Transcriptomic analysis between self- and cross-pollinated pistils of tea plants (Camellia sinensis)

Qingping Ma1, Changsong Chen2, Zhongping Zeng1, Zhongwei Zou3, Huan Li1, Qiongqiong Zhou1, Xuan Chen1, Kang Sun1 and Xinghui Li1*

Abstract

Background: Self-incompatibility (SI) is a major barrier that obstructs the breeding process in most horticultural plants including tea plants (Camellia sinensis). The aim of this study was to elucidate the molecular mechanism of SI in tea plants through a high throughput transcriptome analysis.

Results: In this study, the transcriptomes of self- and cross-pollinated pistils of two tea cultivars ‘Fudingdabai’ and ‘Yulv’ were compared to elucidate the SI mechanism of tea plants. In addition, the ion components and pollen tube growth in self- and cross-pollinated pistils were investigated. Our results revealed that both cultivars had similar pollen activities and cross-pollination could promote the pollen tube growth. In tea pistils, the highest ion content was potassium (K+), followed by calcium (Ca2+), magnesium (Mg2+) and phosphorus (P5+). Ca2+ content increased after self-pollination but decreased after cross-pollination, while K+ showed reverse trend with Ca2+. A total of 990 and 3 common differentially expressed genes (DEGs) were identified in un-pollinated vs. pollinated pistils and self- vs. cross-pollinated groups after 48 h, respectively. Function annotation indicated that three genes encoding UDP-glycosyltransferase 74B1 (UGT74B1), Mitochondrial calcium uniporter protein 2 (MCU2) and G-type lectin S-receptor-like serine/threonine-protein kinase (G-type RLK) might play important roles during SI process in tea plants.

Conclusion: Ca2+ and K+ are important signal for SI in tea plants, and three genes including UGT74B1, MCU2 and G-type RLK play essential roles during SI signal transduction.

Keywords: Self-incompatibility, Ion components, Pollen tube growth, Transcriptome

Background

Self-incompatibility (SI) is a common phenomenon in plant reproduction system, which prevents self-fertilization in flowering plants. There are two classical known mechanisms for SI, namely, homomorphic gametophytic self-incompatibility (GSI) and homomorphic sporophytic self-incompatibility (SSI). In GSI system, the pollen incompatibility (haploid male gametophyte) is controlled by the S allele, pollen and pistils bearing the same S allele trigger an incompatible reaction [1]. While in SSI system, incompatibility is determined by both S alleles of the (diploid-sporophyte) pollen parents [2].

GSI has been found in many plant species, such as Solanaceae [3, 4] and Rosaceae [5-7], while SSI is typically found in Brassicaceae [8]. Both GSI and SSI have male or female determinate conditions which are regulated by different prominent genes [9]. In GSI system, S locus-encoded F-box (SLF/SFB) proteins control the pollen recognition of S-RNase based SI [10-12]. In SSI systems, S-locus receptor kinase (SRK) gene and S-locus cysteine-rich protein (SCR)/S-locus protein-11 (SP11) function as a receptor-ligand pair to recognize self-pollens at the surface of stigma epidermal papilla cells [13]. The SRK is a membrane-spanning receptor protein in stigma containing an extracellular domain (S-domain) for recognition of SP11, a transmembrane domain, and an intracellular serine/threonine kinase domain [14]. S-locus glycoprotein gene (SLG) and SRK exhibit series characteristics which are...
associated with the female determinant of SSI in *Brassica*
[15]. The *S* domain of *SRK* is highly similar to the *SLG*,
which is the first *S*-locus gene to be identified and a soluble
glycoprotein secreted to the stigma surface [16, 17]. Besides,
the pollen coat protein *SCR/SP11* controls pollen deter-
minant of SSI in *Brassica* [8, 18], and many *SP11, SRK,* and
*SLG* alleles were inherited together to term different *S*
haplotypes.

Self-incompatibility mechanism remains unclear in tea
plant. Previous studies suggested that tea plant SI might
be in late-acting self-incompatibility system (LSI), in that
self-pollinated pollen tubes elongated through the style
but failed in fertilization [19, 20]. This has made it
almost impossible to obtain fruits in self-pollinated tea
plants (*Camellia sinensis*); thus, breeding process in tea
plant is not encouraged. LSI is a novel SI system in
plants, but the molecular mechanism of this system is
still unclear. Recently, Zhang et al. [21] found that tea
plant SI might be categorized to GSI through transcrip-
tome analysis. Therefore, the SI mechanism in tea plants
is still controversial and needs further exploration.

To understand the mechanism of SI in tea plant, the
ion components and pollen tube growth in self- and
cross-pollinated pistils were investigated. Furthermore,
the transcriptome of self- and cross-pollinated pistils of
two tea cultivars ‘Fudingdabai’ and ‘Yulv’ was compared
to figure out the DEGs which may be involved in SI of
tea plant. ‘Fudingdabai’ is a national superior clone and
cultivated widely in China because of its good quality,
high yield, and excellent stress resistance, while ‘Yulv’ is a
high-quality cultivar selected from the hybrid offsprings of
‘Yabukita’. Both cultivars are self-incompatible and show
high fruiting rates after cross-pollination. This study will
provide reference for understanding SI mechanism of tea
plant.

**Methods**

**Plant materials and treatments**

Two ten-year-old tea cultivars, namely, *C. sinensis* cv.
Fudingdabai and *C. sinensis* cv. Yulv, cultivated in tea
germpasmus repository of Tea Research Institute of Fujian
Academy of Agricultural Sciences were used in this study.
Flowers from both of the two tea cultivars have three
petals and trifid stigmas. The stigmas of ‘Fudingdabai’
divided at the base but ‘Yulv’ at the upper part (Fig. 1).

Flower buds of the two tea cultivars were harvested at
4:00 pm for pollens collection. Besides, the remaining
flower buds of two cultivars were emasculated and used
for artificial pollination next morning. A total of four
pollination combinations were conducted: ‘Fudingdabai’
(♂) × ‘Fudingdabai’ (♀), ‘Yulv’ (♂) × ‘Yulv’ (♀), ‘Fudingdabai’
(♂) × ‘Yulv’ (♀), and ‘Yulv’ (♂) × ‘Fudingdabai’ (♀), as
shown in Fig. 2. The un-pollinated and pollinated pistils at
8, 24, 48 and 72 h were picked from each combination
and frozen quickly in liquid nitrogen and stored at −80 °C
for RNA extraction. Three biological replicates were
conducted with at least five pistils for each replicate.

**Pollen culture in vitro**

Pollen culture medium was prepared with the following
substances: 0.59 g MES, 0.02 g H3BO3, 0.05 g Ca(NO3)
·4H2O, 5 g sucrose and 5 g polyethylene glycol 4000
(PEG 4000), diluted with distilled water to 100 mL. Pollens
were cultured in the medium in the dark and observed
by Olympus light microscope (Olympus, Tokyo, Japan).
Pollen germination rate and mean pollen tube length at 1,
2, and 4 h were calculated based on eight visual fields.
Fluorescence activity of pollen tube

Fresh un-pollinated and pollinated styles were fixed in FAA fixative buffer (5 mL formalin, 6 mL acetic acid, and 89 mL 50% ethanol) for 24 h [19]. The styles were washed with deionized water and then softened by 2 M NaOH overnight. The softened styles were stained by 0.1% aniline blue solution dissolved with 0.15 M K2HPO4. Finally, the styles were observed under Leica DM6B fluorescence microscope (Leica, Bannockburn, USA) after 15 min staining. At least five styles were observed for each sample.

Ion components of pistils

Un-pollinated and pollinated styles were dried at 80 °C for 4 h. A total of 0.1 g dried samples (at least 15 pistils) were ground and digested in 5 ml nitric acid by ETHOS One high performance microwave digestion system (Milestone, Bergamo, Italy) for 1 h. The digested samples were diluted with nitric acid to 25 mL and analyzed by inductively coupled plasma-optical emission spectrometer (PerkinElmer Optima 2100DV, Massachusetts, USA). A total of nine ions were detected, including potassium (K+), calcium (Ca2+), magnesium (Mg2+), phosphorus (P5+), zinc (Zn2+), boron (B3+), Ferrous (Fe2+), aluminium (Al3+), and manganese (Mn2+). The contents of the ions were quantified by establishing standard curve.

RNA extraction, library construction and sequencing

Total RNA was extracted using Plant RNA extraction kit (Bioteke, China) according to the manual. RNA quality and concentration were assessed by 1% agarose gels, Qubit®2.0 Fluorometer (Invitrogen, Carlsbad, USA), and Agilent Bioanalyzer 2100 system (Agilent, Palo Alto, USA). A total of 3 μg RNA per sample was used for sequencing libraries preparation by NEBNext®Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following manufacturer’s instructions and library quality was assessed on the Agilent Bioanalyzer 2100 system (Agilent Technologies, Palo Alto, CA). The clustering of samples was performed using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) according to the manufacturer’s instructions. Finally, sequencing analysis was carried out with an Illumina Hiseq 2500 platform to generate pair-end reads.

Genome alignment and gene annotation

Raw data of fastq format were processed, and then were cleaned by trimming the adapter sequences, ploy-A containing reads and low quality reads. The clean reads were aligned to the reference genome (http://www.plantking-domgb.com/tea_tree/) by TopHat2 using the default parameters [22]. The mapped reads were assembled into possible transcripts by Cufflinks [23]. The unannotated transcripts were annotated by BLAST [24] based on the following databases: NR (NCBI non-redundant protein sequences) [25], COG (Clusters of Orthologous Groups of proteins) [26], Swiss-Prot (A manually annotated and reviewed protein sequence database) [27], KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) [28].

Identification of differentially expressed genes

Fragments per Kilobase of transcript per Million mapped reads (FPKM) estimates produced by RNA-Seq by Cuffquant and Cuffnorm of Cufflinks was used to evaluate the expression of transcripts [29]. The transcriptome comparisons of un-pollinated vs. pollinated groups and self-pollinated vs. cross-pollinated groups were conducted to find the differentially expressed genes (DEGs). Differential expression analyses were performed using the DESeq R package 1.10.1 [30], which provides statistical routines to determine DEGs based on a negative binomial distribution model. The P values were adjusted by the Benjamini and Hochberg’s approach for controlling the false discovery rate [31]. False discovery rate < 0.01 and fold change > 2 was considered to be significantly differentially expressed. Pearson’s Correlation Coefficient was used to evaluate the correlation of biological repeats [30].

Quantitative real time PCR verification

The first-strand cDNA was synthesized from 1 μg of total RNA by using the RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific, MA, USA) according to the manual. Quantitative real time PCR (QRT-PCR) was performed using SYBR Premix EX Taq (Takara, Japan) on Roche LightCycler® 480II (Switzerland) as instruction specified. The qRT-PCR primers (Table 1) were designed by using Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA). The GAPDH (GenBank: GE651107) from tea plant was used as the reference gene. All of the PCR reactions were conducted in triplicate and the average expression values were calculated. The relative expression level of each gene was calculated with the 2^(-ΔΔCT) method [32].

Statistical analysis

The statistical analysis was conducted using Excel 2016 and GraphPad Prism 5.0 (San Diego, USA). The significance analysis of difference between two samples was evaluated with Student t-test and multiple comparisons were analyzed using One-way ANOVA and P < 0.05 was considered to be statistically significant. The results were displayed as mean ± standard deviation.

Results

Pollen germination in vitro and fluorescence of pollen tubes

In order to evaluate the pollen vitality, pollen germination rate and tube growth between ‘Fudingdabai’ and ‘Yulv’ were assessed and compared. As shown in Additional
file 1: Figure S1A, the pollen appearance between two cultivars has no significant difference. Pollen germination rate and mean length of pollen tubes of the two cultivars were similar and increased gradually with prolongation of the growth time (Additional file 1: Figure S1B).

The fluorescence of pollen tube was observed to examine the growth of pollens in pistils (Additional file 2: Figure S2). After 8 h self-fertilization of ‘Fudingdabai’, the pollens germinated at stigma but no fluorescence was seen in styles. After 24 h, a few pollen tubes entered styles and the fluorescence on the base of styles was observed firstly after 48 h. Pollen tubes of ‘Yulv’ (♂) × ‘Fudingdabai’ (♀) cross-fertilized pistils showed higher growth rate than ‘Fudingdabai’ self-fertilization. The pollen tubes arrived at the base of styles after 24 and 8 h in self-fertilized ‘Yulv’ pistils and ‘Fudingdabai’ (♂) × ‘Yulv’ (♀) cross-fertilized pistils, respectively. Taken these results together, pollens from other cultivars would grow faster in pistils than that from themselves. This result was similar to that by Zhang et al. [21]. In addition, reciprocal cross-pollination showed that the pollen tube growth was slower when ‘Fudingdabai’ was used as maternal parent. However, pollen tubes in all of the self- and cross-pollinated samples reached the base of styles after 48 h.

**Ion components in self- and cross-fertilized pistils**

Ion components, especially Ca²⁺, are an indicator of self-incompatibility [33]. In tea pistils, the highest level of ion component observed in the tea pistil was K⁺, followed by Ca²⁺, Mg²⁺ and P³⁺ in sequence (Fig. 3a). Pistils of ‘Fudingdabai’ contained more K⁺ but less Ca²⁺ than those in ‘Yulv’. Ca²⁺ content in self-pollinated pistils of ‘Yulv’ (YLS) was higher than that in cross-pollinated pistils of ‘Yulv’ (YLC), but no apparent difference between self- (FDS) and cross-pollinated pistils of ‘Fudingdabai’ (FDC). In FDS pistils, the K⁺ content was higher than in FDC pistils, suggesting that Ca²⁺ and K⁺ may be involved in potential signal transduction in SI.

**Transcriptome assembly and function annotation**

A total of 18 samples were sequenced and 122.75 Gb clean data were obtained. The percentages of clean reads having a base quality greater or equal than Q30 were above 85.01% indicating that the data produced by sequencing are of high quality. The clean reads from the

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Table 1 The primers used for qRT-PCR verification

| ID      | Forward (5'-3')       | Reverse (5'-3')   |
|---------|------------------------|-------------------|
| CSA006398 | GGCGTATCCAACAATCTTATCG | CCAAACCAATCATCTCCA |
| CSA005891 | GAACGTTGTTGTCATTGTAT  | CATAAATGTCATGCGGAAG |
| CSA028406 | GAGATCCAGCAGCTGCCGTTG | AGACCCACATTTTTCATTAGC |
| CSA024717 | CCACGCGCCACTTGCTCAGTTT | GAGITGGCCACCGTGAATTCG |
| CSA002728 | GTGGTCACAGCCTGTTTCTAC  | GCCAGTTAGTTGTCATAGAGA |
| CSA026098 | GGCTTCCCTTCTTTTCTTATG | CCACATCAATTTTCTCCCTTG |
| CSA024379 | TCCCATATTAGCCTGCAAC  | ATCCCATCTACGCCCAATAAC |
| GAPDH   | TGGGCATGTGGGATCTCT   | CAGTGGAACACGGAAAGC |

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Fig. 3 Ion components in self- and cross-pollinated pistils of tea plants. a 1 un-pollinated ‘Fudingdabai’ pistils; 2–5 self-pollinated ‘Fudingdabai’ pistils at 8 h, 24 h, 48 h and 72 h; 6–9 ‘Yulv’ (♂) × ‘Fudingdabai’ (♀) cross-pollinated pistils at 8 h, 24 h, 48 h and 72 h; 10 un-pollinated ‘Yulv’ pistils; 11–14 self-pollinated ‘Yulv’ pistils at 8 h, 24 h, 48 h and 72 h; 15–18 ‘Yulv’ (♂) × ‘Fudingdabai’ (♀) cross-pollinated pistils. b The Ca²⁺ and K⁺ content during self- and cross-pollinated pistils.
18 samples showed alignment ratios between 47.77% and 54.96% (SRA accession: SRP110788, Table 2). Based on the alignment with the reference genome of tea, 8136 unannotated genes were found, and 6621 of these genes were annotated after BLAST, with 1481 unigenes in COG database, 3465 in GO database, 2301 in KEGG database, 4285 in Swissprot database and 6588 in Nr database.

**Differently expressed genes analysis**

Correlation analysis showed that T02 of self-pollinated 'Fudingdabai' at 48 h (FDS48) revealed low correlation to other two FDS48 samples (T07 and T12) with R^2 of 0.33 and 0.43, respectively. T15 of self-pollinated 'Yulv' pistil sample at 48 h (YLS48) deviated from other two replicates (T05 and T10) with R^2 of 0.46 and 0.52, respectively. These two samples (T02 and T15) were therefore removed in further DEG analysis. All replicates of remaining samples showed high correlation (Fig. 4).

A total of 1948, 3399, 3927, 3682, 145, 2061, 1343, 600 and 1859 genes were found to be differentially expressed between each of un-pollinated 'Fudingdabai' pistils (FD0) vs. FDS48, FD0 vs. cross-pollinated 'Fudingdabai' pistils after 48 h (FDC48), un-pollinated 'Yulv' pistils (YL0) vs. YLS48, YL0 vs. cross-pollinated 'Yulv' pistils after 48 h (YLC48), FDS48 vs. FDC48, FDS48 vs. YLC48, YLS48 vs. FDC48, YLS48 vs. YLC48 and FDC48 vs. YLC48, respectively (Fig. 5a). By comparing the pollinated groups with un-pollinated groups, 990 common DEGs were found (Fig. 5b). COG classification of these DEGs showed that 'General function prediction only' enriched most of DEGs, followed by 'Transcription,' 'Signal transduction mechanisms,' 'Replication, recombination and repair' and 'Secondary metabolites biosynthesis, transport and catabolism' (Fig. 5c).

GO enrichment analysis revealed that metabolic process in biological process, cell part in cellular component and catalytic activity in molecular function enriched the most DEGs (Additional file 3: Figure S3).

**Differently expressed genes between self- and cross-pollinated groups**

In comparison of self- and cross-pollinated groups, only three common DEGs were found (Fig. 6a). The 1160 common DEGs identified at least in two comparisons were therefore considered in further analysis. COG function classification revealed that cross-fertilization caused a series of responses in transcriptional level. In addition, the three common DEGs in all comparisons were UDP-glycosyltransferase 74B1 (UGT74B1, CSA001819), Mitochondrial calcium uniporter protein 2 (MCU2, CSA014152) and G-type lectin S-receptor-like serine/threonine-protein kinase RLK1 (G-lecRLK, Camellia_sinensis_newGene_13508). These genes showed similar expression patterns in un-pollinated and pollinated pistils. They also expressed at same levels during reciprocal cross-pollinations, but adversely expressed during self-

| Table 2 The alignment of transcriptomic reads on genome |
|-------------------------------------------------------|
| Sample  | Total Reads | Mapped Reads (%) | Unique Mapped Reads | Multiple Map Reads |
|---------|-------------|------------------|---------------------|--------------------|
| FD0–1   | 41,191,716  | 21,483,151 (52.15%) | 49.14% | 3.01% |
| FD0–2   | 47,556,326  | 22,717,253 (47.77%) | 43.74% | 4.03% |
| FD0–3   | 42,082,490  | 22,243,075 (52.86%) | 49.14% | 3.44% |
| FDS48–1 | 42,900,034  | 22,929,028 (53.45%) | 49.59% | 3.86% |
| FDS48–2 | 47,034,306  | 23,852,770 (51.76%) | 46.94% | 3.77% |
| FDS48–3 | 45,609,388  | 23,605,185 (51.76%) | 48.43% | 3.33% |
| FDC48–1 | 48,440,032  | 25,699,526 (53.41%) | 50.72% | 2.68% |
| FDC48–2 | 41,896,840  | 21,354,194 (50.97%) | 47.74% | 3.23% |
| FDC48–3 | 46,099,838  | 25,641,271 (51.93%) | 49.57% | 2.37% |
| YL0–1   | 46,464,662  | 23,481,565 (50.54%) | 47.86% | 2.68% |
| YL0–2   | 43,549,168  | 23,651,982 (53.41%) | 52.27% | 2.04% |
| YL0–3   | 41,549,084  | 21,639,699 (52.08%) | 49.26% | 2.82% |
| YLS48–1 | 53,246,958  | 26,180,870 (49.17%) | 46.35% | 2.82% |
| YLS48–2 | 50,605,784  | 27,367,119 (54.08%) | 51.17% | 2.91% |
| YLS48–3 | 40,743,908  | 21,133,266 (51.87%) | 45.29% | 6.57% |
| YLC48–1 | 45,900,260  | 25,068,461 (54.62%) | 51.87% | 2.74% |
| YLC48–2 | 53,049,730  | 29,151,764 (54.96%) | 52.29% | 2.67% |
| YLC48–3 | 47,106,846  | 24,086,169 (51.13%) | 48.40% | 2.64% |
pollinations of the two cultivars (Fig. 6b). Function annotation found that G-lecRLK was functioned on ‘Signal transduction mechanisms’. MCU2 worked on ‘Energy production and conversion’ and ‘Carbohydrate transport and metabolism’. Finally, UGT74B1 was annotated to ‘General function prediction only’.

In order to compare our results to the previous study [21], the data from self-pollinated (FDS48–1, SRR3290055) and cross-pollinated ‘Fudingdabai’ samples (FDC48–1, SRR3290084) at 48 h were downloaded and re-analyzed based on genome of tea plants. A total of 4262 DEGs were identified between FDS48–1 and FDC48–1 comparison. According to the large number of DEGs, we suggested that ‘Fudingdabai’ should be used as the paternal parent in the study of Zhang et al. [21]. As shown in Fig. 7, in comparison of self- and cross-pollinated groups (FDS48 vs. FDC48, FDS48 vs. YLC48 and FDS48–1 vs. FDC48–1), only five common DEGs were filtered. Therefore, the common DEGs identified at least in two comparisons were concerned. COG function classification revealed similar result to the four groups comparisons in our study (Fig. 7).

Fig. 4 Correlation analysis of the samples for differential expression analysis. Different sample numbers represent un-pollinated ‘Fudingdabai’ pistils (FD0), self-pollinated ‘Fudingdabai’ pistils at 48 h (FDS48), ‘Yulv’(♂) × ‘Fudingdabai’(♀) at 48 h (FDC48), un-pollinated ‘Yulv’ pistils (YL0), self-pollinated ‘Yulv’ pistils at 48 h (YLS48) and ‘Yulv’(♂) × ‘Fudingdabai’(♀) at 48 h (YLC48), respectively. Bold values are R² for replicates of each sample.

DEGs between reciprocal cross-pollinations

In the present study, 1859 DEGs were identified in FDC48 vs. YLC48 comparison. Except for ‘general function prediction only’, the classes of ‘Replication, recombination and repair’, ‘Transcription’ and ‘Signal transduction’ enriched most of the DEGs. In addition, G-lecRLK, MCU2 and UGT74B1 were found in these common DEGs, suggesting that these DEGs played vital roles during SI process. In the five overlapping genes, MCU2 and UGT74B1 were found. G-lecRLK was only expressed in the comparisons in our study because it was annotated by database blast but not genome mapping.
Verification of differentially expressed genes

In order to verify the reliability of RNA-Seq data, eight DEGs were selected for qRT-PCR analysis. As shown in Fig. 9, most of the DEGs showed similar expression trend compared to the RNA-Seq analysis. Therefore, the RNA-Seq analysis is credible.

Discussion

Self-incompatibility is a common phenomenon in angiosperm. In order to understand the SI mechanism of tea plants, we studied the ion components and pollen growth in self- and cross-pollinated pistils from two tea cultivars.

The results showed that pollen tubes grew faster in cross-pollinated pistils than those in self-pollinated pistils. Furthermore, Ca\textsuperscript{2+} in pistils increased after self-pollinations but decreased after cross-pollinations. In addition, comparative transcriptome analysis showed that G-type LecRLK, UGT74B1, and MCU2 genes might contribute the SI signal transduction mechanism in tea plant.

Signal transduction during self-incompatibility in tea plants

Self-incompatibility is genetically regulated by a multi-allelic S-locus which links pollen and pistil S-determinants and resulting in self-recognition. Interactions between pollen and pistil in the same haplotype triggered a SI response, which inhibits pollen tube growth and leads to failure of fertilization [34]. During SI process, a series of signal changes occurred in plants. The earliest identified physiological event caused by SI recognition is the increase of Ca\textsuperscript{2+} in incompatible pollen tubes or stigma papilla cells [35, 36]. In the present study, Ca\textsuperscript{2+} changes suggest a potential correlation between pollen tube growth and Ca\textsuperscript{2+} content. Furthermore, the opposing trend of Ca\textsuperscript{2+} ion content of the two cultivars between self- and cross-pollinated pistils reveals that Ca\textsuperscript{2+} may be an important signal for SI in tea plants.

In the present study, a DEG MCU2 was identified between self- and cross-pollination, which undertook the mitochondrial Ca\textsuperscript{2+} uptake [37]. In animals, Ca\textsuperscript{2+} uptake could regulate the mitochondrial energy production that is a stimulation of sperm-induced Ca\textsuperscript{2+} release [38]. There is evidence that the Ca\textsuperscript{2+} uptake also occurred at fertilization in mammalian eggs [39]. Inhibition of the mitochondrial function also disrupted the sperm-induced Ca\textsuperscript{2+} oscillatory pattern and intracellular Ca\textsuperscript{2+} homeostasis, and resulted in low developmental competence in mammals.
Unlike in animals, the functional mechanism of MCU in tea plants has been less studied, and therefore, needs verification except for Ca$^{2+}$, K$^+$ is also sensitive to SI. In *Papaver rhoeas*, conductance of some monovalent cations, such as K$^+$ and NH$_4^+$ were also stimulated by SI. Interestingly, content of K$^+$ changes was opposite to Ca$^{2+}$ after pollination (Fig. 3). We can therefore propose that SI activates a nonspecific ion channel in tea plants.

**Role of self-incompatibility related genes in tea plants**

LecRLK family has been classified to three subfamilies: L-type, G-type and C-type LecRLKs. This classification...
is supported by the structure analysis of these proteins. L-type LecRLK contains a legume lectin-like extracellular domain, and G-type LecRLK has a α-mannose binding bulb lectin domain, while C-type LecRLKs are characterized due to the presence of calcium-dependent carbohydrate-binding domain [42]. G-type LecRLKs were historically known as SRKs, since they hold the D-mannose binding lectin (B_lectin) and catalytic domain of the serine/threonine kinases. SRK genes have a S domain which is responsible for SI in Brassicaceae [15, 43]. Recently, these genes were also reported to confer abiotic stress tolerance and delay dark-induced leaf senescence in rice [44]. Here, we screened a similar SRK gene (Camellia_sinensis_newGene_13508) from tea plant which differently expressed between self- and cross-pollinated pistils and might contribute to the signal transduction of SI in tea plant. In general, the SRK genes function in SI through the diversity of S domain. Therefore, the S domain of the SRK gene should be identified in different tea cultivars to explore the role of SRK on SI process of tea.

The previous studies have identified a LSI or an ovarian sterility (OS) type controlling self-incompatibility in tea plants [19, 20]. The same phenomenon was also observed in our study. However, the molecular mechanism of this SI system remained unclear until Zhang et al. [21, 45]
proposed a gametophytic SI mechanism based on S-RNase control in tea plant. Unexpectedly, S-RNase gene was not found in tea plant in the present study, but three DEGs were identified in comparison between self- and cross-pollinated pistils: G-type LecRLK, MCU2 and UGT74B1. Pollen tube reception, the crosstalk between the male and female gametophytes when pollen tubes arrive at the synergid cells of the ovule in flowering plants, mutation of TURAN (TUN) and EVAN (EVN) genes led to overgrowth of the pollen tubes inside the female gametophyte and inhibited the rupture of pollen tubes. TUN encodes a UGT superfamily protein and is required for pollen tube growth and integrity by affecting the stability of the pollen-specific FERONIA RLKs [46, 47]. In this work, whether the UGT74B1 and G-type RLK genes work together on fertilization in tea plant remains unknown. Nevertheless, we can suggest that both of the genes may codetermine the SI mechanism in tea plant.

It is difficult to explain that G-type LecRLK, MCU2 and UGT74B1 showed so different expression patterns between self-pollinations. It may be due to the variety difference of tea plants. More cultivars should be adopted to detect the expression of these genes in self- and cross-pollinations to interpret their roles in SI. Besides, function analysis through transgenic test to clarify the mechanism of these two genes in SI will be a good way in the future if more studies can be conducted to overcome the barriers in tea plant transformation. Our study suggests a distinctive mode of action of SI in tea, and the results therein provide new guidance and reference for exploration of SI mechanism in tea.
Conclusion
The present study revealed that cross-pollination could promote the growth of polllens in styles and Ca^{2+} and K^{+} are involved in signal transduction in SI process of tea plants, and also G-type LecRLK and UGT74B1 may function together in controlling SI in tea plants. However, the specific role of these genes in SI process needs further identification. Our study will help understand the SI mechanism of tea plant further.

Additional files

Additional file 1: Figure S1. Pollen appearance and activity between ‘Fudingdabai’ and ‘Yulv’ in vitro. A Pollen germination and phenotype of ‘Fudingdabai’ and ‘Yulv’. B Pollen germination rate and average length of pollen tubes of ‘Fudingdabai’ and ‘Yulv’. (TIF 1137 kb)

Additional file 2: Figure S2. Fluorescence of pollen tubes in self- and cross-pollinated pistils of tea plants at 48 h. “Top” and “Base” means the stigma and the base of the style of tea flower, respectively. Arrows indicate the pollen tubes with fluorescence. (TIF 4517 kb)

Additional file 3: Figure S3. Gene Ontology enrich analysis of DEGs between unpollinated and pollinated samples. (TIF 755 kb)

Abbreviations
DEGs: Differentially expressed genes; GSI: Gametophytic self-incompatibility; G-type RLK: G-type lectin Ser/Thr-protein kinase; LSI: Late-acting self-incompatibility system; MCU2: Mitochondrial calcium uniporter protein 2; SI: Self-incompatibility; SLG: S-Locus glycoprotein gene; SRK: S-Locus receptor kinase; SSI: Sporophytic self-incompatibility; UGT74B1: UDP-glycosyltransferase 74B1

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Availability of data and materials
The datasets generated and/or analysed during the current study are available in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/) with the accession number SRR590055.

Authors’ contributions
QH performed pollen pollination experiment, RNA extraction, RNA-Seq analysis and drafted the manuscript. CC cultivated the tea plants and participated in pollination experiment and samples collection. ZZ was involved in samples collection and detected the pollen activity of tea flowers. ZZ contributed to samples collection and observation of pollen tubes fluorescence. HL conducted the ion assay and edited the figures in the manuscript. QZ carried out qRT-PCR experiment. XC was involved in samples collection. ZZ contributed to samples collection and helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Tea plants used in this study were cultivated in tea germplasm repository of Tea Research Institute of Fujian Academy of Agricultural Sciences (FAAS). This study was permitted by FAAS and conducted in accordance with stipulations of FAAS.

Competing interests
The authors declare that they have no competing financial interests.

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Author details
1Tea Research Institute, Nanjing Agricultural University, Nanjing 210095, China. 2Tea Research Institute, Fujian Academy of Agricultural Sciences, Ningle 355015, China. 3Department of Plant Science, University of Manitoba, Winnipeg R3T 2N2, Canada.

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