Transcriptome profiling of the fertile parent and sterile hybrid in tea plant flower buds

Linbo Chen¹²†, Hao Qu¹²†, Lifei Xia¹², Yue Liu¹², Huiling Jiang¹², Yunnan Sun¹², Mingzhi Liang¹²* and Changjun Jiang³*

Abstract

Background: The tea plant is a crucial economic crop. The floral organ development consumes a large amount of nutrients, which affects the leaf yield. To understand the mechanism by which the tea plant produces sterile floral buds, we obtained a sterile tea plant by artificial hybridization. RNA-sequencing based transcriptome analysis was implemented in three samples to determine the differentially expressed genes (DEGs) related to flower development.

Results: In this study, a total of 1991 DEGs were identified; 1057 genes were up-regulated and 934 genes were down-regulated in sterile hybrid floral buds. These were mainly distributed in the regulation of biological and metabolic processes. Significantly, auxin biosynthesis genes YUCCA, AUX1 and PIN were dramatically down-regulated, and ARF gene was up-regulated in the sterile hybrid floral buds, and flower development-related genes API, AP2 and SPL were changed. A total of 12 energy transfer-related genes were significantly decreased. Furthermore, the expression of 11 transcription factor genes was significantly different.

Conclusion: The transcriptome analysis suggested that the production of sterile floral buds is a complex bioprocess, and that low auxin-related gene levels result in the formation of sterile floral buds in the tea plant.

Keywords: Tea plant, Sterile floral buds, Differentially expressed genes, Auxin

Introduction

Sterility is a complex phenomenon in plants, the main factors of which include the accumulation of reactive oxygen species, abnormalities in energy metabolism, programmed cell death, imbalance in endogenous hormones and changes in environmental conditions [1–3]. In Arabidopsis and cereal grains, floral organ degeneration and abiotic stresses result in sterility. Abortion or degeneration of developing stamens and pistil is the key mechanism used by plants to produce sterile flowers [4].

Among the sterility mechanisms of the plant, flower development is one of the main concerns. It requires early and later organ identity genes. PINFORMED (PIN1), PINOID (PID), YUCCA (YUC) and NAKED PINS IN YUC MUTANT (NPY) are necessary for flower development [5, 6]. Organ identity genes APETALA1 (AP1), APETALA2 (AP2), APETALA3 (AP3), PISTILLATA (PI) and AGAMOUS (AG) are essential for the ABC model in Arabidopsis [7]. The mutation of these genes causes more petals, fewer stamens, fused floral organs, and valveless gynoecia. In addition, important genes for flower development include SPL, TPD1, AMS, DYT1, SHP, WUS [8–13]. Brassinosteroid and jasmonic acid play a positive role in promoting the formation of stamens and pollen. Gibberellin deficiencies are related to male sterility, and the formation of female flowers requires the presence of ethylene [14–16]. Moreover, flower development is regulated by the coordinated interaction of the transcription factor LEAFY and auxin [17]. However, molecular data are limited for the tea plant; the molecular mechanism of flower development remains unknown.

The tea plant is a crucial cash crop widely distributed around the world. Tea leaves have been used to produce various tea beverages. The floral organ development results in the fall of the yield of tea leaves by consuming a large amount of nutrients. Cultivation of the sterile tea plant is key to increasing the yield. The tea plant is self-incompatible,
thus we performed transcriptome sequencing and comparative analysis on three samples, including Foxiang2 (FBH), Fudingbaicha (MBH) and hybrid sterile flowers (ZDH). The aim was to analyze the differentially expressed genes between the fertile and sterile floral buds, and to identify their related bioprocesses and correlative factors. Our results will help to reveal important information on the mechanism of sterility in the tea plant.

Materials and methods

Plant materials

The plant materials were five-year-old tea plants (C. sinensis (L.) O. Kuntze) from the Tea Research Institute, Yunnan Academy of Agricultural Sciences called 'Foxiang2', used as FBH, 'Fudingbaicha', used as MBH, and a sterile hybrid, used as ZDH. Flower buds were separately stripped from three tea plants. All of the experiments were performed using three biological replicates. Flower buds were picked on October 16, 2016, after which they were frozen using liquid nitrogen and stored in a freezer at −80 °C for subsequent mRNA analysis.

Table 1 Primer Sequences for q-PCR

| Gene name | Primer Sequence (5’ to 3’) |
|-----------|----------------------------|
| AP2       | TACAGAGGAGTAACAAGGCATCA     |
| JAR1      | CGTCAAAAGTCGCTCAGCA        |
| ARF       | TGAAACAGAGGAGTCAGGCA       |
| IAA7      | TCCAATGAGAAGAAAGACCCTG     |
| AUX1      | ACTGAGGCGTGGTGTTGGTA       |
| ATL3      | CACACTAACCCTACCATCAGCA     |
| GAPDH     | GATAGTGTTCACGGTCAATGGA     |

Fig. 1 Morphological characteristics of the tea flower. a The morphologies of FBH, MBH and ZDH. b The morphologies of the fertile and sterile floral organ.
cDNA library construction and sequencing
The construction of cDNA libraries and transcriptome sequencing were completed by Beijing Novogene Technology (Beijing, China). Total RNA was used as the starting sample and was directly added to the 3’-terminal hydroxyl group and the uniquely structured complete phosphoryl groups at the 5’-terminal of the sRNA, followed by reverse transcription to synthesize cDNA. After polymerase chain reaction amplification, polyacrylamide gel electrophoresis was used to separate the target DNA fragments, and the gel was recovered, completing the cDNA library. The effective concentration of the library was > 2 nmol/L, and sequencing was performed using Illumina HiSeq 2000 after the library was certified. All of the experiments were performed using three replicates.

Transcriptome assembly
The original image data files obtained from the Illumina HiSeq 2000 were subjected to base calling analysis and converted into raw reads. Among the raw reads obtained from the sequencing, the low-quality reads with adaptors were processed to obtain clean reads. The clean reads were assembled separately, and TGICL was used to get the longest non-redundant unigenes. The transcriptome data were deposited to the NCBI SRA database (SRA accession: PRJNA503652).

Differentially expressed genes (DEGs) test
Differentially expressed genes (DEGs) analysis using DEGseq (three biological replicates per group). DESeq provides statistical routines for determining differential

### Table 2 Summary Dataset of Transcriptome Assembly

|                        | male parent (FBH) | female parent (MBH) | sterile flowers (ZDH) |
|------------------------|-------------------|---------------------|-----------------------|
| Clean reads            | 64,267,724        | 60,674,496          | 74,575,474            |
| GC content             | 44.00%            | 44.17%              | 43.67%                |
| Q30                    | 92.41%            | 92.09%              | 92.53%                |
| Mapped reads ratio     | 73.64%            | 75.27%              | 71.43%                |

**Fig. 2** Cluster analysis of DEGs. **a** Heat map showing expression of the DEGs. High expression genes appear red, while low expression genes appear blue on the heat map. **b** The Venn diagram showing the number of DEGs between FBH vs ZDH, MBH vs ZDH, FBH vs MBH

Chen et al. Hereditas (2019) 156:12
expression in digital gene expression data, using a model based on negative binomial distribution. The resulting \( P \)-values were adjusted using the Benjamin and Hochberg approach for controlling the false discovery rate. The genes with \( P \)-value < 0.05 found by DESeq were differentially expressed.

**GO and KEGG analysis**

DEGs were characterized according to Gene Ontology enrichment analysis. GO annotations were provided by the Blast2GO program. Then the GO classification graph was generated by the WEGO.

KEGG was used to analyze the biological process and unigenes annotation of pathway. The results were comparatively analyzed between the KEGG integrated database resource and our data.

**Quantitative real-time PCR assays**

Total RNA was isolated using TRIpure reagent (BioTeke, China) according to the manufacturer's instructions. cDNA was synthesized from total RNA using a PrimeScript RT reagent kit (TaKaRa, Japan). The obtained cDNA was used as a template in SYBR green-based q-PCR (CFX-96, Bio-Rad, Hercules, CA, USA). GAPDH was used for normalization. The primers are shown in Table 1.

**Results**

**Morphological characteristics of sterile flower buds**

The floral organ of the tea plant consists of a complete bisexual flower composed of a thalamus, calyces, petals, stamens, and a pistil (Fig. 1a). In our study, the sterile flower buds were smaller than the male and female parent flower. Petals were improperly unfolding during the developmental process until flower buds abscission (Fig. 1a). The filaments were shorter than those of fertile flowers, and the anthers contained no pollen (Fig. 1b). In addition, sterile flowers contained two to four imperfect pistils, which were split into two to five smaller stigma (Fig. 1b).

**Transcriptome profiling of the male/female parent and the hybrid bud**

We used the Illumina HiSeq 2000 platform to create three cDNA libraries, FBH, MBH, and ZDH. As a result, a total of 64.2, 60.6 and 74.6 million clean reads were generated. The quality score (Q30) percentage was above 92%, with the GC content of each clean data above 43%. The ratio of mapped reads was 73.64, 75.27 and 71.43%, respectively (Table 2). Afterwards, Trinity was used in splicing for clean reads. A total of 268,289 transcripts were obtained, and the longest transcript for each gene was selected as the unigene, of which 173,248 were screened for differential expression analysis (DEGs).

**DEGs and functional characterization**

A total of 6395 DEGs were detected in FBH, MBH and ZDH. 1914 DEGs were screened out between FBH and MBH. Between ZDH and FBH, the number of DEGs was 5438, whereas it was 3208 between ZDH and MBH. 1991 DEGs were screened out in ZDH, the expression quantity was different with FBH and MBH (Fig. 2).

The unique DEGs were characterized using the GO and KEGG databases. The GO enrichment analysis categorized 1991 unigenes, which contained 47 subcategories of biological processes, cellular components, and molecular functions. For the biological process, “biological regulation”, “cellular process”, “metabolic process”, and “single
organism process” were the most representative groups. In the subcategory of cellular component, “cell”, “cell part”, “macromolecular complex”, and “organelle” were the predominant groups. For the molecular function, “binding”, and “catalytic activity” were the most common. Moreover, “reproduction”, “reproductive process”, and “growth” were enriched in 24, 16, and 3 unigenes, respectively (Fig. 3).

The KEGG analysis revealed that 764 unigenes were mapped into KEGG, containing cellular processes, environmental information processes, generic information processes, metabolism, and organismal systems. The main KEGG pathways included “Carbon metabolism”, “Biosynthesis of amino acids”, “Starch and sucrose metabolism”, “Plant hormone signal transduction”, “Plant pathogen interaction”, and “Glycolysis/Gluconeogenesis” (Fig. 4).

Identification of auxin-related genes involved in DEGs
Auxin is essential for flower organ development [18]. Among DEGs, the auxin biosynthesis gene YUCCA related to floral organ formation was down-regulated. The auxin flux-related PIN homolog gene related to gynoecium formation was down-regulated. In addition, we identified ten auxin response factors ARF (Cluster-23,036.113917, Cluster-23,036.14480, Cluster-23,036.14481, Cluster-23,036.85241, Cluster-23,036.27862, Cluster-23,036.29441, Cluster-23,036.70956, Cluster-23,036.66525, Cluster-23,036.54073 and Cluster-23,036.87364) related to flower maturation was up-regulated (Table 3).

Identification of flower development-related genes involved in DEGs
Abnormal flower development is the key factor responsible for the sterility of the tea plant. The formation of flowers is a key step in the plant life cycle, which is a complex process [19]. Each stage of the process is regulated by flower development-related genes, specifically the ABC floral organ-identity genes [20]. We identified the A-class genes API and AP2 were up-regulated. In
addition, flower development-related genes, such as floral organ formation SPL homolog genes (Cluster-23,036.89600, Cluster-23,036.17164, Cluster-23,036.96383, Cluster-23,036.48034 and Cluster-23,036.89141) were up-regulated (Table 4).

Identification of energy transfer-related genes in DEGs

Energy transfer is an important process of plant growth and development. It is the foundation of the plant’s life [21]. We identified that some genes involved in energy transfer were differentially expressed, including ABC transporter B family member 1 (ABCB1), six transporting ATPase-related genes (ATPeF0D, ATPeF0O, ATPeF1B, ATPeV0A, ATPeV1C, and ATPeV1B), and five solute carrier-related genes (SLC2A8, SLC15A3, SLC25A11, SLC32A, and SLC35B3) (Table 5). A total of 12 genes were down-regulated. In particular, transporting ATPase subunit beta (ATPeF1B), solute carrier family 32 (SLC32A) and

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**Table 3** DEGs Related to IAA Signal Transduction

| Gene ID       | KO name | Annotation                                  | Log[2] ratio |
|---------------|---------|---------------------------------------------|--------------|
| Cluster-23,036.78079 | YUCCA   | indole-3-pyruvate monooxygenase YUCCA10    | 2.50  2.57   |
| Cluster-23,036.68881 | JAR1     | Indole-3-acetamide amino synthetase GH3.5  | 4.81  4.59   |
| Cluster-23,036.78790 | ILR1     | IAA-amino acid hydrolase ILR1-like 6     | 2.65  2.00   |
| Cluster-23,036.63096 | ILR1     | IAA-amino acid hydrolase ILR1-like 4     | 4.29  3.59   |
| Cluster-23,036.77706 | PIN     | auxin efflux carrier protein               | 3.34  2.87   |
| Cluster-23,036.72193 | AUX1/LAX | auxin transporter-like protein 4         | 2.45  2.44   |
| Cluster-23,036.74178 | AUX1/LAX | auxin transporter-like protein 3        | 2.99  2.69   |
| Cluster-23,036.53622 | Aux/IAA | Auxin-responsive protein IAA7            | 6.17  5.43   |
| Cluster-23,036.68884 | Aux/IAA | auxin-induced protein 22D-like            | 3.48  3.33   |
| Cluster-23,036.74950 | Aux/IAA | auxin-induced protein 22D-like            | 5.43  5.34   |
| Cluster-23,036.6113917 | ARF      | auxin response factor 9                    | −4.76 −4.09  |
| Cluster-23,036.614480 | ARF      | auxin response factor 5                    | −7.27 −4.02  |
| Cluster-23,036.614418 | ARF      | auxin response factor 5                    | −6.00 −4.96  |
| Cluster-23,036.685241 | ARF      | auxin response factor 4                    | −2.60 −2.02  |
| Cluster-23,036.617862 | ARF      | auxin response factor 28                   | −3.23 −2.24  |
| Cluster-23,036.694411 | ARF      | auxin response factor 28                   | −1.90 −1.09  |
| Cluster-23,036.649560 | ARF      | auxin response factor 2                    | −1.41 −1.11  |
| Cluster-23,036.665252 | ARF      | auxin response factor 19-like              | −1.38 −1.26  |
| Cluster-23,036.540738 | ARF      | auxin response factor 19-like              | −1.68 −1.49  |
| Cluster-23,036.873645 | ARF      | auxin response factor 1 isoform X1         | −1.98 −1.77  |

**Table 4** DEGs Related to Flower Development

| Gene ID       | KO name | Annotation                                  | Log[2] ratio |
|---------------|---------|---------------------------------------------|--------------|
| Cluster-23,036.89600 | SPL8     | squamosa promoter-binding-like protein 8     | −3.52 −2.36  |
| Cluster-23,036.17164 | SPL6     | squamosa promoter-binding-like protein 6    | −2.32 −2.00  |
| Cluster-23,036.96383 | SPL3     | squamosa promoter-binding-like protein 3    | −4.85 −3.30  |
| Cluster-23,036.48034 | SPL12    | squamosa promoter-binding-like protein 12   | −1.68 −2.86  |
| Cluster-23,036.89141 | SPL9     | squamosa promoter-binding-like protein 9     | −2.75 −3.19  |
| Cluster-23,036.697868 | AP1      | MADS-box transcription factor APETALA1-like | −1.92 −1.36  |
| Cluster-23,036.704173 | AP2      | AP2-like ethylene-responsive transcription factor RAP2–7 | −2.32 −2.26 |
| Cluster-23,036.11884 | AP2      | AP2-like ethylene-responsive transcription factor ANT | −8.76 −7.94 |
| Cluster-23,036.902545 | AGO5     | protein argonaute 5                         | −3.32 −4.08  |
| Cluster-23,036.852038 | AGO4     | protein argonaute 4                         | −2.48 −1.69  |
| Cluster-23,036.998834 | AGO2     | protein argonaute 2                         | −1.61 −7.52  |
| Cluster-23,036.954339 | AGO10    | protein argonaute 10                        | −3.76 −2.27  |
solute carrier family 35 (SLC35B3) were down-regulated 65.7, 60.9 and 54.9-fold, respectively, in ZDH compared with FBH. The expression levels of ATPeF1B, SLC32A and SLC35B3 were also down-regulated 96.8, 34.7 and 65.4-fold, respectively, in ZDH compared with MBH.

### Identification of other transcription factor genes in DEGs

We also identified some transcription factor genes. Among these genes, the expression levels of ATPeF1B, SLC32A and SLC35B3 were also down-regulated 96.8, 34.7 and 65.4-fold, respectively, in ZDH compared with MBH.

#### qPCR analysis of DEGs

To confirm the transcriptome sequencing results, six DEGs were selected for qPCR analysis. Among these genes, Cluster-23,036.57048 (AP2) and Cluster-23,036.14480 (ARF) genes were up-regulated, and Cluster-23,036.68881 (JAR1), Cluster-23,036.53622 (IAA7), Cluster-23,036.69380 (AUX1) and Cluster-23,036.74178 (ATL3) genes were down-regulated (Fig. 5). qRT-PCR results showed a consistent expression tendency compared with the RNA-Seq. The result further confirmed the reliability and accuracy of the transcriptome sequencing.

### Discussion

Here, we used transcriptome sequencing to explore candidate genes associated with sterile floral buds in the tea plant. The method has been applied to the study of the genome for the tea plant [22–25]. A total of 1991 DEGs

| Table 5 DEGs Related to Energy Transfer |
|----------------------------------------|
| Gene ID | KO name | Annotation | Log 2 ratio |
|---------|---------|------------|-------------|
|        |         |            | FBH vs ZDH  | MBH vs ZDH |
| Cluster-23,036.67567 | ABCB1 | ABC transporter B family member 1 | 1.86 | 1.56 |
| Cluster-23,036.73251 | ATPeF0D | transporting ATPase subunit d | 1.82 | 1.71 |
| Cluster-23,036.68425 | ATPeF0O | transporting ATPase subunit O | 1.85 | 1.65 |
| Cluster-23,036.67744 | ATPeF1B | transporting ATPase subunit beta | 6.03 | 6.58 |
| Cluster-23,036.71789 | ATPeVOA | transporting ATPase subunit a | 1.92 | 1.46 |
| Cluster-23,036.61724 | ATPeV1C | transporting ATPase subunit C | 1.77 | 1.37 |
| Cluster-23,036.73958 | ATPeV1B | transporting ATPase subunit B | 1.49 | 1.21 |
| Cluster-23,036.75094 | SLC2A8 | solute carrier family 2 | 2.86 | 2.31 |
| Cluster-23,036.55114 | SLC15A3 | solute carrier family 15 | 1.87 | 1.49 |
| Cluster-23,036.73478 | SLC25A11 | solute carrier family 25 | 2.48 | 1.87 |
| Cluster-23,036.72951 | SLC32A | solute carrier family 32 | 5.92 | 5.15 |
| Cluster-23,036.49241 | SLC35B3 | solute carrier family 35 | 5.77 | 6.03 |

| Table 6 DEGs Related to Transcription factors |
|---------------------------------------------|
| Gene ID | KO name | Annotation | Log 2 ratio |
|---------|---------|------------|-------------|
|        |         |            | FBH vs ZDH  | MBH vs ZDH |
| Cluster-23,036.105297 | AS1 | Transcription factor AS1 | −2.43 | −1.6 |
| Cluster-23,036.65747 | K09264 | MADS-box transcription factor, plant | 1.63 | 1.48 |
| Cluster-23,036.17098 | CPC | Transcription factor CPC | −3.25 | −3.1 |
| Cluster-23,036.14144 | NFYA | nuclear transcription factor Y, alpha | −5.52 | −6.14 |
| Cluster-23,036.62850 | bHLH77 | Transcription factor bHLH77 | 2.68 | 2.92 |
| Cluster-23,036.69683 | bHLH79 | Transcription factor bHLH79 | 6.53 | 5.59 |
| Cluster-23,036.16861 | GAMYB | Transcription factor GAMYB | 2.46 | 2.42 |
| Cluster-23,036.65315 | GTE2 | Transcription factor GTE2 | 5.82 | 6.92 |
| Cluster-23,036.76553 | MYBP | Transcription factor MYB108 | 7.47 | 6.01 |
| Cluster-23,036.71827 | MYB21 | Transcription factor MYB21 | 4.64 | 4.09 |
| Cluster-23,036.70389 | TGA6 | Transcription factor TGA6 | 5.48 | 5.2 |
were screened out from the comparison among the three cDNA libraries (Fig. 2). The complexity of DEGs function was demonstrated by Go and KEGG analysis (Figs. 3 and 4). Based on the analysis, 452 metabolism-related pathways were identified (Fig. 4), suggesting that metabolism may be crucial for the sterility mechanism in the tea plant.

In general, the flavin monooxygenase (YUCCA) is involved in the tryptophan-dependent pathway of auxin biosynthesis [26], mutants of YUCCA present aberrant flower phenotypes, short stamen filaments, and thus sterility [27]. Our results show that the expression levels of YUCCA were down-regulated significantly (Table 3), the flower phenotype also appears as short stamen filaments (Fig. 1b). Thus, inferring that the low expression of YUCCA is a key factor affecting the biosynthesis of auxin leading to flower sterility. In addition, we also found that the expression of SPL homolog genes as an inhibiting factor of YUCCA was up-regulated in sterile floral buds (Table 3). This is possibly due to the high expression of ARF inhibiting auxin signaling targets [30]. Moreover, ARF acts as a positive or negative regulator by binding to the auxin response element TGTCTC [31], and it is possible that the Aux/IAA inhibitor is able to inhibit transcription through interaction with ARF [32].

Interestingly, we found that the A-class of ABC floral organ-identity genes AP1 and AP2 were up-regulated in sterile floral buds (Table 4). It has been reported that the initial expression of AP1 and AP2 was restricted to the first and second whorls, and was inhibited in the third and fourth whorls of flower development [33]. Thus inferring that high expression of AP1 and AP2 represses flower development in first and second whorls. Detailed mechanisms will require further study.

As an important regulator of flower development, auxin is transported to each tissue through carriers and the AUX1/LAX [34]. We found that the expression levels of ARF play pivotal roles in the growth of inflorescences, stamens, anthers, and pistils. Mutations in ARF6 and ARF8 caused male and female infertility [29]. However, we found that the expression level of ten ARF homolog genes was up-regulated in sterile floral buds (Table 3). This is possibly due to the high expression of ARF inhibiting auxin signaling targets [30]. Moreover, ARF acts as a positive or negative regulator by binding to the auxin response element TGTCTC [31], and it is possible that the Aux/IAA inhibitor is able to inhibit transcription through interaction with ARF [32].

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![Fig. 5 qPCR analysis of selected DEGs. Data represent the means ± SD, n = 3 independent experiments. ***p < 0.001 versus control](image-url)
Competing interests
Not applicable.

Consent for publication
Ethics approval and consent to participate
LC, ML and CJ conceived and designed the experiments. YL, HB and YS
666201, China. 2Yunnan Provincial Key Laboratory of Tea Science, Menghai
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57x105; SKLTOF20170115).
Availability of data and materials
We have provided detailed information about the materials and methods in
Authors’ contributions
LC, ML and CJ conceived and designed the experiments. YL, HB and YS
Ethics approval and consent to participate
Not applicable.
Consent for publication
Not applicable.
Competing interests
The authors declare that they have no competing interests
Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in
Author details
1Tea Research Institute, Yunnan Academy of Agricultural Sciences, Menghai
666201, China. 2Yunnan Provincial Key Laboratory of Tea Science, Menghai
Received: 28 December 2018 Accepted: 11 April 2019
Published online: 18 April 2019

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ALIXI/LAX (Table 3) and twelve energy transfer-related
genes (Table 5) were down-regulated, suggesting that the
transport of auxin may be impeded. This may also be an
important factor in the sterile floral buds of the tea plant.
In summary, the present work provides four key factors
for the development of sterile floral buds in the tea plant.
We thus present a theoretical basis for further study of
mechanisms by which sterile floral buds are produced.

Abbreviations
DEGs: Differentially expressed genes; FBH: Foxiang2; GO: Gene Ontology;
KEGG: Kyoto Encyclopedia of Genes and Genomes; MBH: Fudingbaicha;
ZDH: Hybrid sterile flowers

Acknowledgements
This work was supported by the National Natural Science Foundation of
China (31460216, Personnel Training Program of Yunnan Province
(2015HB105) and State Key Laboratory of Tea Plant Biology and Utilization
(SKLTOF20150105, SKLTOF20170115).

Funding
This work was funded by NSFC31460216, 2015HB105, SKLTOF20150105, and
SKLTOF20170115.

Availability of data and materials
We have provided detailed information about the materials and methods in
our manuscript.

Authors’ contributions
LC, ML and CJ conceived and designed the experiments. YL, HB and YS
performed the experiments. LC, LX and HQ analyzed the data. HQ and LC
wrote the paper.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in
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Author details
1Tea Research Institute, Yunnan Academy of Agricultural Sciences, Menghai
666201, China. 2Yunnan Provincial Key Laboratory of Tea Science, Menghai
666201, China. 3State Key Laboratory of Tea Plant Biology and Utilization,
Anhui Agricultural University, Hefei 230036, China.

Received: 28 December 2018 Accepted: 11 April 2019
Published online: 18 April 2019
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