Transcriptome Analysis and EST-SSR Molecular Marker Development of *Anemone shikokiana* under Heterogeneous Habitats (Postprint)

Authors: Shan Xiaohan, Well-being, Zhou Chunxia, Zhang Xin, Pang Yujuan, Li Lixia, Bian Fuhua

Date: 2024-03-26T00:00:00+00:00

Abstract

To investigate the ecological adaptation mechanisms of *Anemone shikokiana* in two distinct habitats—full-light mountain-top shrubland and shaded coniferous-broadleaf mixed forest understory—and to develop EST-SSR molecular markers for this species, this study utilized Illumina high-throughput sequencing technology to conduct transcriptome sequencing on leaves of flowering-stage *A. shikokiana*, obtaining functional annotation and differentially expressed genes. The results demonstrated: (1) Transcriptome sequencing yielded a total of 53,536 Unigene sequences, of which 27,448 were successfully annotated. (2) There were 5,635 differentially expressed genes, with 1,600 up-regulated and the remaining 4,035 down-regulated in *A. shikokiana* from the mountain-top shrubland. A total of 2,460 differentially expressed genes were annotated to 2,533 level-3 terms in the GO database, and 1,051 differentially expressed genes were annotated to 113 metabolic pathways in the KEGG database. (3) The metabolic pathways underlying *A. shikokiana*' s adaptation to heterogeneous habitats primarily involved the photosynthesis-antenna protein pathway and the flavonoid biosynthesis pathway. In the photosynthesis-antenna protein pathway, the *lhca5* gene was up-regulated while the *lhca1-3* genes were down-regulated; in the flavonoid biosynthesis pathway, the *chs*, *c4h*, *f3' h*, *f3h*, *fls*, *ans*, *chi*, *ccoaomt*, and *hct* genes were all up-regulated. (4) A total of 7,146 SSR loci were identified from the transcriptome data of *A. shikokiana*, distributed across 6,006 Unigene sequences, comprising 106 types of repeat motifs, with mononucleotide repeats being the dominant repeat motif. Among 100 pairs of designed and synthesized EST-SSR primers, 68 pairs were validated as effective, of which 11 pairs exhibited polymorphism, amplifying a total of 24 polymorphic fragments. These findings contribute to a deeper understanding of the adaptive regulatory mechanisms of *A. shikokiana* in different habitats and, for the first time, develop EST-SSR molecular markers for this species, filling a gap in this area

and providing important molecular marker resources for the conservation and utilization of this organism.

Full Text

Transcriptome Analysis and Development of EST-SSR Molecular Markers in *Anemone shikokiana* under Heterogeneous Habitats

SHAN Xiaohan, AN Kang, ZHOU Chunxia, ZHANG Xin, PANG Yujuan, LI Lixia, BIAN Fuhua*

College of Life Sciences, Yantai University, Yantai 264005, Shandong, China

Abstract: To investigate the ecological adaptation mechanisms of *Anemone shikokiana* in two distinct habitats—full-light hilltop scrub and shady mixed broadleaved-coniferous forest—and to develop EST-SSR molecular markers for this species, we performed transcriptome sequencing on leaves collected during the flowering stage using Illumina high-throughput sequencing technology. The results revealed: (1) A total of 53,536 Unigene sequences were obtained, of which 27,448 were successfully annotated. (2) We identified 5,635 differentially expressed genes (DEGs), with 1,600 up-regulated and 4,035 down-regulated in hilltop scrub populations compared to forest understory populations. Among these, 2,460 DEGs were annotated to 2,533 GO tertiary terms, and 1,051 DEGs were mapped to 113 metabolic pathways in the KEGG database. (3) The primary metabolic pathways involved in adaptation to heterogeneous habitats were photosynthesis-antenna proteins and flavonoid biosynthesis. In the photosynthesis-antenna protein pathway, *lhca5* was up-regulated while *lhca1-3* were down-regulated. In the flavonoid biosynthesis pathway, *chs*, *c4h*, *f3′ h*, *f3h*, *fls*, *ans*, *chi*, *ccoaomt*, and *hct* were all up-regulated. (4) From the transcriptome data, we identified 7,146 SSR loci distributed across 6,006 Unigenes, encompassing 106 repeat motif types, with mononucleotide repeats being the most abundant. Among 100 synthesized EST-SSR primer pairs, 68 showed effective amplification, with 11 exhibiting polymorphism and amplifying a total of 24 polymorphic fragments. These findings enhance our understanding of the adaptive regulatory mechanisms of *A. shikokiana* in different habitats and represent the first development of EST-SSR molecular markers for this species, filling a critical research gap and providing valuable molecular resources for its conservation and utilization.

Keywords: heterogeneous habitat, *Anemone shikokiana*, transcriptome, EST-SSR, ecological adaptation mechanisms

---

Anemone shikokiana is a perennial herbaceous plant in the family Ranunculaceae, disjunctly distributed between the Jiaodong Peninsula of China and Shikoku Island of Japan (Wang et al., 2014). In China, it is listed as Vulnera-

ble (VU) on the IUCN Red List of Threatened Species and represents a typical rare species (Chen et al., 2018). The plant has medicinal value, with its roots used for clearing heat, detoxifying, and stopping bleeding (Liu, 2014), and holds potential for horticultural development due to its elegant growth habit, large flowers, and extended flowering period (Hou and Liu, 2010).

This species inhabits two heterogeneous habitats above 600 m elevation: hilltop scrub with year-round full sunlight and sandy soil composed primarily of gravel and sand particles, and mixed broadleaved-coniferous forest understory that is dark, humid, and rich in loose humus soil (Pang et al., 2020). Previous studies have demonstrated that plants adapt to heterogeneous water conditions by adjusting individual size and leaf traits (Yue et al., 2023), and that phenotypic plasticity enables adaptation to extreme environments (Wang et al., 2023). *A. shikokiana* adapts to these contrasting environments through modifications in leaf and root morphology, structure, and physiology (Yu et al., 2019; Pang et al., 2020; Pang et al., 2021). However, the molecular mechanisms underlying its adaptation to heterogeneous habitats remain unclear. Plant adaptation fundamentally results from gene-specific expression, which is largely regulated at the transcriptional level (Zhang et al., 2007). The transcriptome, representing the complete set of mRNAs in a specific tissue or developmental stage, serves as an effective tool for studying gene function and structure (Lu et al., 2021). Illumina high-throughput sequencing enables efficient and accurate acquisition of gene expression profiles from specific tissues without a reference genome (Guo et al., 2021), and has been widely applied to investigate plant adaptation to different habitats in species such as *Coreopsis tinctoria* (Sun et al., 2022) and *Medicago ruthenica* (Wu et al., 2022).

Molecular markers are essential for phylogenetic analysis, functional gene tagging, and molecular-assisted breeding. With advances in high-throughput sequencing, EST-SSR markers derived from transcriptome data have become one of the most commonly used molecular marker technologies due to their codominant inheritance, stability, and high accuracy (Yue et al., 2022). EST-SSR markers are cost-effective to develop and applicable to species without reference genomes, facilitating genetic diversity analysis, fingerprint construction, germplasm conservation, and cultivar identification (Yang et al., 2021). They have been successfully developed and applied in various plants including hemp (Bian et al., 2023), pepper (Tian et al., 2022), and *Liquidambar formosana* (Li et al., 2023). However, few molecular markers have been reported for *A. shikokiana*, with no publicly available SSR markers, limiting molecular research on this species.

This study investigated *A. shikokiana* from heterogeneous habitats using Illumina high-throughput sequencing to obtain transcriptome data, perform assembly and functional annotation, identify differentially expressed genes and SSR loci, and design and validate EST-SSR primers. Our objectives were to elucidate the ecological adaptation mechanisms of *A. shikokiana* in heterogeneous habitats and develop applicable EST-SSR molecular markers to fill research

gaps, thereby establishing a foundation for future studies on genetic diversity, germplasm evaluation, functional gene tagging, and molecular-assisted breeding for conservation and utilization.

1.1 Experimental Materials

All experimental materials consisted of *A. shikokiana* leaves collected during the flowering stage. Transcriptome sequencing samples were collected in June 2021 from Taibo Peak and Hanfeng Ridge in the Kunyu Mountain National Nature Reserve, Yantai, Shandong (Table 1). Primer screening materials were collected in September 2021 from Laoshan, Qingdao and Kunyu Mountain, Yantai (Table 2). After collection, samples were washed with distilled water, immediately frozen in liquid nitrogen, and stored at -80°C. Transcriptome sequencing was completed by Shanghai OE Biotech Co., Ltd. For primer screening, genomic DNA was extracted using a modified CTAB method [16], checked via 1% agarose gel electrophoresis, diluted to 20 ng $\cdot$ L^{-1}, and stored at -20°C.

1.2 Transcriptome Analysis

De novo assembly: Trinity software (Manfred G et al., 2011) was used for paired-end assembly to generate transcript sequences. The longest sequence was selected as a Unigene based on sequence similarity and length, followed by clustering and redundancy removal using CD-HIT software to obtain a final set of Unigenes for subsequent analysis.

Functional annotation: Unigenes were functionally annotated by aligning them against the NR, KOG, GO, Swiss-Prot, eggNOG, and KEGG databases using diamond software (Benjamin et al., 2015), and against the Pfam database using HMMER software.

Differential expression gene screening: DESeq software (Anders S et al., 2012) was used to normalize gene counts across samples. BaseMean values estimated expression levels, and differential fold changes were calculated. Significance testing was performed using negative binomial distribution (NB) on read counts. Differentially expressed genes were filtered using criteria of $|\log_2\text{FoldChange}| > 1$ and $p < 0.05$.

1.3 Primer Design and Screening

Based on the transcriptome sequencing results, MISA software (Sebastian B et al., 2017) was used for SSR prediction with parameters set for repeat motif lengths of 1–6 bp and minimum repeat numbers of 10, 6, 5, 5, 5, and 5, respectively. Compound microsatellites were defined as those with inter-SSR sequences $ $100 bp. SSR polymorphism correlates with length, with markers $ $20 bp showing high polymorphism, 12–20 bp moderate, and <12 bp low polymorphism (Temnykh et al., 2001). Following initial screening for SSR length $ $20 bp, removal of compound SSRs, and exclusion of A/T repeats prone to polyadenylation artifacts, 100 SSR loci were randomly selected for primer design

using Primer3 software (Rozen S & Skaletsky H, 2000), designated ANS001-ANS100, and synthesized by Beijing Liuhe BGI.

Primer effectiveness was initially screened using samples from Qingdao Laoshan Slippery Pass via 1% agarose gel electrophoresis. The 20 μL PCR reaction contained 2×Master Mix 4 μL, forward and reverse primers (10 $\mu mol \cdot L^{-1}$) each 2 μL, DNA template 2 μL, and ddH_2O 10 μL. Cycling conditions were: 94°C for 3 min; 35 cycles of 94°C for 30 s, 55°C for 35 s, 72°C for 60 s; final extension at 72°C for 10 min; and storage at 4°C. Primers failing to amplify or producing unexpected fragment sizes were eliminated. Validated primers were then tested for polymorphism using samples YD1 (Yantai Kunyu Mountain Taibo Peak), YL1 (Yantai Kunyu Mountain Laotie Mountain), QD1 (Qingdao Laoshan Laodeng Scenic Area Danfeng Peak), and QL1 (Qingdao Laoshan Slippery Pass) via 8% non-denaturing polyacrylamide gel electrophoresis with silver staining.

2.1.1 Overall Characteristics

De novo transcriptome analysis of *A. shikokiana* yielded 42.34 Gb of clean data, with effective data per sample ranging from 6.98 to 7.10 Gb and Q30 bases between 93.68% and 94.20%, confirming high sequencing accuracy suitable for downstream analysis. Assembly produced 53,536 Unigenes with a total length of 57,909,453 bp, ranging from 301 to 15,502 bp and averaging 1,081.69 bp. Pearson correlation coefficients indicated strong correlations between biological replicates (>0.8689 for group D, >0.8318 for group L), validating the data for subsequent analysis.

2.1.2 Functional Annotation

Among the 53,536 assembled Unigenes, 3,505 (6.55%) were successfully annotated in all seven databases, while 27,448 (51.27%) were annotated in at least one database (Figure 1 [Figure 1: see original paper]). The NR database provided the most annotations (27,044, 50.52%), followed by eggNOG (23,934, 44.71%), Swiss-Prot (19,637, 36.68%), Pfam (17,859, 33.36%), GO (17,540, 32.76%), KOG (5,481, 29.19%), and KEGG (5,481, 10.24%). NR database comparisons revealed highest similarity with *Aquilegia coerulea* (56.72%), followed by *Macleaya cordata* (6.08%), *Papaver somniferum* (4.06%), *Nelumbo nucifera* (3.29%), *Vitis vinifera* (1.92%), *Quercus suber* (0.94%), *Rosa chinensis* (0.9%), *Sphaerulina musiva* SO2202 (0.73%), *Actinidia chinensis* var. *chinensis* (0.58%), *Helianthus annuus* (0.55%), and other species (24.24%).

2.2.1 Differential Expression Unigene Screening

Transcriptome data mining identified 5,635 differentially expressed Unigenes, indicating substantial environmental influence on gene expression. Compared to understory (L) populations, hilltop (D) populations showed 1,600 up-regulated and 4,035 down-regulated genes (Figure 2 [Figure 2: see original paper]).

2.2.2 Differential Expression Unigene Enrichment Analysis

GO and KEGG enrichment analysis revealed that 2,460 DEGs were annotated to 2,533 GO tertiary terms, and 1,051 DEGs were mapped to 113 KEGG metabolic pathways. In the three GO categories—biological process (BP), cellular component (CC), and molecular function (MF)—DEGs were significantly enriched in processes such as abscisic acid metabolic process (GO:0009687), menthol biosynthetic process (GO:0031525), and sulfolipid biosynthetic process (GO:0046506); cellular components including cytosolic large ribosomal subunit (GO:0022625), cytosolic small ribosomal subunit (GO:0022627), and ribosome (GO:0005840); and molecular functions such as aryl-alcohol dehydrogenase (NADP+) activity (GO:0047681), isopiperitenol dehydrogenase activity (GO:0018458), and carveol dehydrogenase activity (GO:0018459).

KEGG pathway analysis categorized DEGs into six classes, with metabolic pathways containing the most Unigenes (480, 45.67%), followed by genetic information processing (425, 40.44%), environmental information processing (58, 5.52%), organismal systems (50, 4.76%), and cellular processes (37, 3.52%). Ten KEGG pathways were significantly enriched ($P<0.01$): ribosome (ko03010), photosynthesis-antenna proteins (ko00196), flavonoid biosynthesis (ko00941), sesquiterpenoid and triterpenoid biosynthesis (ko00909), synthesis and degradation of ketone bodies (ko00072), glutathione metabolism (ko00480), plant-pathogen interaction (ko04626), MAPK signaling pathway-plant (ko04016), flavone and flavonol biosynthesis (ko00944), and stilbenoid, diarylheptanoid and gingerol biosynthesis (ko00945).

2.2.3 Key Differential Expression Unigene Information

From the enriched pathways, we identified key metabolic pathways related to heterogeneous habitat adaptation (Table 3). The photosynthesis-antenna protein pathway (ko00196) contained 15 DEGs: one up-regulated gene *lhca5* (light-harvesting complex I chlorophyll a/b binding protein 5) and 14 down-regulated genes including seven *lhcb1* (light-harvesting complex II chlorophyll a/b binding protein 1), six *lhcb2* (light-harvesting complex II chlorophyll a/b binding protein 2), and one *lhcb3* (light-harvesting complex II chlorophyll a/b binding protein 3). The flavonoid biosynthesis pathway (ko00941) contained 11 up-regulated DEGs: *chs* (chalcone synthase), *c4h* (trans-cinnamate 4-monooxygenase), two *f3' h* (flavonoid 3' -monooxygenase), *f3h* (naringenin 3-dioxygenase), *fls* (flavonol synthase), *ans* (anthocyanidin synthase), *chi* (chalcone isomerase), *ccoaomt* (caffeoyl-CoA O-methyltransferase), and two *hct* (shikimate O-hydroxycinnamoyltransferase).

2.3.1 SSR Locus Overall Characteristics

Screening of 53,536 Unigenes identified 7,146 SSR loci in 6,006 Unigenes, with an average distribution distance of 8.1 kb. SSR length ranged from 10 to 225 bp, with an occurrence frequency of 13.35% (SSR count/total Unigenes) and a

distribution frequency of 11.22% (SSR-containing Unigenes/total Unigenes). A total of 907 sequences contained two or more SSRs (1.68%), and 420 sequences harbored compound SSR types (0.78%). The SSRs comprised 106 repeat motif types (Table 4), with mononucleotide repeats being dominant (3,248, 45.59%), followed by trinucleotide (2,041, 28.56%), dinucleotide (1,645, 23.02%), hexanucleotide (101, 1.41%), tetranucleotide (81, 1.13%), and pentanucleotide repeats (20, 0.28%) (Figure 5 [Figure 5: see original paper]).

2.3.2 EST-SSR Primer Development and Screening

Initial screening of 100 EST-SSR primer pairs via 1% agarose gel electrophoresis yielded 68 effectively amplifying primers. Polymorphism testing of these 68 primers using samples from YD1, YL1, QD1, and QL1 via 8% non-denaturing polyacrylamide gel electrophoresis revealed that all produced clear bands, with 11 primers showing polymorphism (Table 5) and amplifying 24 polymorphic fragments. These results demonstrate that developing EST-SSR primers from transcriptome data is an economical and feasible approach, providing stable, reproducible, and polymorphic markers through simple PCR validation.

3.1 Analysis of Adaptation Mechanisms in Heterogeneous Habitats

High-throughput sequencing technology enables comprehensive and rapid acquisition of plant transcript sequences, greatly facilitating gene discovery and secondary metabolite regulation studies (Lu et al., 2010). Our transcriptome analysis of *A. shikokiana* from heterogeneous habitats revealed that adaptation mechanisms primarily involve photosynthesis and flavonoid biosynthesis pathways.

Photosynthesis, the physiological basis for plant growth and metabolism, is influenced by environmental factors including light, water, and temperature (Fang et al., 2022). Light-harvesting complex II chlorophyll a/b binding proteins (LHC) play crucial roles in regulating photosynthesis by rapidly transferring light energy to PS I and PS II reaction centers for photochemical conversion (Helena et al., 2023). The *lhc* gene family comprises two evolutionary groups: *lhca* encodes LHC I proteins in photosystem I with large light-harvesting cross-sections capable of capturing different wavelengths (Mozzo et al., 2010), while *lhcb* encodes LHC II proteins in photosystem II that bind 50% of thylakoid pigments to form the most abundant antenna proteins (Wang, 2020). We found *lhca5* significantly up-regulated in hilltop scrub populations, consistent with Ganeteg et al. (2004) showing increased *lhca5* expression under high light. Conversely, *lhcb1*, *lhcb2*, and *lhcb3* were significantly down-regulated. Jiang (2019) reported that down-regulation of *lhcb* genes in water celery under drought stress prevents excessive light energy absorption and reduces photosynthesis, while Rahele et al. (2022) demonstrated that decreased *lhcb* expression reduces chlorophyll content in tomatoes under drought. We hypothesize that down-regulation of *lhcb1*-

3 reduces light-harvesting antenna protein synthesis, weakening photosynthesis and water consumption to mitigate drought effects. The contrasting regulation of *lhca* and *lhcb* genes thus enables *A. shikokiana* to cope with high light and low soil moisture in hilltop scrub, representing an elegant abiotic stress response.

Flavonoids are ubiquitous secondary metabolites that absorb UV radiation (280–315 nm), protecting plant organs, particularly photosynthetic tissues, from radiation damage (Zou et al., 2004), and perform multiple physiological functions during development and abiotic stress responses (Chen et al., 2023; Guo et al., 2023; Zhuang et al., 2023). Flavonoid biosynthesis derives from phenylpropanoid metabolism and is regulated by key enzymes including chalcone synthase (CHS), chalcone isomerase (CHI), flavonol synthase (FLS), flavanone 3-hydroxylase (F3H), flavonoid 3' -hydroxylase (F3' H), and anthocyanidin synthase (ANS) (Shen et al., 2022; Dai, 2022; Xiong et al., 2016). All related enzyme-encoding genes were significantly up-regulated in hilltop scrub populations. Xiao et al. (2020) reported positive correlations between *chs* and *chi* expression and flavonoid content, suggesting enhanced flavonoid synthesis in hilltop populations. The intense sunlight in hilltop scrub includes harmful UV radiation and midday heat, which can damage DNA and other cellular structures (Batschauer, 1993). Increased flavonoid synthesis likely provides UV protection and mitigates heat stress, enabling survival in this harsh environment.

3.2 SSR Locus Analysis and Primer Development in *Anemone shikokiana*

The SSR types in *A. shikokiana* are relatively diverse, ranging from mono- to hexanucleotide repeats. Studies indicate that AAG/CTT is the predominant trinucleotide motif (Xiang et al., 2023), consistent with our findings and suggesting high conservation in EST-SSR evolution. EST-SSR markers derived from expressed sequences offer advantages including high conservation and good transferability across species and genera (Lou et al., 2023). Based on high-quality transcriptome data, we designed and synthesized 100 primer pairs, of which 68 amplified effectively and 11 showed polymorphism across different geographic populations. These polymorphic EST-SSR markers can be applied to genetic diversity analysis and molecular marker-assisted breeding. Due to the scarcity of *A. shikokiana* materials in China and limited access to Japanese populations, we could not perform clustering analysis or construct phylogenetic trees in this study. Future work will validate the transferability of these polymorphic EST-SSR primers across *Anemone* species and design additional primers from differentially expressed genes to distinguish populations from different habitats, providing a foundation for evolutionary studies and germplasm conservation.

In summary, our transcriptome analysis of *A. shikokiana* from hilltop scrub and forest understory habitats reveals adaptation mechanisms involving regulation of photosynthesis and flavonoid biosynthesis genes. We have developed the first set of polymorphic EST-SSR markers for this species, filling a critical research gap and providing important resources for its conservation and utilization.

References

Bian J, Wang XN, Cao K, et al., 2023. Genetic structure analysis and fingerprint construction of hemp by EST-SSR analysis[J]. J Plant Genet Resour: 1–16[2023-10-20]. https://doi.org/10.13430/j.cnki.jpgr.20230531001.

Chen LC, Gu DZ, Liu Y, et al., 2018. Establishment of tissue culture regeneration system of *Anemone shikokiana*[J]. Jiangsu Agric Sci, 46(24): 47–50.

Dai MJ, 2022. Molecular cloning and functional analysis of CHIL and F3H in flavonoid biosynthesis pathway in mulberry[D]. Zhenjiang: Jiangsu Univ of Sci and Technology.

Guo LA, Mo RY, Tan J, et al., 2021. Analysis on differential expressed genes in the transcriptome of *Atractylodes macrocephala* from different producing areas[J]. Chin Med Mat, 44(12): 2787–2792.

Hou YT, Liu B, 2010. *Anemone shikokiana* (Makino) Makino[J]. Encyclopedic Knowl, (12): 40.

Jiang Q, 2019. Genomic analysis and response factors under abiotic stress in *Oenanthe javanica*[D]. Nanjing: Nanjing Agricultural University.

Li H, Feng YH, Tang SS, et al., 2023. Development and validity evaluation of *Liquidambar formosana* EST-SSR primers based on transcriptome sequencing[J]. Guihaia, 43(2): 327–335.

Liu Q, 2014. Research on genetic diversity of *Anemone shikokiana*[D]. Jinan: Shandong Normal University.

Lou YF, Gan R, Zhu CL, et al., 2022. Development and application of EST-SSR markers based on transcriptome of *Phyllostachys vivax* McClure f. *aureocaulis* N. X. Ma[J]. Plant Sci J, 40(3): 355–364.

Lu YH, Yu FB, Guo JH, et al., 2021. Analysis of transcriptomic differences between winged and wingless bird cherry-oat aphid *Rhopalosiphum padi* on wheat[J]. Acta Phytophy Sin, 48(6): 1303–1309.

Pang YJ, Sun W, Wang XS, et al., 2022. Influence of soil heterogeneity on root structure of *Anemone shikokiana*[J]. J Yantai Univ, 35(1): 28–34.

Sun HN, Liu DY, Li MY, et al., 2022. Transcriptome data analysis of *Coreopsis tinctoria* based on high-throughput sequencing[J]. Plant Breed: 1–18[2023-10-20]. http://kns.cnki.net/kcms/detail/46.1068.S.20221017.1205.010.html.

Tian HZ, Guo H, Tian H, et al., 2022. Transcriptome characteristics analysis and EST-SSR marker development of *Capsicum annuum* under waterlogging stress[J/OL]. Guihaia: 1–14[2023-10-20]. http://kns.cnki.net/kcms/detail/45.1134.q.20221227.1548.004.html.

Wang YF, Zhu RJ, Cheng XP, 2023. Research on phenotypic plasticity of different diameter class *Bombax ceiba* in heterogeneous habitats[J]. Ecol Sci, 42(3): 61–66.

Wang YH, 2020. Identification and expression profiling of Lhc genes in Ramie[D]. Wuhan: Huazhong Agricultural University.

Wang Z, Pang YJ, Liu CL, et al., 2014. Distribution pattern and analysis of influencing factors of rare plant *Anemone shikokiana* (Makino) Makino[J]. Bot Stud, 34(4): 440–445.

Wei CB, Gu W, Tian R, et al., 2022. Transcriptomic data analysis of *Alisma orientale* tubers grew in different ecological environments by high-throughput sequencing[J]. Mod Chin Med, 24(12): 2342-2349.

Wu RN, Xu B, Shi FL, 2022. Analysis of SSR characteristics for *Medicago ruthenica* 'Zhilixing' based on transcriptome data[J]. J Northwest A & F Univ (Nat Sci Ed), 50(5): 1–8.

Xiao YZ, Han SM, Qin Z, et al., 2020. Analysis of transcriptome sequencing and related genes of flavonoids biosynthesis from *Polygonatum kingianum*[J]. J Henan Agric Univ, 54(6): 931-940.

Yan MM, Wei GC, Pan XH, et al., 2008. A method suitable for extracting genomic DNA from animal and plant—modified CTAB method[J]. J Anhui Agric Sci, (20): 8488+8558.

Yang X, Yang N, Yuan QH, et al., 2021. Development and application of EST-SSR molecular markers in *Pinus bungeana*[J]. J Beijing For Univ, 43(7): 111.

Yu WY, Gao Y, Pang YJ, et al., 2019. Response of leaf morphology and structure of *Anemone shikokiana* to heterogeneous habitats and altitude changes[J]. Acta Ecol Sin, 39 (12): 4413–4420.

Yue XY, Yue W, Yan K, 2023. Adaptation of functional traits of *Phragmites australis* to different water habitats in the Yellow River Delta[J]. J NE For Univ, 51(3): 18–21.

Yue YH, Yan LJ, Huang X, et al., 2022. Development EST-SSR markers based on transcriptome sequences of *Tilia miqueliana* Maxim[J]. Plant Breed: 1-18[2023-10-20]. http://kns.cnki.net/kcms/detail/46.1068.S.20220715.1525.008.html.

Zhang CY, Long Y, Feng J, et al., 2007. Transcriptional regulation of plant genes and its significance in biology[J]. Hereditas, (7): 793–799.

Zheng Y, Jin GL, Wu WR, 2012. Relationship between sequence completeness and polymorphism of microsatellites[J]. Genom Appl Biol, 31(6): 587–591.

Zhou CM, Jiang LP, Dong GQ, et al., 2022. Analysis of differential expression genes of *Chrysanthemum indicum* var. *aromaticum* associated with different altitude habitats using RNA-seq technology[J]. World Sci Technol-Mod Chin Med, 24(4): 1356–1368.

Zou FL, Shou SY, Ye ZZ, et al., 2004. Advances in the research on flavonoid biosynthesis and plant stress response[J]. Chin J Cell Biol, (1): 39–44.

Batschauer A, 1993. A plant gene for photolyase: an enzyme catalyzing the repair of UV-light-induced DNA damage[J]. Plant J, 4(4): 705–709.

Buchfink Benjamin, Chao Xie, Daniel H Huson, 2015. Fast and sensitive protein alignment using DIAMOND[J]. Nat Methods, 12: 59–60.

Chen YY, Lu HQ, Jiang KX, et al., 2023. The Flavonoid Biosynthesis and Regulation in *Brassica napus*: A Review[J]. Int J Mol Sci, 24, 357.

Fang L, Martre P, Jin K, et al., 2022. Neglecting acclimation of photosynthesis under drought can cause significant errors in predicting leaf photosynthesis in wheat[J]. Glob Change Biol, 29, 505–521.

Ganeteg U, Kimmel F, Jansson S, 2004. Lhca5-an LHC-type protein associated with photosystem I[J]. Plant Mol Biol, 54(5): 641–51.

Ghanbari MSR, Tohidfar M, Ahmadikha A, 2022. Gene profile analysis and molecular-physiological evaluation of tomato genotypes under drought stress[J]. J Appl Biotechnol Rep, 9(3): 747–62.

Guo LA, Tan J, Deng XS, et al., 2023. Integrated analysis of metabolome and transcriptome reveals key candidate genes involved in flavonoid biosynthesis in *Pinellia ternata* under heat stress[J]. J Plant Res, 136(3): 359–369.

Helena S, Makio Y, Atsushi T, et al., 2023. Reversible down-regulation of photosystems I and II leads to fast photosynthesis recovery after long-term drought in *Jatropha curcas*[J]. J Exp Bot, 336–351.

Lucinsk R, Jackowski G, 2006. The structure, functions and degradation of pigment-binding proteins of Photosystem II[J]. Acta Biochim Pol, 53 (4): 693–708.

Manfred G, Brian J, Moran Y, et al., 2011. Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data[J]. Nat Biotechnol, 29(7): 644.

Mozzo M, Mantelli M, Passarini F, et al., 2010. Functional analysis of Photosystem I light-harvesting complexes (Lhca) gene products of *Chlamydomonas reinhardtii*[J]. Biochim Biophys Acta, 1797: 212–221.

Pang YJ, Li LX, Bian FH, 2020. Photosynthetic and ultrastructural adaptability of *Anemone shikokiana* leaves to heterogeneous habitats[J]. Braz J Bot, 43, 979–988.

Rozen S, Skaletsky H, 2000. Primer3 on the WWW for general users and for biologist programmers[J]. Methods Mol Biol, 132: 365–86.

Sebastian B, Thomas T, Thomas M, et al., 2017. MISA-web: a web server for microsatellite prediction[J]. Bioinform, 33(16): 2583–2585.

Shen N, Wang TF, Gan Q, et al., 2022. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity[J]. Food Che, 132531. https://pubmed.ncbi.nlm.nih.gov/35413752/.

Simon A, Wolfgang H, 2012. Differential expression of RNA-Seq data at the gene level EMBL. https://www.bioconductor.org/help/course-materials/2012/BioC2012/DESeq.pdf.

Storf S, Stauber EJ, Hippler M, et al., 2004. Proteomic analysis of the photosystem I light-harvesting antenna in tomato (*Lycopersicon esculentum*)[J]. Biochemistry-USA, 43(28): 9214-9224.

Temnykh S, Declerck G, Lukashova A, 2001. Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): Frequency, length variation, transposon associations, and genetic marker potential[J]. Genom Res, 11(8): 1441-1452.

Xiang NY, Lu BJ, Yuan T, et al., 2023. De Novo transcriptome assembly and EST-SSR marker development and application in *Chrysosplenium macrophyllum*[J]. Genesis, 14: 279.

Xiong S, Tian N, Long JH, et al., 2016. Molecular cloning and characterization of a flavanone 3-hydroxylase gene from *Artemisia annua* L.[J]. Plant Physiol Biochem, 105(5): 29-36.

Zhuang WB, Li YH, Shu XC, et al., 2023. The classification, molecular structure and biological biosynthesis of flavonoids, and their roles in biotic and abiotic stresses[J]. Molecules, 28(8): 3599.

Note: Figure translations are in progress. See original paper for figures.