Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2022.019630

ARTICLE

Comparative Transcriptome Analysis Reveals Different Mechanisms of Adaptation to Environment among Three Species of *Saussurea* DC.

Weimin Zhao[1], Fengzhen Li[1], Guomin Shi[3], Jialei Guo[1], Guifang He[1] and Tao He[2,3,*]

[1]School of Ecol-Environmental Engineering, Qinghai University, Xining, 810016, China

[2]State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, 810016, China

[3]Key Laboratory of Landscape Plants of Qinghai Province, Qinghai University, Xining, 810016, China

[*]Corresponding Author: Tao He. Email: hetaoxn@yahoo.com

Received: 03 October 2021 Accepted: 16 December 2021

ABSTRACT

Saussurea medusa, *Saussurea hypsipeta* and *Saussurea obvallata* are typical alpine snowline plants growing in the Qinghai-Tibet plateau. They are characterized by a specialized morphology. *S. medusa* and *S. hypsipeta* have very dense trichomes on whole plant, whereas *S. obvallata* has transparent bracts covered inflorescences. The different forms reflect their adaptation to cold environments. To investigate the different mechanisms of adaptation of these species to cold temperatures, transcriptome sequencing was performed in three species of *Saussurea* DC. A total of 116394 137237 and 113879 Unigenes were identified from *S. medusa*, *S. hypsipeta* and *S. obvallata*, respectively. Of these, 55909 (48.03%), 65519 (47.74%) and 51889 (45.56%) Unigenes were matched in public databases. GO analysis identified that most of annotated Unigenes in the three species of plants were related to cellular, metabolic, and single−organism processes, and binding and catalytic activities. The differential expression of 37 genes related to environmental adaptation were discovered by pairwise comparisons. Of these, two candidate genes (*Interaptin-like and CSLB3*) related to trichome development were identified only in *S. medusa* and *S. hypsipeta*, which was consistent with their distinct morphology. Our data can provide a valuable resource for the further studies on the adaptive mechanisms of molecular and functional ecology in *Saussurea* DC.

KEYWORDS

Saussurea DC.; transcriptome; adaptive mechanisms

1 Introduction

Saussurea medusa Maxim., *Saussurea hypsipeta* Diels. and *Saussurea obvallata* (DC.) Edgew. are perennial herbaceous plants in the family Asteraceae, distributed mainly in the alpine zone of the Qinghai-Tibet Plateau at altitudes from 3800 to 5000 m [1,2]. Many of the high-altitude *Saussurea* are known for their spectacular growth forms. *S. medusa* and *S. hypsipeta*, which are called "woolly" plants, have dense layers of woolly trichomes on their stems, leaves, bracts, and inflorescences [2]. *S. obvallata* is called called "glasshouse" plant, which encloses their inflorescences in large translucent bracts [3]. These spectacular growth forms are thought to represent an evolutionary response to cold and windy environments [4,5]. However, how are the morphological differences among three the species of *Saussurea* DC. produced? What are the molecular mechanisms behind these adaptations?

Transcriptomes are the whole RNA transcripts in cells or tissues. They reflect expressed genes at different life stages, tissue types, physiological state and environmental conditions. Transcriptome-based techniques provide useful means of studying gene expression and gene structure, and revealing the molecular mechanism involved in a specific biological process [6]. Recently, this technique has been widely used in the study of molecular mechanisms of plant responses to drought [7], waterlogging [8], low temperature [9], salt [10], high light [11], and irradiation stresses [12]. By comparative transcriptome analysis, new genes are found and molecular mechanisms are revealed in these plants.

Most studies of *Saussurea* plants growing in the Qinghai-Tibet plateau have focused on their adaptive shapes and anatomies as well as physiological functions [1–3]. Little is known about molecular mechanisms behind these plant adaptations [13]. The present work was performed to examine the comparative transcriptome analysis by transcriptome sequencing and gene function annotation on three species of *Saussurea* plants growing in the Daban Mountain in the northeastern Qinghai-Tibet Plateau. The studies of molecular mechanisms in alpine plant species will contribute to further understand the adaptation of plants to alpine environments since their responses are associated with cold climates; it is probably not useful to know them in the context of a warming climate.

2 Materials and Methods

2.1 Materials Collection

S. medusa, *S. hypsipeta* and *S. obvallata* were collected at flowering stag from Qilian Mountains (37°5′-59′N, 100°55′-102°41′E, 4,200 m a.s.l.) in the northeastern Qinghai-Tibet plateau, China. Growth form of these species is depicted in Fig. 1. This field site features typical plateau continental climate with an annual average (a) temperature of –2°C, (b) rainfall of 482 mm, (c) solar radiation of 6.46 × 109 Jm^{-2}, and (d) barometric pressure of 684.2 hPa. Three plants were collected from each species. The roots, stems, leaves, and flowers obtained from one individual were pooled into a single sample. These materials were flash-frozen in liquid nitrogen.

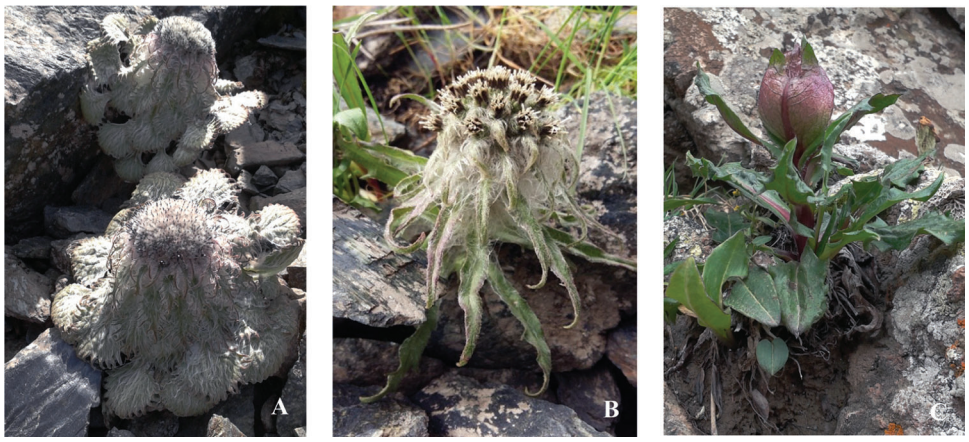

Figure 1: *Saussurea* plants in the high-elevation scree or rock fields. (A) *S. medusa*; (B) *S. hypsipeta*; (C) *S. obvallata*

2.2 RNA Extraction and Transcriptome Sequencing

Trizol® (Invitrogen-Thermo Fisher Scientific, Carlsbad, CA, USA) was used to extract total RNA on the three species. All RNA was treated with DNase I. The NanoPhotometer® spectrophotometer

(IMPLEN, CA, USA) was used to test RNA purity; the Assay Kit in Qubit® 2.0 Flurometer (Life Technologies, CA, USA) was used to determine RNA concentrations; the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA) was used to assess RNA integrity, and RNA Integrated Number (RIN) values ≥8.

We used NEBNext® Ultra™ RNA Library Prep Kit from Illumina® (NEB, USA) to construct the sequences library and the Agilent Bioanalyzer 2100 system to evaluate library quality. We sequenced library preparation to obtain raw reads by Illumina HiSeq™ 2500; clean reads were obtained by removing reads containing adapter, reads containing poly-N and low-quality reads from raw reads. At the same time, Q20, Q30, GC-content and sequence duplication level of the clean data were calculated. All the downstream analyses were based on clean data with high quality. Transcriptome was obtained by splicing clean reads with Trinity [14]. The longest transcript in each gene was taken as the Unigene for subsequent analysis.

2.3 Unigene Functional Annotation

Unigenes of *S. medusa*, *S. hypsipeta* and *S. obvallata* were searched in the public databases (Nr, Nt, Pfam, KOG/COG, Swiss-Prot, KEGG, GO), and gene function was annotated according to gene similarity. The E-value in Nr is less than or equal to 1E-5 (Nt, ≤1E-5; Pfam, ≤0.01, KOG/COG, ≤1E-3; Swiss-Prot, ≤1E-5; KEGG, ≤1E-10; GO, ≤1E-6).

2.4 Orthology Genes Screening and Analysis

Orthology genes were searched for full-length CDS sequences by OrthoMCL, and one-to-one orthology genes were screened out [15]. Nonsynonymous substitution rate (Ka), synonymous substitution rate (Ks) and Ka/Ks of orthology genes were calculated by PALM [16]. GO gene enrichment analysis was carried out for the orthology genes (Ka/Ks>1) that were positively selected by GOseq [17]. Pathway significant enrichment analysis was carried out on the orthology genes positively selected by the hypergeometric test, with KEGG Pathway as the unit [18].

2.5 Analysis of Environmental Adaptation Related Unigenes

By using the classification results of Unigene in the GO database and the functional annotations in the seven public databases, the environmental adaptation-related Unigenes of the three species were analysed by interspecies reduction.

3 Results

3.1 Transcriptome Data Assembly of the Three Species

S. medusa, *S. obvallata* and *S. hypsipeta* obtained 150217, 162036 and 192813 transcripts, respectively (Table 1). The average length of the transcript of *S. medusa* was 748 bp and N50 was 1292 bp. The average length of the transcript and N50 of *S. obvallata* and *S. hypsipeta* were 760 bp, 1270 bp and 698 bp, 1156 bp, respectively. The longest transcript in each gene was taken as Unigene for subsequent analysis. *S. medusa* obtained 116394 Unigenes with an average length of 623 bp and N50 of 966 bp. *S. obvallata* showed 113879 Unigenes with an average length of 635 bp and N50 of 1000 bp. *S. hypsipeta* obtained 13723 Unigenes with an average length of 581 bp and N50 of 857 bp. The results showed that the sequencing quality was sufficient for future analysis needs.

Table 1: Transcript and Unigene assemblies of *S. medusa*, *S. hypsipeta* and *S. obvallata*

Sequencing indicators	*S. medusa*	*S. hypsipeta*	*S. obvallata*
Valid sequences number	127 827 892	130 802 486	134 018 004
Base number (bp)	19.18 G	19.62 G	20.11 G
Q30 (%)	92.07	92.34	92.45
GC (%)	43.83	44.08	43.95
Length range of transcript (bp)	201~13 922	201~14 016	201~13 915
Average length of transcript (bp)	748	698	760
N50 of transcript (bp)	1 292	1 156	1 270
Transcript number	150 217	192 813	162 036
Length range of Unigene (bp)	201~13 922	201~140 16	201~13 915
Average length of Unigene (bp)	623	581	635
N50 of Unigene (bp)	966	857	1 000
Unigene number	116 394	137 237	113 879

3.2 Functional Annotation and Classification of the Three Species' Unigene

3.2.1 Unigene Annotation in the Public Database

S. medusa, S. hypaipeta and *S. obvalata* had 7620, 7245 and 9366 Unigenes, respectively, that can be matched in all databases (Table 2).

Table 2: Unigene function annotation of *S. medusa*, *S. hypsipeta* and *S. obvallata*

Database annotation	Annotation quantity and percentage (%) of *S. medusa*	Annotation quantity and percentage (%) of *S. hypsipeta*	Annotation quantity and percentage (%) of *S. obvallata*
NR database annotation	44606 (38.32)	43860 (38.51)	53750 (39.16)
NT database annotation	26144 (22.46)	26818 (23.54)	30959 (22.55)
KO database annotation	18548 (15.93)	16832 (14.78)	22152 (16.14)
Swiss-Prot database annotation	37253 (32)	33242 (29.19)	43245 (31.51)
Pfam database annotation	34996 (30.06)	31559 (27.71)	40440 (29.46)
GO database annotation	35840 (30.79)	32236 (28.3)	41307 (30.09)
KOG database annotation	21053 (18.08)	17117 (15.03)	24003 (17.49)
All databases annotation	7620 (6.54)	7245 (6.36)	9366 (6.82)
At least one databases annotation	55909 (48.03)	51889 (45.56)	65519 (47.74)

3.2.2 GO Functional Classification of Unigene

The Unigenes of *S. medusa*, *S. obvallata* and *S. hypsipeta* were divided into 55, 56 and 56 types of function respectively, and the 55 types of function were the same (Fig. 2). The Unigene of *S. obvallata* and *S. hypsipeta* found a gene related to cell aggregation. Cell agglutination is associated to the resistance of organisms to environmental UV stress [19].

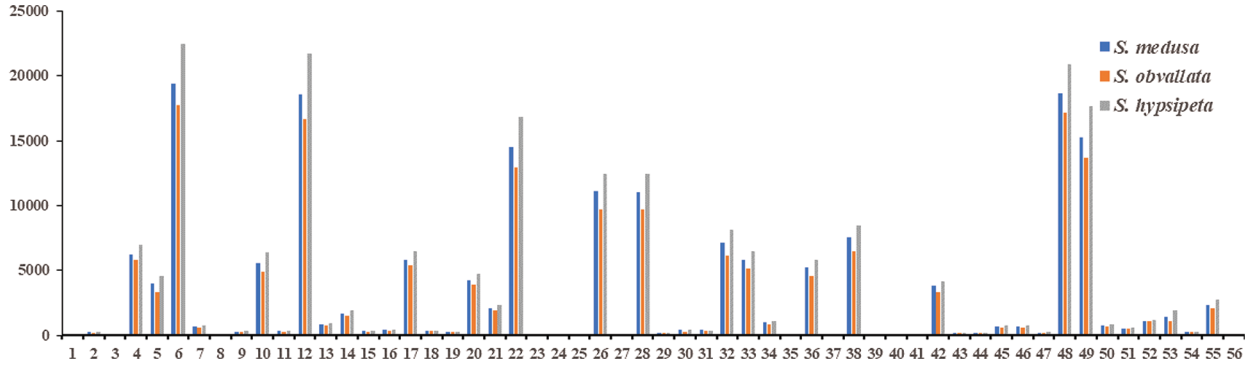

Figure 2: The number of GO function annotated for Unigene in *S. medusa*, *S. hypsipeta* and *S. obvallata*
Notes: 1: behavior; 2: biological adhesion; 3: biological phase; 4: biological regulation; 5: cellular component organization or biogenesis; 6: cellular process; 7: developmental process; 8: growth; 9: immune system process; 10: localization; 11: locomotion; 12: metabolic process; 13: multicellular organismal process; 14: multi−organism process; 15: negative regulation of biological process; 16: positive regulation of biological process; 17: regulation of biological process; 18: reproduction; 19: reproductive process; 20: response to stimulus; 21: signaling; 22: single-organism process; 23: rhythmic process; 24: cell killing; 25: cell aggregation; 26: cell; 27: cell junction; 28: cell part; 29: extracellular matrix; 30: extracellular region; 31: extracellular region part; 32: macromolecular complex; 33: membrane; 34: membrane-enclosed lumen; 35: synapse part; 36: membrane part; 37: synapse; 38: organelle; 39: extracellular matrix component; 40: nucleoid; 41: symplast; 42: organelle part; 43: other organism; 44: other organism part; 45: virion; 46: virion part; 47: antioxidant activity; 48: binding; 49: catalytic activity; 50: molecular function regulator; 51: molecular transducer activity; 52: nucleic acid binding transcription factor activity; 53: structural molecule activity; 54: transcription factor activity and protein binding; 55: transporter activity; 56: metallochaperone activity; 1~25, 26~46 and 47~56 correspond to secondary functional classification of Biological Process, Cellular Component and Molecular Function, respectively.

3.2.3 KOG Functional Classification of Unigene

The KOG annotation results of Unigene of *S. medusa*, *S. hypsipeta* and *S. obvallata* were similar. There were 21053 Unigenes of *S. medusa*, 17117 Unigenes of *S. hypsipeta* and 24003 Unigenes of *S. obvallata* annotated on 25 KOG classifications. Firstly, the general function prediction only accounted for most of the Unigenes, which were 16.41%, 18.05% and 16.46% in *S. medusa*, *S. hypsipeta* and *S. obvallata*, respectively. Next, there were posttranslational modification, proteins turnover and chaperones. The proportion of *S. medusa*, *S. hypsipeta* and *S. obvallata* in this category was 14.32%, 14.41% and 11.64%, respectively. Signal transduction mechanisms were 8.74%, 8.54% and 7.65%, in *S. medusa*, *S. hypsipeta* and *S. obvallata*, respectively (Fig. 3). They accounted for the least Unigene in the classification of Cell motility, Extracellular structures and Nuclear structures.

3.2.4 KEGG Functional Classification of Unigene

The Unigene annotated by the three species belonged to Cellular Processes, Environmental Information Processing, Genetic Information Processing, Metabolism and Organismal Systems, including 19 secondary pathways (Fig. 4). Thereinto, 7 types of pathway Unigene accounted for a large proportion (>50%), and they included Cellular Processes, Folding and sorting and degradation, Translation, Amino acid metabolism, Carbohydrate metabolism, Energy metabolism, and Overview, respectively. The KEGG classification results indicated that the Unigene functions were mainly related to protein translation and material metabolism.

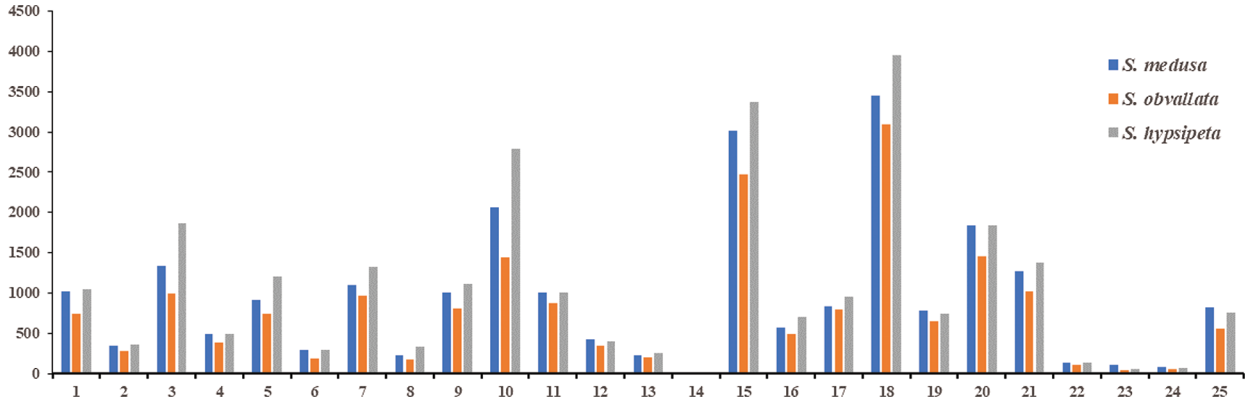

Figure 3: The number of KOG function annotated for Unigene in *S. medusa*, *S. hypsipeta* and *S. obvallata*
Note: 1: RNA processing and modification; 2: chromatin structure and dynamics; 3: energy production and conversion; 4: cell cycle control or cell division or chromosome partitioning; 5: amino acid transport and metabolism; 6: nucleotide transport and metabolism; 7: carbohydrate transport and metabolism; 8: coenzyme transport and metabolism; 9: lipid transport and metabolism; 10: translation or ribosomal structure or biogenesis; 11: transcription; 12: replication and recombination and repair; 13: cell wall or membrane or envelope biogenesis; 14: cell motility; 15: posttranslational or modification or protein turnover, chaperones; 16: inorganic ion transport and metabolism; 17: secondary metabolites biosynthesis or transport and catabolism; 18: general function prediction only; 19: function unknown; 20: signal transduction mechanisms; 21: intracellular trafficking or secretion or vesicular transport; 22: defense mechanisms; 23: extracellular structures; 24: nuclear structure; 25: cytoskeleton.

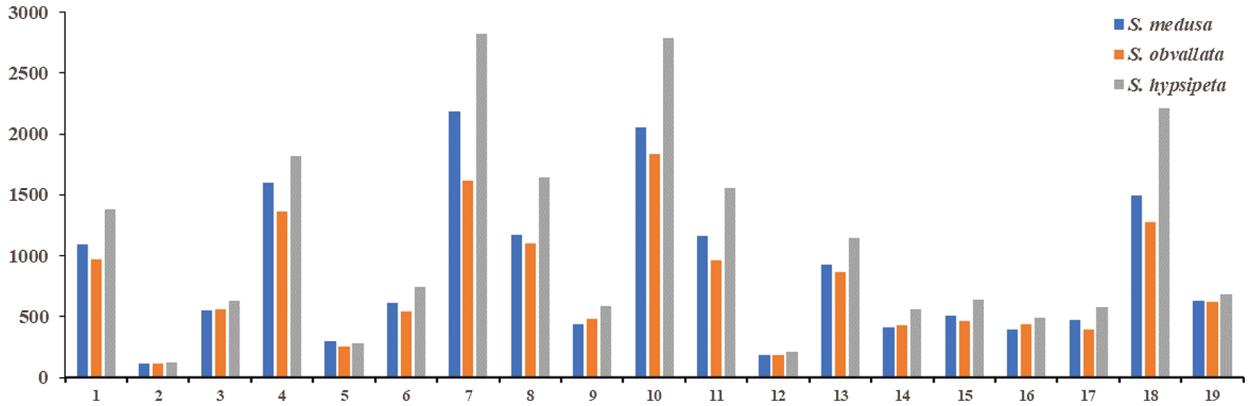

Figure 4: The number of KEGG function annotated for Unigene in *S. medusa*, *S. hypsipeta* and *S. obvallata*
Notes: 1: transport and catabolism; 2: membrane transport; 3: signal transduction; 4: folding and sorting and degradation; 5: replication and repair; 6: transcription; 7: translation; 8: amino acid metabolism; 9: biosynthesis of other secondary metabolites; 10: carbohydrate metabolism; 11: energy metabolism; 12: glycan biosynthesis and metabolism; 13: lipid metabolism; 14: metabolism of cofactors and vitamins; 15: metabolism of other amino acids; 16: metabolism of terpenoids and polyketides; 17: nucleotide metabolism; 18: overview; 19: environmental adaptation; 1, 2~3, 4~7, 8~18 and 19 correspond to secondary functional classification of cellular processes, environmental information processing, genetic information processing, metabolism, and Organismal systems, respectively.

3.3 Orthology Genes Screening of the Three Species

10811 sets of orthologous genes were obtained through OrthoMCL in the three species. There were 463 sets of orthologous genes which Ka/Ks>1, indicating that these genes were rapidly divergent (Fig. 5).

The GO functional enrichment classification was carried out for the orthologous genes of positive selection in the three species. We did not find functional enrichment genes, but 78 genes were related to plant resistance. The resistance genes included peroxidase, ubiquitin, zinc-finger protein and

acyl-CoA-binding protein, which play an important role in plant environmental stress. The KEGG gene enrichment analysis revealed that Ribosome had the largest number of genes enriched in this pathway, with a total of 14 genes, revealing that the genes related to Ribosome synthesis in these three species are subject to strong positive selection (Fig. 6).

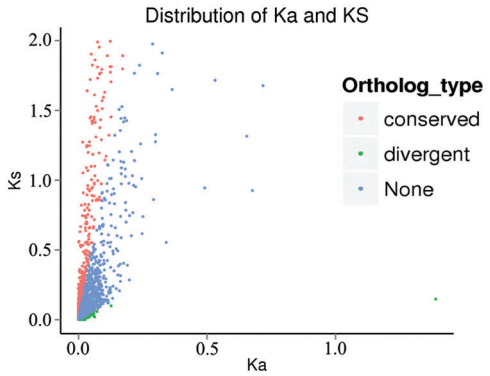

Figure 5: Distribution of Ka and Ks

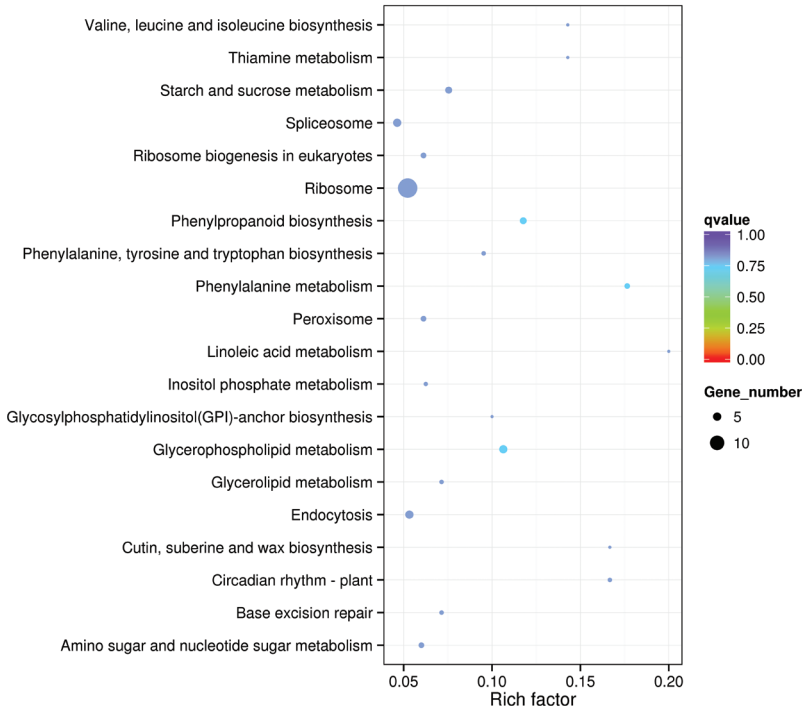

Figure 6: Statistic of KEGG enrichment

3.4 Analysis of Environmental Adaptation Related Unigenes

Based on the classification of the three species Unigenes in GO database, and analysis of Unigene about environmental adaptation, the results showed that the genes related to environmental adaptation in the three species were mainly molecular chaperone, ubiquitin, calmodulin, enzyme and ribosomes (Table 3). *HSC82*, *CLPB* and *HSP17.9A* can enhance the effect of plants on high temperature and salt stresses [20–22]. *Dna J2* and *osigba0134h18.3* were annotated by GO, which are involved in the adaptation of plants to high

temperature stress. *PER50* is one of the enzymes with which plants respond to oxidative stress [23], while *S2P* corresponds to salt stress [24]. *ATPK2* is involved in plant adaptation to cold and high salt stresses [25], while *CRK29* and *CIPK7* are in connection with plant immune responses and adaptation to cold stress [26,27]. *FEN1* is one of the key enzymes in DNA replication and repair in eukaryotes [28]. *RIN1* annotated by GO is involved in plant defensive responses to fungi. *Malate oxidoreductase* can regulate plant growth and respiration [29]. Gene expression is regulated by *SAHH1* through gene methylation modification [30]. *GABA-TP1* participates in plant adaptation to temperature stress [31]. *SAMT* is related to plants' defense responses to biologic stimulation [32,33]. *Calm3* mediates the regulation of enzymes and ion channels through calcium ions [34]. *IAA13* regulates growth and development of plants through ARFs [35]. *H0901F07.20* annotated by GO involves in the binding of lipoyl coenzyme A. *ATG8* is related to the formation of autophagosomes in plants [36]. *NIP3* is correlated with the transport of the heavy metal arsenic in plants [37]. *OJ1754_E06.16* is associated with the processes of protein secretion and signal transmission. *FAF* takes part in plant ABA activation signaling pathway and phosphorylation regulation. *MutS* participates in DNA mismatch repair. *GF14A* is connected with plant adaptation to drought stress [38]. *NIMIN-2* is related to the acquisition of systemic resistance in plants [39], while *ATL4M* is related to abiotic stress responses [40]. *Interaptin-like* links the intracellular system to the cytoskeleton, which regulates the morphogenesis of the multicellular trichomes [41,42]. *CSLB3* is related to the formation of non-cellulosic polysaccharide skeletons in plant cell walls. *GBF* is one of the transcription factors in which cells respond to signals [43]. *AFG2* is associated with the maturation of 60S ribosomal subunit [44]. *PGPS/NH15* annotated by GO participates in the regulation of oxidation-reduction enzyme activities.

Table 3: Differential expression genes for environmental adaptation of *S. medusa*, *S. hypsipeta* and *S. obvallata*

Name and function of gene		Unigene annotation and expression		
		S. hypsipeta	*S. medusa*	*S. obvallata*
CLPB	Molecular chaperone	Yes	Yes	No
OSIGBa0134H18.3	Molecular chaperone	Yes	Yes	No
PER50	Peroxidase	Yes	Yes	No
S2P	Metalloproteinase	Yes	Yes	No
CIPK7	CBL-interacting protein kinase 07	Yes	Yes	No
FEN1	Flap endonuclease 1	Yes	Yes	No
RIN1	RuvB-like helicase 1	Yes	Yes	No
OJ1754_E06.16	Ethylene-responsive small GTP-binding protein	Yes	Yes	No
ATL4M	RING-H2 finger protein ATL4M	Yes	Yes	No
Interaptin-like	Interaptin-like	Yes	Yes	No
CSLB3	Cellulose synthase-like protein B3	Yes	Yes	No
PGPS/NH15	PGPS/NH15	Yes	Yes	No
Dna J2	Molecular chaperone	No	No	Yes
IAA13	Auxin	No	No	Yes
RPL26B	Ribosomal 26 subunit	No	No	Yes

(Continued)

Table 3 (continued)

Name and function of gene		Unigene annotation and expression		
		S. hypsipeta	*S. medusa*	*S. obvallata*
H0901F07.20	Aceyl coenzyme	No	No	Yes
NIP3	Aquaporins	No	No	Yes
GBF	G-box binding factor bZIP transcription factor	No	No	Yes
HSC82	Molecular chaperone	No	Yes	No
HSP17.9A	Molecular chaperone	No	Yes	No
UBC1	Ubiquitin ligase	No	Yes	No
KCTD9	Ubiquitin	No	Yes	No
SAMT	Salicylate O-methyltransferase	No	Yes	No
ATPK2	Protein kinase	Yes	No	Yes
CRK29	Protein kinase	Yes	No	Yes
	Malate oxidoreductase oxidoreductase	Yes	No	Yes
GABA-TP1	Gamma-aminobutyrate transaminase 1	Yes	No	Yes
SAMT	Salicylic acid carboxy methyl transferase	Yes	No	Yes
ATG8	Autophagy	Yes	No	Yes
NIMIN-2	Protein NIM1-INTERACTING 2	Yes	No	Yes
UBE2J2	Ubiquitin ligase	Yes	No	No
SAHH1	Hyperhomocysteinase	Yes	No	No
Calm3	Calmodulin	Yes	No	No
AFG2	ATPase family gene 2 protein	Yes	No	No
FAF	Protein FAF	No	Yes	Yes
MutS	DNA mismatch repair mutS	No	Yes	Yes
GF14A	14-3-3-like protein GF14-A	No	Yes	Yes

4 Discussion

Our sequencing results showed that Unigene N50 of the three species were 857~1000 bp, the base quality value Q30 was over 92%, and the GC content was over 43%. The amount of sequencing data and the number of Unigene spliced were relatively large, which showed that the sequencing quality was good, and the sequence information generated was sufficient and effective [45]. The results of Go, KOG and KEGG of three species Unigenes were very similar, indicating that their genetic relationship was very similar.

The temperature in the collecting area was between 0–30°C, UV radiation up to 7000 $\mu W \cdot cm^{-2}$, and the oxygen partial pressure was 13.25 kPa for the three species. Stresses of low oxygen, strong radiation and large temperature differences made that makes the plants growing in this area formed special stress resistance mechanisms in the long-term to achieve collaborative resilience, such as trichomes, ABA signaling pathway, molecular chaperones and ubiquitin pathway. In our study, there was a gene expression related to cell aggregation in both *S. hypsipeta* and *S. obvallata*. Some research shows that

Gloeocapsa sp. cells with low metabolic activity aggregate to form a lamellar biofilm to protect organisms against ultraviolet radiation [19]. Then, we compared leaf structure among the three species; *S. medusa* had the longest, thickest and white trichomes on the leaf epidermis. This structure can effectively resist and reflect ultraviolet radiation. *S. hypsipeta* and *S. obvallata* may resist the ultraviolet radiation stress through cell aggregation, but the specific mechanism needs to be verified in future experiments. Interaptin protein was the binding protein between the components of the inner membrane and the actin of the cytoskeleton, Microtubules and actin promote the development of trichomes [41,42]. *CSLB3* participates in the formation of the noncellulose polysaccharide skeleton in plant cell wall. In this study, we found that the *Interaptin-like* and *CSLB3* genes were both expressed in *S. hypsipeta* and *S. medusa*. It is speculated that the expression of these two genes can promote the occurrence of trichomes and improve the resistance of these two plant species to radiation and temperature stresses. This finding was also consistent with the structural characteristics of the three species. *S. medusa* and *S. hypsipeta* are densely covered with trichomes. The ground tissues of *S. obvallata* showed no trichomes, and the inflorescences were enclosed in translucent bracts. At the same time, we found that the three plant species expressed different molecular chaperones, ubiquitin, protease and other genes to allow the adaptation of plants to the environmental stresses, and their mechanisms need further study.

Funding Statement: This work was financially supported by the National Natural Science Foundation of China (31960222, 31360095).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Tsukaya, H., Fujikawa, K., Wu, S. (2002). Thermal insulation and accumulation of heat in the downy inflorescences of *Saussurea medusa* (Asteraceae) at high elevation in Yunnan, China. *Journal Plant Research, 115(4),* 263–268. DOI 10.1007/s10265-002-0030-1.
2. Yang, Y., KÖrner, C., Sun, H. (2008). The ecological significance of pubescence in *Saussurea medusa*, a high-elevation himalayan woolly plant. *Arctic Antarctic and Alpine Research, 40(1),* 250–255. DOI 10.1657/1523-0430(07-009)[YANG]2.0.CO;2.
3. Semwal, P., Pauw, A., Palni L.M., S., Thapliyal, A. (2019). Bumblebees (*Bombus rufofasciatus* Smith) pollinate the enclosed inflorescences of the endangered Brahma's lotus (*Saussurea obvallata*: Asteraceae) of the Indian Himalaya. *South African Journal of Botany, 121,* 435–441. DOI 10.1016/j.sajb.2018.12.015.
4. Tsukaya, H., Tsuge, T. (2001). Morphological adaptation of inflorescence in plants that develops at low temperature in early spring: The convergent evolution of "downy plants". *Plant Biology, 3,* 536–543. DOI 10.1055/s-2001-17727.
5. KÖrner, C. (2003). *Alpine plant life*. Berlin, Berlin Heidelberg: Springer-Verlag.
6. Garber, M., Grabherr, M. G., Guttman, M., Trapnell, C. (2011). Computational methods for transcriptome annotation and quantification using RNA-seq. *Nature Methods, 8(6),* 469–477. DOI 10.1038/nmeth.1613.
7. Kumar, J., Gunapati, S., Kianian, S. F., Singh, S. P. (2018). Comparative analysis of transcriptome in two wheat genotypes with contrasting levels of drought tolerance. *Protoplasma, 255(5),* 1487–1504. DOI 10.1007/s00709-018-1237-x.
8. Zhao, N., Li, C., Yan, Y., Cao, W., Song, A. et al. (2018). Comparative transcriptome analysis of waterlogging-sensitive and waterlogging-tolerant Chrysanthemum morifolium cultivars under waterlogging stress and reoxygenation conditions. *International Journal Molecular Sciences, 19(5),* 1455. DOI 10.3390/ijms19051455.
9. Asiche, W. O., Mitalo, O. W., Kasahara, Y., Tosa, Y., Mworia, E. G. et al. (2018). Comparative transcriptome analysis reveals distinct ethylene-independent regulation of ripening in response to low temperature in kiwifruit. *BMC Plant Biology, 18(1),* 47. DOI 10.1186/s12870-018-1264-y.

10. Ma, W., Kim, J. K., Jia, C., Yin, F., Kim, H. J. et al. (2019). Comparative transcriptome and metabolic profiling analysis of buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) under salinity stress. *Metabolites, 9(10),* 225. DOI 10.3390/metabo9100225.
11. Cui, D., Hu, C., Zou, Z., Sun, X., Shi, J. et al. (2020). Comparative transcriptome analysis unveils mechanisms underlying the promoting effect of potassium iodide on astaxanthin accumulation in *Haematococcus pluvialis* under high light stress. *Aquaculture, 525,* 735279. DOI 10.1016/j.aquaculture.2020.735279.
12. Xiong, H., Guo, H., Xie, Y., Gu, J., Zhao, L. et al. (2020). Comparative transcriptome analysis of two common wheat varieties induced by ^{7}Li-ion beam irradiation reveals mutation hotspot regions and associated pathways. *Radiation Physics and Chemistry, 170,* 108650. DOI 10.1016/j.radphyschem.2019.108650.
13. Li, H., Qiu, J., Chen, F., Lv, X., Fu, C. et al. (2012). Molecular characterization and expression analysis of dihydroflavonol 4-reductase (DFR) gene in *Saussurea medusa*. *Molecular Biology Reports, 39(3),* 2991–2999. DOI 10.1007/s11033-011-1061-2.
14. Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A. et al. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology, 29(7),* 644–652. DOI 10.1038/nbt.1883.
15. Li, L., Stoeckert Jr, C. J., Roos, D. S. (2003). OrthoMCL: Identification of ortholog groups for eukaryotic genomes. *Genome Research, 13(9),* 2178–2189. DOI 10.1101/gr.1224503.
16. Yang, Z. (2007). PAML 4: Phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution, 24(8),* 1586–1591. DOI 10.1093/molbev/msm088.
17. Young, M. D., Wakefield, M. J., Smyth, G. K., Oshlack, A. (2010). Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biology, 11(2),* R14. DOI 10.1186/gb-2010-11-2-r14.
18. Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M. et al. (2008). KEGG for linking genomes to life and the environment. *Nucleic Acids Research, 36(suppl_1),* D480–D484. DOI 10.1093/nar/gkm882.
19. Wadsworth, J., Rettberg, P., Cockell, C. S. (2019). Aggregated cell masses provide protection against space extremes and a microhabitat for hitchhiking co-inhabitants. *Astrobiology, 19(8),* 995–1007. DOI 10.1089/ast.2018.1924.
20. Wang, W., Vinocur, B., Shoseyov, O., Altman, A. (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in Plant Science, 9(5),* 244–252. DOI 10.1016/j.tplants.2004.03.006.
21. Imai, J., Yahara, I. (2001). Role of HSP90 in salt stress tolerance via stabilization and regulation of calcineurin. *Molecular and Cellular Biology, 20(24),* 9262–9270. DOI 10.1128/MCB.20.24.9262-9270.2000.
22. Lee, U., Rioflorido, I., Hong, S. W., Larkindale, J., Waters, E. R. et al. (2007). The *Arabidopsis* ClpB/Hsp_{100} family of proteins: Chaperones for stress and chloroplast development. *Plant Journal, 49(1),* 115–127. DOI 10.1111/j.1365-313X.2006.02940.x.
23. Schenk, P. M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T. et al. (2000). Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Plant Biology, 97(21),* 11655–11660. DOI 10.1073/pnas.97.21.11655.
24. Che, P., Bussell, J. D., Zhou, W., Estavillo, G. M., Pogson, B. J. et al. (2010). Signaling from the endoplasmic reticulum activates brassinosteroid signaling and promotes acclimation to stress in *Arabidopsis*. *Science Signaling, 3(141)*. DOI 10.1126/scisignal.2001140.
25. Lee, D. H., Park, S. J., Ahn, C. S., Pai, H. S. (2017). MRF family genes are involved in translation control, especially under energy-deficient conditions, and their expression and functions are modulated by the TOR signaling pathway. *Plant Cell, 29(11),* 2895–2920. DOI 10.1105/tpc.17.00563.
26. Yadeta, K. A., Elmore, J. M., Creer, A. Y., Feng, B. M., Franco, J. Y. et al. (2017). A cysteine-rich protein kinase associates with a membrane immune complex and the cysteine residues are required for cell death. *Plant Physiology, 173(1),* 771–787. DOI 10.1104/pp.16.01404.
27. Huang, C., Ding, S., Zhang, H., Du, H., An, L. (2011). $CIPK_7$ is involved in cold response by interacting with CBL_1 in *Arabidopsis thaliana*. *Plant Science, 181(1),* 57–64. DOI 10.1016/j.plantsci.2011.03.011.

28. Liu, Y., Kao, H. I., Bambara, R. A. (2004). Flap Endonuclease 1: A central component of DNA metabolism. *Annual Review of Biochemistry, 73,* 589–615. DOI 10.1146/annurev.biochem.73.012803.092453.
29. Tomaz, T., Bagard, M., Pracharoenwattana, I., Lindén, P., Lee, C. P. et al. (2010). Mitochondrial malate dehydrogenase lowers leaf respiration and alters photorespiration and plant growth in *Arabidopsis*. *Plant Physiology, 154(3),* 1143–1157. DOI 10.1104/pp.110.161612.
30. Rocha, P. S. C. F., Sheikh, M., Melchiorre, R., Fagard, M., Boutet, S. et al. (2005). The *Arabidopsis HOMOLOGY-DEPENDENT GENE SILENCING1* gene codes for an S-adenosyl-L-homocysteine hydrolase required for DNA methylation-dependent gene silencing. *Plant Cell, 17,* 404–417. DOI 10.1105/tpc.104.028332.
31. Akihiro, T., Koike, S., Tani, R., Tominaga, T., Watanabe, S. et al. (2008). Biochemical mechanism on GABA accumulation during fruit development in tomato. *Plant Cell Physiology, 49(9),* 1378–1389. DOI 10.1093/pcp/pcn113.
32. Ament, K., Krasikov, V., Allmann, S., Rep, M., Takken, F. L. W. et al. (2010). Methyl salicylate production in tomato affects biotic interactions. *Plant Journal, 62,* 124–134. DOI 10.1111/j.1365-313x.2010.04132.x.
33. Leng, F., Lin, Q., Wu, D., Wang, S., Wang, D. et al. (2016). Comparative transcriptomic analysis of grape berry in response to root restriction during developmental stages. *Molecules, 21(11),* 1431. DOI 10.3390/molecules21111431.
34. Takahashi, F., Mizoguchi, T., Yoshida, R., Ichimura, K., Shinozaki, K. (2011). Calmodulin-dependent activation of MAP kinase for ROS homeostasis in *Arabidopsis*. *Molecule Cell, 41(6),* 649–660. DOI 10.1016/j.molcel.2011.02.029.
35. Liscum, E., Reed, J. W. (2002). Genetics of Aux/IAA and ARF action in plant growth and development. *Plant Molecular Biology, 49,* 387–400. DOI 10.1023/a:1015255030047.
36. Yamaguchi, M., Noda, N. N., Nakatogawa, H., Kumeta, H., Ohsumi, Y. et al. (2010). Autophagy-related protein 8 (Atg8) family interacting motif in Atg3 mediates the Atg3-Atg8 interaction and is crucial for the cytoplasm-to-vacuole targeting pathway. *Journal of Biological Chemistry, 285(38),* 29599–29607. DOI 10.1074/jbc.M110.113670.
37. Xu, W., Dai, W., Yan, H., Li, S., Shen, H. et al. (2015). *Arabidopsis* NIP3;1 plays an important role in arsenic uptake and root-to-shoot translocation under arsenite stress conditions. *Molecular Plant, 8(5),* 722–733. DOI 10.1016/j.molp.2015.01.005.
38. Yang, L., You, J., Wang, Y., Li, J., Quan, W. et al. (2017). Systematic analysis of the G-box Factor 14-3-3 gene family and functional characterization of GF_{14a} in Brachypodium distachyon. *Plant Physiology and Biochemistry, 117,* 1–11. DOI 10.1016/j.plaphy.2017.05.013.
39. Weigel, R. R., Bäuscher, C., Pfitzner, A. J. P., Pfitzner, U. M. (2001). NIMIN-1, NIMIN-2 and NIMIN-3, members of a novel family of proteins from Arabidopsis that interact with NPR1/NIM1, a key regulator of systemic acquired resistance in plants. *Plant Molecular Biology, 46,* 43–160. DOI 10.1023/a:1010652620115.
40. Song, J., Xing, Y., Munir, S., Yu, C., Song, L. et al. (2016). An ATL_{78}-Like RING-H_2 finger protein confers abiotic stress tolerance through interacting with RAV_2 and CSN_5B in tomato. *Frontiers in Plant Science, 7,* 1305. DOI 10.3389/fpls.2016.01305.
41. Rivero, F., Kuspa, A., Brokamp, R., Matzner, M., Noegel, A. A. (1998). Interaptin, an actin-binding protein of the α-actinin superfamily in *Dictyostelium discoideum*, is developmentally and cAMP-regulated and associates with intracellular membrane compartments. *Journal of Cell Biology, 142(3),* 735–750. DOI 10.1083/jcb.142.3.735.
42. Chang, J., Xu, Z., Li, M., Yang, M., Qin, H. et al. (2019). Spatiotemporal cytoskeleton organizations determine morphogenesis of multicellular trichomes in tomato. *PLoS Genetics, 15(10),* e1008438. DOI 10.1371/journal.pgen.1008438.
43. Sibéril, Y., Doireau, P., Gantet, P. (2001). Plant bZIP G-box binding factors modular structure and activation mechanisms. *European Journal of Biochemistry, 268,* 5655–5666. DOI 10.1046/j.0014-2956.2001.02552.x.
44. Pertschy, B., Saveanu, C., Zisser, G., Lebreton, A., Tengg, M. et al. (2007). Cytoplasmic recycling of 60S preribosomal factors depends on the AAA protein Drg1. *Molecular and Cellular Biology, 27(19),* 6581–6659. DOI 10.1128/mcb.00668-07.
45. Ai, B., Gao, Y., Zhang, X., Tao, J., Kang, M. et al. (2015). Comparative transcriptome resources of eleven *Primulina* species, a group of stone plants from a biodiversity hotspot. *Molecular Ecology Resources, 15(3),* 619–632. DOI 10.1111/1755-0998.12333.