Full-length SMRT transcriptome sequencing and microsatellite characterization in *Paulownia catalpifolia*

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*Paulownia catalpifolia* is an important, fast-growing timber species known for its high density, color and texture. However, few transcriptomic and genetic studies have been conducted in *P. catalpifolia*. In this study, single-molecule real-time sequencing technology was applied to obtain the full-length transcriptome of *P. catalpifolia* leaves treated with varying degrees of drought stress. The sequencing data were then used to search for microsatellites, or simple sequence repeats (SSRs). A total of 28.83 Gb data were generated, 25,969 high-quality (HQ) transcripts with an average length of 1624 bp were acquired after removing the redundant reads, and 25,602 HQ transcripts (98.59%) were annotated using public databases. Among the HQ transcripts, 16,722 intact coding sequences, 149 long non-coding RNAs and 179 alternative splicing events were predicted, respectively. A total of 7367 SSR loci were distributed throughout 6293 HQ transcripts, of which 763 complex SSRs and 6604 complete SSRs. The SSR appearance frequency was 28.37%, and the average distribution distance was 5.59 kb. Among the 6604 complete SSR loci, 1–3 nucleotide repeats were dominant, occupying 97.85% of the total SSR loci, of which mono-, di- and tri-nucleotide repeats were 44.68%, 33.86% and 19.31%, respectively. We detected 112 repeat motifs, of which A/T (42.64%), AG/CT (12.22%), GA/TC (9.63%), GAA/TTC (1.57%) and CCA/TGG (1.54%) were most common in mono-, di- and tri-nucleotide repeats, respectively. The length of the repeat SSR motifs was 10–88 bp, and 4997 (75.67%) were ≤ 20 bp. This study provides a novel full-length transcriptome reference for *P. catalpifolia* and will facilitate the identification of germplasm resources and breeding of new drought-resistant *P. catalpifolia* varieties.

*Paulownia*, one of the most important fast-growing timber species around the world, is native to China and widely grown in subtropical and warm temperate regions, which have acted an important part of timber supply, ecological environmental construction, soil improvement and so forth. *Paulownia catalpifolia* is a typical and important species of Genus Paulownia in northern China, it exhibits some drought resistance and is renowned for its high density, good color, and beautiful texture. Recently, droughts and water shortages have seriously affected *P. catalpifolia* growth, causing mass deaths in some *P. catalpifolia* plantations. Therefore, high-quality and drought-resistant *P. catalpifolia* varieties are urgently needed. Conventional plant breeding methods, such as cross-breeding and selection breeding, have yielded little success in improving the traits of plants; this is due to genetic resistance, reproductive isolation and long generation cycles, among other factors. Previous studies on *P. catalpifolia* have focused on the chemical composition of its fruits and seeds, as well as on tissue culture; however, molecular studies of *P. catalpifolia* are lacking.

Microsatellites, also known as simple sequence repeats (SSRs), are DNA sequences consisting of continuously repeating motifs, which are composed of 1–6 bases. The type and number of repeat motifs differ among SSRs, resulting in polymorphisms at each SSR locus. SSR molecular markers are widely distributed throughout plant genomes and are characterized by codominance, high polymorphism and good repeatability. SSR loci are

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transcriptome sequencing studies in animals, plants and insects. Furthermore, full-length transcriptome NAs are vital for regulating the neighboring gene expression. A total of 149 common lncRNAs were identified.

Non-coding RNAs (lncRNAs) are not translated into protein and their length are more than 200 nucleotides. LncRNAs and SSRs are more conserved, better universality, lower cost and more interspecific transferability. Moreover, EST-SSR polymorphisms may be directly related to gene function and can be used for researches of other related species. In recent years, EST-SSR markers have been developed and applied in various tree species, including Eucalyptus globulus, Euphrates Poplar, the rubber tree, Robinia pseudoacacia, Fraxinus velutina, and Pinus koraiensis.

Single-molecule real-time (SMRT) sequencing technology (Pacific Biosciences), also known as third generation sequencing technology, can efficiently and accurately obtain high-quality (HQ) long and intact transcripts containing 5′- and 3′-untranslated regions and polyadenosine tails without assembly. SMRT sequencing can be used to accurately identify features such as fusion genes, gene families, long non-coding RNAs (lncRNAs) and alternative splicing (AS) events. SMRT sequencing technology is a reliable method for obtaining full-length transcripts that can be used to study the transcriptomes of non-model plants which lack reference genomes, such as Paulownia and Chinese catalpa. SMRT sequencing technology has been successfully applied to full-length transcriptome sequencing studies in animals, plants and insects. Furthermore, full-length transcriptome sequences obtained using SMRT sequencing contain numerous EST SSRs, which can be used for genetic analyses of the sequenced species and their related species, as well as for studies of conservation biology and molecular assisted breeding. To the best of our knowledge, no full-length transcriptome sequence of P. catalpifolia has been reported.

In this study, we performed a full-length transcriptomic analysis of mixed P. catalpifolia leaves treated with varying degrees of drought stress using SMRT sequencing. We then performed function annotation analyses using publicly available databases and used various bioinformatics software to predict AS, lncRNAs and SSRs and to further analyze SSRs characteristics deeply. In the absence of Paulownia reference genome, the full-length transcriptome sequence acquired in our study not only can be used as a reference sequence for transcriptome sequencing, but also will support further genetic analyses in Paulownia species. In addition, the SSRs predicted in our study will facilitate the development of drought-resistant SSR markers, the discovery of drought-resistant genes and the study of the genetic relationships between P. catalpifolia and other related species.

**Results**

**SMRT sequencing of the full-length transcriptome.** We acquired full-length transcriptomic of P. catalpifolia using SMRT sequencing technology and obtained 28.83 Gb sequencing data. After removing the adapter sequences, approximately 454,554 polymerase reads remained, which then formed 19,052,345 subreads with an average read length of 1470 bp. After self-correction and merging, the subreads formed 405,034 circular consensus sequences (CCSs) (Fig. 1a) with an average length of 1693 bp, and 349,745 full-length non-chimeric sequences (FLNCs) (Fig. 1b). A total of 30,953 transcripts were obtained after clustering and removal of redundant sequences using the PacBio SMRT LINK Cluster tool, and 30,928 HQ transcripts with ≥ 99% accuracy and a full-length read support ≥ 2 were sequenced (Fig. 1c). The length range of the HQ transcripts was 362–7922 bp, the N50 was 1768 bp, and the mean transcript length was 1618 bp. Of the HQ transcripts, 10.47% and 86.07% were 362–900 bp and 1000–3000 bp in length, respectively. Long-length HQ transcripts (> 3000 bp) constituted 3.46% of the total HQ transcripts. After error correction and removal of all 100% identical sequences, 25,969 HQ transcripts remained, its individual transcript length ranging from 362 to 7922 bp, the average length of 1624 bp, and N50 of 1781 bp, which were used in subsequent analyses.

**Functional annotation of the full-length transcriptome sequences.** The functional annotation of the HQ transcripts was then performed. Of the 25,969 transcripts analyzed, 367 could not be functionally assigned by any of the databases used (Table 1). A total of 25,591 (98.54%) HQ transcripts were annotated using the NCBI non-redundant protein database and exhibited homology with known proteins of various species, including Sesamum indicum (75.38%), Erythranthe guttata (12.87%) and Dorcoceras hygrometricum (1.71%) (Fig. 2). The HQ transcripts were then searched against the gene ontology (GO) database to analyze their functions; 18,501 (71.24%) of the HQ transcripts were categorized into 50 GO group, which were divided into three broad classes: biological processes (37,536 HQ transcripts, 38.38%), cellular components (38,888, 39.76%) and molecular functions (21,377, 21.86%) (Fig. 3a). Following searches against the eukaryotic orthologous groups (KOG) database, the HQ transcripts were clustered into 26 KOG terms (Fig. 3b). Furthermore, 13,829 HQ transcripts were identified in the Kyoto encyclopedia of genes and genomes (KEGG) database and grouped into 129 KEGG pathways, which were divided into five broad categories: cellular processes (779 HQ transcripts, 5.63%), environmental information processing (523, 3.78%), genetic information processing (3207, 23.19%), metabolism (8962, 64.81%) and organismal systems (358, 2.59%) (Fig. 3c). Using Swiss-Prot, 22,606 (87.05%) HQ transcripts were annotated.

**Identification of long non-coding RNAs, coding sequences and alternative splicing.** The long non-coding RNAs (lncRNAs) are not translated into protein and its length are more than 200 nucleotides. LncRNAs are vital for regulating the neighboring gene expression. A total of 149 common lncRNAs were identified in P. catalpifolia using four methods (CPC2, CPAT, PLEK and CNCI) (Fig. 4a). TransDecoder software was used to predict 24,982 coding sequences (CDSS), of which 16,722 were intact. The lengths of the amino acids encoded by the intact CDSS were in the range of 100–1840, with the number of amino acids decreasing as the length increased except 100–300 (Fig. 4b). Alternative splicing (AS) is one of crucial biological phenornons, and it is helpful to produce different mature transcripts using the same RNA sequence. AS is highly correlated with...
biological function and a major source of proteomic diversity. A total of 179 AS events were predicted without reference to genomic information in our research.

Identification and characteristic analysis of SSRs. SSR loci were identified within the *P. catalpifolia* full-length transcriptome using MISA microsatellite software. A total of 7367 SSRs were identified, including

![Figure 1](https://example.com/figure1.png)

**Figure 1.** SMRT sequencing of *P. catalpifolia* leaf transcriptomes. (a) Length distribution of CCSs. (b) Length distribution of FLNCs. (c) Length distribution of high-quality transcripts. Figure was made by Microsoft Office Excel 2013 software.

| Database                   | Number of HQ transcripts | Percentage (%) |
|----------------------------|--------------------------|----------------|
| Annotated in NR            | 25,591                   | 98.54          |
| Annotated in GO            | 18,501                   | 71.24          |
| Annotated in KOG           | 12,350                   | 47.56          |
| Annotated in Swiss-Prot    | 22,606                   | 87.05          |
| Annotated in KEGG          | 13,829                   | 53.25          |
| Unannotated                | 367                      | 1.41           |
| Total HQ isoforms          | 25,969                   | 100            |

**Table 1.** Results of the functional annotation of 25,969 HQ transcripts.
Figure 2. The Homologous species distribution of *P. catalpifolia* HQ transcripts. Figure was made by Microsoft Office Excel 2013 software.

**Figure 3.** Gene ontology (GO), eukaryotic orthologous groups (KOG) and Kyoto encyclopedia of genes and genomes (KEGG) functional classifications of high-quality (HQ) transcripts. (a) GO classification of HQ transcripts. (b) KOG classification of HQ transcripts. (c) KEGG classification of HQ transcripts.

763 complex SSRs and 6604 complete SSRs. The total number of HQ transcripts containing SSRs was 6293, of which 747 contained ≥ 2 SSRs. SSRs occurred at a frequency of 24.23% (100% × total number of HQ transcripts examined). The average distribution distance was 5.59 kb and the SSR appearance frequency was 28.37% (100% × total number of SSRs identified / total number of HQ transcripts examined). The complete SSR lengths ranged from 10 to 88 bp, with a mean of 15.99 bp. The number of repeat SSR motifs ranged from 5 to 44, with a mean of 10.03. We found that SSRs with 6 motif repeats were the most common and accounted for 13.64% of the total SSR loci, followed by SSRs with 10 repeats (1263, 17.51%), 5 repeats (1131, 15.34%) and 11 repeats (1057, 14.57%). The total number of HQ transcripts containing SSRs was 6293, of which 2951 mononucleotide (44.68%), 2236 dinucleotide (33.86%), 1275 trinucleotide (19.31%), 50 tetranucleotide (0.76%), 24 pentanucleotide (0.36%) and 68 hexanucleotide SSRs (1.03%) (Fig. 4). The complete SSR lengths ranged from 10 to 88 bp, with a mean of 15.99 bp. The number of repeat SSR motifs ranged from 5 to 44, with a mean of 10.03. We found that SSRs with 6 motif repeats were the most common and accounted for 13.64% of the total SSR loci, followed by SSRs with 10 repeats (1263, 17.51%), 5 repeats (1131, 15.34%) and 11 repeats (1057, 14.57%).

A total of 112 repeat motifs were identified among the complete SSRs, of which there were 2 mononucleotides, 8 dinucleotides, 30 trinucleotides, 24 tetranucleotides, 12 pentanucleotides and 36 hexanucleotides, respectively. Although SSR repeat types from mononucleotide to hexanucleotide all existed and they were also abundant, their occurrence frequency was quite different. The proportion of mononucleotide repeats dominated...
Figure 4. The identification of IncRNAs and the proteins length distribution of the *P. catalpifolia* transcriptome. (a) The Venn diagram of the number of IncRNAs predicted by CPC2, CPAT, PLEK and CNCI. (b) The length distribution of the proteins translated using predicted intact CDSs.

Table 2. Occurrence of microsatellites in the full-length transcriptome of *P. catalpifolia*.

| Item                              | Number |
|-----------------------------------|--------|
| Total number of HQ transcripts examined | 25,969 |
| Total size of the examined HQ transcripts (bp) | 42,183,906 |
| Total number of HQ transcripts containing SSRs | 6293 |
| Total number of SSRs identified  | 7367   |
| Total number of complex SSRs identified | 763     |
| Number of HQ transcripts containing more than one SSR | 747     |

Figure 5. The types and numbers of complete SSRs in *P. catalpifolia*. Figure was made by Microsoft Office Excel 2013 software.
| Repeat motif length | Repeat number | Total number | Frequency (%) |
|---------------------|---------------|--------------|---------------|
|                     | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | > 12 |            |             |
| A/T                | 704| 577| 403| 1132|     |     |     |     |     |     | 2816       | 42.64       |
| C/G                | 16 | 26 | 18 | 75 |     |     |     |     |     |     | 135        | 2.04        |
| AC/GT              | 35 | 28 | 32 | 44 | 27 | 13 | 14 | 41 |     |     | 234        | 3.54        |
| CA/TG              | 78 | 33 | 33 | 15 | 7  | 16 | 5  | 58 |     |     | 215        | 3.26        |
| AG/CT              | 230| 121| 93 | 52 | 58 | 46 | 37 | 170|     |     | 807        | 12.22       |
| GA/TC              | 180| 96 | 66 | 56 | 48 | 38 | 22 | 130|     |     | 656        | 9.63        |
| AT/AT              | 45 | 22 | 19 | 31 | 13 | 14 | 14 | 13 |     |     | 171        | 2.59        |
| TA/TA              | 38 | 34 | 29 | 21 | 12 | 10 | 9  | 12 |     |     | 165        | 2.50        |
| GC/GC              | 2  | 2  |     |     |     |     |     |     |     |     | 4          | 0.06        |
| CG/CG              | 4  |     |     |     |     |     |     |     |     |     | 4          | 0.06        |
| AAC/GTT            | 2  | 3  | 1  | 1  |     |     |     |     |     |     | 7          | 0.11        |
| AAG/CTT            | 49 | 11 | 5  | 5  | 1  | 1  | 1  | 1  |     |     | 78         | 1.18        |
| AAT/ATT            | 8  | 5  | 5  | 1  | 1  |     |     |     |     |     | 21         | 0.32        |
| ACA/TGT            | 4  | 1  |     |     |     |     |     |     |     |     | 5          | 0.08        |
| ACC/GGT            | 26 | 14 | 5  | 2  | 1  |     |     |     |     |     | 48         | 0.73        |
| ACG/CGT            | 1  | 2  | 3  |     |     |     |     |     |     |     | 6          | 0.09        |
| ACT/AGT            | 12 | 4  | 1  |     |     |     |     |     |     |     | 17         | 0.26        |
| AGA/TCT            | 42 | 14 | 5  | 7  | 5  | 2  | 4  | 1  | 2  |     | 82         | 1.24        |
| AGC/GCT            | 34 | 5  | 5  | 5  | 1  |     |     |     |     |     | 50         | 0.76        |
| AGG/CCT            | 27 | 8  | 5  | 1  |     |     |     |     |     |     | 41         | 0.62        |
| ATA/TAT            | 11 | 2  | 1  | 1  |     |     |     |     |     | 16         | 0.24        |
| ATC/GAT            | 26 | 5  | 9  | 1  | 1  |     |     |     |     |     | 42         | 0.64        |
| ATG/CAT            | 34 | 17 | 1  | 5  | 3  |     |     |     |     |     | 60         | 0.91        |
| CAA/TTG            | 13 | 2  | 2  | 2  | 1  |     |     |     |     |     | 20         | 0.30        |
| CAC/GTG            | 30 | 17 | 6  | 5  | 2  |     |     |     |     |     | 60         | 0.91        |
| CAG/CTG            | 40 | 12 | 3  | 19 | 4  | 2  | 1  | 81  |     |     | 102        | 1.54        |
| CCA/TGG            | 72 | 17 | 4  | 5  | 3  | 1  |     |     |     |     | 102        | 1.54        |
| CCG/CGG            | 34 | 24 | 11 | 5  | 4  |     |     |     |     |     | 78         | 1.18        |
| CGA/TCG            | 2  | 6  |     |     |     |     |     |     |     |     | 8          | 0.12        |
| CGC/GCG            | 26 | 1  | 2  | 5  | 1  |     |     |     |     |     | 35         | 0.53        |
| CTA/TAG            | 9  | 1  |     |     |     |     |     |     |     |     | 10         | 0.15        |
| CTC/GAG            | 38 | 16 | 4  | 1  | 1  |     |     |     |     |     | 60         | 0.91        |
| GAA/TTC            | 50 | 25 | 13 | 8  | 2  | 3  | 1  | 1  | 1  |     | 104        | 1.57        |
| GAC/GTC            | 7  | 2  | 1  |     |     |     |     |     |     |     | 10         | 0.15        |
| GCA/TGC            | 24 | 5  | 6  | 5  |     |     |     |     |     |     | 40         | 0.61        |
| GCC/GGC            | 35 | 18 | 4  | 1  |     |     |     |     |     |     | 58         | 0.88        |
| GGA/TCC            | 26 | 10 | 6  | 3  | 3  |     |     |     |     |     | 48         | 0.73        |
| GTA/TAC            | 1  | 1  |     |     |     |     |     |     |     |     | 2          | 0.03        |
| TAA/TTA            | 6  | 5  | 1  | 5  | 1  |     |     |     |     |     | 18         | 0.27        |
| TCA/TGA            | 49 | 6  | 6  | 6  | 1  |     |     |     |     |     | 68         | 1.03        |
| ATCA/TGAT          | 1  |     |     |     |     |     |     |     |     |     | 1          | 0.02        |
| TTTG/CAA           | 3  |     |     |     |     |     |     |     |     |     | 3          | 0.05        |
| AAAT/ATT           | 2  | 1  |     |     |     |     |     |     |     |     | 3          | 0.05        |
| GGAA/TTCC          | 1  |     |     |     |     |     |     |     |     |     | 1          | 0.02        |
| CCCT/AGGG          | 1  | 7  |     |     |     |     |     |     |     |     | 8          | 0.12        |
| TTTA/TAAA          | 2  |     |     |     |     |     |     |     |     |     | 2          | 0.03        |
| TGTA/TACA          | 1  |     |     |     |     |     |     |     |     |     | 1          | 0.02        |
| TTCT/AGAA          | 2  |     |     |     |     |     |     |     |     |     | 2          | 0.03        |
| ACAG/CIGT          | 2  |     |     |     |     |     |     |     |     |     | 2          | 0.03        |
| GAAA/TTTC          | 2  | 1  |     |     |     |     |     |     |     |     | 3          | 0.05        |
| TGA/TCCA           | 4  |     |     |     |     |     |     |     |     |     | 4          | 0.06        |
| TCTT/AAGA          | 1  |     |     |     |     |     |     |     |     |     | 1          | 0.02        |
| ATGT/ACAT          | 1  |     |     |     |     |     |     |     |     |     | 1          | 0.02        |
| CGTG/CAGG          | 1  |     |     |     |     |     |     |     |     |     | 1          | 0.02        |
| GATT/AATC          | 4  |     |     |     |     |     |     |     |     |     | 4          | 0.06        |

Continued
| Repeat motif length | Repeat number | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | >12 | Total number | Frequency (%) |
|---------------------|---------------|---|---|---|---|---|-----|-----|-----|-----|-------------|--------------|
| TCTA/TAGA           |               | 1 |   |   |   |   |     |     |     |     | 1           | 0.02         |
| CTTT/AAAG          |               | 2 | 1 |   |   |   |     |     |     |     | 3           | 0.05         |
| ATAC/GTAT           |               |   |   |   |   |   | 1   |     |     |     | 1           | 0.02         |
| TTGT/ACAA          |               |   |   |   |   |   |     | 1   |     |     | 1           | 0.02         |
| GCCC/GGGC          |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| GGAG/CTCC          |               |   |   |   | 2 | 1 |     |     |     |     | 3           | 0.05         |
| CAAC/GTTG          |               |   |   |   |   |   | 1   |     |     |     | 1           | 0.02         |
| AATA/TATT          |               |   |   |   |   |   |     | 1   |     |     | 1           | 0.02         |
| AAAC/TTTT          |               | 1 |   |   |   |   |     |     |     |     | 2           | 0.03         |
| CCACC/GGTTG        |               |   |   |   |   | 9 |     |     |     |     | 9           | 0.14         |
| TGATG/CATCA        |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| TCCTC/GAGGA        |               |   |   |   | 2 | 2 |     |     |     |     | 4           | 0.06         |
| CCACA/TGGGG        |               |   |   |   |   |   | 1   |     |     |     | 1           | 0.02         |
| CCTTT/AAAGAG       |               |   |   |   | 1 | 1 |     |     |     |     | 2           | 0.03         |
| CACCT/AAATG        |               |   |   |   |   |   | 1   |     |     |     | 1           | 0.02         |
| TTCTT/AAAGAG       |               |   |   |   |   |   | 1   |     |     |     | 1           | 0.02         |
| TATTT/AAAGTA       |               |   |   |   |   |   | 1   |     |     |     | 1           | 0.02         |
| CACCC/GGGGTG       |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CCCAC/GTGGGG       |               |   |   |   |   |   | 1   |     |     |     | 1           | 0.02         |
| CTCTT/AAAGAG       |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| AGCTT/AAAGCT       |               |   |   |   |   |   | 1   |     |     |     | 1           | 0.02         |
| AAAAAG/CTTTTT      |               |   |   |   |   | 2 |     |     |     |     | 2           | 0.03         |
| AAGGAGA/TCTTTT     |               |   |   |   |   | 8 |     |     |     |     | 8           | 0.12         |
| ACAGGGG/CCCTGT     |               |   |   |   | 2 | 2 |     |     |     |     | 2           | 0.03         |
| ACTCCG/GGGGTG      |               |   |   |   |   | 3 |     |     |     |     | 3           | 0.05         |
| AGGAAA/TTTCTC      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| AGGGA/TCTTCTC      |               |   |   |   |   | 3 |     |     |     |     | 3           | 0.05         |
| AGGCCC/GAGCCCT     |               |   |   |   |   | 2 |     |     |     |     | 2           | 0.03         |
| ATGGGC/GCCCAT      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| ATTTCT/GAAAAAT     |               |   |   |   |   | 3 |     |     |     |     | 3           | 0.05         |
| CACCAG/CCTGGGG     |               |   |   |   |   | 2 |     |     |     |     | 2           | 0.03         |
| CACCC/GGGGGT       |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CAGGCA/TGCCTT      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CAGGAA/TTCTTG      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CATCTT/AAATGT      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CCATCT/AGATGT      |               |   |   |   |   | 2 |     |     |     |     | 2           | 0.03         |
| CCCACT/AGCTGG      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CCCCCTT/AAAGGG     |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CCGCCA/TGGCGG      |               |   |   |   | 2 | 1 |     |     |     |     | 3           | 0.05         |
| CCGGGGA/TCCCGG     |               |   |   |   |   | 3 |     |     |     |     | 3           | 0.05         |
| CCTCCCC/GGGGAG     |               |   |   |   |   | 3 |     |     |     |     | 3           | 0.05         |
| CCTTCTC/GAGAGG     |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CCTTTT/AAAGAG      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CTCAAC/GTGGAG      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CCTCAC/GTGGAG      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| CTCCAT/ATGGAG      |               |   |   |   |   | 2 |     |     |     |     | 2           | 0.03         |
| GAACCA/TGCTTC      |               |   |   |   |   | 2 |     |     |     |     | 2           | 0.03         |
| GAGCCG/GGACT       |               |   |   |   |   | 2 |     |     |     |     | 2           | 0.03         |
| GGAGA/GATTC       |               |   |   |   |   | 2 |     |     |     |     | 2           | 0.03         |
| GAGGAT/ATGCC       |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| GGAATG/CATCC       |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| GAGGCA/TGCTCC      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| GTGGGA/TCCACC      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| TCGGCC/GGCGGA      |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| TCTTT/AAAGGA       |               |   |   |   |   | 1 |     |     |     |     | 1           | 0.02         |
| TTCTTT/AAAGAA      |               | 6 |   |   |   |   |     |     |     |     | 6           | 0.09         |
| Continued          |               |   |   |   |   |   |     |     |     |     |             |              |
by A/T type was the highest (2951, 44.68%), and then dinucleotide repeats dominated by AG/CT and GA/TC (2236, 33.86%), trinucleotide repeats dominated by GAA/TTC and CCA/TGG (1275, 19.31%) and hexanucleotide repeats (68, 1.03%). Tetranucleotide and pentanucleotide repeat motifs exhibited relatively low frequencies, accounting for 0.76% and 0.36% of the total motif types, respectively. The statistical analysis of all SSR loci showed that the 5 repeat motif types with the highest occurrence frequency were in order as follows: A/T (2816, 42.64%), AG/CT (807, 12.22%), GA/TC (636, 9.63%), AC/GT (234, 3.54%) and CA/TG (215, 3.26%) (Table 3).

In *P. catalpifolia*, A/T was the most common mononucleotide repeat motif, accounting for 95.43% (2816) of all mononucleotide repeats, while C/G represented only 4.57% (135) (Fig. 6a). Of the dinucleotide repeats, AG/CT motif was the most frequent (807, 36.09%), followed by GA/TC (636, 28.44%), AC/GT (234, 10.47%) and CA/TG (215, 9.62%). The fewest dinucleotide motifs were GC/GC and CG/CG, each representing 1.79% (4) of the total dinucleotide repeats (Fig. 6b). There were 30 trinucleotide motifs present, of which GAA/TTC and CCA/TGG were the most frequent, accounting for 8.16% (104) and 8.00% (102) of the trinucleotide motifs, respectively, followed by AGA/TCT (82, 6.43%), CAG/CTG (81, 6.35%), AAG/CTT (78, 6.12%), CCG/CGG (78, 6.12%) and TCA/TGA (68, 5.33%). The fewest trinucleotide motifs were ACG/CGT (6, 0.47%), ACA/TGT (5, 0.39%) and GTA/TAC (2, 0.16%) (Table 3). Of the 24 tetranucleotide repeat motifs, CCCT/AGGG was the most frequent (8, 16%), followed by TGAA/TTCA (4, 8%) and GATT/AATC (4, 8%). The number of TTTG/CAAA, AAAT/ATTT, GAAA/TTTC, CTTT/AAAG and GGAG/CTCC all had 3 and accounted for 6%, 3 tetranucleotide motifs all with the number of 2 and another 13 repeat motif all with the number of 1. Within the 12 pentanucleotide repeat motifs, CCACC/GGTGG was the most frequent (9, 37.50%), followed by TCCTC/GAGGA (4, 16.67%) and CTTTT/AAAAG (2, 8.33%); the number of remaining 9 repeat motifs all were 1. Of the 36 hexanucleotide repeat motifs, AAG AGA /TCT CTT  was the most frequent (8, 11.76%), followed by TTT  CTT /AAG AAA  (6, 8.82%). The number of 6 repeat motif types were all 3, 8 each were 2 and the remaining 20 each were 1 (Table 3).

**Discussion**

The lack of reference genome has impeded basic genetic research in *P. catalpifolia* and its related species. However, SMRT sequencing technology can generate full-length transcript sequences without a reference genome and has been widely used to predict and validate gene models related to some unique traits in species. In this study, we used the SMRT technique to perform full-length transcriptome sequencing in *P. catalpifolia* using PacBio RS II platform. In total, 28.83 Gb sequencing data were obtained including 349,745 full-length non-chimeric sequence reads, which was similar to the number of FLNC reads in *Rhododendron lapponicum*.

After subjecting the reads to clustering, error correction and redundant sequence removal, a total of 25,969 HQ transcripts were finally obtained. Very-long-read sequences were generated using the SMRT sequencing technology, and one read is considered a full-length transcript under normal circumstances. The HQ transcripts generated using SMRT sequencing were longer in length than those generated using an Illumina system. In this study, the average length of the HQ transcripts in *P. catalpifolia* was 1624 bp, while the mean unigene length was 945 bp in tung tree, 808 bp in *Pueraria lobata* and 690 bp in *Eucommia ulmoides*, each of which were sequenced using an Illumina system. In addition, we found that HQ transcripts > 1000 bp in length accounted for 84.04% of all HQ transcripts in our research, which was much higher than that in *P. australis* (40.09%) and *P. tomentosa* (42.16%) using Illumina sequencing technique. Our results demonstrated that SMRT sequencing is a reliable and efficient method to obtain full-length transcript sequences in species without an annotated reference genome.

### Table 3.

| Repeat motif length | Repeat number | Total number | Frequency (%) |
|---------------------|---------------|--------------|---------------|
| 5                   | 6             | 7            | 8             | 9            | 10           | 11           | 12           | > 12         |
| TTTTCT/AGAAAA       | 1             | 1            | 0.02          |
| TTTTGC/GAAAA        | 1             | 1            | 0.02          |
| Total number        | 834           | 901          | 456           | 378          | 247          | 897          | 757          | 527          | 1607         |
| Frequency (%)       | 12.63         | 13.64        | 6.90          | 5.72         | 3.74         | 13.58        | 11.46        | 7.98         | 24.33        | 100.00       |

Figure 6. The types and numbers of mononucleotide and dinucleotide repeat motifs.
We annotated 25,602 HQ *P. catalpifolia* transcripts using five public databases. The annotated HQ transcripts accounted for 98.59% of all HQ transcripts, a similar rate to those of transcriptomics studies in *R. lapponicum* and *Medicago sativa*. The 367 HQ transcripts with no predicted functions are likely to be species-specific or unknown genes in *P. catalpifolia*. GO classification of the HQ transcripts indicated that the majority were associated with the GO terms metabolic processes, binding, catalytic activity, cellular processes, cell and cell part. HQ transcript annotation using KOG indicated that a large number of transcripts were involved in posttranslational modifications, protein turnover, chaperones, translation, and ribosomal structure and biogenesis. A total of 13,829 HQ transcripts were assigned to specific KEGG pathways, such as carbohydrate metabolism, energy metabolism, translation, folding, sorting and degradation. We also found that many HQ transcripts exhibited multiple molecular functions and participated in diverse biological pathways. Our study provides a wealth of genetic information for molecular research into the growth and development of *P. catalpifolia* leaves, particularly in response to drought stress.

In recent years, SSR molecular markers have been widely used for genetic map construction, genetic diversity analyses and functional gene mining. However, the traditional methods of SSR primer development are time-consuming, complex and costly, thus hindering their development seriously. While the SSR primers developed on the basis of transcriptome sequencing data information are economical, efficient, and abundant, which has gradually become one of important methods. Furthermore, SSR molecular markers are rapidly being developed alongside recent advancements in transcriptome sequencing technology. In our study, a total of 7367 SSR loci were detected from 25,969 HQ transcripts, including 763 complex SSRs and 6604 complete SSRs. The frequency of the SSRs was 28.37%, and the average distribution distance was 5.59 kb. Among the 6604 complete SSRs, the most abundant and frequent mononucleotide, dinucleotide and trinucleotide motifs were A/T, AG/CT and GA/TTC, respectively. Studies examining SSRs in *Hevea brasiliensis*, Chinese cabbage and *R. lapponicum* produced similar results. A/T was the most abundant mononucleotide motif (2816; 95.43%), which was consistent with a study performed by Lagercrantz et al. AG/CT (807; 36.09%) and GA/TC (636; 28.44%) were the most abundant dinucleotide motifs, and CT repeats usually existed in transcriptional regions that might take part in antisense transcription and have an effect on gene regulation. There were differences in SSR abundance of different plant species in diverse researches, and repeat number of 6, 10, 5, 11, and 12 occupied 59.30% of the total complete SSR loci in our study. The SSR markers that we have developed in this work will facilitate mining for drought resistance genes, breeding drought resistant varieties, genetic diversity analyses and genetic map construction in *P. catalpifolia*. Of course, the SSRs found in this study were predicted theoretically and should be verified experimentally before further using.

**Materials and methods**

**Plant materials and RNA extraction.** *P. catalpifolia* seedlings were planted in separate pots at Mengzhou Forest Farm at the Paulownia Research and Development Center of State Administration of Forestry and Grasslands (Jiaozuo, Henan, China, 112° 42′ 58″ E, 34° 51′ 38″ N). The third and fourth fully expanded functional leaves from the top of the stem were collected at 0, 8 and 16 days after drought stress, respectively. The leaves were immediately frozen in liquid nitrogen and stored at −80°C until the experiment.

The leaves from the top of the stem were collected at 0, 8 and 16 days after drought stress, respectively. The leaves were immediately frozen in liquid nitrogen and stored at −80°C until the experiment. The *Paulownia catalpifolia* used in this study were identified by Paulownia Research and Development Center of State Administration of Forestry and Grassland, and the collection and use of *Paulownia catalpifolia* samples in our experiment comply with the guidelines of Paulownia Research and Development Center of State Administration of Forestry and Grassland. Total RNAs extraction were performed using the EZ-10 DNAway RNA mini-prep kit (Sangon Biotech Co., Shanghai, China) following the manufacturer’s instructions. The total RNAs of three samples above were mixed equally according to the method of Mao et al. to form the sample 5 for transcriptome sequencing. The degree of RNA degradation and contamination were evaluated using 1% agarose gels. The RNA purity and concentration were checked using the NanoPhotometer spectrophotometer (Implen, CA, USA) and Qubit RNA Assay Kit (Life Technologies, CA, USA), respectively. RNA integrity was analyzed using an Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The resulting high-quality RNA was used for full-length transcriptome sequencing.

**cDNA library construction and SMRT sequencing of the full-length transcriptome.** Full-length cDNA was synthesised from 1.0 μg purified mRNA using the SMARTer PCR cDNA Synthesis Kit (Clontech, USA) according to the manufacturer’s protocol, its size were selected using the BluePippin Size-Selection System (Sage Science, USA) and then PCR amplified again. The cDNA library was constructed after repairing the ends, connecting dumbbell-shaped SMRT adapters, performing exonuclease digestions and conducting a secondary screening using BluePippin. After the cDNA library had passed quality control using the Qubit 2.0 and Agilent 2100, full-length transcriptome sequencing of *P. catalpifolia* was performed using the PacBio RS II platform, based on the target data volume.

**Quality control and functional annotation of the full-length transcriptome.** The raw SMRT data were pre-processed using the SMRT Pipe analysis workflow within the PacBio SMRT Analysis software suite. Examination of the polyadenosine signal and 5′ and 3′ adaptors, as well as error correction, were performed following the methods similar to the one described. Full-length SMRT transcripts were identified, and non-redundant HQ transcripts were acquired using CD-HIT-EST software. Clustering and removal of redundant sequences were performed using the PacBio SMRT LINK Cluster tool, and all HQ transcripts were aligned to nucleotide and protein databases using BLASTX. The databases used in this study were NCBI non-redundant, gene ontology (GO), eukaryotic orthologous groups (KOG), Kyoto encyclopedia of genes and genomes (KEGG) and Swiss-Prot.
Identification of lncRNAs, coding sequences (CDSs) and AS variants. LncRNA candidates were identified using the following software: coding potential calculator 2 (CPC2), coding potential assessment tool (CPAT), predictor of long non-coding RNAs and messenger RNAs based on an improved k-mer scheme (PLEK), and the coding–non-coding index (CNCI), respectively. LncRNAs with > 200 nucleotides were selected. Trans-Decoder version 3.0.0 was used to identify candidate coding sequences (CDSs) in the full-length transcriptome of *P. catalpifolia*. All non-redundant HQ transcripts were aligned using a previously described method. Candidate AS events were identified using the selection criteria described by Diao et al.

Identification and characterization of SSRs. The microsatellite identification tool (MISA) was used to identify SSRs within the 25,969 HQ transcripts, and the characteristics of the repeated motif types were further analyzed statistically. In this study, the SSR locus were identified according to the criteria below: the repeat motifs were ≥ 6, 5, 5, 5 and 5, respectively.

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Author contributions
Y.Z.F. and J.Q. performed the study conception and experiments. J.J.Z., B.P.W. and C.W.Y. collected samples, prepared materials and analyzed the data. Y.Z.F. and H.J.Z. collected and analyzed the data. Y.Z.F. and Y.Z. written the original draft of the manuscript. J.Q. reviewed and edited of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

Additional information

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