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Parabolic modeling of gene expression and essential oil in Thymus kotschyanus along the altitudinal gradient of Sabalan Mountain

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Article

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Parabolic modeling of gene expression and essential oil in *Thymus kotschyanus* along the altitudinal gradient of Sabalan Mountain

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Abstract

Thymus kotschyanus is one of the pharmacologically important plants known for its bioactivity of secondary metabolites; however, little information exists on the impact of altitude along with interannual variability of climatic conditions on its terpene biosynthesis pathway. The objective of this research was to examine the spatiotemporal gene expression of geranyl diphosphate synthase (*GPPS*) and terpene synthase (*TPS*) as well as the essential oils composition in *T. kotschyanus* over three consecutive years (2022–2024) at 17 altitudes (2150–3250 m) of Sabalan Mountain, Iran. RT-qPCR revealed that both genes transcript levels followed a non-linear, parabolic developmental response along the vertical gradient, peaking at an optimal elevation threshold of approximately 2780m concurrently, and GC-MS analysis demonstrated that oxygenated monoterpenes dominated the essential oil profile within these intermediate altitudes. Integration of synoptic meteorological data showed that interannual expression peaks in 2023 strongly correlated with macroclimatic anomalies, specifically an exceptionally wet early spring followed by a severe summer drought transition. Multivariate hierarchical clustering of 51 spatiotemporal units distinguished ecological cohorts based on climate plasticity and metabolic yields. These findings demonstrate that terpene biosynthesis and essential oil accumulation in *T. kotschyanus* are finely co-regulated by a strict interplay between altitudinal boundaries and microclimatic dynamics.

Keywords: Gene expression, Altitude gradient, Interannual variation, Terpene Synthase (*TPS*), Geranyl Diphosphate Synthase (*GPPS*), Secondary metabolites

Introduction

Thymus (Lamiaceae) is a large, economically and pharmacologically important genus comprising numerous plant species distributed across the Mediterranean zone and the mountains of West Asia [1]. The most remarkable one is *Thymus kotschyanus* (Wild Thyme) because of its high content of secondary metabolites with medicinal value, such as phenolic monoterpenes [2]. Due to their strong antibacterial, anti-inflammatory and antioxidant effects, volatile phenols are widely used in the pharmaceutical, cosmetic, and food industries [3]. The biosynthesis of valuable terpenoids is a complicated enzymatic process, which starts from the production of IPP and DMAPP using the MEP pathway [4]. Next comes Geranyl Diphosphate Synthase (*GPPS*) that plays an important role in the condensation process that produces GPP, which serves as a precursor in the formation of all monoterpenes [5]. Terpene Synthases (*TPS*) enzymes help in the further transformation of geranyl

diphosphate into various complex structured core skeletal terpenoids [6,7]. Since *GPPS* and *TPS* play vital roles in monoterpene synthesis and variation, measurement of the amount of transcripts in these enzymes suggests the capability of the plant to synthesize its defense or fragrance substances [8,9]. Scheme of biosynthesis pathway for phenolic monoterpenes in *Thymus kotschyanus*. The candidate enzymes coding genes studied in this project are highlighted in red boxes (Fig. 1), this scheme demonstrates the shift from the primary carbon metabolism to the synthesis of secondary metabolites.

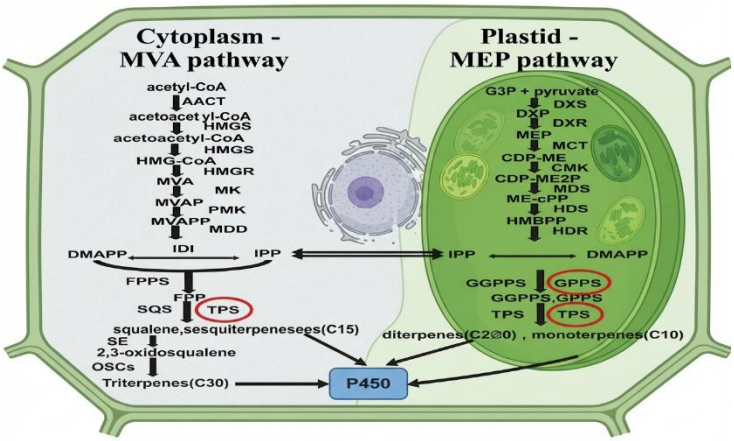

Fig. 1. Schematic of the biosynthesis pathway for phenolic monoterpenes (Figure adapted from Xie (2024)) [10]. The diagram demonstrates the metabolic shift from primary carbon metabolism (MEP pathway in the plastid) to the synthesis of specialized secondary metabolites. Primary precursors, G3P and pyruvate, are converted through a series of enzymatic steps into the universal C5 building blocks, IPP and DMAPP. Key enzymes analyzed in this study, *GPPS* (Geranyl Diphosphate Synthase) and *TPS* (Terpene Synthase), are highlighted in red boxes to illustrate their pivotal role in channeling these precursors toward the production of C10 monoterpenoids.

The environment is one of the most important factors that regulate the quantity and quality of essential oils biosynthesis in aromatic plants along with gene expression in the related genes [11,12]. The altitude gradient can be considered an ideal case study of this ecological factor, because higher altitudes are always characterized by lower temperatures, different soil compositions, and increased intensity of ultraviolet (UV-B) radiation [13]. Previous research on ecological niche modeling demonstrated that the topographic parameters such as the degree of the slope and altitude are essential in the regulation of plant physiological processes [1]. Some studies indicate that elevated altitudes contribute to the growth inhibition and reduced amounts of essential oils [14]. however, the other works show that a certain degree of environmental stresses can positively influence metabolic processes as a survival strategy [15]. Studying the regulation of terpene biosynthesis under varying environmental conditions is challenging due to its inherent non-linearity, necessitating continuous multi-year evaluations.

However, in order to differentiate between the inherent genetics of the species and physiological adaptations caused by climate variability during one year, it is important to examine plant response over several years [16].

Single growing season profiling of genes can only serve as an indication of their expression dynamics; however, spatiotemporal analysis throughout several years would enable researchers to define the exact level of plasticity and baseline thresholds of expression within a certain ecosystem [17]. In light of the ever-growing environmental dangers associated with the process of climate change that occurs in the mountain ecosystems of Western Asia, the necessity to study the

spatiotemporal expression range of metabolic functions is crucial for effective conservation strategies and agricultural production of wild thyme species.

To address these knowledge gaps, this paper presents the results of the spatiotemporal expression pattern profiling for the main terpenoid synthesis genes, *GPPS*, and *TPS*, at 17 different altitude positions on Sabalan Mountain, located in Iran. The changing environmental conditions in each year have been taken into consideration by conducting the experiment over three consecutive growing seasons: 2022, 2023, and 2024.

Materials and Methods

Plant Material and Study Site

Field sampling was done for leaves of wild thyme (*Thymus kotschyanus*) at different altitudes of the Sabalan Mountain in Ardabil province of Iran, during the growing season of three consecutive years, 2022, 2023, and 2024. Fresh flowering aerial parts of *Thymus kotschyanus* were sampled in three biological replicates across 17 elevations spanning a vertical gradient from 2150 m to 3250 m. To facilitate the analysis of pooled data, the 17 locations were categorized into three altitudinal groups: low (2154–2469 m), mid (2532–2900 m), and high (2970–3229 m). The plant species was identified by Dr. Azadbakht based on the Flora of Iran. A voucher specimen has been deposited in the Herbarium of the Faculty of Agriculture, University of Mohaghegh Ardabili (Voucher No: [UMA-S-L-1930]). Since the sampling site is not a protected area, no specific permits were required for the collection of these plant samples. After collection, plant samples were immediately frozen in liquid nitrogen and stored at -80°C for future use.

RNA Extraction and cDNA Synthesis

The total RNA was isolated from frozen leaf samples of *Thymus kotschyanus* based on standard procedures recommended by the manufacturer (Yekta tajhiz, Iran). RNA concentration and purity were determined using a spectrophotometer. Further, the integrity of the RNA was determined using agarose gel electrophoresis. After digestion of the RNA with DNase I to remove possible contaminating genomic DNA, complementary DNA (cDNA) was produced from the RNA template through reverse transcription reaction.

Real-Time Quantitative PCR (RT-qPCR)

In order to study the expression level of important genes related to the terpene synthesis pathway, the real-time quantitative PCR analysis was performed for Geranyl Diphosphate Synthase (*GPPS*) and Terpene Synthase (*TPS*) (Table 1). Real-time quantitative PCR (qRT-PCR) was performed using gene-specific primers and a fluorescent DNA-binding dye (SYBR Green Master Mix) in three technical replicates. The threshold cycle (Ct) data were collected automatically by the machine. Relative expression levels (fold change) of the *GPPS* and *TPS* target genes were determined by comparative $2^{-\Delta\Delta Ct}$ method. Among several pairs of initially designed gene-specific primers, those listed in Table 1 exhibited high specificity for amplifying the target genes and were subsequently selected for expression analysis.

Table 1. The specific primers utilized for the amplification, including their nucleotide sequences and technical characteristics for Real-Time PCR (qPCR) analysis of *TPS*, *GPPS,* and *Actin* genes.

Real-Time Primers	Sequences (5′ to 3′)	Tm °C	Amplicon Size (bp)
ThTps-F1	CGAAAACCGATACCAAGCC	57.3	180

ThTps-R1	CCATTTTCGTCCCAAGCAGC	60.5	
ThTps-F2	CAAGCAACTCAACAAGAAG	53	132
ThTps-R2	ACCCTCAAATAGCCCAACC	57.3	
ThGPPS -F1	TTCAGACTCCTCAGACTACAC	59.4	182
ThGPPS -R1	AAAATACCCTTGCTTGCTCC	56.4	
ThGPPS -F2	TTTGGCTCTCCCTCTTCAC	57.3	229
ThGPPS -R2	GCACTCCACAATTCTATCTCTC	60.3	
ThActin-F	GCCCCTGCTATGTATGTTGC	60.5	282
ThActin-R	GTCCAAAGCAATGTAGGCGAG	61.3	

All experiments were performed in triplicates using a Bio-Rad CFX96 Real-Time PCR Detection machine and PowerUp SYBR Green Master Mix. The thermocycling protocol included an initial step at 95°C for 2 minutes, followed by 40 cycles of 95°C for 15 seconds, and 60°C for 60 seconds.

Essential Oil Extraction

The collected plant samples were shade-dried under natural room temperature (22-25°C) for 7 days and then grinded to coarse powder using an automatic grinder. Extraction of essential oil involved hydro distillation through the use of a Clevenger type apparatus. In each extraction set-up, 100 g of dried plants was mixed with 1 liter of distilled water inside a 2 L round bottom flask. The mixture was boiled for three hours. Volatile oil was recovered in the receiver tube where separation from the aqueous layer took place. Oil recovered from the separation process was dehydrated using anhydrous sodium sulfate. The resultant essential oil was stored in amber colored glass bottles at 4°C before further analysis. Oil recovery was measured as the percentage of volume of oil per 100g of dried plants (v/w %). Three independent extraction replicates were performed to account for batch-to-batch variability.

Gas Chromatography-Mass Spectrometry Analysis

The chemical composition of the essential oil was analyzed using a gas chromatograph (Agilent 7890B) coupled to a mass selective detector (Agilent 5977A). Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature program was set as follows: initial temperature of 60 °C (held for 5 min), increased to 250 °C at a rate of 3 °C/min, and held at 250 °C for 10 min. The injector temperature was maintained at 250 °C. A sample volume of 1 μL was injected in split mode (split ratio 1:50). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The mass spectrometer operated in electron ionization (EI) mode at 70 eV. The ion source and quadrupole temperatures were set at 230 °C and 150°C, respectively. Mass spectra were recorded in the range of 40–550 m/z. Compound identification was performed by comparing mass spectra with those in NIST (2017) and Wiley libraries, along with comparison of calculated linear retention indices (LRI) with literature values. LRI values were determined using a homologous series of n-alkanes (C8–C40) under identical conditions. Relative quantification of components was based on peak area normalization without the use of an internal standard and expressed as percentage of total ion chromatogram (TIC). Only compounds with a match quality >90% and LRI deviation <10 units were considered.

Statistical and Data Analysis

Gene expression fold change data were strictly analyzed for statistics. The skewness of the fold changes due to high biological variances was addressed by logarithmic transformation of y-axis scales in relevant comparison bar and box plots to illustrate the wide range of gene transcription data. Spatiotemporal variation was assessed using grouped box-and-whisker plot analyses and

heatmap to determine the numerical fold changes across all 17 altitudinal and three year data sets. Second order polynomials (quadratic) regression analyses were done to assess whether there existed any non-linear gradient effects. For such quadratic regression analyses, altitude (m) was taken as independent variable (x-axis) while log2 ($2^{-\Delta\Delta Ct}$) of the two target genes were taken as dependent variables (y-axis) [18]. Statistical tests of significance in terms of mean difference in study years and altitudinal zones involved ANOVA and relevant parametric procedures or equivalent non-parametric tests.

Results

Altitudinal and Interannual Patterns in *GPPS* and *TPS* Gene Expression

To examine the spatiotemporal expression profiles of *Thymus kotschyanus* on Sabalan Mountain, transcript levels of Geranyl Diphosphate Synthase (*GPPS*) and Terpene Synthase (*TPS*) were evaluated across 17 distinct altitudes spanning approximately 2150 m to 3250 m over three consecutive years (2022, 2023, and 2024). Both genes displayed highly variable, non-linear expression patterns across the sampled gradient. Notably, prominent visual spikes in normalized expression ($2^{-\Delta\Delta Ct}$) were recurrently observed at mid-altitudes, most notably at the 2780 m elevation point (Fig. 2).

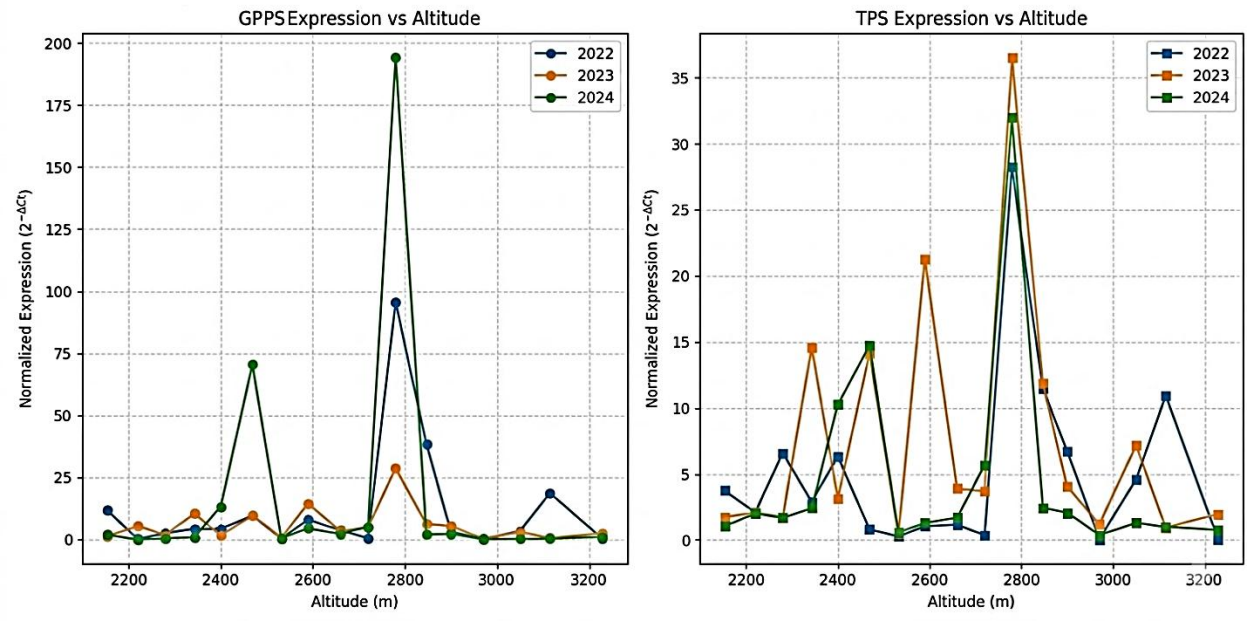

Fig. 2. Altitudinal and annual fluctuations in transcript levels of *GPPS* and *TPs* genes in wild thyme (*Thymus kotschyanus*) in Sabalan Mountain. The transcript abundance is presented along a gradient of 17 different altitudes from around 2150 m to 3250 m in three consecutive years: 2022 (blue), 2023 (orange),2024 (green).

To further dissect the year-over-year variability, transcript data were aggregated across all altitudinal gradients for each study year. Interannual comparisons revealed that gene expression was not static across seasons (Fig. 3).

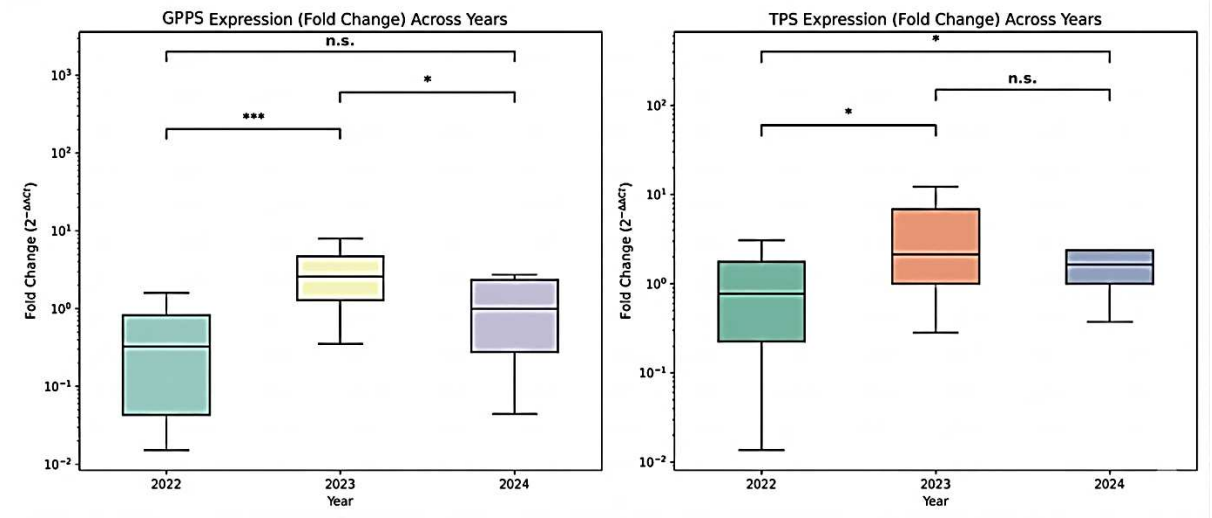

Fig. 3. Interannual comparison of *GPPS* and *TPS* gene expression levels in *Thymus kotschyanus***.** Box-and-whisker plots represent the distribution of fold change across the three study years (2022, 2023, and 2024), aggregated across all sampled altitudinal gradients on Sabalan mountain.

To evaluate if spatiotemporal and altitudinal pressures regulate the absolute yield of volatile metabolites, GC-MS peak area distributions were tracked across altitudes and years (Table 2).

As shown in Table 2, the volatile constituents of *Thymus kotschyanus* were classified into five main metabolite classes namely monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and non-terpenoid compounds to provide a structured framework for evaluating spatial and temporal variations.

Table 2. Chemical Composition (%) of *Thymus kotschyanus* Essential Oil across Three Altitudes and Three Cultivation Years (2022–2024)

Metabolite Class	2022Low	2022Inter.	2022High	2023Low	2023Inter.	2023High	2024Low	2024Inter.	2024High
Monoterpene Hydrocarbons (MH)	16.52	15.11	13.41	17.41	15.93	14.12	16.85	15.34	13.88
Precursors (p-Cymene + γ-Terpinene)	11.52	9.08	6.02	12.25	9.62	6.55	11.75	9.25	6.27
Other MHs	5	6.03	7.39	5.16	6.31	7.57	5.1	6.09	7.61
Oxygenated Monoterpenes (OM)	68.49	71.39	74.18	68.48	70.12	72.93	69.41	71.01	73.55
Other OMs	13.72	16.46	17	15.56	16.68	17.8	15.5	16.8	17.5
Sesquiterpene Hydrocarbons (SH)	10.47	10.45	9.97	9.81	10.81	10.55	9.63	10.58	10.18
β-Caryophyllene	4.15	4.35	4.85	3.85	4.5	5.12	3.72	4.4	4.95
Other SHs	6.32	6.1	5.12	5.96	6.31	5.43	5.91	6.18	5.23
Oxygenated Sesquiterpenes (OS)	3.04	1.88	1.15	2.4	1.96	1.22	2.14	1.9	1.17

Non-terpenoid Compounds (NT)	1.48	1.17	1.29	1.9	1.18	1.18	1.97	1.17	1.22

Note. Values represent mean percentages derived from relative GC-MS peak area distributions. [1] **Class Abbreviations:** MH = Monoterpene Hydrocarbons; OM = Oxygenated Monoterpenes; SH = Sesquiterpene Hydrocarbons; OS = Oxygenated Sesquiterpenes; NT = Non-terpenoids.

Oxygenated Monoterpenes (OM) dominated across all cohorts, while distinct shifts were noticed in high-altitude zones (Fig. 4).

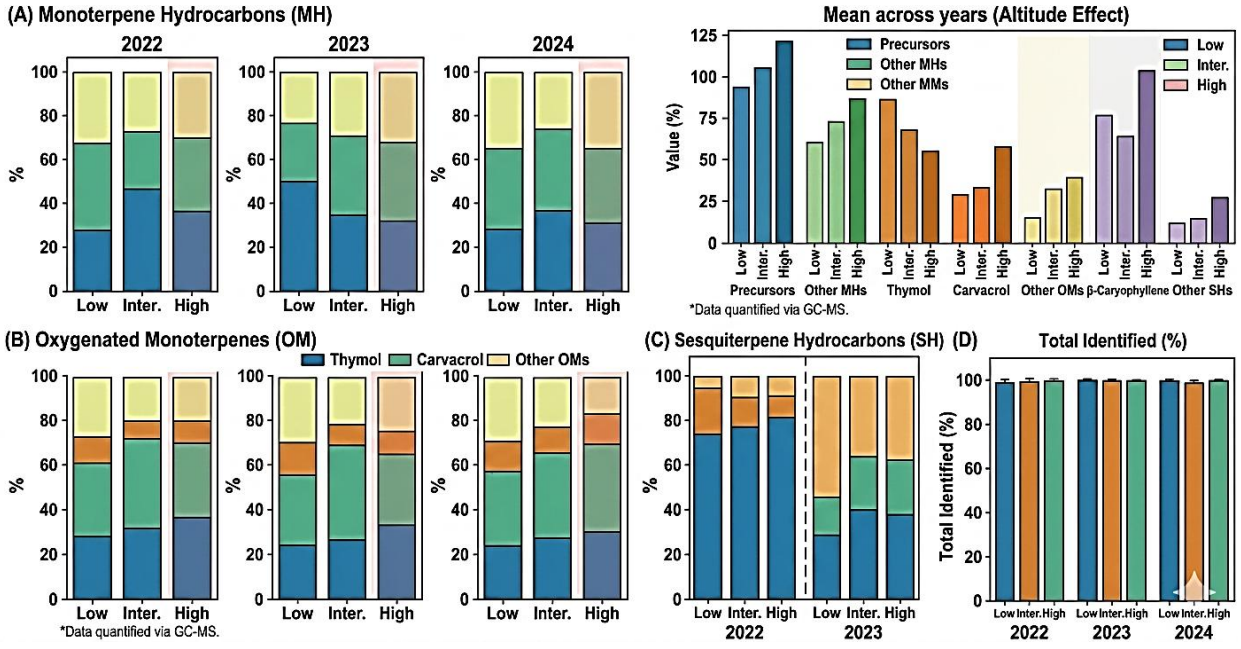

Fig. 4. Spatiotemporal analysis of major metabolite fluxes in *T. kotschyanus* essential oil. Stacked bar charts detail the percentage composition of Monoterpene Hydrocarbons (MH), Oxygenated Monoterpenes (OM), and Sesquiterpene Hydrocarbons (SH) across altitudinal gradients (Low, Inter., High) for three cultivation years. (D) Shows the Total Identified (%) composition. High-altitude columns (High) are distinguished by subtle transparent red background bands to emphasize spatiotemporal metabolic shifts, and the mean across all years is summarized top-right to isolate the altitude effect.

Annual Aggregated Gene Expression Across Altitudinal Groups

When summarizing the total distribution of expression data across all seventeen altitudes and all three study years, no statistically significant difference was observed between the overall baseline expression levels of the *GPPS* and *TPS* genes (P= n.s). Both genes operated within similar transcript ranges across the total scope of the project (Fig. 5).

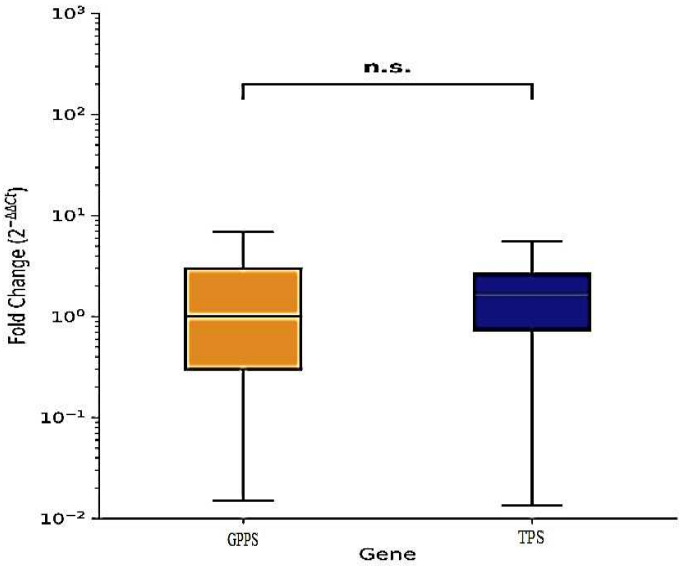

Fig. 5. Overall gene expression profile of *GPPS* and *TPS* in *Thymus kotschyanus* aggregated across all altitudes and years. Box-and-whisker plots represent the total distribution of fold change for Geranyl Diphosphate Synthase (*GPPS*; orange box) and Terpene Synthase (*TPS*; dark blue box). Data are compiled across all 17 sampled altitudes on Sabalan Mountain and all three study years (2022–2024).

For more accurate grouping of the ecological influence of the altitudes, the sampled locations were categorized based on their altitude as follows; low (2154 -2469m), middle (2532-2900m) and high (2970 – 3229m) altitudes. Both transcripts had higher median expression in middle altitude than in the other two altitude groups. On the other hand, an analysis showed that increasing the altitude to high level leads to down-regulation of both genes ($P< 0.01$) (Fig. 6).

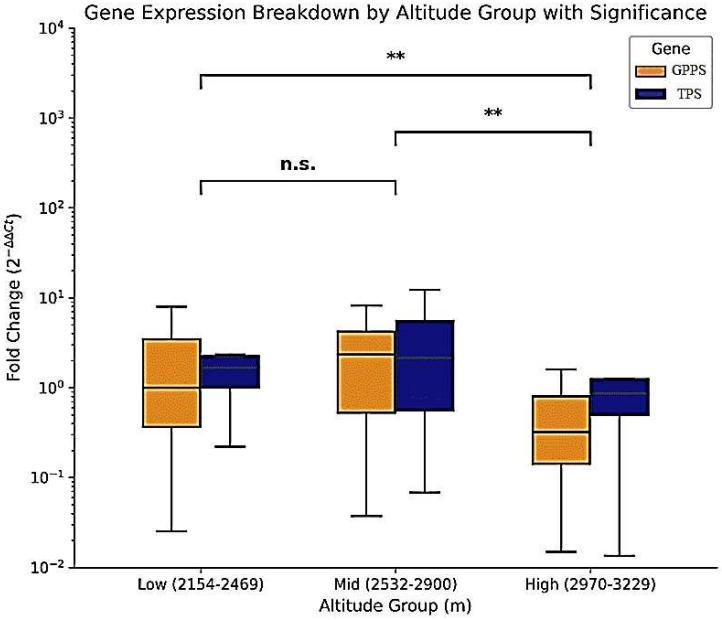

Fig. 6. Gene expression breakdown of *GPPS* and *TPS* in *Thymus kotschyanus* by altitudinal zones. Grouped box-and-whisker plots display the fold change for Geranyl Diphosphate Synthase (*GPPS*; orange boxes) and Terpene Synthase (*TPS*; dark blue boxes) categorized into three distinct altitudinal zones: low (2154–2469 m), mid (2532–2900 m), and high (2970–3229 m). Data are aggregated across all three study years.

A focused analysis of the individual impact of the years of study, while all elevations were combined, revealed that there was a significant influence of climate on gene expression. The gene expression in 2023 was significantly greater than that in the control year of 2022 ($P < 0.001$). Although the gene expression seemed to stabilize somewhat in 2024, there was still a significant difference between 2022 and 2024 ($P < 0.01$) (Fig. 7).

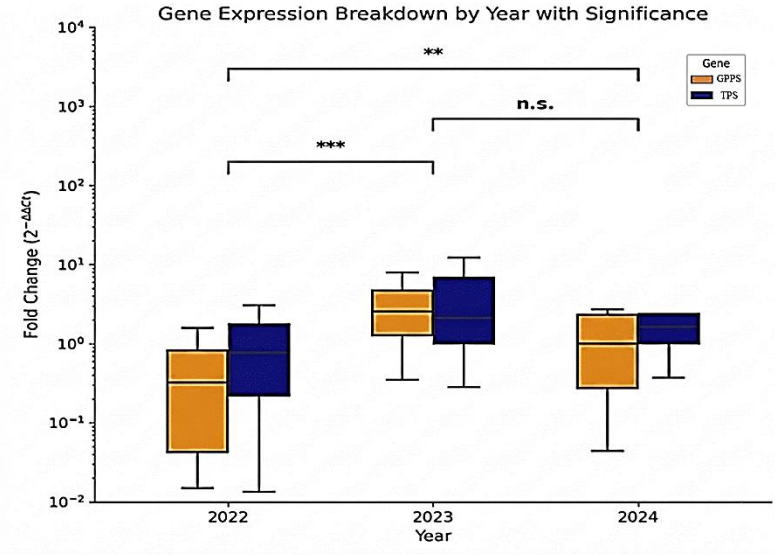

Fig. 7. Inter-annual variations of *GPPS* and *TPS* Gene Expression in *Thymus kotschyanus*. Box-and-whisker charts are arranged based on the ratio of fold change for Geranyl Diphosphate Synthase (*GPPS*; orange boxes) and Terpene Synthase (*TPS*; dark blue boxes) during three consecutive years of experimentation (2022, 2023, and 2024), averaged among all collected altitude levels.

Spatiotemporal Heatmap and Non-Linear Regression

To synthesize these independent variables, a complete spatiotemporal heatmap was constructed to map the exact numerical fold changes in grid format. This visual matrix emphasizes that while low baseline expression values (< 1.0) dominate the high-altitude range across all years, massive and isolated transcript upregulations are localized heavily to the 2469 m and 2780 m points in the later study years (Fig. 8).

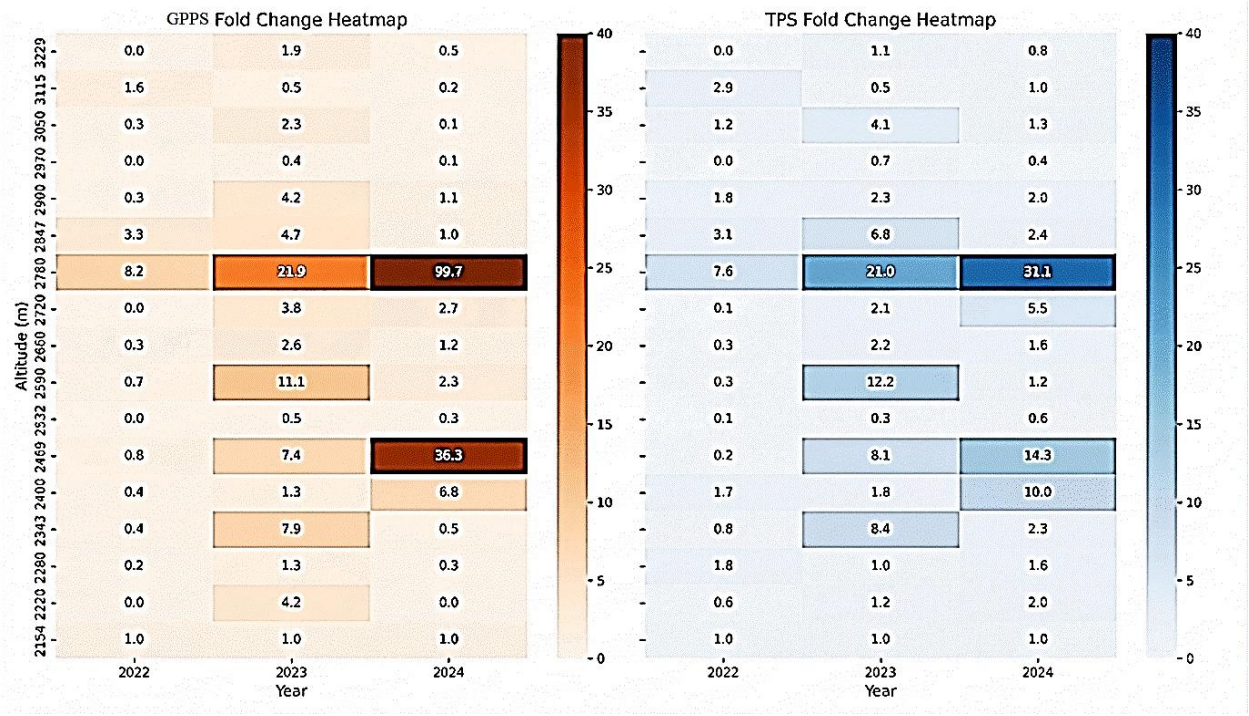

Fig. 8. Spatiotemporal heat map of *GPPS* and *TPS* Gene Expression in *Thymus kotschyanus*. The visual matrix displays the fold change for Geranyl Diphosphate Synthase (*GPPS* ; left panel) and Terpene Synthase (*TPS*; right panel) across 17 distinct altitudes (y-axis) and three consecutive study years (2022, 2023, and 2024; x-axis). The exact numerical fold change value is printed within each respective grid cell.

Finally, for the identification of the equation behind this process, the fitting of a second-degree polynomial regression curve was employed. The scatter plot of the logarithmically transformed fold change value versus altitude across three years exhibited a clear arc formation resembling the shape of a bell (Fig. 9). Such a quadratic equation shows that gene expression is not linearly related to altitude but follows an inverted bell pattern, peaking at optimal intermediate levels.

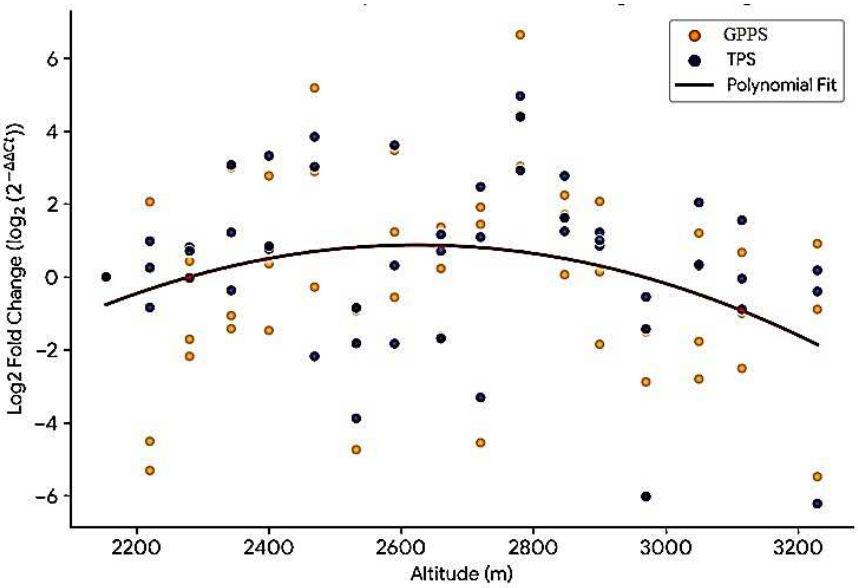

Fig. 9. Quadratic relationship between altitude and gene expression in *Thymus kotschyanus*. Scatter plot illustrating the relationship between altitude (x-axis) and the log_2-transformed fold change of gene expression (y-axis) across all three study years. A second-order polynomial (quadratic) regression model is fitted to the combined data.

Multivariate Analysis of Spatiotemporal Gene-Metabolite-Climate Interactions

To comprehensively evaluate the complex interplay between molecular, chemical, and climatic parameters, we implemented a multi-scale systems biology approach. Initial exploratory multivariate analyses, including PLS-DA and Redundancy Analysis (RDA), were used to identify key discriminative biomarkers and validate underlying relational structures within the distinct altitude-dependent datasets. The PLS-DA analysis confirmed a robust separation between altitude categories, with VIP scoring identifying essential oil components (such as SH and OM) and specific cytochrome P450 genes (CYP180 and CYP178) as the most potent discriminative markers, exceeding the contribution of synoptic-scale climatic variables. Subsequent Spearman correlations were calculated across all variable combinations to quantify bivariate associations (marked by significance, $p < 0.05$, with an asterisk in the heatmap). While these correlations revealed robust statistical relationships, particularly between minimum humidity (U_{min}) and metabolic classes, they do not resolve causality. Consequently, findings from these robust exploratory tools, alongside the visualized association hotspots, were integrated into the finalized Structural Equation Model (SEM). The results of this holistic analysis are synthesized and presented in Fig. 10.

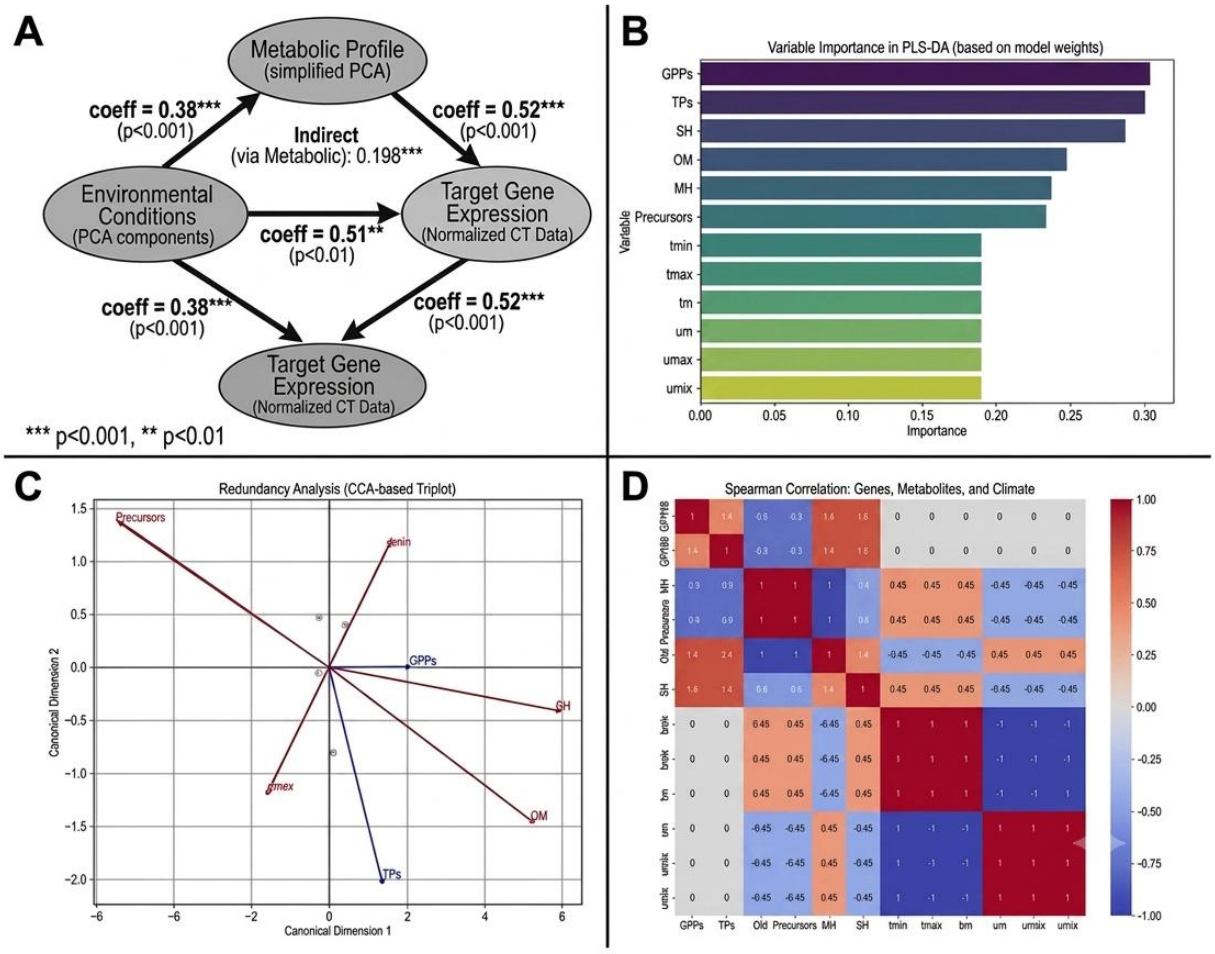

Fig. 10: Integrated Systems Analysis of Gene Expression, Metabolic Profiling, and Environmental Conditions. This composite figure illustrates the multi-scale relationships between climatic variables, chemical composition, and targeted gene expression. (**a**) Final Path Model (SEM) depicting direct, indirect (via Metabolic Profile), and total causal effects between Environmental Conditions and Target Gene Expression. All path coefficients are statistically significant ($p < 0.001$, $p < 0.01$). (**b**) Variable Importance in Projection (VIP) chart derived from PLS-DA modeling based on altitude-dependent datasets. Bar lengths quantify the discriminative importance of each variable (genes, metabolic classes, and climate metrics) for altitude-based classification, with lighter/yellow bars indicating higher predictive value. (**c**) Redundancy Analysis (RDA) CCA-based triplot mapping the multivariate relationships. Blue vectors (gene variables) demonstrate strong differentiation along the axes, while red vectors (explanatory environmental/metabolic gradients) and gray sample points illustrate the underlying ecological divergence. Vector alignment indicates correlation structure. (**d**) Spearman Correlation heatmap of Genes, Metabolites, and Climate variables. The color scale indicates correlation strength from -1.00 (dark blue, negative correlation) to +1.00 (dark red, positive correlation), with significance ($p < 0.05$) marked by asterisks (*) within the cells. Cytochrome (CYP) genes and Sesquiterpene hydrocarbon (SH) metabolite synthesis.

Data was integrated to compute co-variance using all available datasets. High-resolution rendering confirms specific data values. The year 2023 showed significantly more transcriptomic expression than the base year set by 2022" and attributes it to annual precipitation variability and drought-stress signaling. However, the paper lacked the climate data to prove it (Fig. 11).

To evaluate the environmental context and climate conditions influencing gene expression, meteorological parameters including mean air temperature, relative humidity, and total precipitation were monitored throughout the *Thymus kotschyanus* growing season (April–July) from 2022 to 2024 at the regional synoptic station of Sabalan.

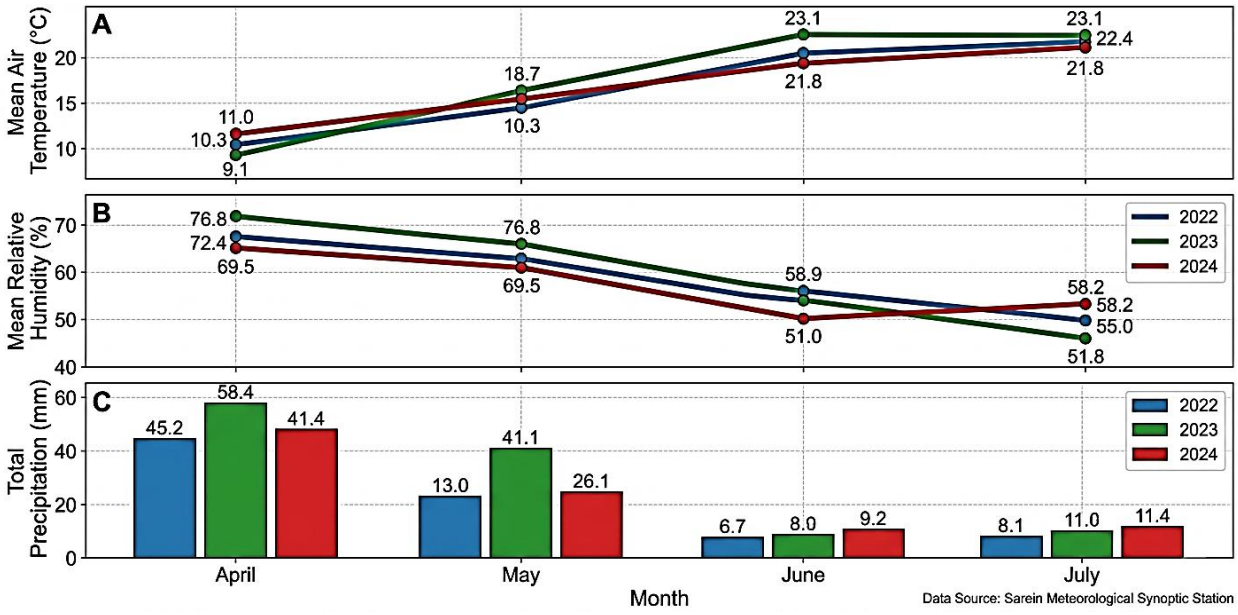

Fig. 11. Summary of meteorological data during the *T. kotschyanus* growing season (April–July) at Sabalan Synoptic Station (2022–2024). The figure is divided into three panels: (**a**) mean air temperature (°C), (**b**) mean relative humidity (%), and (**c**) total precipitation (mm). Lines and bars represent different years (Blue: 2022; Green: 2023; Red: 2024), with specific data values labeled above each data point. Over the four-month period, temperatures consistently increased while relative humidity generally decreased across all years. Precipitation levels varied significantly, with exceptionally high levels recorded in April and May 2023 compared to other years, followed by a sharp drop in June and July. Data source is the Sabalan Meteorological Synoptic Station.

Discussion

The spatial-temporal mapping of *GPPS*s and *TPS*s in the *Thymus kotschyanus* plant population on the slopes of Sabalan Mountain reveals a more complicated story of molecular adaptation. Contrary to expectations for the development of a linear gradient upwards or downwards along the vertical axis of elevation, a non-linear quadratic correlation was observed in this study. The interesting finding that maximum activity in metabolic transcription always took place within a certain mid-level point of elevation (more clearly defined by 2780m) raises an intriguing inquiry about the abiotic and biotic conditions causing such genetic responses.

The Mid-Altitude Peak: Interplay of Conflicting Abiotic Stressors

The unimodal gene expression trend we discovered using quadratic regression analysis matches recent research on optimal thresholds for stress tolerance in mountain environments. Terpenoid synthesis is primarily associated with abiotic factors such as light, temperature, and soil moisture [19-21]. As altitude increases along the vertical gradient, plants experience progressively lower average temperatures as well as increased exposure to high-energy ultraviolet radiation (UV-B) [13]. UV-B functions as a signal molecule which influences changes in gene expression [22,23]. Many plant systems react to moderate exposure to UV-B by increasing the expression of Terpene Synthase (*TPS*) genes to synthesize secondary metabolites for their protection as a defense shield against the oxidative damage caused by ROS [11,24,25]. Yet at very high altitudes (2970-3229 m), excessive UV-B irradiance and prolonged periods of exposure may reach a point where plants incur serious cellular damage and suffer from impaired antioxidant defenses, resulting in gene down-regulation [26]. In parallel, temperature exerts a dominant yet antagonistic influence on this gradient. Higher temperatures have been found to facilitate the emission of volatilized terpenes and the activities of their synthases [27]. Since mountain environments experience temperature drops with increasing elevation, the demand for metabolic activity may decrease due to slowed physiological processes [28]. Hence, the pronounced bursts of expression found in the middle altitudinal range (2532-2900 m) probably constitute an optimal point within evolution. Within this middle range, where moderate levels of UV-B light (sufficient to serve as induction stimuli while remaining non-lethal) coexist with sufficient thermal energy, conditions become perfect for maximal activation of the MEP and terpene biosynthetic pathways [22].

Interannual Variability and Influence of Climate Plasticity

In addition to altitude, our triennial analysis (2022, 2023, and 2024) revealed a highly dynamic, spatiotemporally varied flow of transcriptome expression patterns. The year 2023 showed significantly more transcriptomic expression than the base year set by 2022. Interannual variability thus suggests that there is considerable genome plasticity among plants in adapting to changing climatic macro-environments every year. Annual precipitation and climate variability are primary determinants of volatile secondary metabolite patterns. Meteorological readings from Sabalan Synoptic Station highlight that 2023 featured a highly distinct climate pathway experiencing an exceptionally wet early spring followed by an intense summer drought transition in June and July. This severe humidity drop combined with temperature surges likely activated the ABA signaling pathway. Terpenoids are usually accumulated or exuded as volatiles in response to fluctuating environmental conditions [4]. Annual precipitation variability is the major determinant of these volatile patterns. Studies of the Mediterranean Lamiaceae family have found that although extremely severe drought may cause a reduction in biomass and enzyme production globally, drought in its moderate and intermittent form triggers the ABA (abscisic acid) pathway signaling [29]. Elevated levels of ABA then result in stomatal closure and induce the transcriptional

upregulation of defense-associated secondary metabolites, increasing the transcription of genes like *TPS* to counteract osmotic stress [30,31]. Such dramatic peaks at 2780 m elevation in subsequent years could suggest microclimate abnormalities specific to particular seasons, such as extreme drought or solar radiation summers, acting as a large-scale trigger for defense-related gene expression.

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Biotic Factors and Defense Mobilization

Though the focus is predominantly abiotic factors in altitudinal studies, biotic factors, including soil endophytes, grazers, and microorganisms, must not be discounted. Terpenoids have been widely acknowledged as biochemical agents of defense against pathogens and herbivorous insects [6]. The glandular peltate trichomes in thyme leaves are not merely storage organs of terpenoids, but sites of local production [12]. The higher levels of herbivory or infection at mid-altitudes where most mountain insects and herbivorous animals find their optimum altitude ranges since foliage quality surpasses those at extreme elevations could result in the isolated induction of *GPPS* and *TPS* for mobilization [33]. Methyl jasmonate (MeJA) signaling is highly activated by physical injury and herbivory, and its effects on the overexpression of *TPS* genes have been demonstrated in different plant species [8]. Therefore, the observed spatial and temporal peaks could also indicate the mobilization of inducible defense response systems within attacked communities [34].

The interpretation of gene expression patterns in relation to secondary metabolite profiles can benefit from integrating transcriptomic and metabolomic information, as changes in transcript abundance do not necessarily correspond directly to proportional changes in metabolite accumulation [34,35].

In conclusion, these discoveries provide a deeper understanding of the implications for the conservation and industrial cultivation of *Thymus kotschyanus*. While terpene pathways are determined genetically, the process can also be dynamically adjusted through interaction with the stresses imposed by the environment. Further study into the specific parameters of moisture content, soil type, and UV index levels at the altitude of 2780 m would allow a better understanding of the molecular trigger responsible for this response in wild thyme.

Conclusion

In this study, a spatiotemporal distribution pattern of *Thymus kotschyanus* metabolic gene expressions has been developed over a three-year period from Sabalan Mountain. It has been shown that the transcription of Geranyl Diphosphate Synthase (*GPPS*) and Terpene Synthase (*TPS*) enzymes does not display a monotonous gradient, but rather a non-linear, quadratic relationship depending on altitude. An important elevation level (around 2780 m primarily) exists, beyond which an increased transcription of terpene-related genes is observed in wild thyme due to a favorable convergence of environmental and thermal factors. Moreover, the large variation in gene expression in thyme during the years 2022, 2023, and 2024 indicates genetic flexibility and sensitivity to changes in the climate each year. In the end, the realization that certain mid-altitude ecological habitats determine the highest level of gene expression proves useful. This is because it provides a biological basis to help conservationists protect natural genetic resources and to enable agriculture to optimize essential oil production.

Data availability

All relevant data generated or analyzed during this study are included in this paper. Any further inquiries can be directed to the corresponding author upon reasonable request.

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Author Contributions

Asgar Sartip Kordedeh conducted the research as part of his PhD dissertation and drafted the manuscript. Ali Asghari and Noraddin Hosseinpour Azad contributed equally to this work, jointly supervising and administering the project, providing conceptualization, and contributing to manuscript writing and editing. (Ali Asghari additionally secured the funding).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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During the preparation of this manuscript, the authors utilized Google Gemini Version, 3.5, to the results interpretation. All AI-generated content was thoroughly reviewed, curated, and verified by the authors to ensure accuracy, scientific integrity, and compliance with copyright laws. The authors take full responsibility for the final content of the manuscript.

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