

Transcriptome analysis of critical genes related to flowering in *Mikania micrantha* at different altitudes provides insights for a potential control

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Abstract

Background: *Mikania micrantha* is a vine with strong invasion ability, and its strong sexual reproduction ability is not only the main factor of harm, but also a serious obstacle to control. Therefore, inhibiting the flowering and seed production of *M. micrantha* is an effective strategy to prevent *M. micrantha* from continuing to invade.

Result: The flowering number of *M. micrantha* is different at different altitudes. A total of 67.01 Gb clean data were obtained from nine cDNA libraries, and more than 83.47% of the clean reads were mapped to the reference genome. In total, 5878 and 7686 significantly differentially expressed genes (DEGs) were found in E2-vs-E9 and E13-vs-E9, respectively. Based on the background annotation and gene expression, some candidate genes related to flowering pathway were initially screened, their expression levels in the three different altitudes in flower bud differentiation showed the same trend, that is, at an altitude of 1300 meters, the flower integration gene and flower meristem gene were down-regulated (such as SOC1 and AP1), and the flowering inhibition gene was up-regulated (such as FRI and SVP). Additional, the results show that there were many DEGs involved in hormone signal transduction pathway in the flower bud differentiation of *M. micrantha* at different altitudes.

Conclusions: Our results provide abundant sequence resources for clarifying the underlying mechanisms of the flower bud differentiation and mining the key factors to inhibit the flowering and seed production of *M. micrantha*, so as to provide technical support for the discovery of an efficient control method.

Background

Mikania micrantha Kunth (Asteraceae family) is an extremely pernicious vine, is one of the top 100 worst invasive alien species in the world [1]. As a noxious invasive plant, *M. micrantha* has caused enormous economic losses and ecological damage [2, 3]. With the in-depth study of *M. micrantha*, chemical control, physical control, and biological control have been used to control it, but it has not been prevented fundamentally [4–6]. The basic reason for it was that *M. micrantha* has a large number of flowers, and its flower biomass accounts for 38.4% ~ 42.8% of the total biomass [7]. *M. micrantha* is also called Mile-A-minute weed, which vividly compares the rapid growth and diffusion of *M. micrantha* [8]. *M. micrantha* can grow and reproduce rapidly in a short time under asexual and sexual reproduction. The seed (sexual reproduction) is small and light in weight. and the mature seeds will begin to spread through artificial and natural methods (wind or water) [9]. Inhibiting the sexual propagation of *M. micrantha* is an effective means to control its rapid spread. Therefore, regulating the flowering and seed production of *M. micrantha* is of great significance for the defense and management. Through the division of the suitable areas of *M. micrantha* in Yunnan Province. He et al. already found that *M. micrantha* in Dehong Prefecture is obviously distributed according to altitude, among which the damage area is the most in the altitude range of 801–1000 m, and the number of flowers is the largest in the altitude area of 900 m [10]. In addition, another previous study found that with the increase of altitude, the harmful area of *M. micrantha* decreased, and the number of *M. micrantha* flower numbers also decreased. The area below

1100 m above sea level is the high-level suitable area of *M. micrantha*; when the altitude exceeds 1100 m, the occurrence probability of *M. micrantha* is gradually decrease, which is an unsuitable area [11].

Flower bud differentiation is the most critical stage in the development of flowering plants and it is a sign of the transformation from vegetative growth to reproductive growth [12]. Plants accurately combine internal signals with external environmental signals and then start the flowering process, which is particularly important for the whole process of plant growth and development [13]. In *Arabidopsis*, six important pathways were identified in the regulation of flower bud differentiation, which include photoperiod pathway, vernalization pathway, thermosensory pathway, autonomous pathway, gibberellin pathway, and aging pathway [14]. These six regulation pathways are independent and cross-linked with each other, forming a precise regulation network of flowering. Photoperiod, vernalization and thermosensory are related to environmental exogenous signals, and plant flowering is regulated by light and temperature. Autonomic, aging and gibberellin pathway are related to the plant itself, which regulates the flowering stage according to its own growth, development and endogenous hormone level [15]. The photoperiod is a very important condition that affects the growth and development of plants, and the entire life cycle of a plant is regulated under the influence of the photoperiod [16]. In the process of responding to the photoperiod, the *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) genes are important flowering promoting factors. The *CO* gene activates the expression of the *FT* gene to promote the flowering in plants [17, 18]. Vernalization is defined by scholars as the ability of plants to obtain or accelerate flower transformation through cold treatment [19]. *FLOWERING LOCUS C* (*FLC*) is the key gene of vernalization pathway, and the process of vernalization is the process of silencing *FLC* gene [20]. In addition to vernalization, the external ambient temperature also directly affects the flowering behavior. Generally speaking, high ambient temperature promotes flowering, while low ambient temperature delays flowering [21]. In *Arabidopsis*, the autonomous pathway does not depend on external conditions such as light and temperature, and is independent of endogenous hormone regulation pathways, which promotes flowering by inhibiting the expression of the *FLC* gene of the flower inhibitor [22]. Previous studies have shown that it not only can stimulate the plant growth and development of plant organs, but also induce the transformation of developmental stages [23]. Zhao have proved that spraying high concentration GA has an obvious effect on the flowering of *M. micrantha* [24]. In addition to GA, other endogenous hormones, such as auxin, cytokinin, ethylene, abscisic acid, brassinosteroids, jasmonic acid and salicylic acid, also play a positive or negative role in the signal network leading to reproductive growth [25, 26].

Transcriptome refers to the sum of all RNA transcribed by specific tissues and cells in a specific function or development period, including mRNA and no coding RNA (ncRNA) [27, 28]. Transcriptome technology has been successfully applied to the study of flower bud differentiation mechanism in many plants, such as *Annona squamosa*, *Litsea cubeba*, *Chrysanthemum morifolium* and so on [29–31]. In the model plant *Arabidopsis thaliana*, the flowering mechanism also has been studied well and to extent in a few other plant species [32]. However, the information available about the molecular basis of flower bud differentiation in *M. micrantha* is very scarce. *M. micrantha* has different flowering numbers at different altitudes. Therefore, understanding the molecular mechanism of flower bud differentiation and mining genes related to flower bud differentiation in *M. micrantha* at different altitudes to formulate the

corresponding control measures are of great significance to the prevention and control of this invasive plant. In the later stage of *M. micrantha* control, the key genes excavated were used to control the flowering of *M. micrantha* by endophytic means. In our study, we have constructed independent cDNA libraries of three different altitudes in *M. micrantha* for Illumina RNA-seq. Analysis of transcriptome data related to bioinformatics was used to characterize flower bud transcriptional pathways at different altitudes of *M. micrantha*. We identified and mined the key differentially expressed genes (DEGs) that were subject to regulation in flower bud differentiation at different altitudes. The transcriptome sequencing of *M. micrantha* flower bud may help understand the role of various hormones, transcription factors, and regulatory pathways in flowering mechanism.

Result

Differences in flowering number of *M. micrantha* at different altitudes

As shown in Figure 1, there were differences in flowering number of *M. micrantha* at different altitudes. We observed that the number of inflorescences and florets at 900 m were significantly higher than those at 200 m and 1300 m. The number of inflorescences at 900 meters is five times that at 1300 meters and is 3.6 times that of 200 meters. In addition, the number of inflorescences and florets at 200 meters is more than that at 1300 meters, but the difference is not significant. Overall, in Dehong Dai and Jingpo autonomous prefecture, Yunnan province, China, the most suitable growth area for *M. micrantha* is 900 meter. High altitude and low altitude are not conducive to the flowering and invasion of *M. micrantha*. In order to further understand the molecular mechanism of flower bud differentiation of *M. micrantha*, we selected the flower buds at the lowest altitude (200 meters), the most suitable altitude (900 meters) and the highest altitude (1300 meters) for transcriptome analysis.

Difference analysis of ecological factors related to altitude

The significant differences of ecological factors in three altitude areas invaded by *Mikania micrantha* were analyzed. The results showed (Figure 2) that the annual average temperature, the average temperature of the hottest month and the radiation temperature in the high-altitude (1300m) and low-altitude (200m) areas invaded by *M. micrantha* were significantly lower than those at an altitude of 900 meters. At an high-altitude areas, the annual sunshine duration is significantly lower than 900 meters. There is no significant difference in annual precipitation, relative humidity and storm runoff among the three altitude regions. Among the environmental factors related to altitude, temperature may be one of the most important factors affecting the ecological adaptability of *Mikania micrantha*. In addition to temperature, light may also significantly affect the flower bud differentiation of *Mikania micrantha*.

Illumina Sequence and assembly

The transcriptome of *M. micrantha* flower buds at an altitude of 200m, 900m and 1300m of three stages was monitored using RNA-Seq. In total, nine cDNA libraries with three repetitions for each altitude were prepared and sequenced. 51,479,370, 46,823,016 and 53,614,214 raw reads were generated for an altitude of 200 meters, 46,985,372, 43,984,916 and 46,915,486 for an altitude of 900 meters, as well as 50,071,842, 58,214,954 and 60,613,424 for an altitude of 1300 meters (Table 1). After removing adaptors, low quality and ambiguous sequences, the clean reads were all higher than 96.61%, and the Q20 values were all over 97.92% (Table 1). The average Q30 and GC percentages were, respectively, 93.92% and 43.1% (Table 1).

Table 1

Statistical analysis of *Mikania micrantha* reads in nine libraries.

Sample	Raw Reads	Clean Reads	Clean Base (G)	Q20 (%)	Q30 (%)	GC Content (%)	Reads Mapped
E2-1	51,479,370	50,184,502	7.53	97.99	93.78	43.10	89.05%
E2-2	46,823,016	45,537,886	6.83	98.14	94.14	43.10	88.84%
E2-3	53,614,214	52,370,142	7.86	98.11	94.07	43.12	88.64%
E9-1	46,985,372	45,730,652	6.86	97.99	93.81	43.08	85.77%
E9-2	43,984,916	42,891,540	6.43	98.03	93.91	43.10	83.92%
E9-3	46,915,486	45,908,178	6.89	98.05	93.96	43.02	86.99%
E13-1	50,071,842	48,757,468	7.31	97.92	93.66	42.68	77.35%
E13-2	58,214,954	56,240,314	8.44	98.02	93.97	43.55	78.16%
E13-3	60,613,424	59,078,680	8.86	98.06	94.00	43.15	72.54%

Q20 percentage the proportion of nucleotides with quality value >20; Q30 percentage the proportion of nucleotides with quality value >30; GC percentage the proportion of guanine and cytosine nucleotides present.

The Pearson correlation coefficients of all gene expression values between each pair of samples were calculated, and the correlation coefficients of the nine datasets are presented in the form of heat maps (Figure 3A). These graphs reflect the correlations of gene expression at three altitudes, the higher the correlation coefficient is, the more similar the gene expression level is. Principal component analysis (PCA) showed that there were significant differences in gene expression profiles among the treatment repeat groups at three altitudes. The PC1 and PC2 explained 37.87% and 25.64% variation, respectively (Figure 3B).

Differential Gene Expression Analysis

In the transcriptomes obtained in different altitudes of *M. micrantha*, we set the following criteria for DEG screening: $|\log_2\text{Fold Change}| \geq 1$ and $Q \text{ value} \leq 0.05$. As shown in Figure 4, in the E2 vs. E9 comparison, 5878 differentially expressed, 2527 up-regulated and 3351 down-regulated unigenes were found. For the E13 vs. E9 comparison, there were 7686 differentially expressed, which 3365 up-regulated and 4321 down-regulated (Figure 4A). Meanwhile, the Venn diagram of the DEGs shows the overlap relationships between the comparison groups, 2477 DEGs were identified in both E2 vs. E9 and E13 vs. E2 (Figure 4B). In the volcano plot, red dots were up-regulated DEGs, green dots were down-regulated DEGs, the number of down-regulated DEGs is more than up-regulated DEGs in E13 vs E9 and E13 vs E9 (Figure 4C and Figure 4D)

GO and KEGG Enrichment Analyses of DEGs

To study the *M. micrantha* buds' transcriptome dynamics and identify the candidate genes, three transcriptomes were generated from different altitude samples (200m, 900m, and 1300m). In this work, GO and KEGG analysis were used to classify the functions of the annotated DEGs under different elevations treatment.

GO and KEGG Pathway enrichment analyses were performed on the DEGs to identify differences in the biological processes and pathways between E2 and E9. Totally, many DEGs between E9 and E2 enriched in GO categories. As shown in Additional file 1, GO enrichment analysis the biological processes category identified a number of categories, including 'secondary metabolite biosynthetic' (GO:0044550), 'hormone biosynthetic process' (GO:0042446) and 'host programmed cell death induced by symbiont' (GO:0034050). For the 'molecular function' category, the dominant terms were 'ADP binding' (GO:00435310), 'oxidoreductase activity, acting on CH-OH group of donors' (GO:0016614), and 'glucosyltransferase activity' (GO:0046527). For the 'cellular component' category, the dominant term was 'chromatin'(GO:0000785) Furthermore, 2064 DEGs enriched for KEGG pathways between E2 and E9 (Additional file 2), 'plant hormone signal transduction' (ko04075), 'biosynthesis of secondary metabolites' (ko01110), 'phenylpropanoid biosynthesis' (ko00940), and 'plant-pathogen interaction' (ko04626) were the main different pathways between these two samples (Figure 5A).

Additionally, many DEGs were enriched in GO categories between E13 and E9. In the 'biological process' category, the dominant terms were the following: 'anion transport' (GO:0006820), 'hormone metabolic process' (GO:0042445), and 'response to cold' (GO:0009409). In the 'cellular component' category, the most representative term was the following: 'plant-type cell wall' (GO:0009505). Finally, in the 'molecular function' category, the most representative terms were the following: 'oxidoreductase activity, acting on CH-OH group of donors' (GO:0016614), 'ADP binding' (GO:0043531), and 'hydrolase activity, hydrolyzing O-glycosyl compounds' (GO:0004553) (Additional file 1). Furthermore, 2682 DEGs enriched for KEGG pathways between E13 and E9 (Additional file 3), 'metabolic pathways' (ko01100), 'biosynthesis of

secondary metabolites' (ko01110), 'plant hormone signal transduction' (ko04075), 'phenylpropanoid biosynthesis' (ko00940), and 'plant-pathogen interaction' (ko04626) were the main enrichment pathways between these two samples (Figure 5B).

Identification of DEGs Involved in Flowering Pathway and Various Flowering-Related Hormones pathways

We further analyzed the expression levels of key DEGs of flowering pathway and flower molecular networks in flower development. Based on a comparative analysis of the NCBI and TAIR databases, many DEGs in *M. micrantha* showed homology to known flowering time-associated genes from other plant species. Most of the flowering time associated genes in *M. micrantha* could be assigned to six classical flowering-related pathway.

In the photoperiod pathway, we identified nine DEGs, *GIGANTEA* (*GI*, 1 DEG), *CYCLIC DOF FACTOR 1* (*CDF1*, 2 DEGs), *CONSTANS* (*CO*, 4 DEGs), and *TEOSINTE BRANCHED 1/CYCLOIDEA/PCF* (*TCP*, 2 DEGs), which were significantly upregulated at the 900m except *CDF1*. (Figure 6A). Additional, in the gibberellin pathway, we identified four key genes, including *GIBBERELLIN RECEPTOR GID1* (*GID1*, 2 unigenes) and the *GA RECEPTOR DELLA PROTEIN GIBBERELLIC ACID INSENSITIVE1* (*GAI*, 2 DEGs). Among of them, *GID1* was significantly up-regulated at 900m, two *GAI* were down-regulated at 900m (Figure 6B). Moreover, three DEGs in *M. micrantha* are involved in the autonomous pathway, such as *FLOWERING TIME CONTROL PROTEIN FY* (*FY*, 1 DEG), *FLOWERING LOCUS D* (*FLD*, 1 DEG), and *CLEAVAGE STIMULATION FACTOR SUBUNIT 77* (*CstF77*, 1 DEG), which were up-regulated at 900m (Figure 6C). Interestingly, We identified that two unigenes that affect the circadian rhythm of the circadian clock, which including *LATE ELONGATED HYPOCOTYL* (*LHY*) and *TIMING OF CAB OF EXPRESSION 1* (*TOC1*), *LHY* was down-regulated at 900m. In addition, several *M. micrantha* DEGs exhibited similarities to genes in the vernalization pathway, including *FRIGIDA* (*FRI*, 1 DEG) and *VERNALIZATION IN-SENSITIVE 3* (*VIN3*, 2 DEGs) (Figure 6D). For the aging pathway, only two DEGs were found, including *SQUAMOSA PROMOTER-BINDING-LIKE PROTEIN 9* (*SPL9*, 1 DEGs) and *APETELA 2* (*AP2*, 1 DEG), which were evidently up-regulated at 900m (Figure 6E). Last, we identified key genes in temperature pathway including *SQUAMOSA PROMOTER-BINDING-LIKE PROTEIN 3* (*SPL3*), *HIGH EXPRESSION OF OS-MOTICALLY RESPONSIVE GENES 1* (*HOS1*), *FLOWERING LOCUS K* (*FLK*), and *PROTEIN EMBRYONIC FLOWER 1* (*EMF1*). However, none of them are DEGs. Among of them they were up-regulated at 900m except *SVP* (Figure 6F). In addition to the genes related to the six pathways, we obtained some DEGs associated with flower integrator genes *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF CONSTANS OVEREXPRESSION 1* (*SOC1*) (Figure 6G), flower meristem identity genes *LEAFY* (*LFY*), flower organ identity genes *CAULIFLOWER* (*CAL*), *APETALA 1* (*AP1*), and *SEPALLATA* (*SEP*) were identified and were found to be mainly up-regulated at 900m (Figure 6H).

Transcriptional levels of hormone signal transduction showed the DEGs involved in the signal transduction pathways of auxin, GA, cytokinin (CK), ethylene, ABA, BR, JA, and SA in three elevations in

M. micrantha. At three elevations, we identified a total of 13 key DEGs in the auxin-signaling pathway, of which ten were significantly down-regulated at 900 m. Whereas, two DEGs i.e., IAA16 and IAA13 were up-regulated at 900 m (Figure 7A and Additional file 4). In the brassinosteroids biosynthetic process, we identified three key genes, including *CYTOCHROME P450 734A1* (*CYP734A1*), *Dwarf 4* (*DWF4*), and *BRASSI-NAZOLE-RESISTANT 1* (*BZR1*). Interestingly, they were up-regulated at 900m (Figure 7B and Additional file 4). We also identified four key genes in gibberellin biosynthetic process and metabolism, of which *GIBBERELLIN 20-OXIDASE* (*GA20OX*) and *ENT-KAURENE OXIDASE* (*KO*) were up-regulated at 900m, while *GIBBEREL-LIN-2-BETA-DIOXYGENASE* (*GA2OX*) were down-regulated at 900m (Figure 7C and Additional file 4). Moreover, compared with E9, we identified many key genes in the ethylene signaling pathway, of which *ETHYLENE INSENSITIVE 3* (*EIN3*), *1-AMINOCYCLOPROPANE-1-CARBOXYLATE OXIDASE HOMOLOG 1* (*ACO1*), *1-AMINOCYCLOPRAPANE-1-CARBOXYLATE SYNTHASE* (*ACS*), *ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR 039* (*ERF39*), *ETHYLENE RESPON-SIVE TRANSCRIPTION FACTOR 016* (*ERF16*), *ETHYLENE RESPONSIVE TRAN-SCRIPTION FACTOR 109* (*ERF109*), *ETHYLENE RESPONSIVE TRANSCRIPTION FACTOR 027* (*ERF27*), and *ETHYLENE RESPONSIVE TRANSCRIPTION FACTOR 07* (*ERF7*) were significantly up-regulated at 1300 m (Figure 7D and Additional file 4). In cytokinin signaling pathway, six key genes were identified, of which *LONELY GUY 7* (*LOG7*) and *LONELY GUY 3* (*LOG3*) were up-regulated at 900m, of which *CYTOKININ DEHYDROGENASE 6* (*CKX6*) and *UDP-GLYCOSYLTRANSFERASE* (*UGT*) were down-related at 900m (Figure 7E and Additional file 4). In the salicylic acid signaling, we found that *PTI-COMPROMISED RECEPTOR-LIKE CYTOPLASMIC KI-NASE 1* (*PCRK1*), and *PTI-COMPROMISED RECEPTOR-LIKE CYTOPLASMIC KI-NASE 2* (*PCRK2*), which were significantly down-regulated at the 900m(Figure 7F and Additional file 4). One *JASMONIC ACID-AMIDO SYNTHETASE* (*JAR1*) gene, *4-COUMARATE-COA LIGASE-LIKE 5* (*4CLL5*) gene, *LIPOXYGENASE 3* (*LOX3*), and *MYC2* were down-regulated at 900m in Jasmonic acid (JA) signaling pathway (Figure 7G and Additional file 4). In the abscisic acid biosynthetic process, ten key genes i.e., *XaNTHOXIN DEHYDROGENASE* (*ABA2*), *9-CIS-EPOXYCAROTENOID DIOXYGENASE 3* (*NCED3*), *9-CIS-EPOXYCAROTENOID DIOXYGENASE 2* (*NCED2*), *ZEAXANTHIN EPOXIDASE* (*ZEP*), *ABSCISIC ACID RECEPTOR PYL8*, *ABSCISIC ACID RECEPTOR PYL4*, *ABSCISIC ACID-INSENSITIVE 5* (*ABI5*), and *ABSCISIC ACID 8'-HYDROXYLASE 2* (*ABAH2*) were down-regulated at 900m (Figure 7H and Additional file 4).

Measurements of Endogenous Hormones Concentrations

There are many kinds of plant hormones in flower buds, and different plant hormones play different regulatory roles in the process of plant flowering. In our results, the results of the KEGG annotation urged us to measure the levels of several plant endogenous hormones in the flower buds of *M. micrantha* at different altitudes To examine the roles of endogenous hormones at flower bud differentiation in *M. micrantha*, the contents of abscisic acid (ABA), 1-Aminocyclopropanecarboxylic acid (ACC), gibberellins GA₅, indole acetic acid (IAA), jasmonic acid (JA), salicylic acid (SA), brassinosteroids (BR), trans-Zeatin (tZ), cis-Zeatin (cZ), and N6-Isopentenyladenine (IP) were measured in *M. micrantha* flower buds at different elevation. The content of JA, SA, BR, tZ, and cZ were too low to be analyzed. Both the GA₁₉, GA₃,

and IP contents reached their highest points at 900m. It was also found that the changes of the contents of the selected a DEG (gene-E3N88_27609) involved in GA₁₉ and GA₃ metabolism at different altitudes were opposite to the changes of GA₁₉ and GA₃ contents at different altitudes. However, the ACC and IAA content reached its lowest point at 900m. The IAA content in E13 area is significantly higher than that in E9 area by 62.74%, the ACC content in E13 area is higher than that in E9area by 4.67%. It was also found that the content changes of the selected two DEGs (gene-E3N88_26993 and gene-E3N88_13343) involved in IAA synthesis and two DEGs (gene-E3N88_07983 and gene-E3N88_30550) involved in ACC synthesis at different altitudes were consistent with the change trend of IAA content at different altitudes. The content of ABA increased significantly from 639.33 $\mu\text{g}\cdot\text{g}^{-1}$ (200 m) to 101.32 $\mu\text{g}\cdot\text{g}^{-1}$ (1300 m), which reached their highest points at 1300m (Figure 8). Both the content of ABA and the expression of DEGs (gene-E3N88_26993 and gene-E3N88_13343) involved ABA synthesis were the lowest in E9. This indicated that various endogenous hormones are closely related to flower bud differentiation of *M. micrantha*.

Bioregulation Analysis and WGCNA of DEGs

Using MapMan software, the biological regulation and metabolism-related roles of the DEGs were analyzed at different altitudes of *M. micrantha* flower buds (Figure 9). The results show that the DEGs mainly responded to IAA, ethylene, ABA, cytokinins, BR, JA, SA, and GAs hormone regulation. There were many up-regulated and down-regulated DEGs associated with various endogenous hormones, the up-regulated DEGs were more than down-regulated ones. The DEGs involved in the ABA pathway and GA pathway were the most abundant, and the DEGs involved in the jasmonate pathway were the least abundant. But beyond that, many DEGs were enriched in the transcription factor (TF), protein modification, and protein degradation categories. TFs are key regulatory proteins of transcription in biological processes, especially in flower buds differentiation. Therefore, we studied the expression dynamics of TF genes in *M. micrantha*. In total, 444 TF genes were identified in E9 vs E2 and 592 TF genes were identified in E9 vs E13. In this study, among the top ten abundance during flower buds differentiation, including the basic helix-loop-helix (bHLH), NAC, B3, MYB, basic leu-cine zipper (bZIP), WRKY, ERF, MADS-box, C2H2, and MYB-related. On redox reactions, the DEGs participated in biological reactions regulated by calcium regulation, receptor kinases, G-proteins, MAP kinases, phosphoinositides, carbon, nutrients, and light. Several DEGs accumulated in a series of oxidoreductase categories, such as the heme, thioredoxin, ascorb/glutathione, glutaredoxin, and dismutase/catalase categories. In addition, the DEGs participated in biological reactions regulated by light, nutrients, carbon, phosphoinositides, MAP kinases, G-proteins, receptor kinases, calcium regulation, and 14.3.3 in redox reactions.

WGCNA (Weighted correlation network analysis) is an analytical method to explore the relationship between modules and concerned phenotypes and to mine key genes in coexpression networks [33]. Many different modules were identified based on pairwise correlations of gene expression across three samples. Eigengenes and clusters were calculated based on the correlations to quantify the co-

expression similarity of entire modules, using a strict cut off of 0.25, corresponding to correlation of 0.75, to merge the optimization into 20 modules (Figure 10).

Observing the correlation coefficients of the modules, it was found that the genes in the darkmagenta, and thistle had higher specificity at E9. The expression trend of differentially expressed genes on the darkmagenta module is up-regulated in E9 and down regulated in E2 and E13. KEGG pathway analysis was performed on the darkmagenta module, and it was found that DEGs were enriched in metabolic pathways, biosynthesis of secondary metabolites, 'starch and sucrose metabolism', and 'plant hormone signal transduction' (Additional file 5). This indicated that the flower bud differentiation of *M. micrantha* needs carbohydrate metabolism to provide energy. In addition, plant hormones also play a very important role in the flower bud differentiation of *M. micrantha*.

Discussion

Illumina sequencing and sequence annotation

We performed comparative analysis of gene expression of three altitudes. We identified a number of DEGs involved in various biological processes, which are likely to be associated with the regulation of the *M. micrantha* flower bud development. The number of down-regulated genes is larger than up-regulated genes in E13 vs E9 and E13 vs E9, indicating that flower buds differentiation requires more gene suppression than activation in *M. micrantha*. And the onset of flower development was accompanied by the repression of many genes similar to previously reported in Arabidopsis during the incipient of flower bud differentiation.

The second-generation molecular sequencing technology RNA-seq has already become the most common method used for studying gene expression and screening and predicting candidate genes [34]. Wang et al (2021) have used transcriptome sequencing to obtain candidate genes related to early flowering and flower development in two flower development stages of two tomato varieties with different flowering periods [34]. Zhang et al (2013) has screened genes with high differential expression and related to flower development in *Cymbidium sinense* from three sub-libraries as candidate genes [35]. The temporal regulation of gene expression plays an important role in plant growth and development.

Identification of DEGs associated with flower bud differentiation and flower development

Based on the regulatory network of flowering pathway related gene involved in Arabidopsis flower bud differentiation, six regulatory pathways were implicated in the response of *M. micrantha* (Figure 11).

Photoperiod pathway includes a the input pathway of the clock, a circadian clock and an output pathway from the clock to flowering [36]. The *LATE ELONGATED HYPOCOTYL (LHY)* gene in the MYB transcription factor family are located upstream in the circadian network and negatively regulate the expression of

TIMING OF CAB EXPRESSION 1 (TOC1) [34]. The circadian clock affects the regulation of light on the circadian clock by regulating some optical signal components, such as *GI (GIGANTEA)*, and finally forms a certain circadian rhythm and affects the expression of downstream genes [37]. Under long conditions, the expression of *CO* is modified by the activity of three proteins: *GI*, *FKF1*, and *CDF1*, which are regulated by circadian clock [38,39]. In our study, *GI* is regulated by circadian rhythm and activates the expression of *CO* and *FT* genes by inhibiting the expression of *CDF1*. The expression of transcription inhibitor *CDF1* was down-regulated at 900 m. Autonomic pathway is the flowering pathway that some plants will bloom after reaching a certain stage of vegetative growth in the absence of external induction [22]. In *Arabidopsis*, it has been cloned in-to *FCA*, *FY*, *FPA*, *FVE*, *FLD*, *FLK*, and *LD*, they inhibit the expression of *FLC* in a parallel manner, thereby promoting flowering [40,41]. In *M. micrantha*, we only identified *FY* and *FLD* were significantly up-regulated at 900m. Therefore, in the autonomous pathway of *Mikania micrantha*, the flower bud differentiation occurring at 900m may be due to the inhibitory effect of the autonomous factors on *FLC*. Vernalization refers to the process by which low temperature induces flowering in plants [14]. In *Arabidopsis*, *FRI* is located upstream of *FLC* and mediates vernalization by regulating *FLC* [42,43]. In our study, we found that *FRI* were up-regulated at 1300m, which may result in high expression of *FLC* at 1300 m. However, we did not detect *FLC* expression at any elevation, which may be due to sampling time. *Chenopodium Quinoa* Willd also did not identify a *FLC* homologous gene [44]. This result implied that the expression of *FRI* is positively regulated to inhibit the differentiation of flower buds of *Mikania micrantha* at 1300m. In biennials and winter annuals, *VIN3* forms the inhibition of *FLC* through vernalization, and *VRN1* and *VRN2* participate in the maintenance of *FLC* inhibition [45,46]. We only found a *VIN3* gene down-regulated at 1300m, Which can promoted *FT* gene expression by inhibiting *FLC* gene in *M. micrantha*. In addition, aging pathway is a key regulation pathway for flowering regulation of *M. micrantha*. The key gene *SPL* in the age pathway is regulated by a small molecule RNA-miRNA156 [47,48]. The expression of RNA-miR156 is regulated by the age pathway and its content gradually decreases with the growth of plants, and the accumulation of its target gene *SPL* transcription factor gradually increases [49]. In *Arabidopsis*, *SPL9* directly induce flowering by activating flower organ characteristic genes *AP1*, *LFY*, and *SOC1* [50]. In *Raphanus sativus*, the genes related to age pathway were also found, such as *SPL1*, *SPL2*, *SPL3*, *SPL9*, *SPL13*, and *SPL15*, which are related to mossing and flowering [51]. In our study, *SPL9* was up-regulated at 900m, which was consistent with the expression trend of the downstream flowering integrator *SOC1* gene. This indicated that *SPL9* gene promotes the flower bud differentiation of *Mikania micrantha* at 900m.

In addition to the low temperature induction in the vernalization pathway, the ambient temperature at the vegetative growth stage of plants is also significant for flower formation [52]. In *Arabidopsis*, even under short-day conditions when the temperature is 25 or 27°C, the flowering time is similar to that under 23°C long-day conditions [53]. The temperature during the vegetative growth period has an important effect on the flowering of plants, showing a trend of high temperature promotion and low temperature inhibiting flowering [52]. In *Narcissus tazetta* var. *chinensis*, the increase of temperature also promoted flowering, and the expression of *FT* homologous gene *NFT1* was up-regulated at high temperature [54]. MYB transcription factor *EFM (EARLY FLOWING MYB PROTEIN)* also mediates the effect of ambient

temperature on flowering, directly inhibits the expression of *FT* in leaf vascular, and its expression is up-regulated by *SVP* [55]. In *M. micrantha*, we only found that a *SVP* homologous gene was down-regulated at 900m, which may be because the area at 900 meters has a higher ambient temperature than that at 1300 m. In other words, the area at 900 meters is more suitable for the survival of *M. micrantha*. In Arabidopsis, previous study show that the microRNA156 (miR156)-*SQUAMOSA PROMOTER BINDING PRO-TEIN-LIKE3* (*SPL3*) module directly regulates *FT* expression to control ambient temperature responsive flowering [56]. In this study, we found that a *SPL3* homologous gene was up-regulated at 900m, which may promote the expression of the downstream florigen *FT* gene at 900 meters. On the other hand, *HOS1* inhibits flowering induced by low temperature, which negatively regulates *FLK* through ubiquitination, and weakens the activation of *FT* by *FLK* [57]. In our study, although *HOS1* was up-regulated at 900 m, the *FLK* homologous gene was regulated at 900 m, which can promoted the *M. micrantha* flower bud differentiation at 900m. Under non-induced short-day conditions, GA is a necessary condition for Arabidopsis to bloom, and GA treatment can promote early flowering of Arabidopsis under short-day [58]. Achard and Genschik (2009) summarized the molecular model of GA signal transduction, active GA ubiquitinates the DELLA protein and degrades it through the 26S proteasome, which is a major way to relieve the inhibition of flowering [59]. In our study, three DELLAs were down regulated at 900m, which lead to the flower bud differentiation of *M. micrantha* at 900m. In the transcriptome data, we identified a large number of DEGs related to photoperiodic pathways and related to two temperature related pathways (temperature pathway and vernalization), which is consistent with the results of our significant analysis of ecological factors. Temperature and light are the main factors affecting flower bud differentiation of *M. micrantha*.

Under the control of complex genetic pathways, plants integrate environmental and endogenous signals to precisely regulate flowering time. The above six genetic pathways of flowering regulation are not isolated, and various signaling pathways ultimately achieve the regulation of flower bud differentiation by regulating integrator genes [60]. At present, three integrator genes *FT*, *LFY* and *SOC1* have been found in Arabidopsis [61]. *FT* is a downstream target gene of the key gene *CO* of the photoperiod pathway, the *FLM* gene of temperature pathway and the *SPL9* gene of aging path-way, which can promote the expression of downstream *LFY* [62,63]. In our study, although we identified three *FT* homologous genes in Mikania micrantha transcriptome data, their expression were up-regulated at 900 m, which was consistent with the ex-pression trend of upstream flowering promoting gene that promoted its flowering. However, the three *FT* homologous genes we identified do not belong to DEGs. *SOC1* gene can integrate flowering signals from various pathways to achieve the purpose of regulating flowering time and flower morphology [64,65]. In our study, the expression level of flower integrator gene *SOC1*, flower meristem identity genes *SPLs* and *LFY*, flower organ identity *AP1*, *CAL*, and *SEP* were significantly upregulated at the 900m in *M. micrantha*, suggesting their important roles in 900cm. Flower meristem genes are highly conserved among plants. The expression of *LFY* in *Camellia sinensis* also affects flower bud formation, flower meristem genes *CAL* and *AP1* were identified in *Ambrosia artemisiifolia* L. to induce flowering [66,67]. In addition, some candidate DEGs expression levels in the three different altitudes showed the same trend, that is, the expression of DEGs that promote flowering (such as *SOC1* and *CO*)

was upregulated at an altitude of 900 meters, and the expression of DEGs that inhibited flowering (such as *SVP* and *FR1*) was decreased at an altitude of 900 meters.

The putative roles of phytohormone crosstalk in *M. micrantha* flower development

In this study, we used transcriptome technology to investigate the changes of DEGs in flower bud differentiation at 200m, 900m, and 1300m. A KEGG pathway enrichment analysis indicated that the DEGs were significantly enriched in the pathways related to phytohormone synthesis and signal transduction processes. The results revealed that DEGs involved in the key pathways of hormone signal transduction and biosynthetic process associated with flower bud differentiation. This enabled us to identify key genes, and further clarify the molecular mechanisms underlying flower buds differentiation in *M. micrantha*.

Auxin is an important signal molecule in plants, which regulates almost all aspects of plant growth and development, including the initiation of flower primordium and the identification of flower organs [68,69]. In our study, the known *AUXIN RESPONSE FACTOR* (ARF) and Aux/IAs were identified, suggesting that the ARF-Aux/IAA regulatory pathway is critical for flower development in *M. micrantha*. In model auxin response is regulated by the ARF family as well as AUX/IAA proteins that repress the activity of some ARFs [70]. In *M. micrantha*, the expression of the ARF was down-regulated and the Auxin/IAA were up-regulated at 900 m, which is consistent with the content of IAA was the lowest at 900 m. In sugar apple, the expression of IAA3 was increased during flower transition and flower development [29]. In *M. micrantha*, IAA3 showed highest expression at 900m, which is consistent with the change of IAA content. In Arabidopsis, previous studies have found that the *YUC* gene family play an important role in the in converting IPA to IAA, while indole-3-acetaldehyde oxidase (AAO) catalyze the conversion of IAAld to IAA [71,72]. Gibberellin (GA) is an integral phytohormone, which plays diverse roles in plant growth and development, including seed germination, stem elongation, and flowering time [73]. GA homeostasis is achieved by tight regulation of both activating enzymes, including GA20ox and GA3ox that catalyze GA biosynthesis, and deactivating enzymes, (GA2ox), that contribute to GA turnover [74]. Interestingly, the GA biosynthesis genes, GA2ox, showed significantly reduced expression at 900m in *M. micrantha*. It was in agreement with the decrease in the contents of GA at 900m. However, the expression of GA20ox1 and KO, another two GA biosynthetic genes, was induced at 900m in *M. micrantha*. In Arabidopsis, GA20ox1 and KO1 play roles in both the synthesis of bioactive GA and environmental stimuli responses [75,76]. It suggested the fact that GA plays important roles in flower bud differentiation in *M. micrantha*. In Arabidopsis, the GA effect on flowering is genetically mediated by five DELLA (Asp-Glu-Leu-Leu-Ala) proteins, REPRESSOR OF GAL (RGA), GA-INSENSITIVE (GAI), RGA-LIKE 1 (RGL1), RGL2, and RGL3 [77,78]. It has been proved in rice that there are gibberellin regulatory pathways that are all regulated by the GID1-DELLA system [79]. In *M. micrantha*, three GAI were identified, which showed down expression at 900m. It indicated that GA paly a positive role in *M. micrantha* flower bud differentiation. The exogenous application of GA₃ promoted the formation of flower buds in Arabidopsis by activating family genes

related to flowering in the apical meristems [23]. In our study, the content of GA₃ reached the highest, which implied GA₃ play a positive role in *M. micrantha*. Cytokinins are mainly involved in some basic developmental processes, and regulate the formation of buds in lower plants [80]. In Arabidopsis, CKXs can reduce the content of cytokinin in plants by removing the unsaturated isopentenyl side chain of cytokinin [81,82]. In our study, CKX6 was identified and were down-regulated at 900m, which was the same trend as the highest content of IP (Isoamyl alkenyl adenine) at an altitude of 900 meters. On the other hand, in the direct activation pathway, LOGs also plays a pivotal role in regulating cytokinin activity during normal growth in Arabidopsis [83]. In *M. micrantha*, LOGs were up-regulated at 900m. It suggested that cytokinin play a positive role in *M. micrantha* at 900m. Ethylene is an important phytohormone, which involved in the regulation of flower transition, fruit ripening, stress responses and so on [84,85]. In Arabidopsis, 1-aminocyclopropane-1-carboxylic acid (ACC), ACC synthase (ACS), and ACC oxidase (ACO) are key enzymes in ethylene biosynthetic process [86]. In *M. micrantha*, ACO and ACS were identified and were down-regulated at 900m, and content of ACC reached its lowest points at 900m, which implied the role of ethylene as a flower repressor in *M. micrantha*. Previously, the role of ABA in flowering regulation is still controversial because its promoting and inhibiting effects have been reported [26,87]. Wang et al (2019) demonstrated that ABA content were upregulated in later stages of flower development in *Lonicera japonica* [88]. In Loquat, genes of ABA signaling pathway and ABA content were significantly upregulated after flower anthesis [89]. In our study, genes of ABA signaling pathway and ABA content were significantly upregulated at 1300m, indicating the fact that ABA plays negative roles in *M. micrantha*. The phenotype of the ABA deficient mutant and the lack of a significant flowering phenotype in the transgenic lines that overexpress the *NCED3* genes [90]. In *M. micrantha*, *NCED3* was up-regulated at 200m, it is consistent with ABA content.

In addition to IAA, GA, and cytokinin, the ethylene ABA, BR, JA, and SA also play import roles in the flower bud differentiation. BR are steroid hormones that are essential for plant growth and development, which control the division, elongation and differentiation of various cell types throughout the entire plant life cycle [91]. The promotive role of BRs in flower transition was proposed based on the late-flowering phenotype of BR-deficient mutants, *DWF4* [92]. In *M. micrantha*, *DWRF4* was down-regulated at 200m. In our study, genes of BR biosynthetic process were significantly upregulated at 900m. Due to complex regulatory mechanism, the corresponding express trend of the hormone-related genes is not very clear. Further experiments are needed to reveal their specific functions in *M. micrantha*. Jasmonic acid (JA) is a lipid plant hormone, the signal pathways of JA derivatives and the response mechanism to adversity stress have been thoroughly studied, but their role in the regulation of flowering is less studied [93]. The key repressors of JA signaling are JASMONATE-ZIM domain (JAZ) proteins, which could positively regulate the expression of FT through their interaction with *MYC2* and *MYC3* [94-96]. However, we did not found the JAZ homologous gene and found a *MYC2* homologous gene down-regulated at 900 meters, which may imply JA is a negative role in flowering regulation. Salicylic acid (SA) is a kind of phenolic phytohormone widely present in plants, and it has a significant regulatory effect on the growth, development and resistance of plants [97]. In Arabidopsis, the accumulation of SA is induced by exogenous application of SA solution or ultraviolet irradiation to inhibit the expression of *FLC*

transcription factors, thereby promoting flower formation [98]. In *Pharbitis nil*, the poor-nutrition stress-induced flowering was inhibited by aminooxy acetic acid, a phenylalanine ammonialyase inhibitor, and this inhibition was almost completely reversed by SA [99]. In our study, genes of SA signaling pathway were significantly down-regulated at 900m. We can infer that SA can promote flowering, but there is insufficient evidence at present, which needs to be proved by later research.

Furthermore, the signal pathways related to various plant hormones do not act independently on the regulation of flowering, but are achieved by changing the expression levels of key flower-forming genes after pooling different hormone signals [100]. For example, ARFs and IAAAs could directly regulate genes encoding GA metabolic enzymes, such as GA20OX [101]. Meanwhile, BR signaling promotes GA biosynthesis through inducing GA20OX expression, DELLAs directly inhibits each other with *BRASSINAZOLE RESISTANT 1 (BZR1)* and *BRI1-EMS-SUPPRESSOR 1 (BES1)* [102,103]. But beyond that, *ACID-INSENSITIVE 4 (ABI4)* and *ABI5* repress flowering by directly promoting the expression of *FLC*, which results in the down-regulated expression of *FT* and *SOC1* [87,104]. In a word, the cross action of various phytohormones signaling pathways affects flower bud differentiation.

In summary, nine independent cDNA libraries from *M. micrantha* flower buds at 200m, 900m, and 1300m were constructed and sequenced. A large number of DEGs were identified in *M. micrantha*. Flowering time-associated and flower development-related genes were characterized based on GO and KEGG. Furthermore, the expression levels of DEGs regulated to hormone signal transduction at different altitudes were also analyzed. The identification and analyses of these hormone-related genes will aid us in elucidating the regulatory mechanisms of hormones.

Materials And Methods

Plant material

All flower buds of *M. micrantha* were collected from three different altitudes: (1) E2, the area at 200m altitude (2020/10/06); (2) E9, the area at 900 altitude (2020/10/06); and (3) E13, the area at 1300 altitude (2020/10/06) from Tongbiguan nature reserve, Dehong Dai and Jingpo Autonomous Prefecture, Yunnan Province, China. During flower buds sample collection, flower buds from at least ten plants were mixed and regarded as one biological replicate representing each altitudes, and three independent replicates were performed. Flower buds materials were frozen in liquid nitrogen immediately after collection and then stored at -80 °C until RNA was extracted. Inflorescences and florets were taken as the investigation objects. A quadrat of 20 cm×20 cm was randomly set for each elevation (200m, 400m, 800m, 900m, 1000m, 1300m) to investigate the number of inflorescences and florets in the quadrat. There were three quadrats of 20 cm×20 cm at each elevation, with three replicates.

Extraction of ecological factors

By using wheatA (<http://www.wheata.cn/>), eight eco-environmental factors were extracted from the three altitude areas of the samples collected from 2017 to 2021 for five consecutive years, including

annual average temperature (°C), average temperature in the hottest month (°C), radiation temperature (°C), annual sunshine duration (h), relative humidity (%), evapotranspiration (mm), annual precipitation (mm), and storm runoff (mm). Based on the ANOVA, the values of ecological factors of three altitudes were significantly analyzed.

Transcriptome sequencing

RNA extraction and construction of Illumina library

The floral buds after removing visible leaves and shoot apex from E2, E9, and E13 was used for RNA-seq. Total RNA was extracted individually using the Trizol Kit (Promega, Beijing, China). The quantity and quality were determined using RNase-free agarose gel electrophoresis and the total RNA concentration was measured using Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA) at 260nm and 280nm. The poly (A) mRNA was isolated from total RNA samples with Magnetic Oligo (dT) Beads and used for mRNA-sequencing library construction. The construction of cDNA library was performed according to the method described by Yang et al [105]. The cDNA was synthesized using the fragmented mRNA and end-repaired followed by a single 'A' base addition. mRNA-Sequencing Sample Preparation Kit (Illumina) was used to pre-prepare the DNA fragments for ligation to the adapters. After purification, the cDNA fragments (200 ± 25 bp) were excised and retrieved. The ligation products were size fractioned by agarose gel electrophoresis, and fragments were excised for PCR amplification.

Sequencing, assembly and functional annotation

The mRNA-sequencing libraries were sequenced using Illumina HiSeq™ 2500 sequencing platform (Illumina Inc., San Diego, USA) by Wuhan MetWare Biotechnology Co., Ltd. (www.metware.cn). For de novo assembly, reads with more than 10% N bases (bases unknown) and those containing adaptor sequences were removed. Low quality reads containing more than 50% of low Q (≤ 20) bases were also removed. Then, the clean reads were assembled using Trinity program to construct unique consensus sequences [106]. The raw sequence data has been submitted to the NCBI Short Read Archive with accession number. The assembled unigenes were aligned to a series of protein databases using the BLASTX alignment algorithm with $E < 0.00001$. Annotation of the assembled unigenes was performed by searching against the NCBI Nr (non-redundant) protein (<http://www.ncbi.nlm.nih.gov>), Swiss-Prot (<http://www.expasy.ch/sprot>), KEGG (<https://www.genome.jp/kegg/>), KOG (<http://www.ncbi.nlm.nih.gov/KOG>), Pfam and TAIR (<https://www.arabidopsis.org/>) databases with an $E\text{-value} \leq 10^{-5}$ for Functional annotation. Sequences were aligned to the KEGG database, significant enrichment analysis was performed and the pathways with Q values ≤ 0.05 were defined as showing

significant enrichment in DEGs. The Blast2GO software was used to obtain gene ontology terms (GO, <http://www.geneontology.org>) analysis. When the results conflicted among databases, then the following order of priority was employed: NR, Swiss-Prot, KEGG, and KOG.

Measurements of Plant Hormone Contents

For the determination of endogenous hormones in the *M. micrantha* buds at different altitudes, individual samples from different altitudes of *M. micrantha* flower buds were harvested and then immediately frozen in liquid nitrogen and stored at -80°C until extraction. The samples from different altitudes used to determine endogenous hormone contents are consistent with the samples from different altitudes used for RNA sequencing. Firstly, 50 mg sample was ground into fine power in liquid nitrogen, which was extracted with methanol/water/formic acid (15: 4: 1, V/V/V). The extracts from three replicates of each treatment were then combined and evaporated to dryness under nitrogen gas stream, reconstituted in 80% methanol (V/V), and filtrated (PTFE, 0.22 µm; Anpel) before LC-MS/MS analysis. The following determination was performed on the AB Sciex QTRAP 6500 LC-MS/MS platform with an electrospray ESI (Agilent Technologies). An aliquot (20µL) was injected into Zobax XDB C18 column (2.1 mm × 100 mm × 1.8 µm, Agilent Technologies) with a flow rate of 0.35 mL/min. The mobile phase was 0.05% formic acid in water (A) and 0.05% formic acid in acetonitrile (B). The gradient was as follows: 0.0 min, A/B (95: 5, V/V); 1.0 min, A/B (95: 5, V/V); 8.0 min, A/B (5: 95, V/V); 9.0 min, A/B (5: 95, V/V); 9.1 min, A/B (95: 5, V/V); 12 min, A/B (95: 5, V/V). We performed three replicates for each assay.

Statistical analyses

For quantification analysis of RNA-Seq, the number of Fragments Per Kilobase of transcript per Million fragments mapped (FPKM) was used to measure the expression levels of unigenes [107]. Significant differences in the contents of hormones was calculated using a one-way ANOVA analysis with a Turkey test and a significance level at $\alpha = 0.05$ in SPSS software. All expression analyses were performed in three replicates. Reported values represent arithmetic averages of three replicates. Data was expressed as mean plus or minus standard deviation (mean $\pm$ SD). Based on the CDS of the *M. micrantha* genome, the mapping file was constructed using the annotation website (<https://www.plabipd.de/portal/mercator-sequence-annotation>), The expression levels of DEGs in the comparison groups of E9-vs-E2 and E9-vs-E13 were uploaded. Then, we chose PageMan functional analysis with MapMan software to annotate the biological functions of the differentially expressed genes between each sample. The WGCNA v1.48 package was used to construct a gene coexpression network. In this study, the expression levels of nine transcriptome samples (three altitudes, three repetitions each) were selected for WGCNA analysis. R with WGCNA package was used to build a coexpression network; the parameters of WGCNA used default settings, except that the power was 5, TOMType was unsigned, minModuleSize was 30, and mergeCutHeight was 0.25 [33]. In order to study the highly correlated modules, the Pearson correlation

coefficient between the sample matrix and the gene module was calculated and statistically tested. The larger is the correlation coefficient, the higher is the correlation between the module and the sample.

Conclusion

In this study, comparative transcriptome analysis show that there were significant differences in the expression of flowering pathway related DEGs and hormone signal transduction pathway related DEGs in the flower bud differentiation of *M. micrantha* at different altitudes. Generally, many DEGs were annotated as genes related to hormone biosynthesis, transport, or signal transduction at flower bud differentiation. Hence, these plant hormones can be used as a control measure to protect forests and other land from *M. micrantha* invasion. Additionally, by considering six different flowering pathways, we analyzed and predicted the functions of these DEGs. Among them, key flower integrator genes and flower organ identity genes were significantly upregulated at 900 m. The flower bud differentiation in *M. micrantha* is regulated by a large number of genes involved in complex molecular pathways. These results play a critical role in illuminating the molecular mechanisms of flower bud differentiation in *M. micrantha* and they will provide abundant candidate genes for studies of prevention, control, and flower-time regulation in *M. micrantha*.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The nucleotide sequences of raw data from this study were submitted to the NCBI sequence read Archive (SRA) under the accession number PRJNA792910.

Author Contributions

C.L., B.Y., and N.Z. conceived and designed the study; Z.Z. and L.L carried out data curation and formal analysis; resources, C.L. and N.Z. participated in writing original draft preparation; Z.Z., S.Z. and M.J. participated in the supervision; L.P and L.F participated in the visualization. N.Z. and B.Y. provided funding, supervised the study and revised the first draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

M. micrantha used in the experiment came from Dehong Dai and Jingpo Autonomous Prefecture, Yunnan Province, China. Before sampling *M. micrantha* in this area, we have obtained permission from Yunnan

Forestry and Grassland Pest Control and Quarantine Bureau. In addition, the specimen of *M. micrantha* has been preserved in the specimen Library of Southwest Forestry University, and its specimen number is SWFU20181006089. Otherwise, the corresponding authors (Bin Yang and Ning Zhao) belong to Key Laboratory of Forest Disaster Warning and Control of Yunnan Province, Southwest Forestry University, this laboratory is specialized in the prevention and control of forest harmful organisms and has the qualification of collecting samples. The authors confirm that all experimental research and field studies on *M. micrantha*, including the collection of *M. micrantha*, must comply with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Lowe, S., Browne, M., Boudjelas, S. *et al.* 100 of the World's Worst Invasive Alien Species: A Selection From the Global Invasive Species Database. (2000).
2. Macanawai, A. R., Day, M., Tumanengdiete, T. *et al.* Impact of *Mikania micrantha* on crop production systems in Viti Levu, Fiji. *Pakistan Journal of Weed Science Research* 18, 357-365 (2012).
3. Yin, L., Liu, B., Wang, H. *et al.* The Rhizosphere Microbiome of *Mikania micrantha* Provides Insight Into Adaptation and Invasion. *Frontiers in microbiology* 11, 1462, doi:10.3389/fmicb.2020.01462 (2020).
4. M., J., W. *et al.* Potential biological control agents for *Mikania micrantha* hbk from the Neotropical region. *Tropical Pest Management* 28, 242-254 (1982).
5. Shao, H., Peng, S., Liu, Y. *et al.* The biological control and the natural enemy of *Mikania micrantha* H.B.K's in China. *Ecologic Science* (2002).
6. Guo, C., Ma, Y., Qin, J. *et al.* Chemical control effects of 30 kinds of herbicides on *Ambrosia artemisiifolia* and *Mikania micrantha*. *Plant Protection* 40, 179-183 (2014).

7. Rui-Ye, L. I. Occurrence and Control of Alien Pest *Mikania micrantha* H.B.K. *Journal of Agricultural Catastrophology* (2012).
8. Waterhouse, D. F. *Biological Control of Weeds: Southeast Asian Prospects*. Monographs (1994).
9. Yong-Pai, M. A. *Control Techniques of Mikania micrantha Woodland in Dehong Prefecture*. Forest Inventory and Planning (2011).
10. Dongbei, H. E., Shanxi N , K. E., Zhou, P. *et al.* Research on Distribution Pattern and Control Policies of *Mikania micrantha* Kunth in Dehong Prefecture. *Central South Forest Inventory and Planning* (2016).
11. Xiao-Yong, F. U., Sang-Zi, Z. E., Zhou, X. *et al.* Distribution prediction and assessment of *Mikania micrantha* in Yunnan province based on Max Ent model. *Guangdong Agricultural sciences* (2015).
12. Bo, Q., Wei, Z., Chen, X. *et al.* Research progress of Flower Bud Differentiation Mechanism of Plant. *Chinese Agricultural Science Bulletin* (2010).
13. Yueping, M. & Silan, D. Flower Bud Differentiation Mechanism of Anthophyta. *Molecular Plant Breeding* (2003).
14. Srikanth, A. & Schmid, M. Regulation of flowering time: all roads lead to Rome. *Cellular and molecular life sciences : CMLS* 68, 2013-2037, doi:10.1007/s00018-011-0673-y (2011).
15. Dorca-Fornell, C., Gregis, V., Grandi, V. *et al.* The Arabidopsis SOC1-like genes AGL42, AGL71 and AGL72 promote flowering in the shoot apical and axillary meristems. *The Plant journal : for cell and molecular biology* 67, 1006-1017, doi:10.1111/j.1365-313X.2011.04653.x (2011).
16. Guo, C. X., Tian, S. B., Zheng, C. S. *et al.* FT Gene of Flowering Determination of Plants in Photoperiod Pathway. *Genomics and Applied Biology* (2009).
17. Putterill, J., Robson, F., Lee, K. *et al.* The CONSTANS gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* 80, 847-857, doi:10.1016/0092-8674(95)90288-0 (1995).
18. Kardailsky, I., Shukla, V. K., Ahn, J. H. *et al.* Activation tagging of the floral inducer FT. *Science* 286, 1962-1965, doi:10.1126/science.286.5446.1962 (1999).
19. Villacorta-Martin, C., Núñez de Cáceres González, F. F., de Haan, J. *et al.* Whole transcriptome profiling of the vernalization process in *Lilium longiflorum* (cultivar White Heaven) bulbs. *BMC Genomics* 16, 550, doi:10.1186/s12864-015-1675-1 (2015).
20. Andrés, F. & Coupland, G. The genetic basis of flowering responses to seasonal cues. *Nature reviews. Genetics* 13, 627-639, doi:10.1038/nrg3291 (2012).
21. Jagadish, S. V., Bahuguna, R. N., Djanaguiraman, M. *et al.* Implications of High Temperature and Elevated CO₂ on Flowering Time in Plants. *Front Plant Sci* 7, 913, doi:10.3389/fpls.2016.00913 (2016).
22. Simpson, G. G. The autonomous pathway: epigenetic and post-transcriptional gene regulation in the control of Arabidopsis flowering time. *Current opinion in plant biology* 7, 570-574, doi:10.1016/j.pbi.2004.07.002 (2004).

23. Mutasa-Göttgens, E. & Hedden, P. Gibberellin as a factor in floral regulatory networks. *J Exp Bot* 60, 1979-1989, doi:10.1093/jxb/erp040 (2009).
24. Zhao, N., Ze, S., Liu, N. *et al.* Exogenous phytohormone application and transcriptome analysis of *Mikania micrantha* provides insights for a potential control strategy. *Genomics* 113, 964-975, doi:10.1016/j.ygeno.2021.02.008 (2021).
25. Davis, S. J. Integrating hormones into the floral-transition pathway of *Arabidopsis thaliana*. *Plant, cell & environment* 32, 1201-1210, doi:10.1111/j.1365-3040.2009.01968.x (2009).
26. Domagalska, M. A., Sarnowska, E., Nagy, F. *et al.* Genetic analyses of interactions among gibberellin, abscisic acid, and brassinosteroids in the control of flowering time in *Arabidopsis thaliana*. *PLoS One* 5, e14012, doi:10.1371/journal.pone.0014012 (2010).
27. Costa, V., Angelini, C., De Feis, I. *et al.* Uncovering the complexity of transcriptomes with RNA-Seq. *J Biomed Biotechnol* 2010, 853916, doi:10.1155/2010/853916 (2010).
28. Wang, L., Zhao, S., Gu, C. *et al.* Deep RNA-Seq uncovers the peach transcriptome landscape. *Plant Mol Biol* 83, 365-377, doi:10.1007/s11103-013-0093-5 (2013).
29. Liu, K., Feng, S., Pan, Y. *et al.* Transcriptome Analysis and Identification of Genes Associated with Floral Transition and Flower Development in Sugar Apple (*Annona squamosa* L.). *Front Plant Sci* 7, 1695, doi:10.3389/fpls.2016.01695 (2016).
30. Ren, L., Liu, T., Cheng, Y. *et al.* Transcriptomic analysis of differentially expressed genes in the floral transition of the summer flowering chrysanthemum. *BMC Genomics* 17, 673, doi:10.1186/s12864-016-3024-4 (2016).
31. He, W., Chen, Y., Gao, M. *et al.* Transcriptome Analysis of *Litsea cubeba* Floral Buds Reveals the Role of Hormones and Transcription Factors in the Differentiation Process. *G3 (Bethesda, Md.)* 8, 1103-1114, doi:10.1534/g3.117.300481 (2018).
32. Melzer, S., Lens, F., Gennen, J. *et al.* Flowering-time genes modulate meristem determinacy and growth form in *Arabidopsis thaliana*. *Nature genetics* 40, 1489-1492, doi:10.1038/ng.253 (2008).
33. Langfelder, P. & Horvath, S. WGCNA: an R package for weighted correlation network analysis. *Bmc Bioinformatics* 9, 559 (2008).
34. Wang, H., Yang, Y., Zhang, Y. *et al.* Transcriptome Analysis of Flower Development and Mining of Genes Related to Flowering Time in Tomato (*Solanum lycopersicum*). *Int J Mol Sci* 22, doi:10.3390/ijms22158128 (2021).
35. Zhang, J., Wu, K., Zeng, S. *et al.* Transcriptome analysis of *Cymbidium sinense* and its application to the identification of genes associated with floral development. *BMC Genomics* 14, 279, doi:10.1186/1471-2164-14-279 (2013).
36. Simpson, G. G. Evolution of flowering in response to day length: flipping the CONSTANS switch. *BioEssays : news and reviews in molecular, cellular and developmental biology* 25, 829-832, doi:10.1002/bies.10330 (2003).
37. Yanovsky, M. J. & Kay, S. A. Signaling networks in the plant circadian system. *Current opinion in plant biology* 4, 429-435, doi:10.1016/s1369-5266(00)00196-5 (2001).

38. Imaizumi, T., Schultz, T. F., Harmon, F. G. *et al.* FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in Arabidopsis. *Science* 309, 293-297, doi:10.1126/science.1110586 (2005).
39. Fornara, F., Panigrahi, K. C., Gissot, L. *et al.* Arabidopsis DOF transcription factors act redundantly to reduce CONSTANS expression and are essential for a photoperiodic flowering response. *Developmental cell* 17, 75-86, doi:10.1016/j.devcel.2009.06.015 (2009).
40. Kim, D. H. & Sung, S. Genetic and epigenetic mechanisms underlying vernalization. *The arabidopsis book* 12, e0171, doi:10.1199/tab.0171 (2014).
41. Cheng, J. Z., Zhou, Y. P., Lv, T. X. *et al.* Research progress on the autonomous flowering time pathway in Arabidopsis. *Physiology and molecular biology of plants : an international journal of functional plant biology* 23, 477-485, doi:10.1007/s12298-017-0458-3 (2017).
42. Johanson, U., West, J., Lister, C. *et al.* Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. *Science* 290, 344-347, doi:10.1126/science.290.5490.344 (2000).
43. Shindo, C., Aranzana, M. J., Lister, C. *et al.* Role of FRIGIDA and FLOWERING LOCUS C in determining variation in flowering time of Arabidopsis. *Plant Physiol* 138, 1163-1173, doi:10.1104/pp.105.061309 (2005).
44. Golicz, A. A., Steinfort, U., Arya, H. *et al.* Analysis of the quinoa genome reveals conservation and divergence of the flowering pathways. *Functional & integrative genomics* 20, 245-258, doi:10.1007/s10142-019-00711-1 (2020).
45. Bastow, R., Mylne, J. S., Lister, C. *et al.* Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature* 427, 164-167, doi:10.1038/nature02269 (2004).
46. Sung, S. & Amasino, R. M. Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. *Nature* 427, 159-164, doi:10.1038/nature02195 (2004).
47. Xu, M., Hu, T., Zhao, J. *et al.* Developmental Functions of miR156-Regulated SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) Genes in Arabidopsis thaliana. *PLoS genetics* 12, e1006263, doi:10.1371/journal.pgen.1006263 (2016).
48. Hyun, Y., Richter, R. & Coupland, G. Competence to Flower: Age-Controlled Sensitivity to Environmental Cues. *Plant Physiol* 173, 36-46, doi:10.1104/pp.16.01523 (2017).
49. Bergonzi, S., Albani, M. C., Ver Loren van Themaat, E. *et al.* Mechanisms of age-dependent response to winter temperature in perennial flowering of Arabis alpina. *Science* 340, 1094-1097, doi:10.1126/science.1234116 (2013).
50. Wang, J. W., Czech, B. & Weigel, D. miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. *Cell* 138, 738-749 (2009).
51. Nie, S., Li, C., Xu, L. *et al.* De novo transcriptome analysis in radish (Raphanus sativus L.) and identification of critical genes involved in bolting and flowering. *BMC Genomics* 17, 389, doi:10.1186/s12864-016-2633-2 (2016).
52. Samach, A. & Wigge, P. A. Ambient temperature perception in plants. *Current opinion in plant biology* 8, 483-486, doi:10.1016/j.pbi.2005.07.011 (2005).

53. Balasubramanian, S., Sureshkumar, S., Lempe, J. *et al.* Potent induction of *Arabidopsis thaliana* flowering by elevated growth temperature. *PLoS genetics* 2, e106, doi:10.1371/journal.pgen.0020106 (2006).
54. Li, X. F., Jia, L. Y., Xu, J. *et al.* FT-like NFT1 gene may play a role in flower transition induced by heat accumulation in *Narcissus tazetta* var. *chinensis*. *Plant & cell physiology* 54, 270-281, doi:10.1093/pcp/pcs181 (2013).
55. Yan, Y., Shen, L., Chen, Y. *et al.* A MYB-domain protein EFM mediates flowering responses to environmental cues in *Arabidopsis*. *Developmental cell* 30, 437-448, doi:10.1016/j.devcel.2014.07.004 (2014).
56. Kim, J. J., Lee, J. H., Kim, W. *et al.* The microRNA156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3 module regulates ambient temperature-responsive flowering via FLOWERING LOCUS T in *Arabidopsis*. *Plant Physiol* 159, 461-478, doi:10.1104/pp.111.192369 (2012).
57. Lee, J. H., Kim, J. J., Kim, S. H. *et al.* The E3 ubiquitin ligase HOS1 regulates low ambient temperature-responsive flowering in *Arabidopsis thaliana*. *Plant & cell physiology* 53, 1802-1814, doi:10.1093/pcp/pcs123 (2012).
58. Wilson, R. N., Heckman, J. W. & Somerville, C. R. Gibberellin Is Required for Flowering in *Arabidopsis thaliana* under Short Days. *Plant Physiol* 100, 403-408, doi:10.1104/pp.100.1.403 (1992).
59. Achard, P. & Genschik, P. Releasing the brakes of plant growth: how GAs shutdown DELLA proteins. *J Exp Bot* 60, 1085-1092, doi:10.1093/jxb/ern301 (2009).
60. Simpson, G. G. & Dean, C. *Arabidopsis*, the Rosetta stone of flowering time? *Science* 296, 285-289, doi:10.1126/science.296.5566.285 (2002).
61. Zeng, Q., Zhao, Z. H. & Zhao, S. Q. [Signal pathways of flowering time regulation in plant]. *Yi chuan = Hereditas* 28, 1031-1036 (2006).
62. Huang, T., Böhlenius, H., Eriksson, S. *et al.* The mRNA of the *Arabidopsis* gene FT moves from leaf to shoot apex and induces flowering. *Science* 309, 1694-1696, doi:10.1126/science.1117768 (2005).
63. Kumar, S. V., Lucyshyn, D., Jaeger, K. E. *et al.* Transcription factor PIF4 controls the thermosensory activation of flowering. *Nature* 484, 242-245, doi:10.1038/nature10928 (2012).
64. Lee, J. & Lee, I. Regulation and function of SOC1, a flowering pathway integrator. *J Exp Bot* 61, 2247-2254, doi:10.1093/jxb/erq098 (2010).
65. Kimura, Y., Aoki, S., Ando, E. *et al.* A flowering integrator, SOC1, affects stomatal opening in *Arabidopsis thaliana*. *Plant & cell physiology* 56, 640-649, doi:10.1093/pcp/pcu214 (2015).
66. Mátyás, K. K., Hegedűs, G., Taller, J. *et al.* Different expression pattern of flowering pathway genes contribute to male or female organ development during floral transition in the monoecious weed *Ambrosia artemisiifolia* L. (Asteraceae). *PeerJ* 7, e7421, doi:10.7717/peerj.7421 (2019).
67. Liu, Y., Hao, X., Lu, Q. *et al.* Genome-wide identification and expression analysis of flowering-related genes reveal putative floral induction and differentiation mechanisms in tea plant (*Camellia sinensis*). *Genomics* 112, 2318-2326, doi:10.1016/j.ygeno.2020.01.003 (2020).

68. Alabadí, D., Blázquez, M. A., Carbonell, J. *et al.* Instructive roles for hormones in plant development. The International journal of developmental biology 53, 1597-1608, doi:10.1387/ijdb.072423da (2009).
69. Casanova-Sáez, R. & Voß, U. Auxin Metabolism Controls Developmental Decisions in Land Plants. Trends in plant science 24, 741-754, doi:10.1016/j.tplants.2019.05.006 (2019).
70. Ljung, K., Bhalerao, R. P. & Sandberg, G. Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. The Plant journal : for cell and molecular biology 28, 465-474, doi:10.1046/j.1365-313x.2001.01173.x (2001).
71. Seo, M., Akaba, S., Oritani, T. *et al.* Higher activity of an aldehyde oxidase in the auxin-overproducing superroot1 mutant of Arabidopsis thaliana. Plant Physiol 116, 687-693, doi:10.1104/pp.116.2.687 (1998).
72. Won, C., Shen, X., Mashiguchi, K. *et al.* Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF ARABIDOPSIS and YUCCAs in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America 108, 18518-18523, doi:10.1073/pnas.1108436108 (2011).
73. Yu, S. & Wang, J. W. The Crosstalk between MicroRNAs and Gibberellin Signaling in Plants. Plant & cell physiology 61, 1880-1890, doi:10.1093/pcp/pcaa079 (2020).
74. Jiang, Z., Sun, L., Wei, Q. *et al.* A New Insight into Flowering Regulation: Molecular Basis of Flowering Initiation in Magnolia × soulangeana 'Changchun'. Genes 11, doi:10.3390/genes11010015 (2019).
75. Helliwell, C. A., Poole, A., Peacock, W. J. *et al.* Arabidopsis ent-kaurene oxidase catalyzes three steps of gibberellin biosynthesis. Plant Physiol 119, 507-510, doi:10.1104/pp.119.2.507 (1999).
76. Rieu, I., Ruiz-Rivero, O., Fernandez-Garcia, N. *et al.* The gibberellin biosynthetic genes AtGA20ox1 and AtGA20ox2 act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. The Plant journal : for cell and molecular biology 53, 488-504, doi:10.1111/j.1365-313X.2007.03356.x (2008).
77. Sun, T. P. & Gubler, F. Molecular mechanism of gibberellin signaling in plants. Annual review of plant biology 55, 197-223, doi:10.1146/annurev.arplant.55.031903.141753 (2004).
78. Davière, J. M. & Achard, P. Gibberellin signaling in plants. Development (Cambridge, England) 140, 1147-1151, doi:10.1242/dev.087650 (2013).
79. Yano, K., Aya, K., Hirano, K. *et al.* Comprehensive gene expression analysis of rice aleurone cells: probing the existence of an alternative gibberellin receptor. Plant Physiol 167, 531-544, doi:10.1104/pp.114.247940 (2015).
80. Mok, D. W. & Mok, M. C. CYTOKININ METABOLISM AND ACTION. Annual review of plant physiology and plant molecular biology 52, 89-118, doi:10.1146/annurev.arplant.52.1.89 (2001).
81. Galuszka, P., Popelková, H., Werner, T. *et al.* Biochemical Characterization of Cytokinin Oxidases/Dehydrogenases from Arabidopsis thaliana Expressed in Nicotiana tabacum L. Journal of Plant Growth Regulation (2007).

82. Bartrina, I., Otto, E., Strnad, M. *et al.* Cytokinin regulates the activity of reproductive meristems, flower organ size, ovule formation, and thus seed yield in *Arabidopsis thaliana*. *Plant Cell* 23, 69-80, doi:10.1105/tpc.110.079079 (2011).
83. Kuroha, T., Tokunaga, H., Kojima, M. *et al.* Functional analyses of LONELY GUY cytokinin-activating enzymes reveal the importance of the direct activation pathway in *Arabidopsis*. *Plant Cell* 21, 3152-3169, doi:10.1105/tpc.109.068676 (2009).
84. Johnson, P. R. & Ecker, J. R. The ethylene gas signal transduction pathway: a molecular perspective. *Annual review of genetics* 32, 227-254, doi:10.1146/annurev.genet.32.1.227 (1998).
85. Achard, P., Baghour, M., Chapple, A. *et al.* The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. *Proceedings of the National Academy of Sciences of the United States of America* 104, 6484-6489, doi:10.1073/pnas.0610717104 (2007).
86. Lin, Z., Zhong, S. & Grierson, D. Recent advances in ethylene research. *J Exp Bot* 60, 3311-3336, doi:10.1093/jxb/erp204 (2009).
87. Wang, Y., Li, L., Ye, T. *et al.* The inhibitory effect of ABA on floral transition is mediated by ABI5 in *Arabidopsis*. *J Exp Bot* 64, 675-684, doi:10.1093/jxb/ers361 (2013).
88. Wang, T., Yang, B., Guan, Q. *et al.* Transcriptional regulation of *Lonicera japonica* Thunb. during flower development as revealed by comprehensive analysis of transcription factors. *BMC plant biology* 19, 198, doi:10.1186/s12870-019-1803-1 (2019).
89. Jing, D., Chen, W., Xia, Y. *et al.* Homeotic transformation from stamen to petal in *Eriobotrya japonica* is associated with hormone signal transduction and reduction of the transcriptional activity of *EjAG*. *Physiologia plantarum* 168, 893-908, doi:10.1111/ppl.13029 (2020).
90. Ruggiero, B., Koiwa, H., Manabe, Y. *et al.* Uncoupling the effects of abscisic acid on plant growth and water relations. Analysis of *sto1/nced3*, an abscisic acid-deficient but salt stress-tolerant mutant in *Arabidopsis*. *Plant Physiol* 136, 3134-3147, doi:10.1104/pp.104.046169 (2004).
91. Planas-Riverola, A., Gupta, A., Betegón-Putze, I. *et al.* Brassinosteroid signaling in plant development and adaptation to stress. *Development (Cambridge, England)* 146, doi:10.1242/dev.151894 (2019).
92. Azpiroz, R., Wu, Y., LoCascio, J. C. *et al.* An *Arabidopsis* brassinosteroid-dependent mutant is blocked in cell elongation. *Plant Cell* 10, 219-230, doi:10.1105/tpc.10.2.219 (1998).
93. Berger, S. Jasmonate-related mutants of *Arabidopsis* as tools for studying stress signaling. *Planta* 214, 497-504, doi:10.1007/s00425-001-0688-y (2002).
94. Chini, A., Fonseca, S., Fernández, G. *et al.* The JAZ family of repressors is the missing link in jasmonate signalling. *Nature* 448, 666-671, doi:10.1038/nature06006 (2007).
95. Cheng, Z., Sun, L., Qi, T. *et al.* The bHLH transcription factor MYC3 interacts with the Jasmonate ZIM-domain proteins to mediate jasmonate response in *Arabidopsis*. *Molecular plant* 4, 279-288, doi:10.1093/mp/ssq073 (2011).
96. Fernández-Calvo, P., Chini, A., Fernández-Barbero, G. *et al.* The *Arabidopsis* bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of

- jasmonate responses. *Plant Cell* 23, 701-715, doi:10.1105/tpc.110.080788 (2011).
97. Campos-Rivero, G., Osorio-Montalvo, P., Sánchez-Borges, R. *et al.* Plant hormone signaling in flowering: An epigenetic point of view. *Journal of plant physiology* 214, 16-27, doi:10.1016/j.jplph.2017.03.018 (2017).
98. Khurana, J. P. & Cleland, C. F. Role of Salicylic Acid and Benzoic Acid in Flowering of a Photoperiod-Insensitive Strain, *Lemna paucicostata* LP6. *Plant Physiol* 100, 1541-1546, doi:10.1104/pp.100.3.1541 (1992).
99. Wada, K. C., Yamada, M., Shiraya, T. *et al.* Salicylic acid and the flowering gene FLOWERING LOCUS T homolog are involved in poor-nutrition stress-induced flowering of *Pharbitis nil*. *Journal of plant physiology* 167, 447-452, doi:10.1016/j.jplph.2009.10.006 (2010).
100. Conti, L. Hormonal control of the floral transition: Can one catch them all? *Developmental biology* 430, 288-301, doi:10.1016/j.ydbio.2017.03.024 (2017).
101. O'Neill, D. P. & Ross, J. J. Auxin regulation of the gibberellin pathway in pea. *Plant Physiol* 130, 1974-1982, doi:10.1104/pp.010587 (2002).
102. Li, Q. F., Wang, C., Jiang, L. *et al.* An interaction between BZR1 and DELLAs mediates direct signaling crosstalk between brassinosteroids and gibberellins in *Arabidopsis*. *Science signaling* 5, ra72, doi:10.1126/scisignal.2002908 (2012).
103. Unterholzner, S. J., Rozhon, W., Papacek, M. *et al.* Brassinosteroids Are Master Regulators of Gibberellin Biosynthesis in *Arabidopsis*. *Plant Cell* 27, 2261-2272, doi:10.1105/tpc.15.00433 (2015).
104. Shu, K., Chen, Q., Wu, Y. *et al.* ABSCISIC ACID-INSENSITIVE 4 negatively regulates flowering through directly promoting *Arabidopsis* FLOWERING LOCUS C transcription. *J Exp Bot* 67, 195-205, doi:10.1093/jxb/erv459 (2016).
105. Yang, B., Guan, Q., Tian, J. *et al.* Transcriptomic and proteomic analyses of leaves from *Clematis terniflora* DC. under high level of ultraviolet-B irradiation followed by dark treatment. *Journal of proteomics* 150, 323-340, doi:10.1016/j.jprot.2016.10.001 (2017).
106. Grabherr, M. G., Haas, B. J., Yassour, M. *et al.* Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature biotechnology* 29, 644-652, doi:10.1038/nbt.1883 (2011).
107. Mortazavi, A., Williams, B. A., McCue, K. *et al.* Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature methods* 5, 621-628, doi:10.1038/nmeth.1226 (2008).

Figures

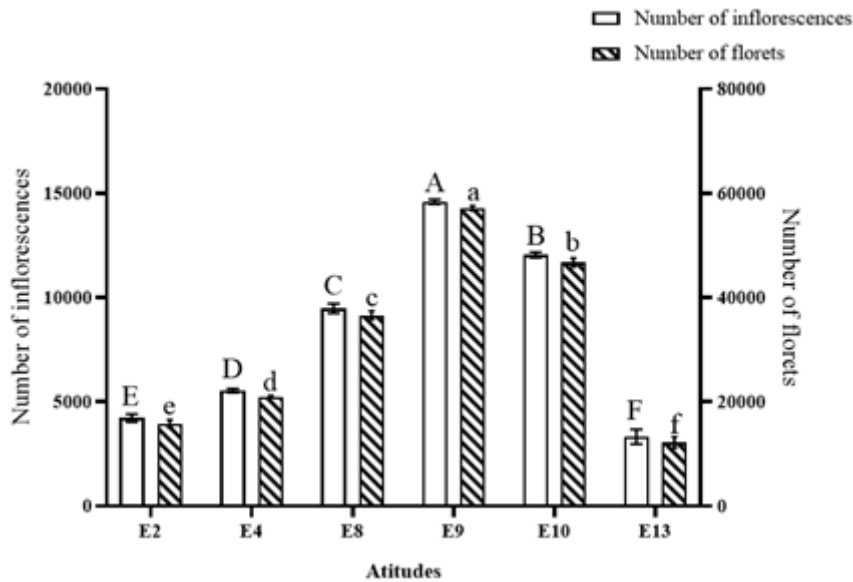


Figure 1

Differences in flowering number of *M. micrantha* at different altitudes. Different capital letters indicate that there were significant differences in the number of inflorescences of *M. micrantha* is different at different altitudes ($p < 0.05$). Different lowercase letters indicate that there were significant differences in the number of florets of *M. micrantha* at different altitudes ($p < 0.05$).

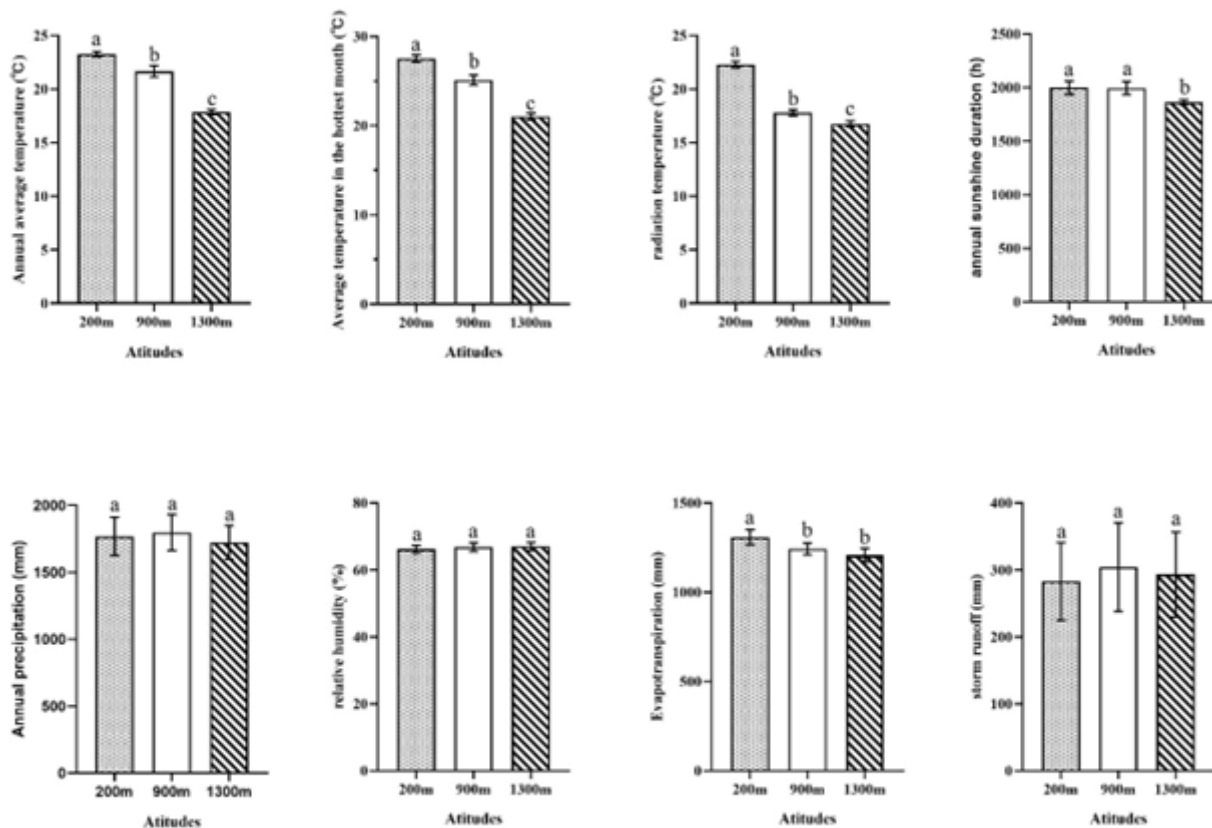


Figure 2

Bar chart of significance analysis of ecological factors in three altitudes. Different lowercase letters indicate that there are significant differences in ecological factors at different altitudes ($p < 0.05$).

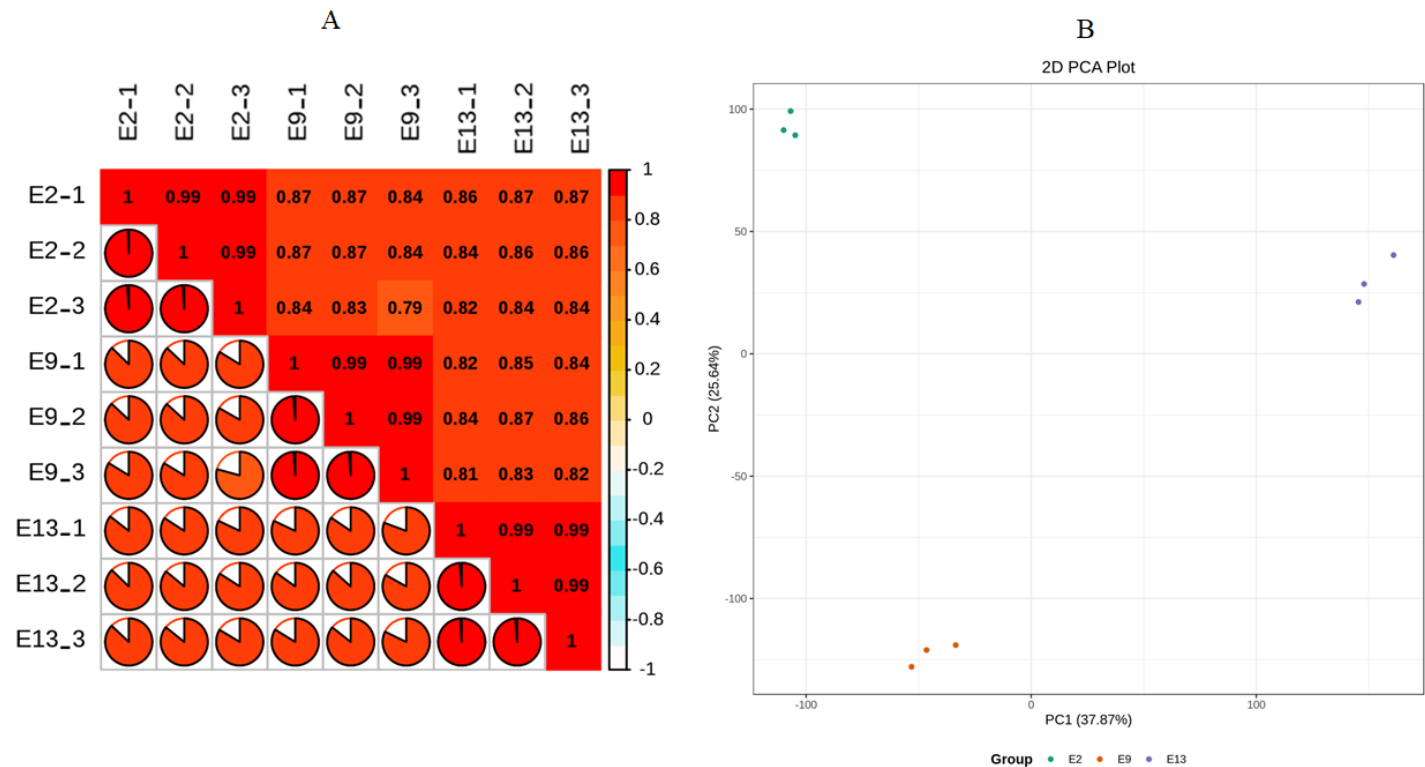


Figure 3

(A) Pearson correlation coefficients of all nine samples. The Pearson correlation coefficients of all gene expression levels at E2, E9, and E13; (B) Principal component analysis of expressed genes in floral bud differentiation of *M. micrantha* with different altitudes i.e., E2, E9, and, E13.

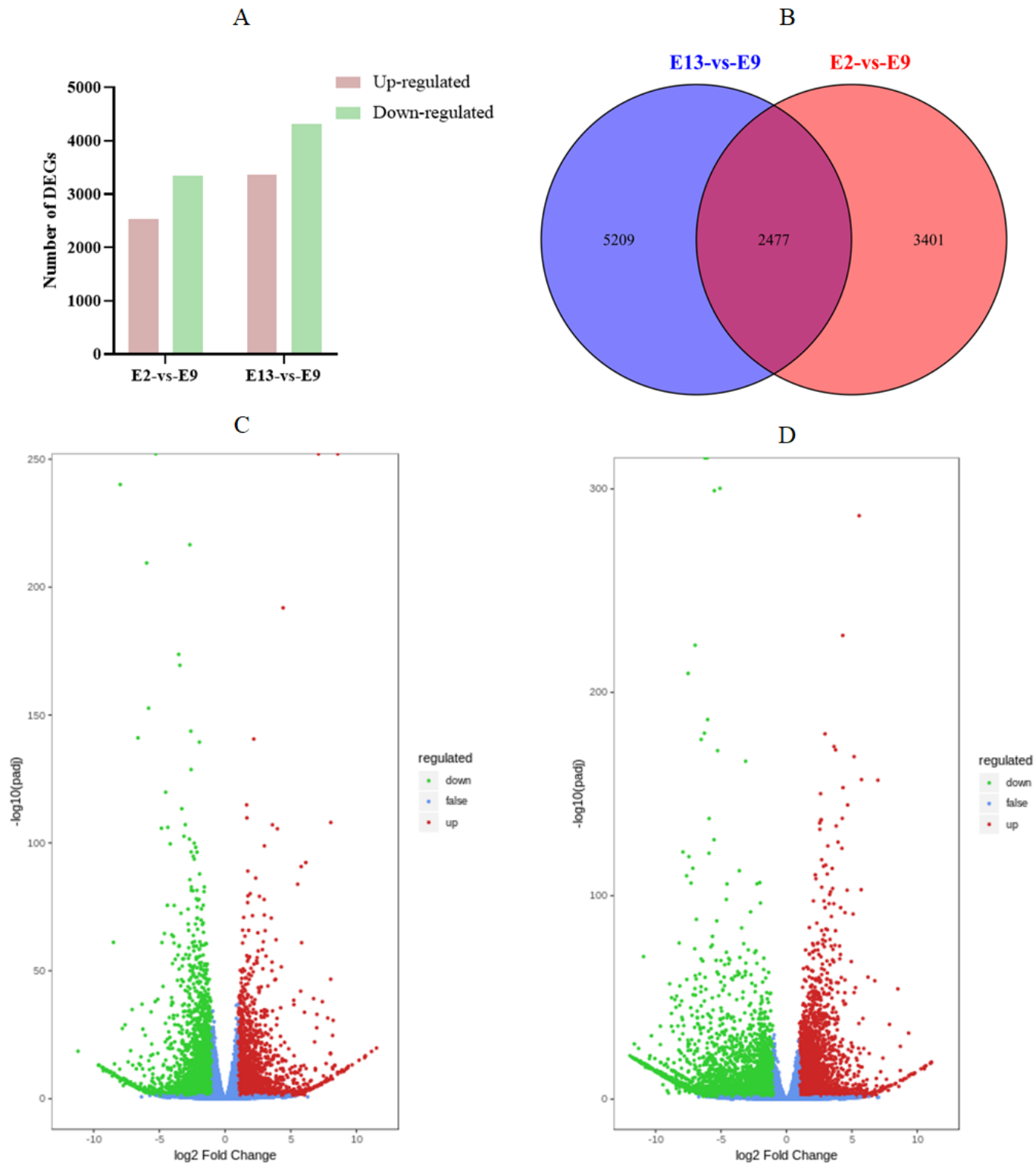


Figure 4

Analysis of the differentially expressed unigenes (DEGs) in Different elevations in *M. micrantha*. (A) Venn diagram of DEGs included in two comparisons. (B) DEGs identified from different comparisons. (C) Volcano map of E2-vs-E9. (D) Volcano map of E13- vs-E9

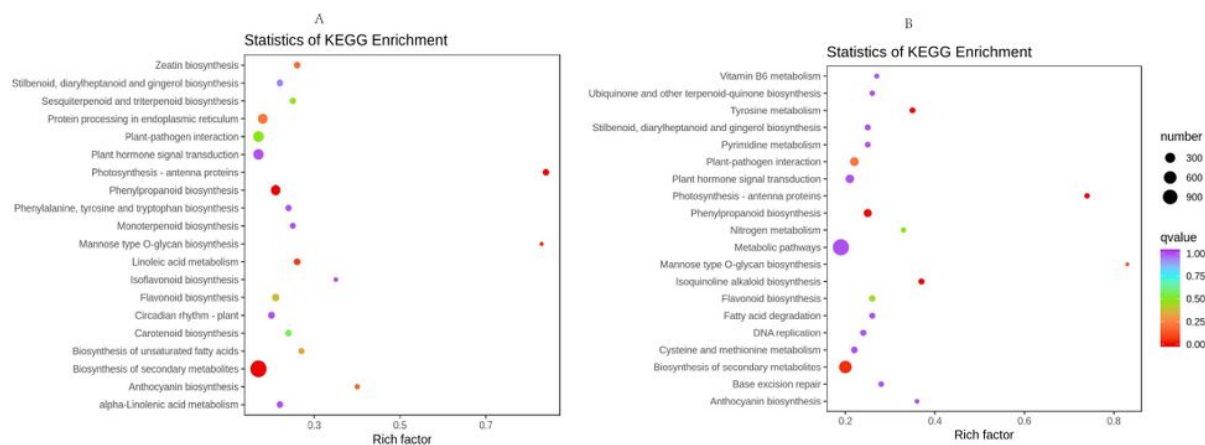


Figure 5

KEGG Enrichment Analyses of DEGs. (A) E9 vs. E2 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment Analyses of DEGs. (B) E9 vs. E13 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment Analyses of DEGs. Rich factor is a ratio of the number of DEGs annotated with a pathway relative to the total number of genes annotated with this pathway. The larger value of the rich factor represented, the greater the enrichment of this KEGG pathway.

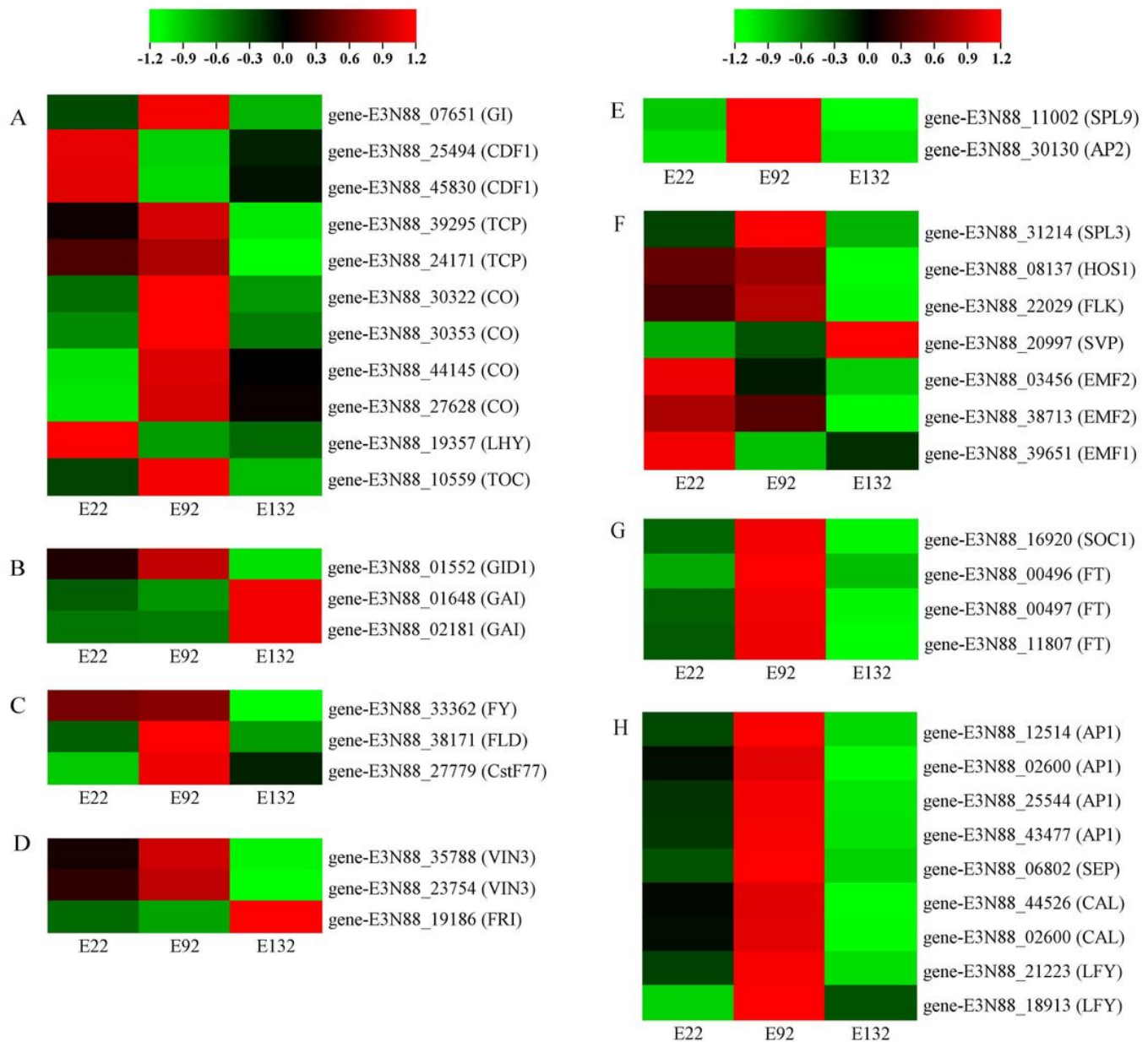


Figure 6

Expression changes of the genes involved in flowering-related pathways. (A) Expression levels of DEGs in photoperiod pathway; (B) Expression levels of DEGs in gibberellin pathway; (C) Expression levels of DEGs in autonomous pathway; (D) Expression levels of DEGs in vernalization pathway; (E) Expression levels of DEGs in aging pathway; (F) Expression levels of DEGs in temperature pathway; (G) Expression levels of DEGs in floral meristem identity and floral organ identity; (H) Expression levels of DEGs in floral integrator.

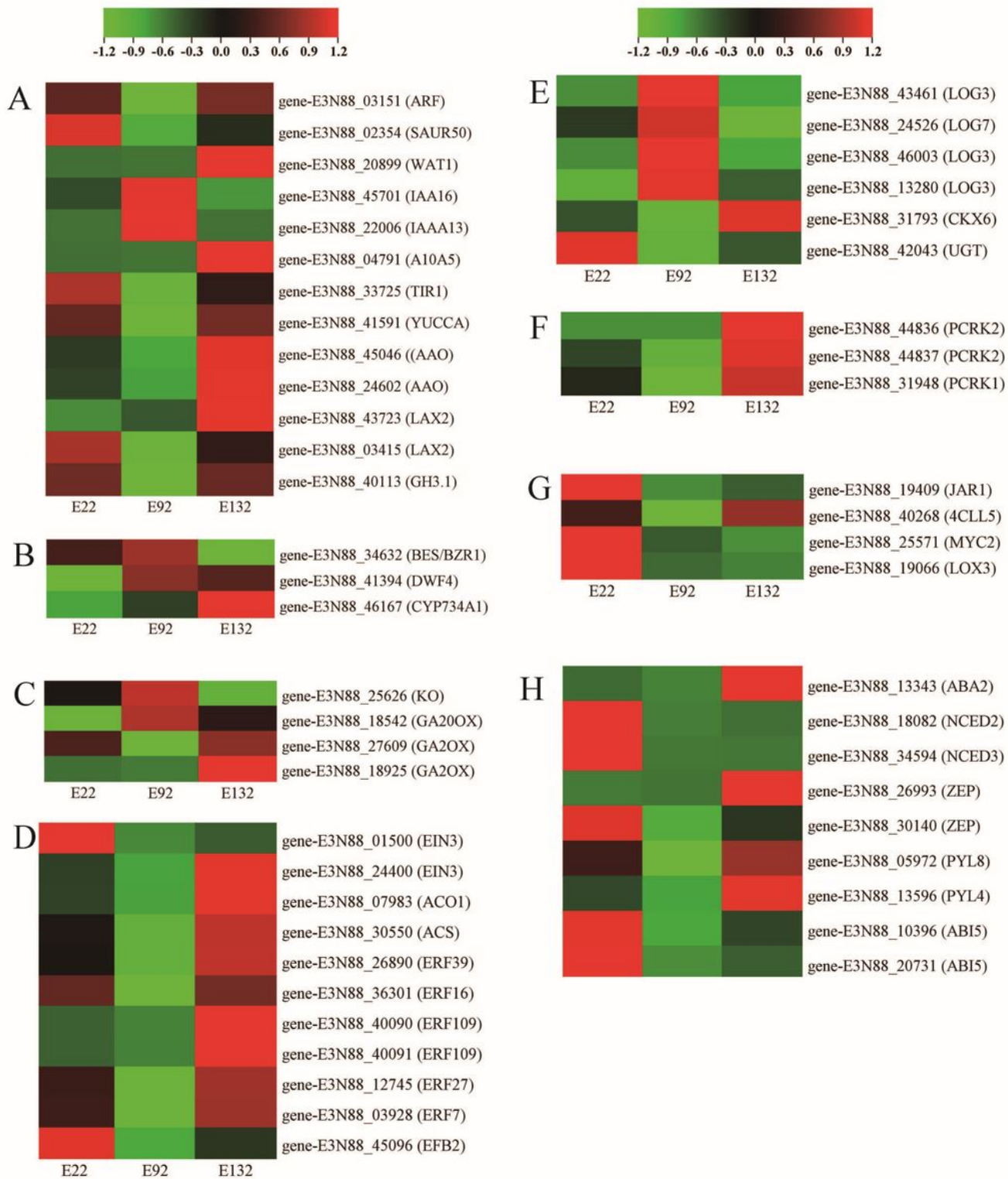


Figure 7

Expression levels of DEGs involved in the auxin signaling, brassinosteroid signaling pathway, gibberellic signaling pathway and ethylene signaling pathway. (A) Expression levels of DEGs of auxin signaling pathway. (B) Expression levels of DEGs of brassinosteroid signaling pathway. (C) Expression levels of DEGs of gibberellic signaling pathway. (D) Expression levels of DEGs of ethylene signaling pathway. (E) Expression levels of DEGs of cytokinin signaling pathway. (F) Expression levels of DEGs of salicylic acid

(SA) signaling pathway. (G) Expression levels of DEGs of Jasmonic acid (JA) signaling pathway. (H) Expression levels of DEGs of abscisic acid signaling pathway.

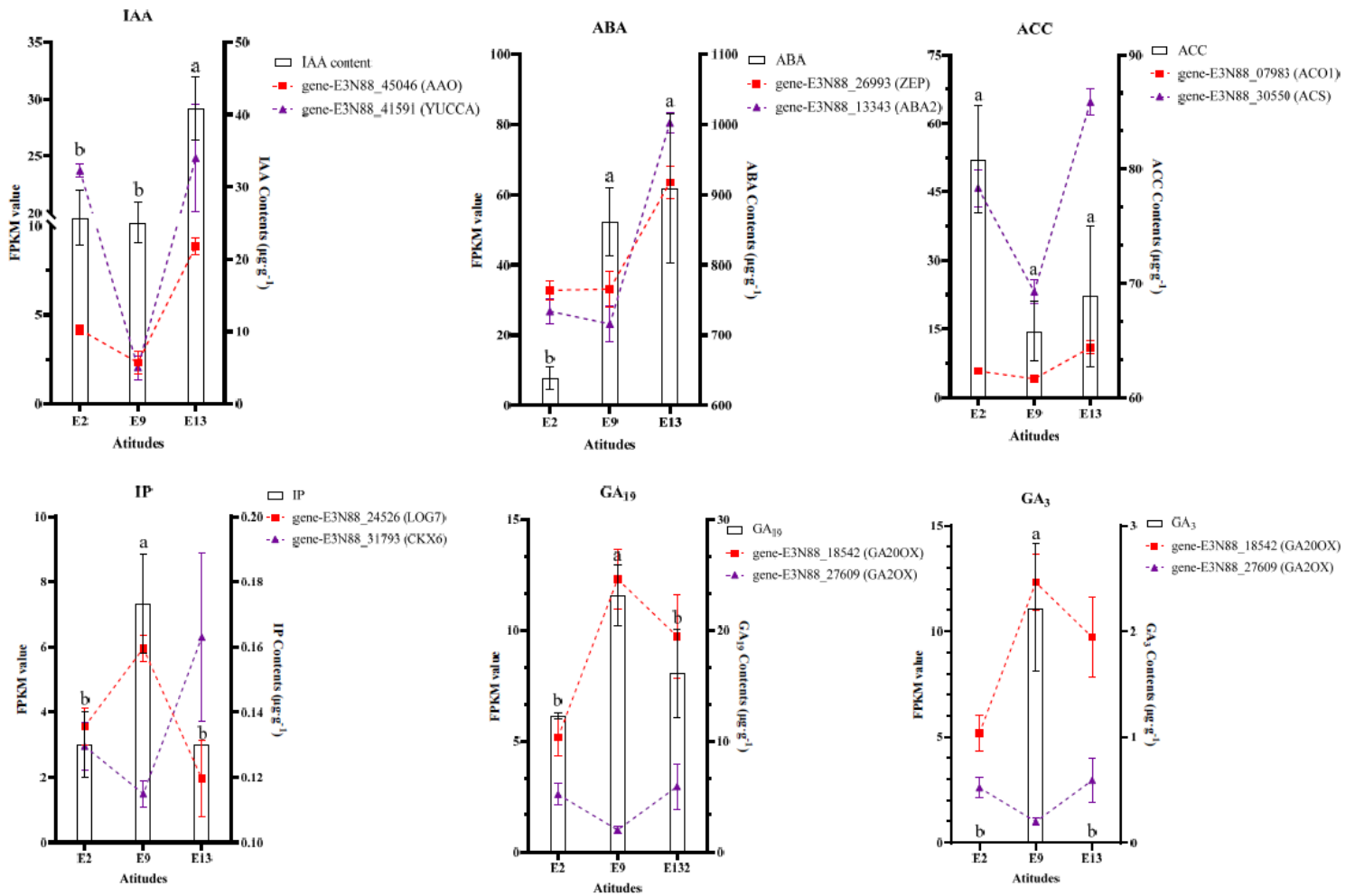


Figure 8

The changes of endogenous hormone content at different altitudes. (A) ABA contents, (B)ACC contents, (C) IAA contents, (D) GA₁₉ contents, (E) GA₃ contents, and (F) IP contents at three different altitudes in *M. micrantha* were measured. Significant differences are indicated by different letters ($p < 0.05$).

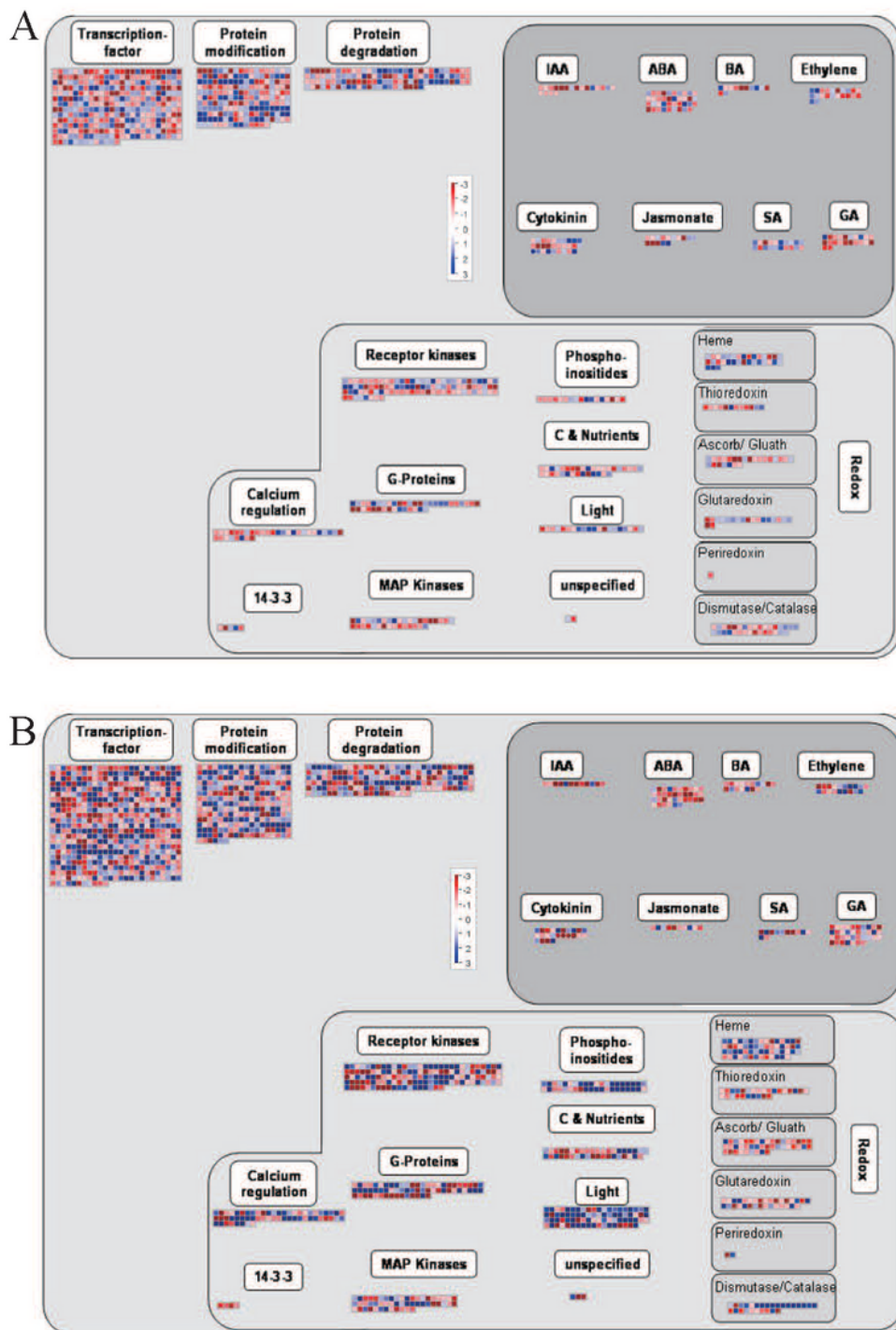


Figure 9

The clustering pattern of DEGs generated with the MapMan tool. (A) Overview of DEG regulation in E9-vs-E2. (B) Overview of DEG regulation in E9-vs-E13. Each square represents a separate gene. Red represents upregulation and blue represents downregulation. The color brightness represents the degree of difference.

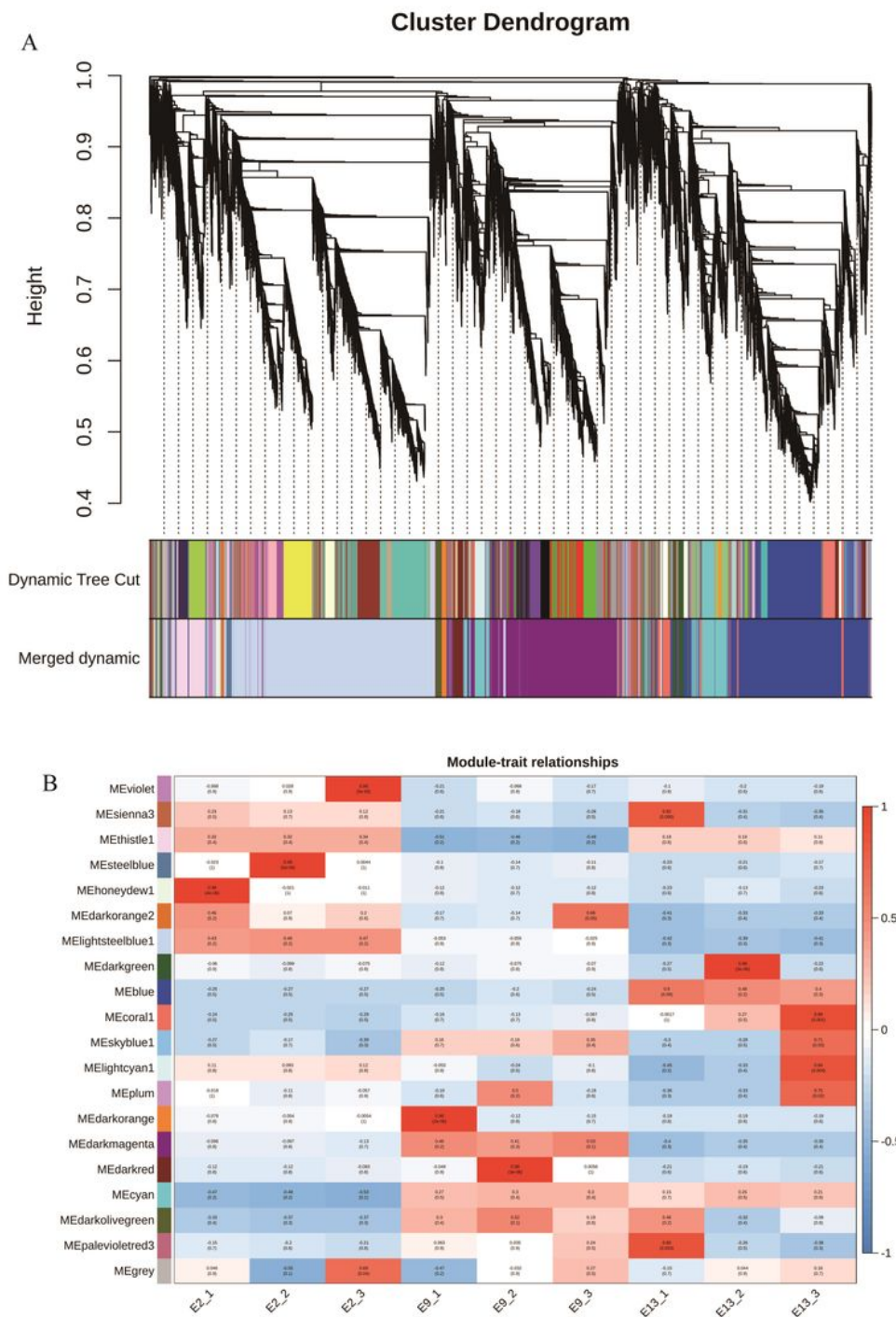


Figure 10

Gene dendrogram module colours and module–trait relationships identified by weighted gene co-expression network analysis (WGCNA) in *M. micrantha* flower buds samples. (A) Gene clustering tree and module division. In the dendrogram, each leaf corresponds to a gene. (B) Module-trait associations. The abscissa represents the samples; the ordinate represents the modules. The upper value in each cell represents the correlation coefficient, and the lower value represents the p value.

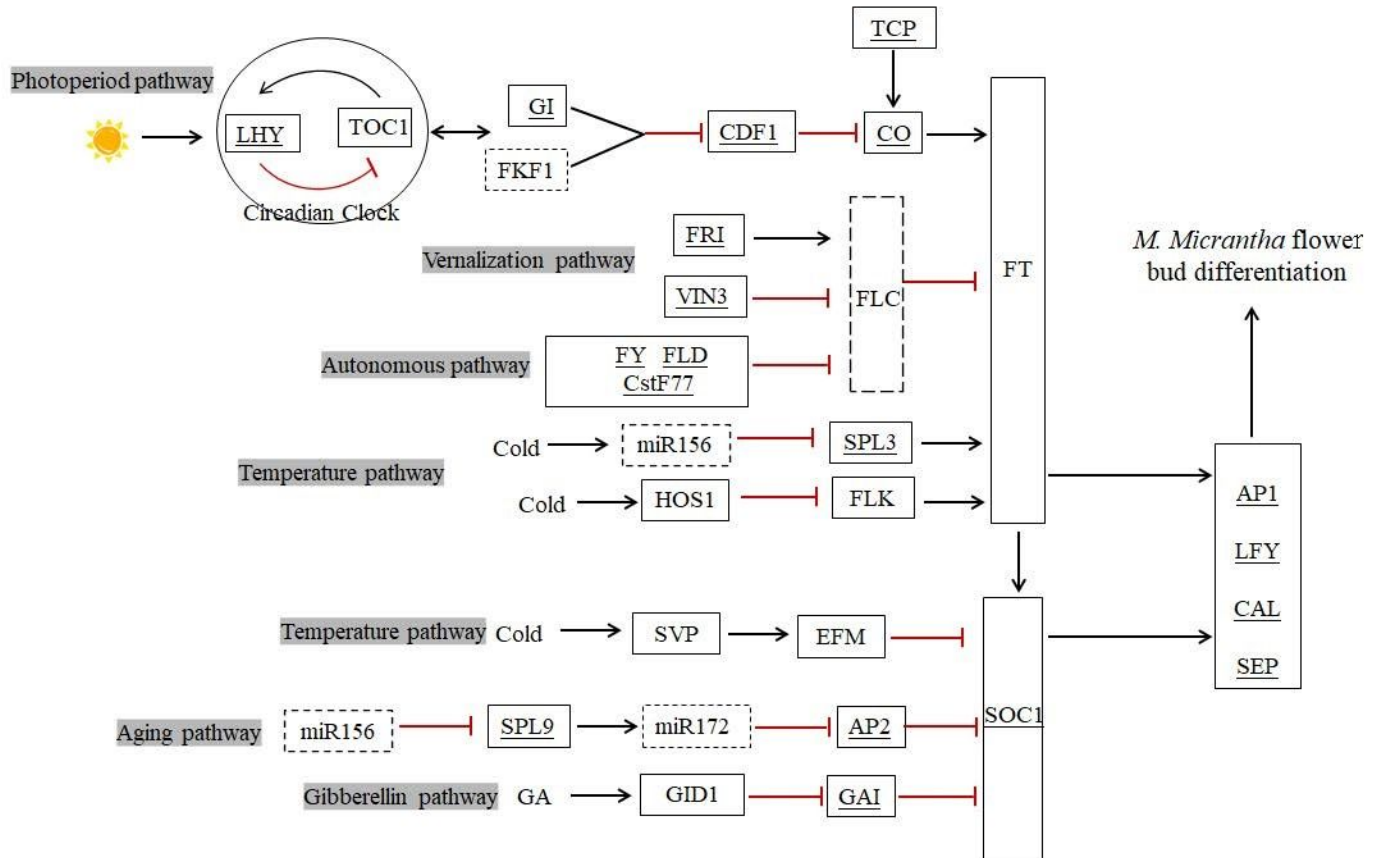


Figure 11

Schematic of the regulatory genes and pathways in floral bud differentiation of *M. micrantha*. The letters in the boxes stand for abbreviation of genes. The gene name with underlines indicate DEGs. The dashed box indicates that this gene was not found in our transcriptome. Lines with arrows indicate activation, and red lines with blunt ends indicate repression.

Supplementary Files

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

- [Supplementarymaterial.zip](#)