The genomic sequence and comparative genomic analysis of cultivated passion fruit(*Passiflora edulis* L.)

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Abstract: Cultivated passion fruit is a fruit tree widely cultivated in southern China, but little is known about its genomics, which seriously restricts the molecular genetics research of passion fruit. In this study, we analyzed the 165.7Mb representative genome sequences. The results showed that the passion fruit genome contained a large number of simple sequence repeats (SSR). Compared to the cassava and peach genomes, the passion fruit genome has 23,053 predicted genes. These genes can be aligned to 282 plant genomes. GO annotation indicated that these genes are involved in metabolic pathways of carbohydrates, organic acids, lipids and other molecules. KEGG pathway enrichment assigned these genes into five major categories and 19 secondary functions. Cluster analysis of gene families showed that 12,767 genes could be clustered into 9,868 gene families and 291 unique gene families. On the evolutionary relationship, the passion fruit is closely related to *Populus trichocarpa* and *Ricinus communis*, but the rate of evolution is slower. In summary, this genomic analysis result is informative, and will facilitate the future studies on gene functions of passion fruit.

Keywords: cultivated passion fruit (*Passiflora edulis* L.); genome; gene annotation; phylogenetic evolution; bioinformatics

1. Introduction

There are more than 530 species of passion fruit, and the most widely cultivated species is *Passiflora edulis*, which belongs to the *Theoideae* suborder, *Passifloraceae* family, and *Passiflora L.* genus [1]. Passion fruit has really high contents of nutrition, including sugar, fat, protein, vitamins and mineral elements [2,3]。

In eukaryotes, the genome is the entire genetic material of a single set of chromosomes in the species. Each cell of a plant contains three distinct genomes: nuclear genome, mitochondrial genome, and plastid genome. Currently, studies are mainly focused on the nuclear genome. Chromosomes are gene carriers, and the gene functions are closely related to the structural components on chromosomes. Genome sequencing can help us better understand the functions and evolution of plant genes.Currently, the genome sequencing study on passion fruit is still focused on the

development of molecular markers. Cerqueira-silva et al. [4] developed 69 pairs of SSR primers using two passion fruit genome microsatellite-enriched libraries. Santos et al. [5] used BAC end sequencing method to obtain 6,194,248 bp of passion fruit genome data, in which 669 microsatellite sequences were found, with an average of one SSR per 9.25 kb genome sequence. Later, Araya et al. [6] developed 816 pairs of SSR primers in the structural and functional regions using parts of the passion fruit genome sequence. The results showed that 53.2% of SSR primers were polymorphic. Recently, Costa et al. [7] sequenced the cDNA of Xanthomonas infected passion fruit, and developed the functional SSR and SNP markers.

With the rapid development of High-throughput sequencing, nearly 200 plants have been sequenced. In May 2017, the Beltsville Agricultural Research Center performed genome-wide sequencing on passion fruit CGPA1 using Illumina GAII sequencing technology, and assembled the sequencing results to the Scaffold level. However, they did not conduct genome analysis on these results. In this study, we performed genome annotation and comparative genomic analysis on passion fruit genome. Our results will facilitate the further studies on molecular mechanisms of passion fruit, and also provide references for the scientific development and efficient utilization of passion fruit.

2. Materials and Methods

2.1. Genomic Sequence of Passion Fruit

The passion fruit genome was uploaded to NCBI (https://www.ncbi.nlm.nih.gov/assembly/GCA_002156105.1/#/st) by the Beltsville Agricultural Research Center.

2.2. Genome Annotation of Passion Fruit

Identification of autonomous DNA transposon: The known autonomous DNA transposons in plants, such as Arabidopsis, were collected from public databases (Swiss-Prot and Repbase). Then, the transposons in passion fruit were identified by the software detectMITE [8].

Gene structure prediction: Homologous prediction was conducted by comparing the protein coding sequence of a known homologous species with the genomic sequence of a new species (the number of homologous species is no more than 5). The gene structures of new species were predicted by softwares such as BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), GeneWise [9], etc. De novo prediction used the software depending on statistical characteristics of genomic sequence data to predict gene structure. The commonly used software includes Augustus [10], Glimmer HMM [11], SNAP (http://homepage. Mac.com/iankorf/), etc. After performing the gene structure prediction, the results were combined with the transcriptome alignment data; then, these data were integrated by the EVidenceModeler software (http://evidencemodeler.sourceforge.net/) to generate a non-redundant, more complete gene set. Finally, the EVM annotation results were corrected using PASA (http://pasa.sourceforge.net/) and the transcriptome assembly data. The information such as UTR and variable cutting sites was added to obtain the final gene set.

Gene function annotation: The gene set obtained by gene structure annotation was compared with a known protein database by comparison software, in order to obtain the gene function information. The commonly used protein databases include SwissProt (http://www.uniprot.org/), KEGG (http://www.genome.jp/kegg/), InterPro (https://www.ebi.ac.uk/interpro), NR (ftp://ftp.ncbi.nlm.nih.gov/blast/db/) and GO (http://www.geneontology.org/).

2.3. Gene Family and Phylogenetic Tree

Gene family identification: The software OthoMCL[12] was used. The default e value was 1e-5 and the expansion coefficient was 1.5.

Phylogenetic analysis: The software MUSCLE [13] was used to compare different gene families. The sequence alignment results went through jModelTest/ProTest [14] software to find the optimal sequence substitution model. Then, the phylogenetic tree of

106 9 species was constructed by PhyML software [15] using the maximum likelihood
107 method.

108 **3. Results**

109 3.1. Assembly of Passion Fruit Genome

110 The research group at Beltsville Agricultural Research Center used Illumina GAII
111 technology to sequence the passion fruit CGPA1 genome. The average sequencing
112 depth was 4.5×, with 225,293,527 reads in total. Finally, 165,656,733 bp of passion
113 fruit genome sequence was obtained, with 235,883 Contig (Contig N50 was 1,303 bp,
114 Contig L50 was 30,212 bp) and 234,012 scaffolds (Scaffold N50 was 1,311 bp,
115 Scaffold L50 was 30,081 bp). The GC content of the genome was 38.6%.

116 3.2. Repeated Sequence Annotation

117 The SSR Search software [16] and homologous annotation were used to annotate
118 the repeated sequences in passion fruit genome. The results showed that there were
119 428,294 full-type SSR and 1,544,549 incomplete- and composite-type SSR [6]. For
120 transposons, there were 59 *Mutator* transposons, 41 *EnSpm* transposons, 49 *hAT*
121 transposons, 221 *PIF* transposons, and 2 *MLE* transposons.

122 3.2. Gene Annotation and Functional Enrichment Analysis of Passion Fruit Genome

123 Genetic structure prediction was conducted using homologous prediction and De
124 novo prediction. Using BLAST, GeneWise, and other alignment softwares, the
125 genomic sequence of passion fruit was compared with the coding sequences of known
126 homologous species *Manihot esculenta* [17] and *Prunus persica* [18] to predict the
127 gene structures in passion fruit genome. These prediction results were then combined
128 with the transcriptome alignment data, and all the gene sets predicted by different
129 methods were integrated by the EvidenceModeler software to generate a non-redundant
130 and more complete gene set. Finally, the EVM annotation results were corrected using
131 PASA and transcriptome assembly results. The information such as UTR and variable
132 cutting sites were added, and 23053 genes were eventually predicted.

The gene set obtained by gene structure prediction was blasted in NR, SwissPort, KEGG, InterPro, Pfam and GO databases, and the gene annotation information was shown in Table 1. In KEGG database, the passion fruit genome had 16,835 genes annotated. The gene length was 61-6994 bp, with an average of 670 bp. The total length of annotated genes was 11,784,169 bp, accounting for 7.1% of the whole genome. The predicted passion fruit genes can be mapped to the genomes of 282 plant species. Among these genes, 3,015 of them were aligned to the *Populus trichocarpa* genome, 2058 genes were mapped to the *Jatropha curcas* genome, 1,644 genes were aligned to the *Ricinus communis* genome, 630 genes were mapped to the *Theobroma cacao* genome [19], and 572 genes were aligned to the *Vitis vinifera* genome [20].

GO analysis was used to classify the functions of annotated genes into categories of Biological process, Cellular component and Molecular Function; then, these functions were further refined into 41 secondary functions (Figure 1). In the Biological process category, there were more genes involved in cellular process (GO: 0009987) and metabolic process (GO: 0008152), accounting for 4,689 and 5,047 genes, respectively; in the Cellular component category, more genes were involved in cell part (GO:0044464) and cell (GO: 0005623), both of which included 1,542 genes; in the Molecular Function category, the catalytic activity (GO: 0003824) and structural molecule activity (GO: 0005198) included more genes, accounting for 5,018 and 5,595, respectively. Since passion fruit has a pleasant aromatic odor and has high contents of sugar, fat, protein, vitamins and minerals [2,3], we focused our study on the metabolic processes of carbohydrates, organic acids, lipids, etc., and found that 1,356 genes were involved in the metabolism of aromatic compounds.

In living organisms, different genes were coordinated to perform biological functions. The same actions between different genes form a pathway, and the pathway-based analysis is helpful for further interpreting the gene functions. KEGG database was used to analyze the gene pathways, and the results showed that the gene

pathways were divided into five categories according to the pathway type (Figure 2): A: Cellular Processes; B: Environmental Information Processing; C: Genetic Information Processing; D: Metabolism; E: Organismal Systems. These five categories can be subdivided into 19 secondary functional classes. Among the 1,1325 genes, 61.6% were associated with metabolic pathways, and the largest group was related to carbohydrate metabolism. Glucose, sucrose, starch and cellulose are the main forms of carbohydrates. Studies have shown that passion fruit is rich in sugars and fats [2,3]. In the passion fruit genome, there were only 570 genes involved in environmental adaptation, suggesting that passion fruit may be less capable to resist biological or non-biological stresses.

3.3. Gene Family and Phylogenetic Analysis

Based on the passion fruit genome annotation results and the previous studies [1,5], we performed gene family analysis using another nine species, which were *Actinidia chinensis* [21], *Theobroma cacao* [19], *Vitis vinifera* [20], *Arabidopsis thaliana* [22], *Populus euphratica* [23], *Prunus persica* [18], *Ricinus communis* [24], and *Oryza sativa L. ssp. japonica* [25]. The number of aligned genes in each species is shown in Table 2. Via cluster analysis of gene families, we found 12,767 genes of passion fruit could be clustered into 9868 gene families, with an average of 1.29 genes per family. Moreover, there were 291 gene families that were unique for passion fruit (Figure 3).

Referring to the study from Santos et al. [5], we selected the genomes from *Actinidia chinensis* [21], *Theobroma cacao* [19], and *Vitis vinifera* [20] to perform homologous analysis with the predicted genes of passion fruit (Figure 4). The results showed that *Theobroma cacao* had the most homologous genes with passion fruit. Using *Oryza sativa L. ssp. japonica* genome as the reference, we also did phylogenetic analysis on the nine species with homologous genes (Figure 5). The cluster analysis showed that the monocots were clearly separated from the dicots. Also, passion fruit was evolutionarily closer to *Populus trichocarpa* and *Ricinus communis*, but the evolution rate was slow.

Discussion

The passion fruit genome is rich in repetitive elements, which can be used to develop molecular markers. In our previous study, We identified 13,104 perfect SSRs in the 165.6 Mb of cultivated passion fruit genome. Then we developed 12,934 pairs of SSR primers using a full-type SSR, and the SSR marker showed good polymorphism [16]. According to the different transposon vectors, transposons can be divided into two types: retrotransposons (Class I) and DNA transposons (Class II). The former is mediated by RNA and the latter is mediated by DNA. MITEs (Miniature Inverted Repeat Transposable Elements) are a special class of non-autonomous DNA transposons that are distributed in high-copy form in the genome of plants. The MITEs transposon marker developed by MITEs can only amplify two bands in general, and the PCR product can be efficiently isolate by conventional agarose gel electrophoresis, so the marker is highly efficient and co-dominant molecular marker. We used softwares to identify the MITEs transposon of the cultivated passion fruit genome, and obtained 372 transposons and their flanking sequences, which was important for the development of MITEs markers.

The 165.7Mb of passion fruit genome sequence was used to perform gene annotation with homologous species *Manihot esculenta* [17] and *Prunus persica* [18], and a total of 23,053 genes were predicted. The passion fruit genome size is 1,230 Mb [26], and the genome size involved in this study is approximately 13.5% of the total genome length. Therefore, we need to assemble the passion fruit genome to a higher level using high-throughput sequencing, especially at the chromosomal level, is particularly important.

By comparing the predicted protein sequences of passion fruit genome with the known protein sequences, we found that there were more genes related to carbohydrate metabolism, consistent with the fact that passion fruit is rich in sugar, fat, protein, vitamins and mineral elements [2,3]. However, there were less genes involved in

environmental adaption in passion fruit genome, indicating that passion fruit may have poor capability to resist biological or non-biological stresses. At present, the main diseases of passion fruit are viral diseases, bacterial diseases and fungal diseases, among which fungal stem rot is particularly serious.

The comparison between passion fruit genome and the genomes of other eight species showed that only a few genes were unique in passion fruit. The unique family mainly contain genes of unkwnown functional proteins, retrovirus-related Pol polyprotein, zinc finger domain (CH2H2) proteins, and putative ribonuclease H protein. A number of genes are associated with retrovirus, which may suggest an important cause of the serious occurrence of viral disease in passion fruit. Specific regulatory sequences on DNA can bind to the corresponding regulatory proteins (transcription factors) and promote the initiation of transcription. In the unique family of passion fruit, the transcription factor family contains many genes, which may indicate that rich gene expression patterns are necessary for the continuous adaptation of passion fruit to the environment and to adjust its growth and metabolism.Moreover, in the evolutionary relationship, passion fruit is closer to *Populus trichocarpa* [23] and *Ricinus communis* [24], but the evolution rate is slower.

ACKNOWLEDGMENTS

We thank Beltsville Agricultural Research Center. This work was supported by the Guangxi Natural Science Foundation of China (2018GXNSFBA281024, 2018GXNSFAA138124) and Guangxi Academy of Agricultural Sciences (2018YT19).

DATA ACCESSIBILITY

All sequence data are gained from Genome Information for *Passiflora edulis* (BioProjects: PRJNA371406).

AUTHOR CONTRIBUTIONS

X.H., Yang, Y.Y., Wu contributed to study design, Q.L., Tian, J.Y., Liu, Y.C., Huang, W.H., Huang contributed to data analysis, X.Z., Xia, H.F., Mou contributed to make tables and figures. All authors read and approve the paper.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Table 1 The annotation cultivated passion fruit genome.

Database	Annotated Num	Annotated Percent(%)
NR	22062	95.7
Swiss-Prot	17974	78
KEGG	16835	73
InterPro	20786	90.2
Pfam	15341	66.5
GO	11108	48.2
Annotated	22200	96.3
Total	23053	-

Table 2 Genes used for gene family clustering in nine species.

Species	Genes number	Genes in families	Unclustered genes	Family number	Unique families	Average genes per family
Arabidopsis thaliana	48321	44484	3837	15029	2081	2.96
Theobroma	30854	29472	1382	15251	439	1.93

cacao						
Actinidia chinensis	33115	29260	3855	14583	629	2.01
Populus trichocarpa	51717	49631	2086	15441	797	3.21
Prunus persica	47089	42308	4781	15830	1115	2.67
Vitis vinifera	29927	21929	7998	14789	729	1.48
Ricinus communis	28584	26580	2004	15009	329	1.77
Oryza sativa	42132	28555	13577	13886	2587	2.06
Passiflora eduis	23053	12767	10286	9868	291	1.29

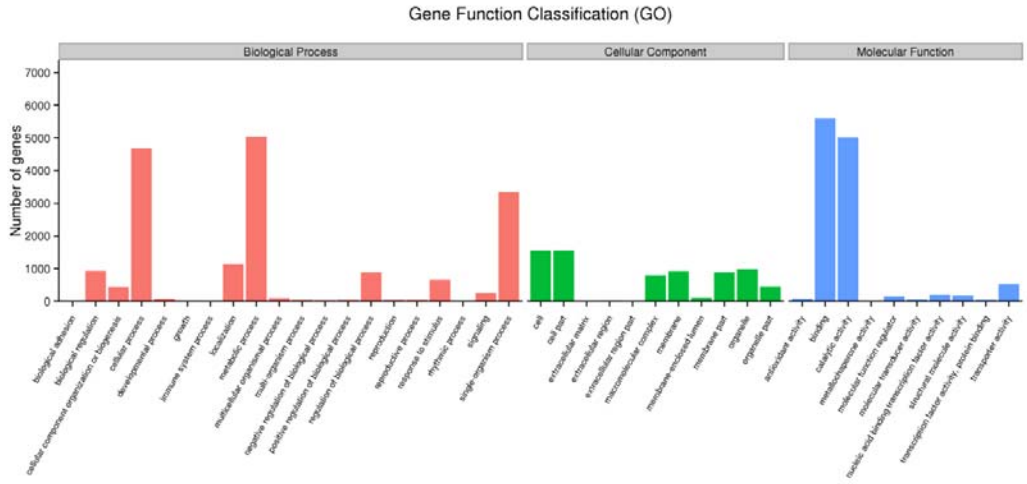

Figure 1. GO function analysis of the annotated genes.

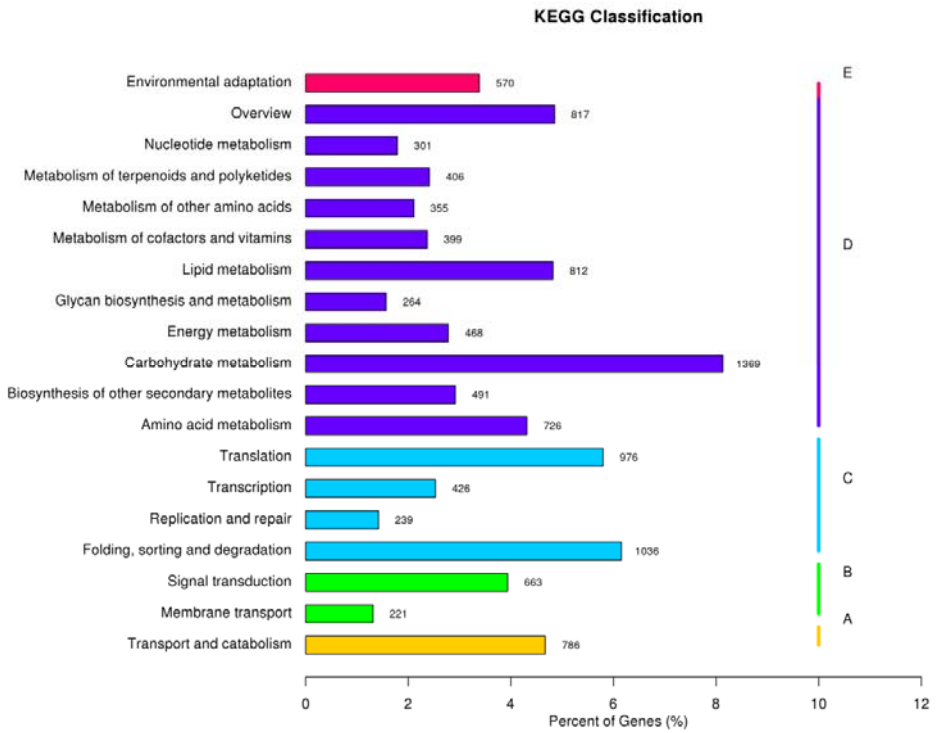

Figure 2. Pathway classification of the annotated genes.

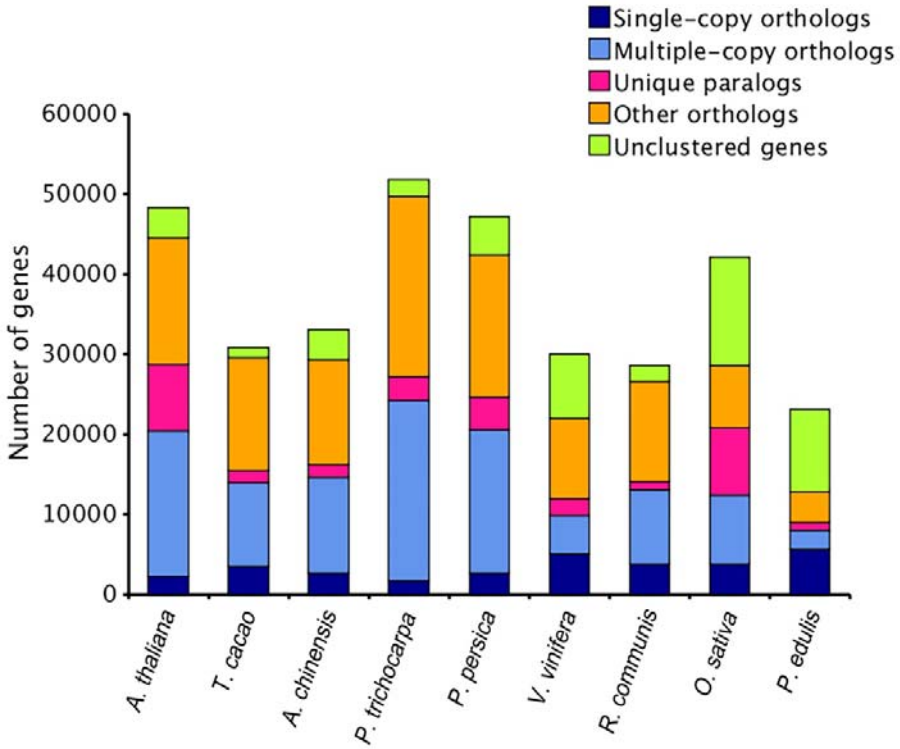

Figure 3. Gene family clustering in 9 species.

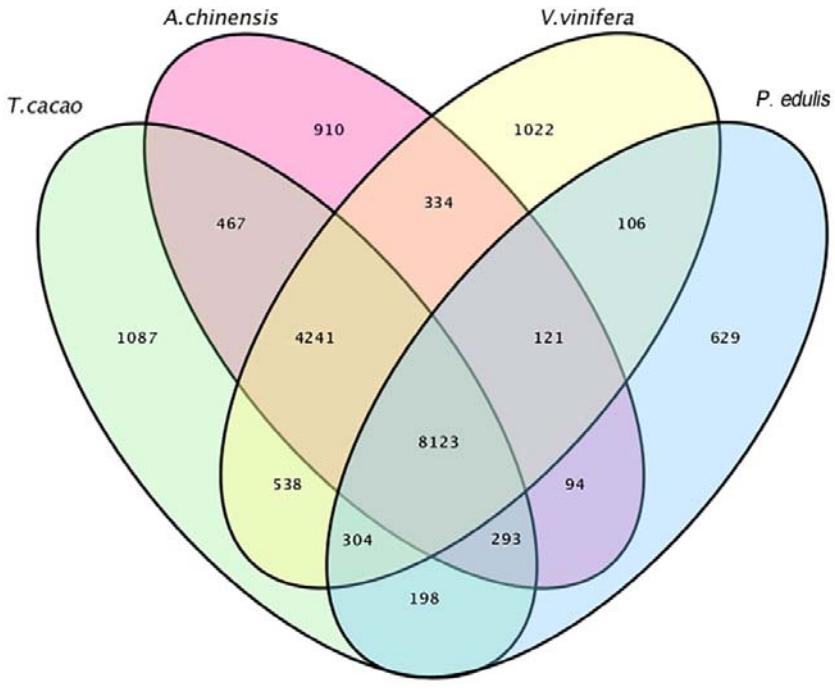

Figure 4. Homology analysis in 4 species.

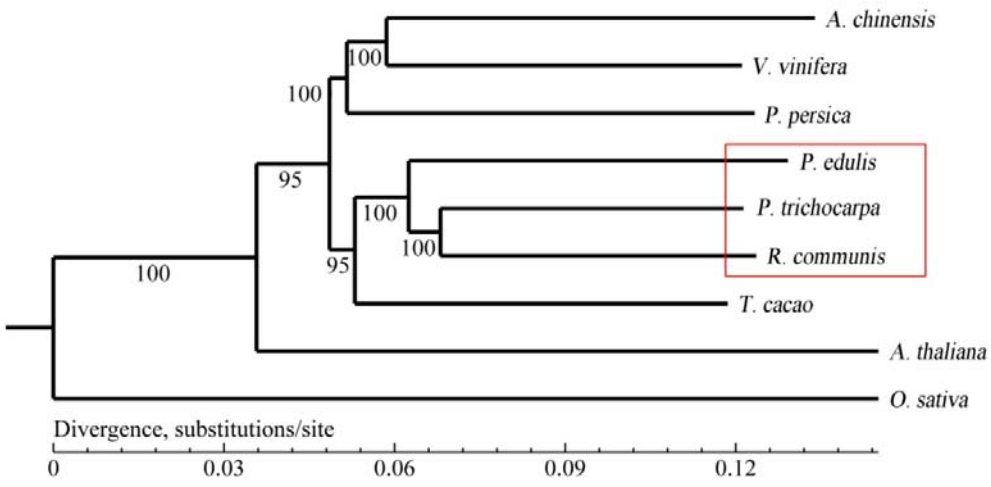

Figure 5. Phylogenetic analysis of 9 species.

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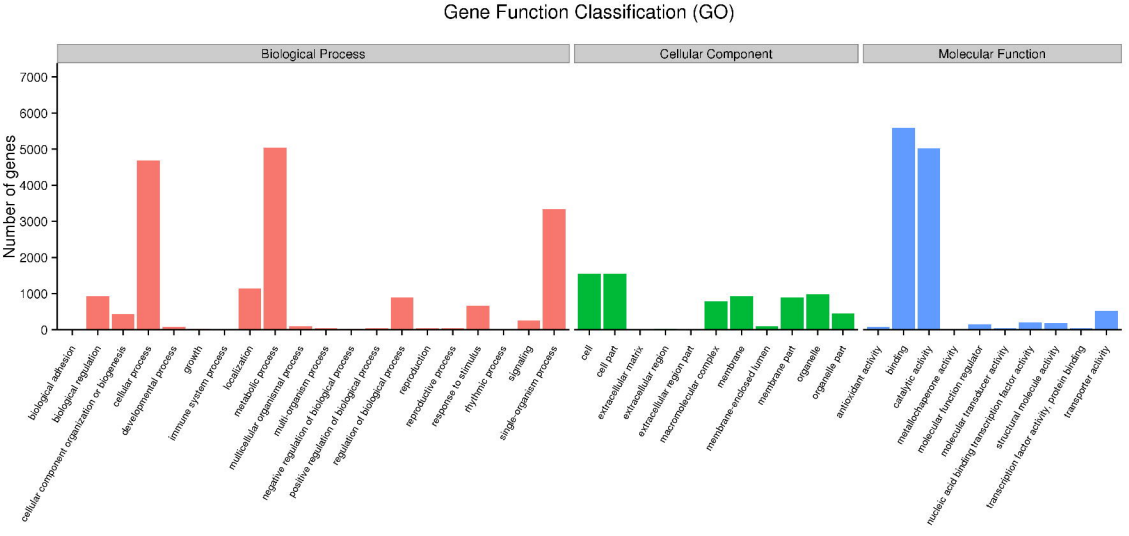
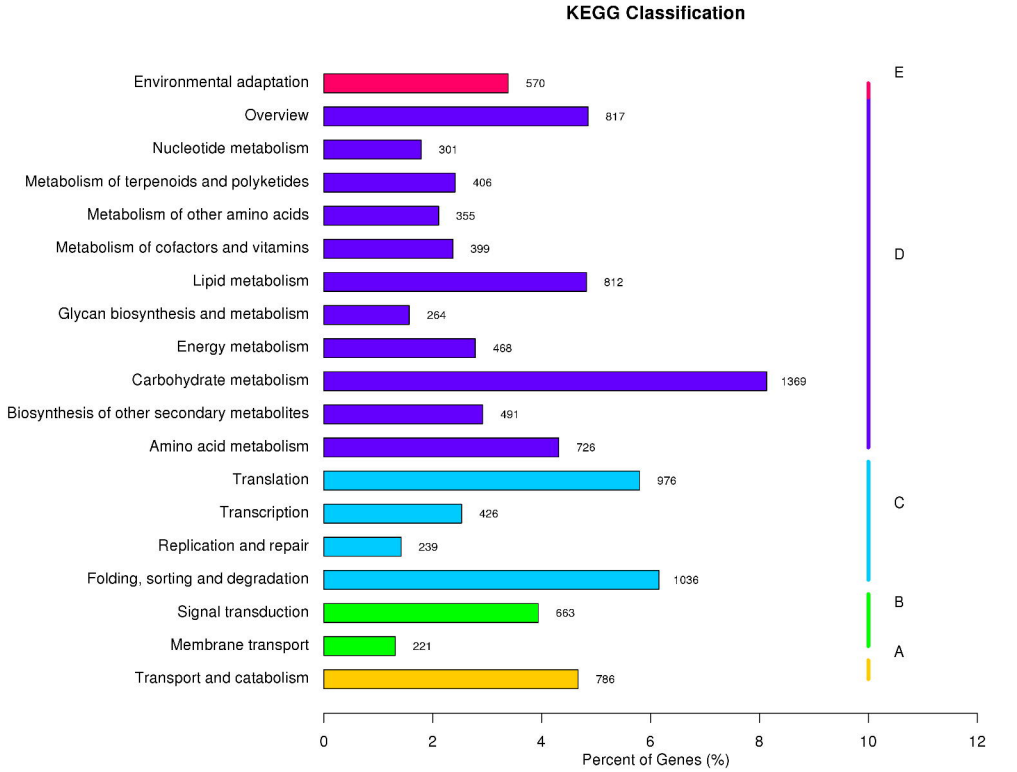

Gene Function Classification (GO)
Biological Process
Cellular Component
Molecular Function
Number of genes
7000
6000
5000
4000
3000
2000
1000
0
biological adhesion
biological regulation
cellular component organization or biogenesis
cellular process
developmental process
growth
immune system process
localization
metabolic process
multicellular organismal process
multi-organism process
negative regulation of biological process
positive regulation of biological process
regulation of biological process
reproduction
reproductive process
response to stimulus
rhythmic process
signaling
single-organism process
cell
cell part
extracellular matrix
extracellular region
extracellular region part
macromolecular complex
membrane
membrane-enclosed lumen
membrane part
organelle
organelle part
antioxidant activity
binding
catalytic activity
metallochaperone activity
molecular function regulator
molecular transducer activity
nucleic acid binding transcription factor activity
structural molecule activity
transcription factor activity, protein binding
transporter activity


KEGG Classification
Environmental adaptation
Overview
Nucleotide metabolism
Metabolism of terpenoids and polyketides
Metabolism of other amino acids
Metabolism of cofactors and vitamins
Lipid metabolism
Glycan biosynthesis and metabolism
Energy metabolism
Carbohydrate metabolism
Biosynthesis of other secondary metabolites
Amino acid metabolism
Translation
Transcription
Replication and repair
Folding, sorting and degradation
Signal transduction
Membrane transport
Transport and catabolism
570
817
301
406
355
399
812
264
468
1369
491
726
976
426
239
1036
663
221
786
E
D
C
B
A
0
2
4
6
8
10
12
Percent of Genes (%)