1 **Physiological and transcriptome analysis of indica rice ‘MH86’ overexpressing**
2 ***OsSPL14***

3 **Running title:** Phenotypic and transcriptomic changes in OE: *OsSPL14* rice

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25 **Highlight**

26 *OsSPL14*-overexpression transgenic plants showed shorter growth period,
27 short-narrow flag leaves, and thick-green leaves. Transcript profile, abscisic acid
28 (ABA) and gibberellin acid (GA₃) level, and culm composition also changed
29 obviously.

Abstract

OsSPL14, identified as *IDEAL PLANT ARCHITECTURE1 (IPA1)* or *WEALTHY FARMER'S PANICLE (WFP)* gene, plays a critical role in regulating rice plant architecture. Here, the study showed that *OsSPL14*-overexpression transgenic rice plants had shorter growth period, short-narrow flag leaves, and thick-green leaves. Compared with wild type plant 'MH86', transgenic plants had higher chlorophyll a (Ca), chlorophyll b (Cb) and carotenoid (Cx) content at both seedling and maturity stage. Meanwhile, transcriptome analysis identified 473 up-regulated and 103 down-regulated genes in transgenic plant. The expression of differentially expressed genes (DEGs) involved in carotenoid biosynthesis, abscisic acid (ABA) metabolism and lignin biosynthesis increased significantly. Most of DEGs participated in "plant hormone signal transduction" and "starch and sucrose metabolism" are also up-regulated in transgenic plant. In addition, there were higher levels of ABA and gibberellin acid (GA₃) in *OsSPL14*-overexpression transgenic plants. Moreover, the content of culm lignin, cellulose, silicon and potassium all increased dramatically. Thus, these results demonstrate that overexpression of *OsSPL14* has influence on leaf development, hormone level and culm composition in rice, which provide more insight into understanding the function of *OsSPL14*.

Key words: Culm composition, Hormone level, Indica rice 'MH86', *OsSPL14*, Overexpression, Phenotype, Transcriptome analysis

Abbreviations

ABA abscisic acid

DEGs differentially expressed genes

FC fold Change

FDR false discovery rate

GA₃ gibberellin acid

GO gene ontology

59 **JA** jasmonate acid
60 **KEGG** kyotoencyclopedia of genes and genomes
61 **qRT-PCR** quantitative real-time PCR

62

63 **Introduction**

64 *OsSPL14* is a member of the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE
65 (SPL) genes. SPL genes encode plant-specific transcription factors containing a
66 highly conserved zinc ion-containing DNA binding domain, known as SQUAMOSA
67 PROMOTER BINDING PROTEIN [SBP]-box (Yamasaki *et al.*, 2004). SPB1 and
68 SPB2 were the original SPL genes identified in *Antirrhinum majus* that bind to the
69 promoter of the floral meristem identity gene SQUAMOSA (Klein *et al.*, 1996).

70 Many SPL genes contain a target site of miRNAs (microRNAs) including
71 miR156/157, and the target sites are located in the coding regions or 3' untranslated
72 region (3' UTR). In *Arabidopsis thaliana*, 10 of 16 SPL genes have been predicted to
73 be targets of miR156 (Rhoades *et al.*, 2002; Schwab R *et al.*, 2005). *SPL3*, *SPL4*, and
74 *SPL5* have a target site for the miR156 in their 3' UTR and are strongly repressed by
75 miR156. Overexpression of *SPL3* accelerated flowering, and plants expressing *SPL3*
76 containing mutated miR156 target site or without *miR156* target site appeared earlier
77 flowering and fewer leaves (Cardon *et al.*, 1997; Wu and Poethig, 2006; Gandikota *et*
78 *al.*, 2007). Besides, paralogous genes *SPL9* and *SPL15* with miR156 target site
79 involved in controlling the juvenile-to-adult phase transition, and *spl9 spl15* double
80 mutants showed shortened plastochron, altered inflorescence architecture and
81 enhanced branching (Schwarz *et al.*, 2008; Wang *et al.*, 2008). In addition, *SPL8* with
82 no miRNA target sites affected megasporogenesis, trichome formation on sepals, and
83 stamen filament elongation (Unte *et al.*, 2003). Accordingly, a series of studies on *SPL*
84 gene in *Arabidopsis thaliana* suggested that *AtSPL* genes mainly associated with plant
85 development and flowering.

86 In rice, there are 19 SPL genes (*OsSPL*) that unevenly distributed in the genome.
87 Among them, 11 *OsSPL* genes were putative targets of OsmiR156. *OsSPL* genes

88 express in various tissues including root, leaf, stem, panicle, stamen, pistil, and most
 89 of them predominantly expressed in the young panicles (xie *et al.*, 2006). Study on the
 90 liguleless mutant indicated that *OsLGI* encoding OsSPL8 participates in building the
 91 laminar joint between leaf blade and leaf sheath boundary, so as to control the
 92 development of ligule and auricle (Lee *et al.*, 2007). *OsSPL14*, also known as the
 93 *IPA1/WFP* gene, is a homologous gene of the Arabidopsis SPL9/SPL15, and it is
 94 regulated by OsmiR156/OsmiR529 (Jeong *et al.*, 2011; Yue *et al.*, 2017). A point
 95 mutation in target site for OsmiR156 resulted in a significantly increased protein level
 96 of OsSPL14, which leads to reduced tiller number, increased grain number and
 97 grain weight, produced stout stems and improved lodging resistance (Jiao *et al.*, 2010).
 98 Additionally, an epigenetic change in the *OsSPL14* promoter resulted in the same
 99 phenotype (Miura *et al.*, 2010). Moreover, the three tandem repeats in the upstream
 100 regions of *IPA1/OsSPL14* enhanced an open chromatin configuration and reduced
 101 DNA methylation at *IPA1/OsSPL14* promoter, resulting in an optimal *OsSPL14*
 102 expression and ideal plant architecture with proper tiller number and large panicle
 103 (Zhang *et al.*, 2017). While, the *ipa1* loss-of-function mutants generated by
 104 CRISPR/Cas9 had a dwarf phenotype with increased tiller number (Li *et al.*, 2016). In
 105 addition, genome-wide expression profiling analysis suggested that *IPA1/OsSPL14*
 106 could directly bind to the SBP-box target motif GTAC and regulated expression of
 107 TEOSINTE BRANCHED1 (TB1) and DENSE AND ERECTPANICLE1 (DEP1), so
 108 as to influence till number, plant height and panicle length (Lu *et al.*, 2013). Then,
 109 according to *IPA1/OsSPL14* effect mentioned above, the studies on mechanism of
 110 gene expression regulation were carried out. The study indicated that a RING-finger
 111 E3 ligase named IPA1 INTERACTING PROTEIN1 (IPI1) could regulate the protein
 112 levels of *IPA1/OsSPL14* in different tissues. Interestingly, IPI1 promotes
 113 accumulation of *IPA1/OsSPL14* in shoot apices, but it promotes degradation of
 114 *IPA1/OsSPL14* in panicles (Wang J *et al.*, 2017). And the other research suggested
 115 that a human ovarian tumor domain-containing ubiquitin aldehyde-binding protein
 116 1(OTUB1)-like deubiquitinase (*OsOTUB1*) could promote the degradation of

117 IPA1/OsSPL14 indirectly (Wang S *et al.*, 2017). Moreover, *DWARF 53 (D53)*, a key
118 repressor of the strigolactone (SL) signaling pathway, suppressed the transcription of
119 *IPA1/OsSPL14*. However, IPA1/OsSPL14 acted in the feedback regulation of *D53*
120 expression (Song *et al.*, 2017). The latest research indicated that phosphorylated
121 IPA1/OsSPL14 activates expression of *WRKY45* and then enhanced disease resistance
122 (Wang *et al.*, 2018). In short, the previous studies revealed that IPA1/OsSPL14 played
123 important role in controlling plant architecture and panicle architecture. Also, gene
124 regulatory networks related to *IPA1/OsSPL14* are complex and the corresponding
125 molecular mechanism requires further clarification.

126 In this study, we introduced *OsSPL14* into indica cultivar ‘MH86’, and obtained
127 transgenic lines overexpressing *OsSPL14* with shorter growth period, short-narrow
128 flag leaf, fewer tiller numbers, strong culm. Transcriptome analysis indicated that
129 genes involved in carotenoid biosynthesis and lignin biosynthesis were up-regulated
130 in transgenic plant. Levels of ABA and GA₃ improved, and the content of culm lignin,
131 cellulose, silicon and potassium increased obviously. Taken together, these
132 discoveries supplement the function description of OsSPL14.

133 **Materials and Methods**

134 **Generation of transgenic rice**

135 The plasmid pCAMBIA1300-*OsSPL14* (Supplementary Fig.S1; *OsSPL14/IPA1*:
136 GenBank GU136674.1, 7229 bp containing the promoter and coding region for
137 mRNA) was introduced into mature rice (*Oryza sativa L.* indica cultivar ‘MH86’)
138 embryos using Agrobacterium-mediated transformation as described by Datta (1997).
139 The transformed cells were selected initially on NB medium (Sivamani *et al.*, 1996)
140 containing 50 mg/L hygromycin (SIGMA-ALORICH). After 3-4 selection cycles
141 with a 14-day duration per cycle, surviving cell clusters were regenerated. The plants
142 that were generated were selected further in the culture solution containing 20 mg/L
143 hygromycin with a 16-h photoperiod at 26 °C for 4 weeks and then transferred to a
144 greenhouse and grown with WT plants under natural sunlight.

145 Genomic DNA was prepared from the bialaphos-resistant plants by the

146 Cetyltrimethylammonium bromide (CTAB) method and used as a template for
 147 Polymerase Chain Reaction (PCR) and southern blot analysis. The presence of the
 148 introduced gene in the regenerated plants was verified by PCR using the Hygromycin
 149 F/R primers. A total of 100 µg DNA per sample was digested overnight with *EcoRI*
 150 restriction enzyme at 37°C. The digested DNA was fractionated in 1% (w/v)
 151 TAE-agarose gels and then transferred to a Hybond-N nylon membrane (Amersham,
 152 Arlington Heights, IL, USA), according to the manufacturer's instructions. A partial
 153 sequence of *Hygromycin* gene was isolated from the plasmid and labeled using an
 154 AlkPhos direct labeling kit (Amersham) in order to make the hybridization probes.
 155 Then the DNA fragments on the Hybond-N nylon membrane were hybridized with the
 156 *Hygromycin* probe.

157 Total RNA was isolated from PCR positive plants and WT using TRIzol reagent
 158 (Invitrogen, USA). First-strand cDNA was synthesized from 2 µg of total RNA using
 159 the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, LTU) following the
 160 manufacturer's instructions. Reverse transcription polymerase chain reaction
 161 (RT-PCR) was performed using Hygromycin F/R and Actin150 F/R primers.

162 The transgenic plants were grown in transgenic experimental field. And stably
 163 inherited transgenic plants possessing two copies of the transgene were selected and
 164 used in this study.

165 **RNA sequencing and transcriptome analysis**

166 Total RNA was isolated from one-month-old seedlings of transgenic plants and
 167 'MH86' using TRIzol reagent. RNA purity and concentration were checked using
 168 NanoDrop microvolume spectrophotometer (Thermo Scientific NanoDrop
 169 Products, Waltham, MA, USA). And RNA integrity was quantified using Agilent 2100
 170 Bioanalyzer® (Agilent Technologies, Palo Alto, Calif.). Then the enrichment of
 171 mRNA containing poly (A) was performed by Oligo (dT) magnetic beads.
 172 Subsequently, the mRNA was fragmented and used to synthesize double-stranded
 173 cDNA (dsDNA). And the dsDNA was purified using AMPure XP beads (Beckmann
 174 Coulter, Pasadena, CA). Next, the "A" tail and paired-end adapters for sequencing

175 were ligated to the end of dsDNA. Different sizes of fragments were selected using
176 AMPure XP beads for PCR amplification to create the cDNA library. The
177 concentration and insert size of the cDNA library were detected using Qubit2.0 and
178 Agilent 2100, respectively. Meanwhile, the effective concentration of the cDNA
179 library was accurately quantified using quantitative PCR. After complete testing, the
180 cDNA library was sequenced using Illumina HiSeq2500 Sequencer. Low quality reads
181 were filtered to get clean reads, then the clean reads were mapped to the reference
182 genome (http://plants.ensembl.org/Oryza_indica/Info/Index) using TopHat2 and
183 mapped reads were obtained.

184 Based on the location information of mapped reads, gene expression levels were
185 calculated by cuffquant and cuffnorm components of cufflinks software using
186 fragments per kilobase of transcript per million fragments mapped (FPKM) as
187 measurement index. According to screening standard of fold Change (FC) ≥ 2 and
188 false discovery rate (FDR) < 0.01 , differentially expressed genes (DEGs) analysis
189 between *OsSPL14*-overexpression transgenic plants and wild type plant 'MH86' was
190 performed by differential expression analysis for sequence count data (DEseq). The
191 log₂FC (fold changes) was used to indicate the significance of expression difference
192 of each gene. The test corrections of P-values were performed using the
193 Benjamini-Hochberg False Discovery Rate method. The numbers of DEGs between
194 samples were counted and plotted in Volcano plot.

195 For gene ontology (GO) enrichment analysis, DEGs were implemented in
196 biological annotation system by Gene Ontology Consortium. And DEGs were
197 classified according to biological process, molecular function and cellular component
198 ontologies. The most enriched GO terms were conducted by KOBAS (2.0), and GO
199 terms with FDR ≤ 0.05 were considered as significantly enriched. The DEGs were
200 subjected to search against Cluster of Orthologous Groups of proteins database to
201 predict and classify their possible functions. Then the pathway enrichment and
202 annotation analysis of DEGs were carried out by comparing to the KEGG (Kyoto
203 Encyclopedia of Genes and Genomes) database. At last, taking pathway in KEGG

204 database for unit, the DEGs significantly enriched metabolic pathways or signal
205 transduction pathways were identified by hypergeometric test.

206 Quantitative Real-time PCR (qRT-PCR)

207 To validate DEGs identified in the transcriptome analysis and analyze some genes
208 from *OsSPL14* gene regulatory networks, qRT-PCR was conducted on selected genes.
209 For qRT-PCR, cDNA was generated from the previously collected RNA using the
210 RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, LTU). The qRT-PCR was
211 performed on a 7500 real-time PCR system (Applied Biosystems) with the Fast Start
212 Universal SYBR Green Master system (Roche, USA), following the manufacturer's
213 protocol. Relative quantification of gene expression was performed using actin gene
214 expression as a reference. The relative quantitative method ($\Delta\Delta CT$) was used to
215 evaluate the quantitative variation in the examined replicates. Primers used in
216 qRT-PCR are listed in Supplementary Table S1. Additionally, TB F/R and DEP F/R
217 primers, D53 F/R primers, and D17 F/R, D14 F/R, D3 F/R primers were referenced to
218 Lu *et al.* (2013), Song *et al.* (2017), Sun *et al.* (2014), respectively.

219 Assay of abscisic acid (ABA), jasmonate acid (JA) and gibberellin acid (GA₃)

220 One-month-old seedlings and plants at tillering stage were collected to reserve. And
221 ABA, JA, GA contents were detected by high performance liquid chromatography
222 (HPLC, Agilent 1100).

223 For extraction of ABA, 0.15 g plant material was manually ground in liquid
224 nitrogen. Samples were taken in 1 mL ABA extraction reagent (Suzhou Comin
225 Biotechnology Co. Ltd) at 4 °C for overnight. Samples were centrifuged for 10 min at
226 8000 ×g, and the residue was extracted with 0.5 mL ABA extraction reagent for 2
227 hours again. After 2 times centrifugation, the supernatant was collected to be dried
228 with 40 °C nitrogen until containing no organic phase. Then 0.5 mL petroleum ether
229 was added to extract and decolorize at 60-90 °C for three times. The samples in the
230 lower layer were collected to be dried with 40 °C nitrogen, then concussed and
231 dissolved in 0.5 mL mobile phase containing acetic acid and methanol. Next, the
232 samples were filtered through minisart filters for HPLC analysis. Chromatographic

experiments were performed on Kromasil C18 column (250mm*4.6mm, 5 μ m) with a mixture acetic acid-methanol as the mobile phase, a flow rate of 0.8 mL/min, a detection wavelength at 254 nm, and column temperature at 30 °C.

JA was extracted using 2 g plant material in Eppendorf tube containing JA extraction reagent (Suzhou Comin Biotechnology Co. Ltd). After 30 min sonication, samples were centrifuged for 10 min at 8000 $\times$ g. The supernatant was collected to be dried with nitrogen in ice-water bath. Then the samples were dissolved in 0.5 mL mobile phase of methanol: 0.1% formic acid (65: 35) and filtered for HPLC analysis. Chromatographic experiments were performed with a flow rate of 0.8 mL/min, a detection wavelength at 210 nm, and column temperature at 35 °C.

GA was extracted using 1.5 g plant material in 1 mL GA extraction reagent (Suzhou Comin Biotechnology Co. Ltd) at 4 °C for overnight. After centrifugation, the supernatant was collected to be dried. And 0.5 mL petroleum ether was added to extract and decolorize at 60-90 °C for three times. Next, the samples were extracted in reagent and dried with nitrogen. Then the samples were dissolved in 0.5 mL mobile phase of 0.1% acetic acid: methanol (60: 40) and filtered for HPLC analysis. Chromatographic experiments were performed Kromasil C18 column with a flow rate of 0.8 mL/min, a detection wavelength at 254 nm, and column temperature at 30 °C.

Scanning electron microscopy (SEM) analysis

At heading stage, a 0.3-cm section was cut with a scalpel in the middle of the third internode and placed in 3% glutaraldehyde for fixing 8-10 h. After rinsed with phosphate buffer (0.1mol/L, PH7.2), samples were fixed with 1% osmic acid for 1h. Samples were rinsed with phosphate buffer, then they were dehydrated in a grade of ethanol series (35%, 50%, 60%, 70%, 95%, 100%, 100%) for 1h per grade. Next, samples were transferred into isoamyl acetate and dried using CO₂ as transitional fluid. Finally, the samples were mounted onto aluminum stubs and coated with carbon gold, then observed with 3500N scanning electron microscope (Hitachi, Tokyo, Japan) to collect images.

Composition analysis of culm

262 At the mature stage, plants culm was collected and placed in an oven at 105°C for 30
263 min, then transferred to 65°C until a constant weight was achieved. Then the plant
264 samples were crushed in a grinder to make powder for test.

265 For determination of cellulose and lignin content, 1 g powder sample was
266 treated with 100 mL 3% sodium dodecyl sulfate containing three drop of decalin and
267 0.5 g anhydrous sodium sulfite in beaker. And the sample solution was heated to
268 boiling for 1 h. After filtration, the precipitate was washed with hot water and
269 acetone, then dried 3 h at 105 °C to weigh. Next, the precipitate was heated and
270 digested with 100 mL 2% cetyltrimethylammonium bromide. The sample solution
271 was filtered and the second precipitate was obtained, then washed and dried to weigh.
272 Finally, the second precipitate was treated with 72% sulfate for 3 h, then desiccated
273 to weigh. The numerical calculation of cellulose and lignin content was reference to
274 method described in GB/T 13885-2003/ISO 6869:2000; 2000.

275 For measurement of potassium content, 2 g powder sample was digested with
276 30mL nitric acid and perchloric acid (5:1) overnight. Then the sample solution was
277 treated by heating digestion method to be clear. After forging, deionized water was
278 added to a constant volume of 100 mL and filtered for detection. According to the
279 standard curves of potassium, potassium content was measured with atomic
280 absorption spectrometry (AAS) at 766.5 nm (GB/T 13885-2003/ISO 6869:2000;
281 2000).

282 1 g powder sample sifted by 60-mesh sieve was using to measure silicon (Si)
283 content. 15 mL concentrated sulfuric acid was gradually added to the powder sample
284 in beaker. 40 min later, 50 mL concentrated nitric acid was gradually added to it and
285 slow heating to precipitate. Then sodium hydroxide was added to dissolve the
286 precipitate, and the silicon (Si) content of culm was determined using the silicon
287 molybdenum blue colorimetric method (Tong *et al.*, 2005; Dai *et al.*, 2005).

288 **Results**

289 **Phenotype of MH86 over-expressing *OsSPL14***

290 We introduced “pCAMBIA1300-*OsSPL14*” into indica cultivar MH86 using

291 agrobacterium-mediated transformation and generated the *OsSPL14*-overexpression
 292 transgenic plants (OE: *OsSPL14*; Supplementary Fig. S2). Remarkably, the growth
 293 period of OE: *OsSPL14* transgenic plants shortened by 5-15 days depended on the
 294 growing areas (Fig. 1A). OE: *OsSPL14* transgenic plants had shorter and narrower
 295 flag leaves. Compared to that of wild type plant ‘MH86’ (WT), the flag leaf length
 296 and width of OE: *OsSPL14* transgenic plant decreased by about one third and one
 297 quarter respectively (Fig. 1B, C, D). Well consistent with previous report (Jiao *et al.*,
 298 2010), OE: *OsSPL14* transgenic plants showed decreased tiller number and strong
 299 culm (Fig. 1E). The culm diameter and culm wall thickness of transgenic plant
 300 increased by about 24% and 20% respectively (Fig. 1F, G). However, the height of
 301 OE: *OsSPL14* transgenic plants was not significantly different from that of ‘MH86’
 302 (Fig. 1H). In addition, it is obvious that OE: *OsSPL14* transgenic plants appeared
 303 greener, especially at the maturity stage. Also, the leaves of transgenic plants were
 304 thicker than that of ‘MH86’. And the result of measurement indicated that the
 305 chlorophyll a (Ca), chlorophyll b (Cb) and carotenoid (Cx) content of leaves
 306 increased obviously at seedling stage and maturity stage (Fig. 1I, J).

307 **Comparative analysis of transcriptome profiling**

308 To investigate the impact of *OsSPL14* overexpression on the rice transcriptome, we
 309 conducted an RNA sequencing experiment using OE: *OsSPL14* transgenic line and
 310 ‘MH86’ (Control). Differentially expressed genes (DEGs) was identified by
 311 screening standard thatfold Change (FC) ≥ 2 and false discovery rate (FDR) < 0.01 .
 312 And the analysis revealed a total of 576 differentially expressed genes containing
 313 473 up-regulated genes and 103 down-regulated genes (Fig. 2A).

314 Subsequently, gene ontology (GO) was used to classify the functions of the
 315 DEGs. The DEGs were categorized into three major GO categories including
 316 biological process, cellular component and molecular function, which totally
 317 contained 51 function groups (Fig. 2B). Meanwhile, the most enriched GO terms
 318 were conducted by KOBAS (2.0) with FDR ≤ 0.05 , and it showed that DEGs were
 319 grouped into 20 different GO terms, including 15 terms for molecular function, 3

terms for biological process and 2 terms for cellular component (Fig. 2C). It implied that DEGs annotated with GO terms were mainly related to “ion binding”, “heme binding”, “sequence-specific DNA binding”, “oxidation-reduction process”, “regulation of transcription” and “cytoplasmic membrane-bounded”.

Moreover, in order to further understand the biological functions of the DEGs, we mapped the DEGs in Kyoto Encyclopedia of Genes and Genomes (KEGG) database and obtained 20 KEGG pathways that the most remarkably enriched (Fig. 2D; Supplement Table S2). And according to enrichment factor, Q value and the number of enriched genes in corresponding pathway, five KEGG pathways were selected to be annotated in details, which showed that most of the DEGs in these pathways were up-regulated (Table 1). In particular, the five DEGs in “Carotenoid biosynthesis” were all up-regulated. Among them, “BGIOGA011548” encoding beta-carotene hydroxylase 2 (DSM2) and “BGIOGA029294” encoding Phytoene synthase (PSY) are the important genes involved in carotenoid biosynthesis. Because carotenoid is the precursor of abscisic acid (ABA), the other genes “BGIOGA025169” and “BGIOGA013214” encoding 9-cis-epoxycarotenoid dioxygenase (NCED), “BGIOGA029635” encoding Abscicic acid 8'-hydroxylase 3 (8'-OH-ABA3) also participate in ABA metabolism. The up-regulated DEGs in “Phenylpropanoid biosynthesis” were “BGIOGA005999” encoding phenylalanine ammonia-lyase (PAL), “BGIOGA006502” and “BGIOGA008177” encoding trans-cinnamate 4-monooxygenase/cinnamate-4-hydroxylase (C4H), “BGIOGA026897” encoding “4-coumarate-CoA ligase” (4CL), which also are key enzyme genes for lignin just because lignin is a phenylpropanoid monomer polymer (Zhong and Ye, 2015). The pathway showed the highest enrichment factor was “Thiamine metabolism”, in which the up-regulated DEG was “BGIOGA025304” encoding 1-deoxy-D-xylulose-5-phosphate synthase 2 (DXS). DXS is the important enzyme involved in terpenoid anabolism, such as the biosynthesis of chlorophylls, tocopherols (VE), carotenoids, ABA, gibberellin (GAs), and so on (Aharoni *et al.*, 2005; Estévez *et al.*, 2001). Additionally, the DEGs in “Plant hormone signal

transduction” are related to jasmonate acid (JA), ethylene and ABA signal transduction. The DEGs in “Starch and sucrose metabolism” are mainly related to sucrose, starch, trehalose, fructan and pectin metabolism.

Gene expression analysis by qRT-PCR

Firstly, we analyzed the expression of *OsSPL14* in OE: *OsSPL14* transgenic lines. As expected, the expression of *OsSPL14* increased obviously in the detected transgenic lines (Fig. 3A). To confirm DEGs identified in the transcriptome analysis, several genes from the five KEGG pathways selected in last part were analyzed by qRT-PCR. And the result suggested that the expression of 15 up-regulated DEGs were higher in OE: *OsSPL14* transgenic plants than that in ‘MH86’ (Fig. 3B). Meanwhile, the expression of five down-regulated DEGs were lower in OE: *OsSPL14* transgenic plants than that in ‘MH86’ (Fig. 3C). In short, the expression profiles of DEGs analyzed by qRT-PCR were well consistent with transcriptome analysis.

According to previous research, *OsSPL14* regulated expression of *TB1* and *DEP1*, and *OsSPL14* may act with *D53* to mediate the Strigolactone (SL)-regulated tiller development (Lu *et al.*, 2013; Song *et al.*, 2017). So the expression profiles of *TB1*, *DEP1* and some genes related to SL biosynthesis or SL signal transduction were also analyzed by qRT-PCR. The result indicated that *TB1* had no obvious change, while *DEP1* was up-regulated slightly in OE: *OsSPL14* transgenic plants. In addition, compared with ‘MH86’, *DWARF 17 (D17)* involved in SL biosynthesis was up-regulated in OE: *OsSPL14* transgenic plants. *DWARF 14 (D14)* and *DWARF 3 (D3)* involved in SL signal transduction were up-regulated in transgenic plants (Fig. 3D). These results suggested that overexpression of *OsSPL14* could indeed influenced on the expression of several genes involved in plant architecture.

Changes in ABA, JA and GA₃ content

In contrast with that of ‘MH86’, the phenotype of OE: *OsSPL14* transgenic plant changed greatly. Coincidentally, according to transcriptome analysis, it is remarkable that the expression of several genes in carotenoid biosynthesis, ABA metabolism, terpenoid anabolism and plant hormone signal transduction pathways are up-regulated. Thus, we are particularly intrigued that whether the content of relevant

379 plant hormones changed in OE: *OsSPL14* transgenic plants. To ascertain it, we
 380 randomly selected two OE: *OsSPL14* transgenic lines to measure ABA, JA and GA₃
 381 content of OE: *OsSPL14* transgenic plants at seeding stage and tillering stage.
 382 Compared to that of 'MH86', ABA content of OE: *OsSPL14* transgenic plants
 383 increased obviously at seeding stage, by 18% and 23% in two transgenic lines
 384 respectively. The ABA content of transgenic plant increased slightly at tillering stage
 385 (Fig. 4A). However, JA content has no change in transgenic plant at seeding stage
 386 and decreased slightly in one transgenic line at the tillering stage (Fig. 4B). In
 387 contrast, the GA₃ content of transgenic plants increased dramatically at the seeding
 388 stage, which was about three times that of 'MH86'. Though both the GA₃ content of
 389 transgenic lines and 'MH86' decreased significantly at tillering stage, the GA₃
 390 content of transgenic lines still increased by 56% and 27% compared to that of
 391 'MH86' (Fig. 4C). Together, the observations indicated that there are higher level of
 392 ABA and GA₃ in OE: *OsSPL14* transgenic plants than that in 'MH86'.

393 **Scanning and composition analysis of culm**

394 One obvious characteristic of OE: *OsSPL14* transgenic plants is their strong culm.
 395 Consequently, the internal organizational structure of culm was observed by
 396 scanning electron microscopy (SEM). The observation showed that OE: *OsSPL14*
 397 transgenic plants had more small vascular bundles and sclerenchyma cells, and also
 398 had larger big vascular bundles. Moreover, it was obvious that the lignified degree of
 399 cortical cell was greater in OE: *OsSPL14* transgenic plant (Fig. 5A).

400 Based on transcriptome analysis in this article and the changes in internal
 401 organizational structure of culm, it is noteworthy that the expression profiles of the
 402 key genes involved in lignin biosynthesis and carbohydrates metabolism changed
 403 significantly. Subsequently, we hypothesized that culm composition of OE:
 404 *OsSPL14* transgenic plant was likely to change. To test this prediction, we measured
 405 the lignin, cellulose, silicon and potassium content of culm. In contrast with that of
 406 'MH86', the lignin content of OE: *OsSPL14* transgenic plant increased distinctly, by
 407 17% and 30% in the two lines respectively (Fig. 5B). And the cellulose content of

transgenic lines also increased by 13% and 16% (Fig. 5C). Remarkably, the silicon content of transgenic plant increased dramatically by 45% and 78% compared to that of 'MH86' (Fig. 5D). In addition, the potassium content also increased obviously by 18% and 19% respectively (Fig. 5E). Evidently, overexpression of OsSPL14 in rice would cause great changes in culm character and composition. Besides, it is well known that culm composition is closely related to culm mechanical strength, which may likely explain the previously findings that the culm mechanical strength of the NIL OsSPL14^{ipa1} plant with high expression of OsSPL14 was significantly increased (Jiao *et al.*, 2010).

Grain quality analysis

It is clear that transgenic lines overexpressing OsSPL14 have large panicle, more branching and more grains per panicle (Jiao *et al.*, 2010; Miura *et al.*, 2010). Here, we performed grain quality analysis of OE: OsSPL14 transgenic plant. The result indicated that brown rice rate, milled rice rate and gel consistency had no obvious change in transgenic plant (Fig. 6A, B, C). While, head rice rate and grain length of OE: *OsSPL14* transgenic plant decreased slightly (Fig6. D, E), and the gelatinization temperature elevated slightly (Fig6. F). The amylose content of transgenic plant decreased obviously (Fig6. G). However, the chalkiness ratio of OE: OsSPL14 transgenic plant was one fold higher than that of 'MH86' (Fig6. H). Meanwhile, the chalkiness degree was 2.2 times that of 'MH86' (Fig6. I). The higher chalkiness ratio and chalkiness degree induced the decreased transparency (Fig6. J). Therefore, it seemed that some grain quality characters of OE: OsSPL14 transgenic plant changed to a certain extent.

Discussion

Os SPL14, as a transcription factor, plays an important role in rice plant architecture. The typical characteristics of transgenic plant overexpressing *OsSPL14* were reduced tiller number and strong culm. Expect for these characteristics, we observed that OE: *OsSPL14* transgenic plant have shorter growth period. It is consistent with previous research that overexpression of SPL3 caused early flowering and SPL9/SPL15

437 controlled the juvenile-to-adult phase transition in *Arabidopsis thaliana* (Cardon *et*
438 *al.*, 1997; Schwarz *et al.*, 2008). Further study suggested that SPL3 impacted on
439 flowering through directly activating plant-specific transcription factor LEAFY
440 (LFY), MADS domain protein FRUITFULL (FUL) and APETALA1 (AP1), which
441 controlled meristem identity (MI) transition (Yamaguchi *et al.*, 2009). A previous
442 study showed that the SPL genes affect flowering as downstream targets of
443 CONSTANS (CO) and flowering time regulator (FT) at the shoot apex (Schmid *et*
444 *al.*, 2003). But the later study indicated that SPLs control flowering in a separate
445 endogenous flowering pathway differed from familiar FT/FD (FD: bZIP
446 transcription factor) flowering model, and the miR156/SPL module promoted
447 flowering in the absence of photoperiodic cues (Wang *et al.*, 2009). Thus, the
448 molecular mechanisms of SPLs pathway controlling flowering are not fully
449 understood, and there is hardly research on the molecular mechanisms in rice.

450 It is obvious that OE: *OsSPL14* transgenic plant showed short-narrow flag
451 leaves and thick-green leaves in this study. In *Arabidopsis thaliana*, most
452 miR156-targeted SPL genes have high levels in the shoot apex (Cardon *et al.*, 1997;
453 Wu and Poethig, 2006). Moreover, Wang *et al.* (2008) indicated that miR156
454 quantitatively modulates SPL expression in leaf primordia and SPL mediates
455 nonautonomous effects of existing leaf primordia on the initiation of new leaf
456 primordia at the shoot apical meristem. And plants overexpressing SPL9 showed a
457 strong plastochron with reduced the leaf initiation rate. The research suggested SPLs
458 function in plastochron length and leaf size, but the mechanism including the
459 downstream target gene of SPLs in leaf development is still unknown. Similarly,
460 OsSPL14 was predominantly expressed in the shoot apex (Jiao *et al.*, 2010; Miura *et*
461 *al.*, 2010). And OsSPL14 mRNA levels decreased in flag leaves of
462 OsmiR156-overexpression transgenic plants (Xie *et al.*, 2006). It suggested the
463 possibility of OsSPL14 involved in development of flag leaf, though there is hardly
464 study on the molecular mechanisms of SPLs in regulating leaf development in rice.
465 Correspondingly, we observed that OE: *OsSPL14* transgenic plants showed greener

and the chlorophyll a, chlorophyll b and carotenoid content were higher at seedling stage and at maturity stage. It may chiefly attributable to the up-regulation of genes involved in terpenoid anabolism as description in transcriptome analysis of this study. Nevertheless, there is hardly research on effects of SPLs on leaf color either in rice or in *Arabidopsis thaliana*.

Plant hormones play a vital role in regulating plant growth, development and adaption to environment change (Santner and Estelle, 2009). ABA is a phytohormone that regulates seed dormancy, seed embryo development and stress tolerance improvement (Nambara and Marion-Poll, 2005; Matilla *et al.*, 2015). And SLs are hormones involved in suppressing lateral shoot branching, root development, response to environmental condition and secondary growth in vascular plants (Ruyter-Spira, 2013; Al-Babili, 2015). It is notable that ABA and SLs are apocarotenoids shared carotenoid as a biosynthetic precursor (Matusova *et al.*, 2005). In accord, our transcriptome analysis revealed that the expression of phytoene synthase gene (*PSY*) and beta-carotene hydroxylase 2 gene (*DSM2*) that the key enzyme genes in carotenoid biosynthesis are up-regulated in OE: *OsSPL14* transgenic plant. Besides, the expression of 9-cis-epoxycarotenoid dioxygenase gene (*NCED*) participated in the rate-limiting step in ABA biosynthesis and genes involved in SLs biosynthesis and SLs signal transduction also changed. Indeed, the carotenoid content of transgenic plant was higher than that of ‘MH86’, and it could provide more precursor for ABA or SLs biosynthesis. According to the common biosynthetic precursor, there is inevitable relation between ABA and SLs. ABA-deficient tomato mutants exhibit greatly reduced ABA and SLs content with the downregulation of SL biosynthetic genes, suggesting a role of ABA in the regulation of SLs biosynthesis (López-Ráez *et al.*, 2010). Similarly, SL deficient showed reduced ABA level under osmotic stress (Liu *et al.*, 2015). And there is evidence that the biosynthetic pathways of ABA and SLs are functionally connected. Haider *et al.* (2018) indicated that SLs interact with ABA during the drought response in rice. SL and ABA pathways are connected with through the SL

495 biosynthetic enzyme DWARF27 (D27), and overexpression of D27 in rice resulted
496 increased ABA levels. Also, a study indicated that enhanced levels of endogenous
497 ABA cause a decrease in SL production and there was an antagonistic interaction
498 between SLs and ABA biosynthesis in barley. Barley plants expressing an RNAi for
499 *ABA 8'-hydroxylase* involved in ABA degradation had higher ABA content but lower
500 SLs content, and led to increased tiller numbers (Wang *et al.* 2018), but it was likely
501 to occur in the extreme situation. In our study, OE: *OsSPL14* transgenic plants with
502 higher ABA contents still showed decreased tiller numbers. And previous study
503 indicated that *OsSPL14* may mediate the SL-regulated tiller development (Song *et*
504 *al.*, 2017). So we conjecture higher ABA could accompany with higher SLs level and
505 they combined effect on plant growth and development.

506 In addition, gibberellin (GA) is another important growth-promoting hormone.
507 GA is one of tetracyclic diterpenoid plant hormones that participate in diverse
508 growth and developmental processes including leaf differentiation, stem elongation,
509 trichome development, seed germination and induction of flowering (Olszewski N *et*
510 *al.*, 2002; Fleet and Sun, 2005). One main function of GA is regulating organs
511 elongation by affecting cell division and elongation. The gibberellin-deficient *dwarf1*
512 (*gdd1*) rice mutant showed a phenotype of greatly reduced length of root, stems,
513 spikes, and seeds, which can be rescued by exogenous GA₃ treatment. Further
514 elucidated that GDD1 with transcription regulation activity affects GA accumulation
515 through regulating ent-kaurene oxidase (KO2) involved in GA biosynthesis (Li *et al.*,
516 2011). In Arabidopsis, *SPL8*, a member of *SPL* family genes, was proved to be a
517 tissue-dependent regulatory role in response to GA in plant development. And
518 change in *SPL8* expression affected transcription of genes involved in GA
519 biosynthesis and signaling (Zhang *et al.*, 2006). In this study, we found that the
520 expression of 1-deoxy-D-xylulose-5-phosphate synthase 2 gene (*DXS*) involved in
521 terpenoid anabolism is up-regulated obviously in OE: *OsSPL14* transgenic plant, and
522 the GA₃ content increased significantly. Previous research showed that rice plant
523 with high *OsSPL14* protein level appeared large panicles and increased plant height

(Jiao *et al.*, 2010; Zhang *et al.*, 2017), which may attribute to high GA₃ level. Moreover, the other main function of GA is promoting flowering. The study indicated that SPL15 was a key component in the integration of age- and GA-derived flowering pathways at the Arabidopsis Shoot Meristem (Hyun *et al.*, 2016). Hence, we conjecture that there was a definite relation between early flowering in OE: *OsSPL14* transgenic plant and high GA₃ level. Based on these, we infer that overexpression of OsSPL14 has influence on several plant hormones biosynthesis so as to regulate plant development. However, the mechanisms are needed to be further elucidated.

In vascular plants, the major component of the secondary cell wall is lignocellulose, which is mainly consisted of lignin, cellulose, hemicellulose, cell wall proteins and so on (Kummar *et al.*, 2016). Lignin is a phenylpropanoid monomer polymer that maintains structural integrity of the cell wall and increases mechanical strength of plant. The pathways of lignin monomer biosynthesis start with deamination of phenylalanine by phenylalanine ammonia-lyase (PAL). Then the product cinnamic acid was transferred to coumaric acid by cinnamic acid 4-hydroxylase (C4H), and further acted by 4-coumarate--CoA ligase (4CL) produces *p*-coumaroyl-CoA (Boerjan *et al.*, 2003; Zhong and Ye, 2015; Kummar *et al.*, 2016). In this study, transcriptome analysis indicated that the expression of PAL, C4H and 4CL were up-regulated in OE: *OsSPL14* transgenic plant. Coincidentally, the lignin content of culm increased dramatically in OE: *OsSPL14* transgenic plant. Additionally, cellulose is a polysaccharide and cellulose microfibrils form the main load-bearing network (Somerville, 2006). Our study suggested that the expression of some genes participated in sugar metabolism changed and the cellulose content of culm increased obviously in OE: *OsSPL14* transgenic plant. Guo *et al.* (2003) indicated that high cellulose and lignin contents within a certain stem enhanced the lodging resistance. Thus, it is clear that higher content of lignin and cellulose would make contributions to enhancement of mechanical strength in *OsSPL14*-overexpression plant. Additionally, quantity of silicon had a close

relationship with mechanical strength of culm. Silicon, mainly existed in epidermal layers of stems, leaf sheaths and vascular bundles, can increase strength and hardness of the plant cell walls (Luo *et al.*, 2007). The study indicated that increasing silicon content of plants could improve the mechanical strength of the basal internode and eventually increase plant resistance to lodging (Jiang *et al.*, 2012). Besides, potassium is another factor impacted on mechanical strength of culm. Yang *et al.* (2004) indicated that thickness of basal stem and anti-fracture correlated with silicon and potassium content. Our result showed that silicon and potassium content increased in OE: *OsSPL14* transgenic plant. It further illustrated that mechanical strength of *OsSPL14*-overexpression plant increased so as to elevate lodging resistance. Together, OsSPL14 is a pleiotropic regulator that participates in several metabolic pathways and plays an important role in growth and development of plants (Fig. 7). Certainly, the regulatory network of OsSPL14 is worth further studying in rice.

Supplementary data

Fig. S1. The schematic diagram of *OsSPL14* expression vector.

Fig. S2. Detection of transgenic plants.

Table S1. Primers used in this study.

Table S2. KEGG enrichment analysis of DEGs.

Acknowledgements

The plasmid pCAMBIA1300-*OsSPL14* was provided by Professor Jiayang Li, the Institute of Genetics and Developmental Biology, Chinese Academy of Science, China. And we thank Prof. Jiayang Li for reading the manuscript. This work was supported by the Key Program of National Transgenic Research of China (Grant no. 2016ZX08001-004) and The Special Foundation of Non-Profit Research Institutes of Fujian Province (Grant no. 2016R1020-8).

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Figure legends

Fig.1. Analysis of plant phenotype.

(A) The plant architecture of wild plant MH86 and OE: *OsSPL14* transgenic plant. (B) Leaf morphological character. (C) Length of flag leaf. (D) Width of flag leaf. (E) Number of tillers. (F) Diameter of antepenultimate internode. (G) Wall thickness of antepenultimate internode. (H) Plant height. (I) Chlorophyll a, Chlorophyll b and carotenoid content of leaf at seedling stage. (J) Chlorophyll a, Chlorophyll b and carotenoid content of flag leaf at maturity stage. Bar=5 cm. (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

Fig.2. Transcriptome analysis.

(A) Volcano plot of DEGs. Larger absolute value of abscissa means greater different multiple, and larger ordinate value means more significantly differential expression. Each dot represents a gene, in detail, green dots represent down-regulated genes, red dots represent up-regulated genes, and blue dots represent non-differentiated genes. (B) Gene ontology classifications of DEGs. The abscissa is GO classification, the left side of the ordinate is the percentage of genes, and the right side is the number of genes. (C) The most enriched GO terms. The ordinate is enriched GO term, and the abscissa is the q-value. The digitals on the column represent the number of differentially expressed genes. Different colors represent biological processes, cellular components and molecular functions respectively. (D) Scatter diagram of KEGG enrichment. Longitudinal axis represents pathway name, and lateral axis represents rich factor. The size of the point indicates the number of differentially expressed genes in the pathway, and the color of the point corresponds to a different Qvalue range.

Fig.3. qRT-PCR analysis.

(A) Expression analysis of *OsSPL14* in WT and OE: *OsSPL14* transgenic lines. (B)

Expression patterns of 15 up-regulated genes were validated by qRT-PCR. C4H (502): BGIOGA006502; C4H (177): BGIOGA008177. (C) Expression patterns of 5 down-regulated genes were validated by qRT-PCR. (D) The expression profiles of *TBI*, *DEP1*, *D17*, *D53*, *D14* and *D3*.

Fig.4. ABA, JA and GA₃ content analysis.

(A) ABA content at seeding stage and tillering stage. (B) JA content at seeding stage and tillering stage. (C) GA₃ content at seeding stage and tillering stage. (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

Fig.5. Scanning and chemical components analysis of culm.

(A) Electron scanning microscopy analysis. BVB: Big vascular bundle; SVB: Small vascular bundles; SC: sclerenchyma cell. (B) Lignin content of culm. (C) Cellulose content of culm. (D) Silicon content of culm. (E) Potassium content of culm.

Fig.6. Grain quality analysis.

(A) Brown rice rate. (B) Milled rice rate. (C) Gel consistency. (D) Head rice rate. (E) Grain length. (F) Gelatinization temperature. (G) Amylose content. (H) Chalkiness ratio. (I) Chalkiness degree. (J) Transparency. (* $P \leq 0.05$, ** $P \leq 0.01$)

Fig.7. A model for the function of OsSPL14 in regulating plant growth and development. Arrows with dotted line mean there are unclear molecular mechanisms. RISC: RNA-induced silencing complex; L: Lignin; C: Cellulose; Si: Silicon; K: Potassium.

Table 1 Annotation of five KEGG-enrichment pathways

*1: Gene ID in *EnsemblPlants*

*2: The Rice Annotation Project Database

KEGG pathway	Gene ID* ¹	Gene ID in RAP* ²	Regulation	Log2FC	Anno
Carotenoid biosynthesis	BGIOSGA011548	Os03g0125100	Up	1.28	Beta- ϵ
	BGIOSGA029294	Os09g0555500	Up	1.40	Phyto
	BGIOSGA013214	Os03g0645900	Up	1.57	9-cis- ϵ
	BGIOSGA025169	Os07g0154100	Up	1.51	9-cis- ϵ
	BGIOSGA029635	Os09g0457100	Up	1.10	Absci
Phenylpropanoid biosynthesis	BGIOSGA005999	Os02g0626532	Up	1.01	Pheny
	BGIOSGA006502	Os02g0466900	Up	1.08	Trans-
	BGIOSGA008177	Os02g0467600	Up	1.41	Trans-
	BGIOSGA026897	Os08g0448000	Up	1.10	4-cou
	BGIOSGA014207	Os04g0651000	Down	-1.13	class l
	BGIOSGA022766	Os06g0306300	Down	-1.62	class l
	BGIOSGA027874	Os08g0113000	Down	-1.21	class l
Thiamine metabolism	BGIOSGA025304	Os07g0190000	Up	1.13	1-deo
	BGIOSGA013327	Os03g0679700	Down	-1.23	Phosp
	BGIOSGA025849	Os07g0529600	Down	-1.17	Cyste
Plant hormone signal transduction	BGIOSGA003839	Os01g0583100	Up	1.24	Protei
	BGIOSGA011032	Os03g0268600	Up	1.05	Protei
	BGIOSGA015611	Os04g0167875	Up	1.54	Protei
	BGIOSGA017925	Os05g0457300	Up	1.06	Protei
	BGIOSGA030517	Os09g0325700	Up	1.61	Protei
	BGIOSGA009392	Os03g0860100	Up	1.04	Ethyle
	BGIOSGA016308	Os04g0395800	Up	2.41	Jasmc
	BGIOSGA011983	Os03g0180900	Up	1.12	Jasmc
	BGIOSGA029689	Os09g0439200	Up	1.80	Jasmc
	BGIOSGA010919	Os03g0297600	Down	-1.36	Absci
Starch and sucrose metabolism	BGIOSGA007345	Os02g0106100	Up	2.03	Beta- α
	BGIOSGA011829	Os03g0141200	Up	1.41	Beta- α
	BGIOSGA031437	Os10g0553300	Up	1.02	Treha
	BGIOSGA031535	Os10g0521000	Up	1.16	alpha,
	BGIOSGA000847	Os01g0743200	Up	1.15	Pectin
	BGIOSGA028767	Os08g0450100	Up	2.01	Pectin
	BGIOSGA007188	Os02g0131400	Up	1.71	Beta- α
	BGIOSGA014898	Os04g0474300	Up	1.14	Beta- α
	BGIOSGA019700	Os05g0361500	Down	-1.14	Pectin
	BGIOSGA032689	Os10g0323500	Down	-1.07	Beta- α

A

WT

OE: OsSPL14

B

WT

OE: OsSPL14

C

D

E

F

G

H

I

J

A **B** **C** **D**

A **B** **C**