and aromatic compounds, and the other related to peptidase and nucleotidyltransferase activities (Figure 5; Table S4). These relationships were further linked to two phenotypic traits of trees: CW and Average Angle of Dips. Precipitation Seasonality and Isothermality directly influenced the genes associated with cyclic and aromatic compound biosynthesis, subsequently affecting CW. In contrast, the mean temperatures of the three environmental variables influenced the gene groups related to peptidase and nucleotidyltransferase activities, which then affected the Average Angle of Dips. Additionally, our model explained 76.15% of the variance in CW and 68.24% of the variation in Average Angle of Dips (Table S5), indicating that we could substantially explain the variations in phenotypic traits. This model helps visualize the complexity of gene responses to environmental cues and their potential impact on phenotypes, emphasizing the significance of specific environmental factors in the adaptive responses of the species.

4 Discussion

The capacity of plants to adapt to environmental stress, particularly under rapidly changing climate conditions, is a central topic in ecological and evolutionary biology research. Previous studies have shown that plants can achieve local adaptation through genetic variation even over short geographical and temporal scales (Medrano et al. 2020; Innes et al. 2023), suggesting that plants can cope with diverse environmental challenges by selecting specific life-history traits (Poncet et al. 2010). However, long-lived and highly specialized species may face issues of adaptive lag, especially pronounced against the backdrop of rapid climate change (Holliday et al. 2017; Sang et al. 2022; Bisbing et al. 2021). In response to this challenge, our study, using the translocation of *C. hodginsii* as an example, explored the potential adaptability of conifer trees under environmental change pressures. Climate change has significantly increased the intensity of droughts in humid regions, posing major challenges to plant growth and development (Ruffault et al. 2013; Grossiord et al. 2020). Temperature and moisture are key factors affecting plant growth and development, with changes in environmental temperature directly impacting plant physiological processes (Porporato et al. 2001; Ding, Shi, and Yang 2020).

The strong El Niño event of 2015-2016, part of the El Niño Southern Oscillation (ENSO) phenomenon, coincided with the transplantation period of our *C. hodginsii* seedlings (Qian 2022). This severe drought could lead to a significant increase in tree mortality (Nepstad et al. 2007), providing a unique environmental test for the growth and development of our plants. Under such harsh conditions, the early growth stages of the plants were likely significantly affected. Notably, transcriptomic analyses were conducted on these four-year-old transplanted *C. hodginsii*. The results showed a range of transcriptional changes closely related to the climate of their seed provenance locations (Figure 2 and Figure 3), especially genes involved in the biosynthesis of cyclic and aromatic compounds (Figure 4). This suggests that *C. hodginsii* might cope with early environmental stress by regulating the synthesis of

aromatic compounds. These compounds not only play roles in signaling between plants, enhancing stress tolerance (Ahammed et al. 2015; 2020), but may also prime surrounding plant tissues for stronger responses to subsequent stresses (Erb et al. 2015). This mechanism may be particularly important for conifers, known for their aromatic wood (Wan et al. 2022), in adapting to rapid environmental changes.

Gymnosperms often have large genome sizes, largely attributed to historical transposon activity causing genomic redundancy (Zhu et al. 2023). In this study, we found a positive correlation between the climate of seed provenance locations and genes involved in peptidase and nucleotidyltransferase activities, enzymes associated with transposon activity (Hickman and Dyda 2015; 2016). This suggests that there might be differences in transposon activity levels among *C. hodginsii* provenances. Additionally, the activity changes in these genes seem to be related to a decrease in the Average Angle of Dips (Figures 4 and 5). Transposon activity plays a unique role in species diversification and gene function variation, even in species with narrower gene pools (Domínguez et al. 2020). Specifically, Genic transposable element (TE) insertions can lead to the production of multiple transcriptional isoforms, thereby increasing the diversity of gene expression (Aktaş et al. 2017; Vandecraen et al. 2017). As a Tertiary relict gymnosperm (Yin et al. 2021), transposon activity in the evolutionary history of *C. hodginsii* has provided significant momentum (Dubin, Scheid, and Becker 2018; Lisch 2012). In the context of dramatic global climate changes, active transposons may offer ongoing dynamism for the environmental adaptability of *C. hodginsii*, facilitating phenotypic variation.

Precipitation and sunlight significantly influence tree architecture, where smaller branch angles and shorter branch lengths help reduce the risk of photodamage and cavitation in the water transport system (Lines et al. 2012; Ceulemans et al. 1990; Haworth and McPherson 1995). Our findings indicate a negative correlation between changes in Precipitation Seasonality and both the CW and Average Angle of Dips of *C. hodginsii* (Figure 5). This suggests that *C. hodginsii* from regions with more seasonal precipitation may be more sensitive to extreme drought conditions. Additionally, transposon activity could indirectly affect branching patterns by regulating the expression of genes related to plant hormone signaling and branch development (Lisch 2009). For instance, a class of aromatic plant hormones, such as strigolactones, can regulate plant architecture (Gomez-Roldan et al. 2008; Crawford et al. 2010). Therefore, we speculate that transposon activity may regulate the branching pattern of *C. hodginsii* by influencing the expression of genes in hormone signaling pathways that regulate plant architecture, thereby helping it better adapt to its growing environment.

While our study provides insights into the potential interactions between environmental factors, transposon activity, and plant hormones, the way these elements collectively influence the adaptive development of relict plants like *C. hodginsii* requires further investigation. In particular, understanding how *C. hodginsii* utilizes these mechanisms to adapt to changing environments remains an open question. Future research should delve into the specific mechanisms of these interactions through genetic and physiological experiments to deepen our understanding of plant adaptive evolution. Moreover, considering that transcriptional regulatory networks and epigenetic mechanisms, such as DNA methylation and histone modification might be associated with transposon activity (Wan et al. 2022),

exploring their roles in rapid environmental adaptation will be crucial. This necessitates not only broader genomic data but also specific studies on the role of epigenetic modifications in adaptive development.

Declarations

Author Contributions

P.-K. Z.: Conceptualization, Data Curation, Formal Analysis, Writing - Original Draft. Y.-P. X.: Data Curation, Investigation. R.-J. D.: Data Curation, Investigation. K. L.: Data Curation, Investigation. T.Y. H.: Funding Acquisition. L.-G. C.: Funding Acquisition. Y.-S. Z.: Resources. L.-Y. C.: Supervision, Writing- Reviewing and Editing.

Acknowledgements

This work was supported by National Key Research and Development Program of China [grant numbers 2021YFD2200501]; Scientific Research Project of Fujian Province [grant numbers 2023J01478]; Fujian Forestry Nursery Technology Tackle Key Issues Project Phase Seven [grant numbers LZKG202207] and Forestry Peak Discipline Construction Project from Fujian Agriculture and Forestry University [grant numbers 72202200205]. The funding agency was not involved in the design of the study, collection, analysis, interpretation of data and writing the manuscript.

Data Availability Statement

The sequencing data and phenotypic measurements in the current study are available in the NGDC (National Genomics Data Center) repository, PRJCA023136. Further information may be requested from the corresponding author.

References

1. Ahammed G, Li X, Liu Ai-rong, Chen S (2020) Brassinosteroids in Plant Tolerance to Abiotic Stress. *J Plant Growth Regul* 1–14. <https://doi.org/10.1007/s00344-020-10098-0>
2. Ahammed G, Xia X, Li X, Shi K, Jingquan Yu, and, Yanhong Zhou (2015) Role of Brassinosteroid in Plant Adaptation to Abiotic Stresses and Its Interplay with Other Hormones. *' Curr Protein Pept Sci* 16 5:462–473. <https://doi.org/10.2174/1389203716666150330141427>
3. Aktaş Tuğçe, İlk İbrahimAvşar, Maticzka D, Bhardwaj V, Rodrigues CP, Mittler G, Manke T, Backofen R, Akhtar A (2017) DHX9 Suppresses RNA Processing Defects Originating from the Alu Invasion of the Human Genome. *Nature* 544(7648):115–119. <https://doi.org/10.1038/nature21715>
4. Anderson JT, Willis JH, Thomas Mitchell-Olds (2011) Evolutionary Genetics of Plant Adaptation. *Trends Genet* 27(7):258–266. <https://doi.org/10.1016/j.tig.2011.04.001>
5. Berger D, Bauerfeind SS, Blanckenhorn WU, Andreas M Schäfer, CRYPTIC GENETIC VARIATION IN A POLYMORPHIC FEMALE SPERM STORAGE ORGAN' (2011) *Evolution* 65(10):2830–2842.

- <https://doi.org/10.1111/j.1558-5646.2011.01392.x>. 'HIGH TEMPERATURES REVEAL
6. Bisbing SM, Alexandra K, Urza, Brian J, Buma DJ, Cooper M, Matocq, Angert AL (2021) Can Long-Lived Species Keep Pace with Climate Change? Evidence of Local Persistence Potential in a Widespread Conifer. *Divers Distrib* 27(2):296–312. <https://doi.org/10.1111/ddi.13191>
 7. Capblancq T, Forester BR (2021) Redundancy Analysis: A Swiss Army Knife for Landscape Genomics. *Methods Ecol Evol* 12(12):2298–2309. <https://doi.org/10.1111/2041-210X.13722>
 8. Ceulemans R, Stettler RF, Hinckley TM, Isebrands JG (1990) and Paul E. Heilman. 'Crown Architecture of Populus Clones as Determined by Branch Orientation and Branch Characteristics.' *Tree Physiology* 7 1_2_3_4: 157–67
 9. Chen S, Zhou Y, Chen Y, Jia, Gu (2018) Fastp: An Ultra-Fast All-in-One FASTQ Preprocessor. *Bioinformatics* 34(17):i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>
 10. Chen Z-H, Soltis DE (2020) Evolution of Environmental Stress Responses in Plants. *Plant Cell Environ* 43(12):2827–2831. <https://doi.org/10.1111/pce.13922>
 11. Crawford SE, Shinohara N, Sieberer T, Williamson L, George G, Hepworth J, Müller Dörte, Domagalska M, Leyser O (2010) Strigolactones Enhance Competition between Shoot Branches by Dampening Auxin Transport. *Development* 137:2905–2913. <https://doi.org/10.1242/dev.051987>
 12. Dauphin B, Rellstab C, Schmid M, Zoller S, Karger DN, Brodbeck S Frédéric Guillaume, and Felix Gugerli. 2021. 'Genomic Vulnerability to Rapid Climate Warming in a Tree Species with a Long Generation Time'. *Glob Change Biol* 27 (6): 1181–1195. <https://doi.org/10.1111/gcb.15469>
 13. Ding Y, Shi Y, and Shuhua Yang (2020) Molecular Regulation of Plant Responses to Environmental Temperatures. *Mol Plant* 13(4):544–564. <https://doi.org/10.1016/j.molp.2020.02.004>
 14. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Thomas R, Gingeras (2013) STAR: Ultrafast Universal RNA-Seq Aligner. *Bioinformatics* 29(1):15–21
 15. Domínguez M, Dugas E, Benchouaia Médine, Leduque B, Jiménez-Gómez JoséM, Colot V, Leandro Quadrana (2020) The Impact of Transposable Elements on Tomato Diversity. *Nat Commun* 11(1):4058. <https://doi.org/10.1038/s41467-020-17874-2>
 16. Dubin M, Mittelsten Scheid O, Becker C (2018) Transposons: A Blessing Curse. *Curr Opin Plant Biol* 42:23–29. <https://doi.org/10.1016/j.pbi.2018.01.003>
 17. Eizaguirre C, Baltazar-Soares M (2014) Evolutionary Conservation—Evaluating the Adaptive Potential of Species. *Evol Appl* 7(9):963–967. <https://doi.org/10.1111/eva.12227>
 18. Ellis N, Smith SJ, Roland Pitcher C (2012) Gradient Forests: Calculating Importance Gradients on Physical Predictors. *Ecology* 93(1):156–168. <https://doi.org/10.1890/11-0252.1>
 19. Erb M, Veyrat N, Robert CAM, Xu H, Frey M, Ton J, Ted CJ, Turlings (2015) Indole Is an Essential Herbivore-Induced Volatile Priming Signal in Maize. *Nat Commun* 6(1):6273. <https://doi.org/10.1038/ncomms7273>
 20. Fick SE, Hijmans RJ (2017) WorldClim 2: New 1-Km Spatial Resolution Climate Surfaces for Global Land Areas. *Int J Climatol* 37(12):4302–4315. <https://doi.org/10.1002/joc.5086>

21. Fournier-Level A, Korte A, Cooper MD, Nordborg M, Schmitt J, Wilczek AM (2011) A Map of Local Adaptation in *Arabidopsis Thaliana*. *Science* 334(6052):86–89.
<https://doi.org/10.1126/science.1209271>
22. Gomez-Roldan V, Fermas S, Brewer PB, Virginie Puech-Pagès, Dun EA, Pillot J-P, Letisse F et al (2008) 'Strigolactone Inhibition of Shoot Branching'. *Nature* 455 (7210): 189–94.
<https://doi.org/10.1038/nature07271>
23. Grossiord C, Buckley TN, Cernusak LA, Kimberly A, Novick B, Poulter RTW, Siegwolf JS, Sperry (2020) and Nate G. McDowell. 'Plant Responses to Rising Vapor Pressure Deficit'. *New Phytologist* 226 (6): 1550–66. <https://doi.org/10.1111/nph.16485>
24. Haworth K, McPherson GR (1995) Effects of *Quercus Emoryi* trees on Precipitation Distribution and Microclimate in a Semi-Arid Savanna. *J Arid Environ* 31(2):153–170.
<https://doi.org/10.1006/jare.1995.0057>
25. Hess, Moritz H, Wildhagen L, Junker, Ensminger I (2016) Transcriptome Responses to Temperature, Water Availability and Photoperiod Are Conserved among Mature Trees of Two Divergent Douglas-Fir Provenances from a Coastal and an Interior Habitat. *BMC Genomics* 17.
<https://doi.org/10.1186/s12864-016-3022-6>
26. Hickman A, Dyda F (2015) Mechanisms of DNA Transposition. *Microbiol Spectr* 3 2.
<https://doi.org/10.1128/microbiolspec.MDNA3-0034-2014>
27. Hickman A, Dyda F (2016) DNA Transposition at Work. *Chem Rev* 116 20:12758–12784.
<https://doi.org/10.1021/acs.chemrev.6b00003>
28. Holliday JA, Sally N, Aitken JEK, Cooke B, Fady SC, González-Martínez et al (2017) Myriam Heuertz, Juan-Pablo Jaramillo-Correa,. 'Advances in Ecological Genomics in Forest Trees and Applications to Genetic Resources Conservation and Breeding'. *Molecular Ecology* 26 (3): 706–17.
<https://doi.org/10.1111/mec.13963>
29. Huang Q, Wang Y (2016) Overexpression of TaNAC2D Displays Opposite Responses to Abiotic Stresses between Seedling and Mature Stage of Transgenic *Arabidopsis*. *Front Plant Sci* 7.
<https://doi.org/10.3389/fpls.2016.01754>
30. Hufford K, Mazer S (2003) Plant Ecotypes: Genetic Differentiation in the Age of Ecological Restoration. *Trends Ecol Evol* 18:147–155. [https://doi.org/10.1016/S0169-5347\(03\)00002-8](https://doi.org/10.1016/S0169-5347(03)00002-8)
31. Hummel I, El-Amrani A, Gouesbet G, Hennion F, Couée I (2004) Involvement of Polyamines in the Interacting Effects of Low Temperature and Mineral Supply on *Pringlea Antiscorbutica* (Kerguelen Cabbage) Seedlings. *J Exp Bot* 55 399:1125–1134. <https://doi.org/10.1093/JXB/ERH126>
32. Innes PA, April M, Goebel, Chris CR, Smith K, Rosenberger, Kane NC (2023) 'Gene Expression and Alternative Splicing Contribute to Adaptive Divergence of Ecotypes'. *Heredity*, December.
<https://doi.org/10.1038/s41437-023-00665-y>
33. Isabel N, Holliday JA, Aitken SN (2020) Forest Genomics: Advancing Climate Adaptation, Forest Health, Productivity, and Conservation. *Evol Appl* 13(1):3–10. <https://doi.org/10.1111/eva.12902>

34. Jari Oksanen GL, Simpson F, Guillaume Blanchet R, Kindt P, Legendre PR, Minchin RB, O'Hara P, Solymos, Henry M, Stevens H (2017) 'Vegan: Community Ecology Package'. 2017. <https://cran.r-project.org/web/packages/vegan/index.html>
35. Kan Y, Mu X-R, Gao J, Lin H-X, and Youshun Lin (2023) The Molecular Basis of Heat Stress Responses in Plants. *Mol Plant* 16(10):1612–1634. <https://doi.org/10.1016/j.molp.2023.09.013>
36. Liao Y, Smyth GK, Wei Shi (2014) featureCounts: An Efficient General Purpose Program for Assigning Sequence Reads to Genomic Features. *Bioinformatics* 30(7):923–930. <https://doi.org/10.1093/bioinformatics/btt656>
37. Lines ER, Miguel A, Zavala, Drew W, Purves, Coomes DA (2012) Predictable Changes in Aboveground Allometry of Trees along Gradients of Temperature, Aridity and Competition. *Glob Ecol Biogeogr* 21(10):1017–1028. <https://doi.org/10.1111/j.1466-8238.2011.00746.x>
38. Lisch D (2009) Epigenetic Regulation of Transposable Elements in Plants. *Annu Rev Plant Biol* 60:43–66. <https://doi.org/10.1146/annurev.arplant.59.032607.092744>
39. Lisch D (2012) How Important Are Transposons for Plant Evolution? *Nat Rev Genet* 14:49–61. <https://doi.org/10.1038/nrg3374>
40. López-Maury L, Marguerat S, Jürg Bähler (2008) Tuning Gene Expression to Changing Environments: From Rapid Responses to Evolutionary Adaptation. *Nat Rev Genet* 9(8):583–593. <https://doi.org/10.1038/nrg2398>
41. Ma Y, Wang J, Hu Q, Li J, Sun Y, Zhang L, Abbott RJ, Liu J, Kangshan Mao (2019) Ancient Introgression Drives Adaptation to Cooler and Drier Mountain Habitats in a Cypress Species Complex. *Commun Biology* 2(1):213. <https://doi.org/10.1038/s42003-019-0445-z>
42. Medrano M, Alonso C, Bazaga P, López E, Herrera C (2020) 'Comparative Genetic and Epigenetic Diversity in Pairs of Sympatric, Closely Related Plants with Contrasting Distribution Ranges in South-Eastern Iberian Mountains'. *AoB Plants* 12. <https://doi.org/10.1093/aobpla/plaa013>
43. Nepstad D, Tohver I, Ray DG, Moutinho P, Cardinot G (2007) 'Mortality of Large Trees and Lianas Following Experimental Drought in an Amazon Forest.' *Ecology* 88 9: 2259–69. <https://doi.org/10.1890/06-1046.1>
44. Pertea G (2020) and Mihaela Pertea. 'GFF Utilities: GffRead and GffCompare'. *F1000Research* 9 (April): 304. <https://doi.org/10.12688/f1000research.23297.1>
45. Poncet B, Herrmann D, Gugerli F, Taberlet P, Holderegger R, Gielly L, Rioux D, Thuiller W, Aubert S, Manel S (2010) 'Tracking Genes of Ecological Relevance Using a Genome Scan in Two Independent Regional Population Samples of *Arabis alpina*'. *Molecular Ecology* 19. <https://doi.org/10.1111/j.1365-294X.2010.04696.x>
46. Porporato A, Laio F, Ridolfi L, Rodríguez-Iturbe I (2001) Plants in Water-Controlled Ecosystems: Active Role in Hydrologic Processes and Response to Water Stress: III. Vegetation Water Stress. *Adv Water Resour* 24:725–744. [https://doi.org/10.1016/S0309-1708\(01\)00006-9](https://doi.org/10.1016/S0309-1708(01)00006-9)
47. Qian Y (2022) 'China: China and the 2015–2016 El Niño'. In *El Niño Ready Nations and Disaster Risk Reduction*, edited by Michael H. Glantz, 29–42. Disaster Studies and Management. Cham: Springer

International Publishing. https://doi.org/10.1007/978-3-030-86503-0_2

48. Razgour O, Forester B, Taggart JB, Bekaert Michaël, Juste J, Ibáñez C, Puechmaille Sébastien J (2019) Roberto Novella-Fernandez, Antton Alberdi, and Stéphanie Manel. 'Considering Adaptive Genetic Variation in Climate Change Vulnerability Assessment Reduces Species Range Loss Projections'. *Proceedings of the National Academy of Sciences* 116 (21): 10418–23. <https://doi.org/10.1073/pnas.1820663116>
49. Rezaie N, Reese F, and Ali Mortazavi (2023) PyWGCNA: A Python Package for Weighted Gene Co-Expression Network Analysis. *Bioinformatics* 39(7):btad415. <https://doi.org/10.1093/bioinformatics/btad415>
50. Ruffault J, Martin-StPaul NK, Rambal S, Mouillot F (2013) Differential Regional Responses in Drought Length, Intensity and Timing to Recent Climate Changes in a Mediterranean Forested Ecosystem. *Clim Change* 117(1):103–117. <https://doi.org/10.1007/s10584-012-0559-5>
51. Sang Y, Dan ZLX, Feng J, Shi T, Jia C, Zhang X et al (2022) Genomic Insights into Local Adaptation and Future Climate-Induced Vulnerability of a Keystone Forest Tree in East Asia. *Nat Commun* 13(1):6541. <https://doi.org/10.1038/s41467-022-34206-8>
52. Tenenhaus M, Vinzi VE, Chatelin Y-M, Lauro C (2005) PLS Path Modeling. *Comput Stat Data Anal* 48(1):159–205. <https://doi.org/10.1016/j.csda.2004.03.005>
53. Vandecraen J, Chandler M, Aertsen A, Van Houdt R (2017) The Impact of Insertion Sequences on Bacterial Genome Plasticity and Adaptability. *Crit Rev Microbiol* 43:709–730. <https://doi.org/10.1080/1040841X.2017.1303661>
54. Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M Ove Hoegh-Guldberg, and Franz Bairlein. 2002. 'Ecological Responses to Recent Climate Change'. *Nature* 416 (6879): 389–395. <https://doi.org/10.1038/416389a>
55. Wan T, Gong Y, Liu Z, YaDong Zhou C, Dai, Wang Q (2022) 'Evolution of Complex Genome Architecture in Gymnosperms'. *GigaScience* 11 (August): giac078. <https://doi.org/10.1093/gigascience/giac078>
56. Weeks A, Sgrò C, Young A, Frankham R, Mitchell N, Miller KA, Byrne M et al (2011) Assessing the Benefits and Risks of Translocations in Changing Environments: A Genetic Perspective. *Evol Appl* 4:709–725. <https://doi.org/10.1111/j.1752-4571.2011.00192.x>
57. Xu Q, Zhu C, Song YFZ, Xing S, Liu W, Tao Sang (2016) Population Transcriptomics Uncovers the Regulation of Gene Expression Variation in Adaptation to Changing Environment. *Sci Rep* 6(1):25536. <https://doi.org/10.1038/srep25536>
58. Yang H, Li J, Milne RI, Tao W, Wang Y, Miao J, Wang W et al (2022) Genomic Insights into the Genotype–Environment Mismatch and Conservation Units of a Qinghai–Tibet Plateau Endemic Cypress under Climate Change. *Evol Appl* 15(6):919–933. <https://doi.org/10.1111/eva.13377>
59. Yin Q-Y, Fan Q, Li P, Truong DV, Zhao Wan-Yi Ren-Chao Zhou, Su-Fang Chen, and Wen-Bo Liao. 2021. 'Neogene and Quaternary Climate Changes Shaped the Lineage Differentiation and Demographic

History of *Fokienia Hodginsii* (Cupressaceae s.l.), a Tertiary Relict in East Asia'. J Syst Evol 59 (5): 1081–1099. <https://doi.org/10.1111/jse.12582>

60. Zhu P, He T, Zheng Y, Chen L (2023) The Need for Masked Genomes in Gymnosperms. Front Plant Sci 14:1309744. <https://doi.org/10.3389/fpls.2023.1309744>

Figures

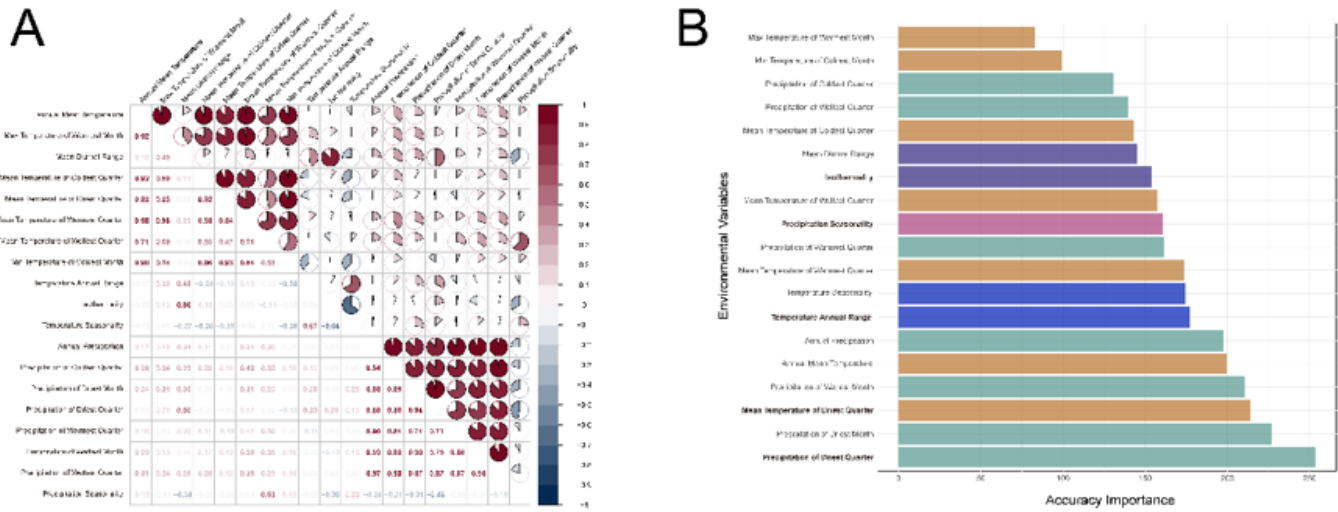


Figure 1

Correlation matrix (A) and importance barplot (B) of the 19 environmental factors. The depth of color and the fill level of the shapes are both used to represent the strength of the correlation, with dark colors and solid fills indicating strong correlations, and white and hollow shapes indicating no correlation. Environmental factor importance was calculated using the random forest algorithm. Within the highly correlated groups (correlation coefficient $|r| > 0.65$), the most important factors are highlighted in bold and the bars for the same factor group are colored the same.

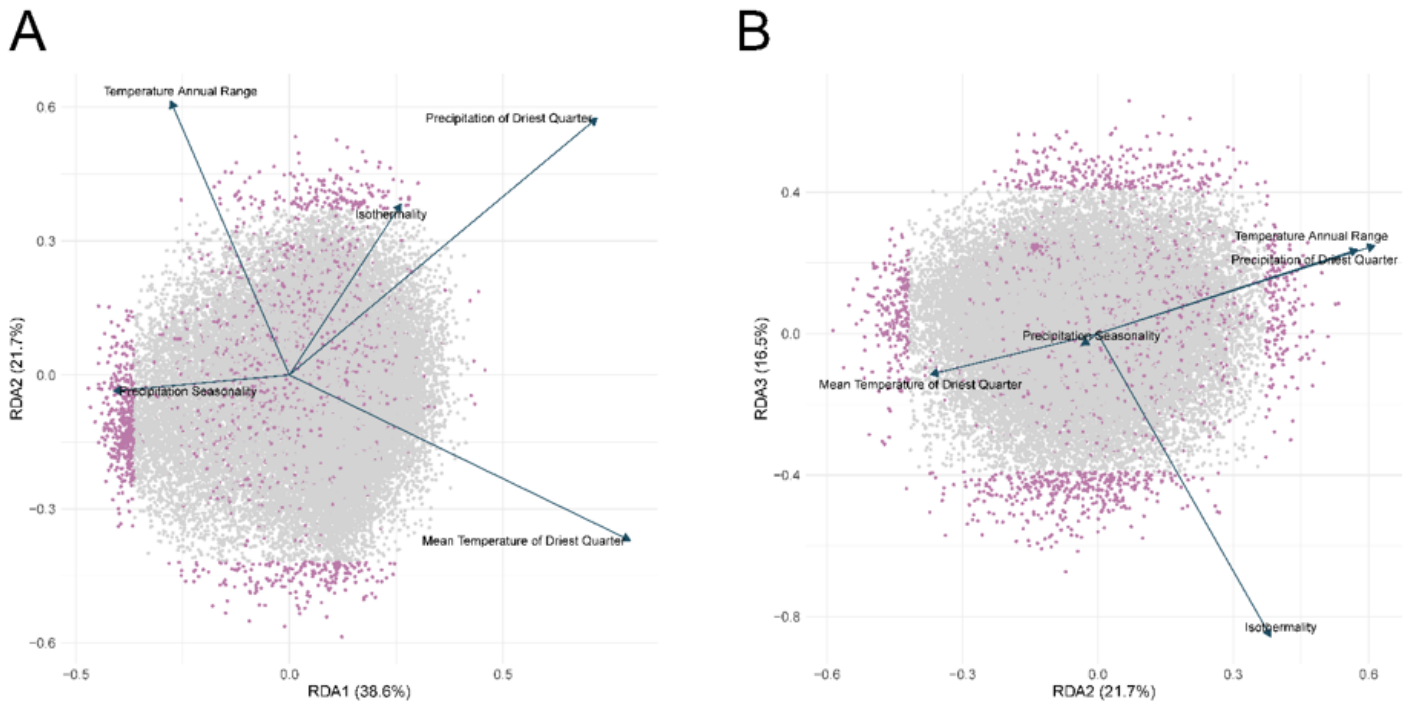


Figure 2

Ordination plots of redundancy analysis (RDA) of gene expression under different environmental conditions, showing RDA1 vs. RDA2 (A) and RDA2 vs. RDA3 (B). In both plots, individual genes are depicted as small points, with those more affected by environmental factors highlighted in purple. The vectors represent the direction and magnitude of the environmental factors' influence on gene expression, with the length of the vector corresponding to the strength of the influence. These plots collectively emphasize the multidimensional nature of gene-environment interactions.

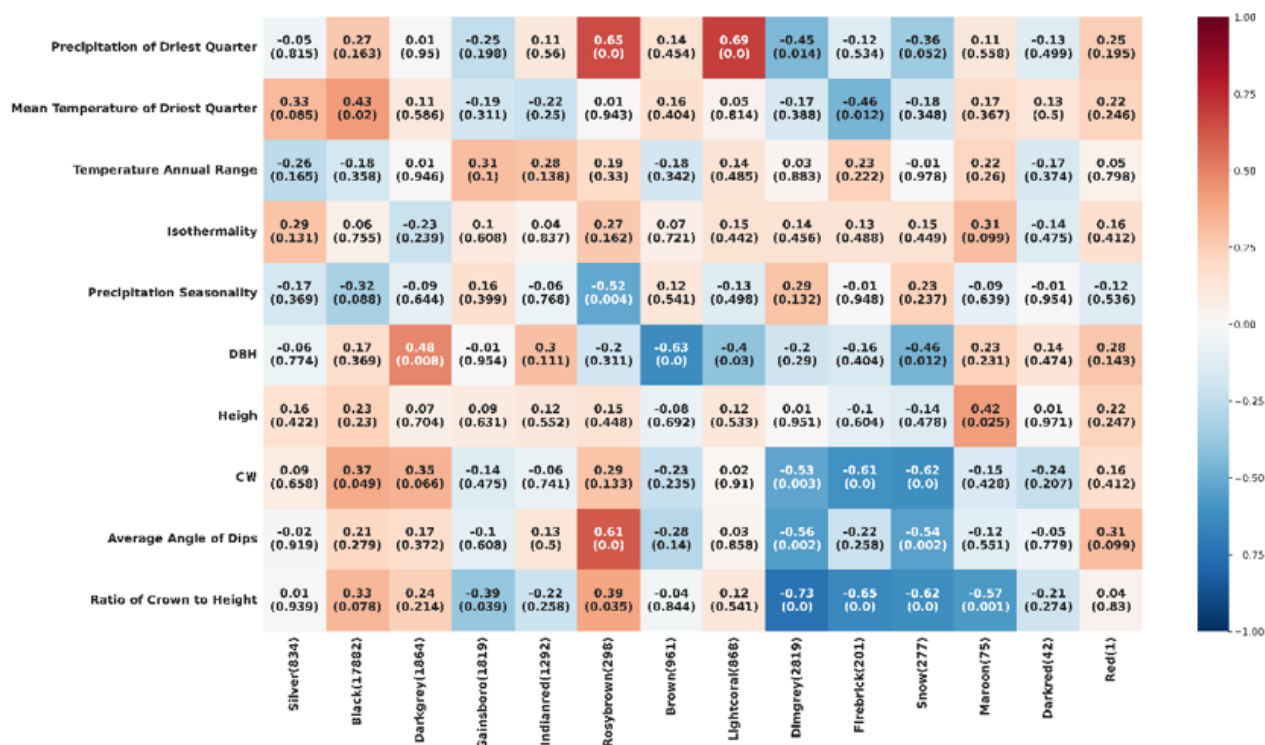


Figure 3

Heatmap showing the results of weighted gene co-expression network analysis (WGCNA) associating gene modules with environmental and phenotypic variables. Each cell in the heatmap represents the correlation coefficient and its significance in parentheses between a gene module and an environmental or phenotypic trait. The color scale on the right indicates the strength of the correlation. This analysis helps to identify potential genetic networks that may contribute to the adaptation to changing environmental conditions.

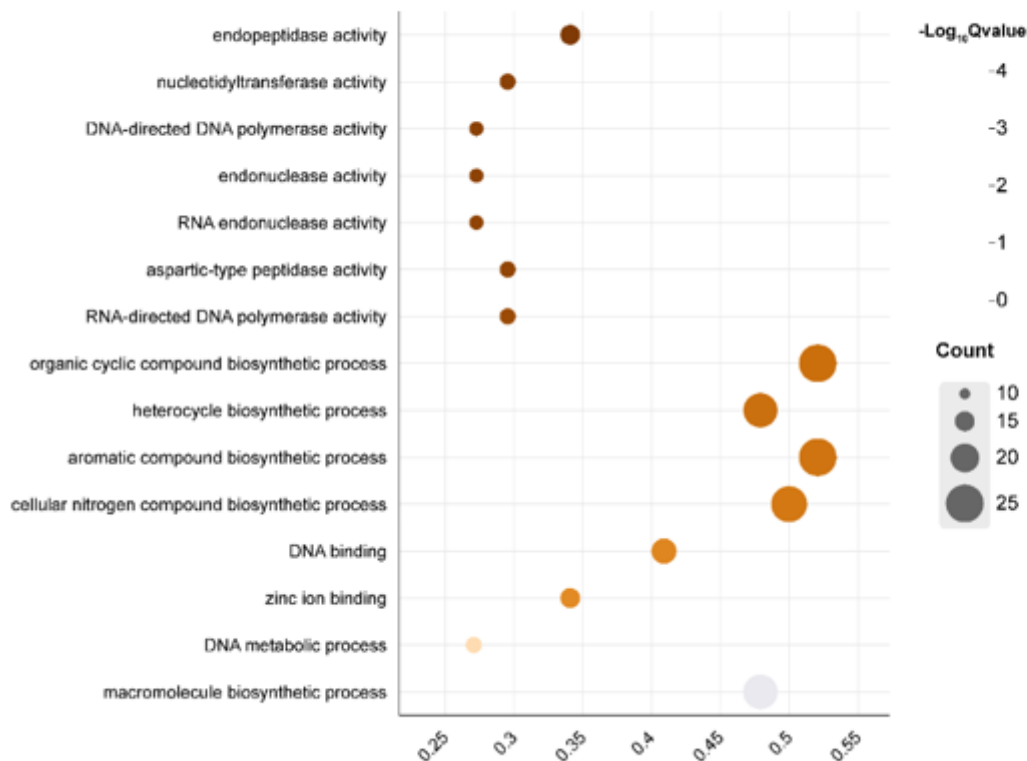


Figure 4

Gene ontology (GO) functional enrichment analysis of key genes identified with KME > 0.6 by WGCNA and RDA. The bubble plot represents various biological processes and molecular functions, with the size of each bubble reflecting the number of genes associated with that term and the color representing the $-\log_{10} Qvalue$ of the enrichment significance. Higher values indicate more significant enrichment. The categories range from endopeptidase activity and nucleotide transferase activity to biosynthetic processes, suggesting that these genes may be crucial for the species to adapt to environmental changes.

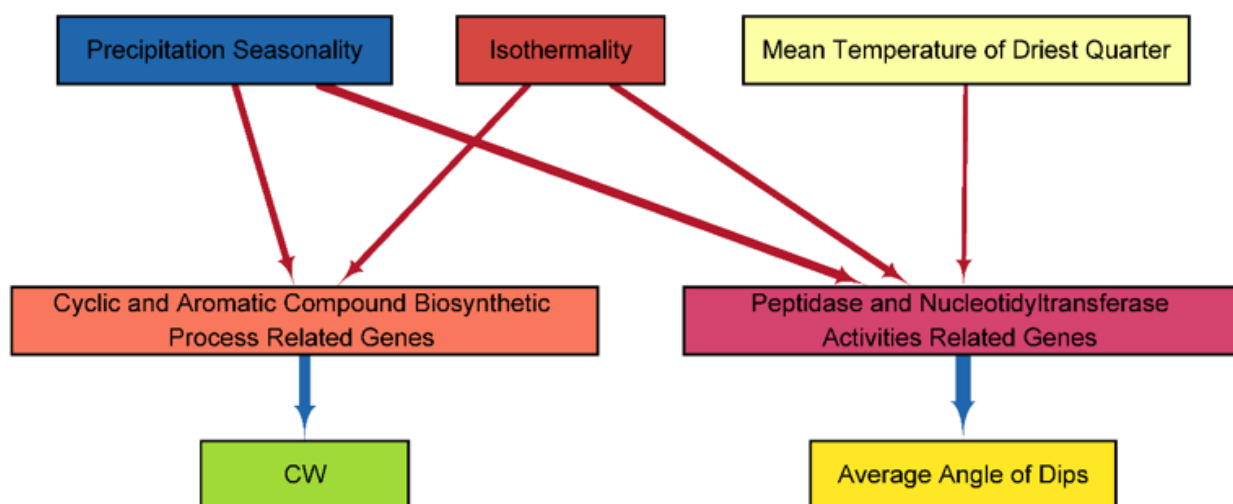


Figure 5

Partial least squares path modeling (PLS-PM) plot showing the relationships between specific functional gene categories and environmental and phenotypic data. These relationships are represented by the color and thickness of the arrows, with red indicating positive effects and blue indicating negative effects, and the thicker the arrow, the stronger the relationship. The structure of the model highlights the hypothesized pathways by which environmental factors may influence gene expression and subsequently the phenotypic traits of the trees.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.xlsx](#)
- [TableS2S5FigureS1S3.docx](#)