Comparative transcriptomic analysis provides an insight into floral organ petaloid in lotus (Nelumbo nucifera)

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Research article

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9   Running title: Comparative transcriptomic analysis of *Nelumbo nucifera* petaloid

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26

27 **Abstract**

28 **Background:** Lotus (*Nelumbo nucifera*) is a famous flower with high ornamental value. Flower color

29 and flower morphology are two main factors for flower lotus breeding. Petaloid is a universal

30 phenomenon in lotus flowers. However, the genetic regulation of floral organ petaloid in lotus still

31 remains unclear.

32 **Result:** In this study, transcriptomic analysis was performed among five organs, including petal,

33 stamen petaloid, stamen, carpel petaloid, and carpel in lotus. Using WGCNA analysis, 37 candidate

34 genes were found to be related to carpel petaloid. Additionally, one floral homeotic gene encoded

35 MADS box transcription factor, *AGAMOUS* (*AG*), was identified as candidate gene for petaloid in

36 lotus.

37 **Conclusion:** The above results explored the candidate genes related to petaloid, setting a theoretical

38 basis for the molecular regulation of petaloid.

39 **Key words:** *Nelumbo nucifera*, Flower morphology, Petaloid, floral organ, Transcriptomic

40

Background

Lotus (*Nelumbo nucifera*) is an aquatic plant, which is widely cultivated as food crop in East Asia. Additionally, it is also one of the famous traditional flowers, especially in China. Based on different breeding purposes, lotus is classified into three groups, namely seed lotus, rhizome lotus, and flower lotus. With its high ornamental value, the aim of breeding in flower lotus is performed under distinct flower colors and shapes. Generally, a flower is constituted by four floral organs, including sepal, petal, stamen, and carpel in angiosperm plant. Thus, the different number of floral organs and diverse organs features form various flower morphologies. In lotus, the peculiar flower patterns are mainly constructed by aberrant floral organs, such as stamen petaloid and carpel petaloid.

The petaloid phenomenon attracted research attention from as early as 286 BC [1]. Petaloid organs in other locations were possessed morphological traits of petal [2]. Based on three floral homeotic mutations and genetic relationships between *Arabidopsis* and *Antirrhinum*, the typical ABC model has been widely accepted since the 1990s [3, 4]. Based on this model, petals are determined by A and B class genes; stamens are determined by B and C class genes; while carpels are determined by C class genes. Most of ABC model genes are MADS box transcription factors, except one A class gene encoded by *APETALA2* (*AP2*). Meanwhile, this model is also applicable to many monocot flowers after modifications, despite their differences in flower morphology [5, 6]. B-function genes, including *APETALA3/PISTILLATA* (*AP3/PI*) are essential in influencing petaloid [5]. The role of *AP3/PI* genes is specification in 'petaloid' identity under the sliding borders or fading borders models, which are different from the earlier models [2]. C and A function genes have antagonism regulation with each other [7]. Loss of C class genes functions results in substitution of petals for stamens and sepals for carpels[8].. The reduction in the expressions of *AGAMOUS* (*AG*), a C-function MADS box gene*, homolog gene could cause the double flower morphology in rose, *Thalictrum thalictroides*, and *Cyclamen persicum* [9-11]. When the expression of *RABBIT EARS* (*RBE*) is down-regulated, the transcripts of *AG* are de-repressed in floral and inflorescence meristems [12]. The interactions between *WUSCHEL* (*WUS*) and *AG* are involved in floral determination while *AG* is a central gene in the genetic network of floral organ development [13]. During flower patterning, *AP3* and *AG* associate with *LEAFY* (*LFY*) after induction [14]. Other transcription factors might regulate the floral organ formation through affecting the ABC model genes. However, the molecular mechanism of petaloid is still not fully understood.

71   Previously, it has been shown that the obscure expression of several candidate genes in boundaries

72   of petal and stamen could result in the stamen petaloid formation [15], which might also be influenced

73   by DNA methylation [16]. Additionally, the latest studies on lotus were comprehensively reviewed

74   which revealed the absence of a detailed study on petaloid formation in *N. nucifera* [17]. To obtain a

75   comprehensive understanding on the petaloid formation in lotus, we used transcriptomic analyses

76   among petal (P), stamen petaloid (Sp), stamen (St), carpel petaloid (Cp), and carpel (C) from a bowl

77   lotus 'Sleeping Beauty'. The results might provide some new insights into improving our

78   understanding about petaloid formation.

79

80   **Results**

81   **Petaloid phenotype of *N. nucifera***

82   Lotus is a famous aquatic flowering plant, which has high ornamental value. The flowers of lotus are

83   beautiful with gorgeous colors and various flower morphologies. With artificial selection, double

84   flower is popular for landscape architecture. Among them, 'Sleeping beauty' is a bowl lotus with long

85   term flowering time and abundant number of flower, which also possess floral aberration. During

86   different flowering stages, a special phenomenon in the flower shape of 'Sleeping beauty' occurrs

87   (**Figure 1**). The abnormal floral organs are stamen petaloid and carpel petaloid, which are defective for

88   reproduction (**Figure 1**). Scanning electron microscope was used to visualize the epidermal cell

89   morphology of petal, stamen petaloid and carpel petaloid. The upper and lower epidermal cells was

90   shown to have similar shape, including mastoid cells and wax crystal, among P, Sp, and Cp (**Figure 2**).

91

92   **Overview of the transcriptomic analysis**

93

94   RNA-seq was performed for five samples, including P, Sp, St, Cp, and C, with each one having three

95   biological replicates (**Figure 1**). Under sequencing quality control, a total of 50.4 Gb clean data was

96   generated. The percentage of Q30 in each sample was no less than 91.61% (**Table S1**). 85.52-94.24%

97   clean reads of each sample were mapped to the lotus genome (http://lotus-db.wbgcas.cn/) [18]. The

98   total number of genes or transcripts from the samples was 30469, out of which 3784 were noted as new

99   genes. DEGs (Differentially Expressed Genes) were screened based on an absolute fold change of no

100   less than two and an FDR (False Discovery Rate) ≤0.05. A total of 8238 (C vs P), 3944 (C vs Cp),

101   4481 (P vs Cp), 4231 (Cp vs Sp), 216 (P vs Sp), 1223 (St vs Sp), and 2450 (St vs P) DEGs were

102   detected, including 3637, 2133, 2932, 1779, 199, 821, and 1095 up-regulated DEGs and 4601, 1811,

103   1549, 2452, 17, 402, and 1355 down-regulated DEGs, respectively (**Figure 3**). Moreover, the pearson

104   relationships were performed with pair-wise comparison among these five tissues with their DEGs

105   (**Figure S1**). We found that P vs Sp had the highest relationship up to 0.8533. The result suggested that

106   petal and stamen petaloid were high similarity.

107   P, Sp, St, Cp, and C were divided into two groups, including carpel petaloid group (C vs P, C vs Cp,

108   and P vs Cp) and stamen petaloid group (St vs P, P vs Sp, and St vs Sp). From the above mentioned

109   DEGs, 21 genes had significant change among petal, stamen petaloid, and stamen; and 1025

110   distinguished genes were identified in petal, carpel petaloid, and carpel (**Figure 3**). Between carpel

111   petaloid group and stamen petaloid group, there were seven common DEGs including NNU_04669,

112   NNU_09105, NNU_10192, NNU_21294, NNU_22371, NNU_23867, and NNU_26585. It is suggested

113   that they are involved in petaloid formation.

114

115   **The reliability of RNA-seq data**

116   To verify the reliability of RNA-seq data, fifteen DEGs were selected and subjected to qRT-PCR

117   analysis in five floral organs (including P, Sp, St, Cp, and C). Most of the selected genes exhibited

118   similar trend with RNA-seq data, except for the NNU_17837 and NNU_21294 (but r still more than

119   0.75, **Figure 4**). Meanwhile, compare the DEGs in Petal, Stamen petaloid, and Stamen of 'Sleeping

120   beauty' with previous study, they have 1140 common DEGs. Their correlation relationship was 0.8160

121   (**Figure S2**). These results further proved the reliability of the transcriptome data.

122

123   **Weighted genes co-expression network analysis (WGCNA)**

124   To identify the patterns of gene expression in petaloid process, WGCNA was performed for analyzing

125   weighted gene co-expression network aimed at further understanding the floral organ formation in lotus.

126   Six modules were obtained, including grey, green, turquoise, yellow, blue, and brown (**Figure 5A)**.

127   Thereinto, the grey module was a collection of genes that could not be assembled into other modules

128   and had 836 genes. There were 1185, 7527, 1857, 3615 and 2595 genes in green, turquoise, yellow,

129   blue, and brown module, respectively. The green module was found to be related to carpel petaloid, of

130   which module-trait relationships value was 0.96 (**Figure 5A and B**). 360 hub genes (module-trait

131   relationships value > 0.9) were selected of which 81 genes were both hub genes and DEGs (**Figure 5C**).

132   Among them, 37 genes ($\log_2$FC > 5 or < -5) were chosen as candidate genes including five

133   transcription factors COL16 (NNU_00499), bHLH51 (NNU_13078), MYB38 (NNU_19814), GATA9

134   (NNU_23627), and bHLH35 (NNU_26108) (**Figure 5D**). One of them is related to plant auxin gene

135   *GH3* (NNU_22327). Additionally, other three genes contained NNU_01046, NNU_12218, and

136   NNU_21373 are the member of P450 family.

137

Identification of DEGs involved in stamen petaloid and carpel petaloid

139   Among P vs Cp, P vs Sp, and Cp vs Sp, they contained 41 common DEGs (**Figure S3**). Meanwhile,

140   1091 out of all DEGs in Cp vs Sp were found to be not overlapped between P vs Cp and P vs Sp. The

141   certain proportion of DEGs was specific in stamen petaloid and carpel petaloid. Additionally, 54 DEGs

142   were common between P vs Cp and P vs Sp. This result suggested that these DEGs might share some

143   conserved gene expression in stamen petaloid and carpel petaloid. After filtering out the genes with a

144   low expression (FPKM <5), the several numbers of DEGs were assigned to transcription factors and

145   plant hormone signal transduction out of 41 common DEGs (**Table S2**). Genes encoding

146   Homeobox-leucine zipper protein, a MADS-box family gene AG, a zinc finger CCHH

147   domain-containing protein, a myb-related protein and a indole-3-acetic acid-amido sythetase GH3.5

148   showed over two-fold decreased in stamen petaloid and carpel petaloid relative to petal. Interestingly, a

149   gene encoding B3 domain-containing protein exhibited over two-fold increased in carpel petaloid

150   compared with petal.

151   Three DEGs (NNU_10192, NNU_12600 and 17837) were reported in our recent and previously

152   study suggesting that they are associated with stamen petaloid [15]. This is more so for NNU_10192

153   being an *AG* homologue gene, a member of MADS-box family belonged to classical ABC model.

154   Classical ABC model members have interesting expression patterns by applying the FPKM value via

155   transcriptome profiling in different floral organs (**Figure 6**). NNU_10192 and NNU_26656 encoding

156   for floral homeotic *AG* gene were found to have the lowest expression in petal compared with other

157   flower tissues. In contrast, A class genes (NNU_04430, NNU_17043, and NNU_13608) and B class

158   genes (NNU_08090, NNU_23351, and NNU_02674) expressed higher in petal. Additionally, based on

159   the expression profiles (**Figure 6**), A class genes, *AP1-like* (NNU_04430) and *AP2-like* (NNU_17043)

160   had a similar expression, being down-regulated in C vs Cp and St vs Sp. C class genes (NNU_10192

161   and NNU_26656) showed the opposite expression.

Discussion

The various phenotypes of lotus flowers have fundamental own critical ornamental value. The morphology of a flower is influenced by the aberrant floral organs, especially petaloid. Cultivar lotus 'Sleeping Beauty' has various abnormal floral organs similar to petal being from homeotic stamen and carpel transformed. To explore the mechanism of petaloid formation, comparative transcriptomic analysis was performed in P, Sp, St, Cp, and C. This will be enabled the expansion of our understanding of flower development and petaloid formation in lotus.

Overview transcriptomic data

Currently, two lotus genome including 'China Antique' and 'Chinese Tai-zi' had been sequenced and released providing a research basis for omic study and breeding [18, 19]. Through transcriptomic analysis in P, Sp, St, Cp, and C, DEGs involved in petaloid were identified. In the pairwise comparison of petal, stamen petaloid, and stamen, DEGs in P vs Sp was fewer than St vs Sp (**Figure 3**). It has been declared that stamen petaloid is more similar to petal than to stamen. This is consistent with stamen petaloid having the petal morphology as previously reported [15]. The number of DEGs between C vs Cp and P vs Cp did not show any significant difference, suggesting that the abnormal flower organ (carpel petaloid) remained more of the carpel trait with petal-like features.

Genes associated with petaloid

A large amount of transcription factors were reported to be involved in floral development in model plants, such as MADS-box, MYB, and bHLH [20-22]. MADS-box transcription factors play key roles in controlling morphogenesis of floral organ. *AGAMOUS* (*AG*) is involved in regulation stamen and carpel formation and development in *Arabidopsis* [8, 23]. Owing to A class genes being expanded to inner whorl, transformation of stamens to petals was as a result of mutation of *AG*. In contrast, ectopic expression of *AG* in outer whorl causes sepal carpeliod and petal stamenoid [24]. *AG* homeotic gene has been universally identified in many plants, such as rose, petunia, *Thalictrum thalictroides*, *Prunus lannesiana* and *Medicago truncatula* [9, 11, 25-27]. These show that the homologous *AG* pattern of expressions and regulation of stamen and carpel identity are conservative. In our study, notably, the candidate gene (NNU_10192) is an *AG* homolog, belonging to MADS-box family member. *AG* was among the DEGs with lower expression in stamen petaloid than in stamen. Meanwhile, its expression in carpel petaloid was less than that in carpel and lowest in petal (**Figure 6**). These results show that a

7

193 declined expression of *AG* in inner whorl results in carpel petaloid formation and breaks down the gene

194 expression boundary. In the previously study, we performed transcriptome analysis for stamen petaloid

195 showing that several MADS-box genes, including *AG* were found to be possibly involved in floral

196 organ specification [15].

197   Using WGCNA, genes implicated in carpel petaloid were screened, including transcription factors

198 and hormone-related genes. Previously, MYB not only improves petal and stamen development, but

199 also induces carpel growth [28]. Here, NNU_19814, which is a *MYB38* ortholog gene, showed highest

200 level in the carpel petaloid, suggesting it being involved in petal development. Different stages of

201 flower development were controlled by *bHLH* gene, including regulated the growth of carpel margin

202 tissues [29]. In lotus, 117 *bHLH* genes were identified with most of their functions have not yet

203 confirmed [30]. In our study, two *bHLH* homologue genes, NNU_13078 and NNU_26108, were

204 specifically expressed in carpel petaloid. HAN encoding a GATA transcription factor that regulates

205 floral organ specification, directly controlling hormone response genes and correlates with the number

206 of petals [31, 32]. *GH3.5* is a direct downstream gene of *HAN*, whose mutant exhibits low transcript of

207 *GH3.5* [31]. This idea is consistent with our result that the expression profile of *GH3* (NNU_22327) is

208 similar with *GATA4* (NNU_22327) and is significantly expressed in carpel petaloid of *N. nucifera*.

209 These candidate genes were reported that they involved in stamen petaloid [15]. These indicate that

210 GH3 and GATA are essential in petal formation and promote to alteration other floral organs to

211 petal-like feature. Additionally, *GH3* gene is regulated by phytochrome B, and modulates light

212 signaling pathway [33]. *CONSTANTS-LIKE* (*COL*) gene homolog NNU_00499, which is annotated as

213 *COL16* belong to a COL family of zinc finger protein transcription factor. A previously reported

214 *COL16* in petunia is involved in the chlorophyll biosynthesis [34].*CYP715* is a homolog of cytochrome

215 P450 family members, which is a key regulator of floral maturation, affecting petal development and

216 maintains flower hormone homeostasis [35]. Overexpression of *KLUH/CYP78A5* leads to an increase

217 in the number of petals and epidermis cells, while they decrease in *klu/cyp78a5* mutant. These suggest

218 that *KLUH/CYP78A5* regulates organ development via cell proliferation [36, 37]. Three genes encode a

219 cytochrome P450, including NNU_01046, NNU_12218 and NNU_21373, had a similar expression

220 pattern, suggesting that they are possibly involved in regulation of petaloid formation. *ANS*

221 (NNU_08856) and *ANR* (NNU_08935) referred to the flavonoids synthesis pathway, were found by

222 tanscriptomic analysis. The results potentially associate with the surface of carpel petaloid with light

223   green color (**Figure 1D**). Glyceraldehyde-3-phosphate dehydrogenase *GAPCP2* (NNU_01252), which

224   is one of the glycolysis/gluconeogenesis pathway genes being related to carbohydrate transport and

225   metabolism plays a key role in maintaining reproductive organs development [38]. Further study on

226   glyceraldehyde-3-phosphate dehydrogenase effects on alteration of carpel to petal needs to be carried

227   out. For the above candidate genes, they are almost certainly involved in the regulatory network of

228   flower development but their connections with floral organ formation have not been verified.

229

230   **The labile boundary in floral organs of *N. nucifera***

231   A few-petalled, double-petalled, duplicate-petalled, and all-double-petalled groups were systematized

232   for the flower morphologies in lotus [39]. The different number of petal in cultivar lotus was generated

233   by breeder domestication. For traditional breeding and production, they only focus on their aims and

234   improving plant potentially values. Factors in the molecular mechanism how modulates being still

235   unclear. In normal floral morphology, floral organs are located in four whorls in order in

236   dicotyledonous plants. Boundaries exhibit between floral organs within whorls [40]. The labile

237   boundary was previously found in rose flower and that the expression pattern of *AG* is responsible for

238   morphological diversity [9].

239   A number of candidate targets of MADS box genes have known function in petal growth [41]. In

240   the floral classic ABC model, petal identity is specified by A and B gene classes, stamen by B and C

241   gene classes and carpel by C gene classes. From **Figure 6**, our results suggest that A, B and C class

242   genes are involved in petaloid formation, which is in agreement with previous studies. Deficiencies in

243   inter-whorl boundaries can result in hybrid structures such as petal-stamens [42]. The stamen petaloid

244   and carpel petaloid in lotus may also cause by defects in inter-whorl boundaries. In accordance with

245   this supposition, the gene model of lotus flower was summary in **Figure 7**. The boundary genes should

246   be conducted to understand how they build the restricted expression pattern and perform functions in

247   their complicated regulation network.

248

249   **Conclusions**

250   This study was carried out to investigate the different transcriptomic dynamics resulting abnormal

251   flower morphology of lotus. Through comprehensive analysis, 1025 DEGs related to carpel petaloid

252   were identified. Fifteen DEGs were validated by qRT-PCR. Several transcription factors were found

253   associated with petaloid formation in this study. Notably, the member of MADS-box family, *AG* being

254   a floral homeotic gene played a key role in floral organ petaloid.

255

256   **Methods**

257   **Plant growth and sample collection**

258   Lotus cultivar 'Sleeping beauty' was acquired from field genebank for lotus in Wuhan Botanical

259   Garden, Chinese Academy of Sciences (WBGCAS), Hubei Province, China. The rhizomes of

260   'Sleeping beauty' were then separated into three plastic buckets (90 cm×90 cm). Petal, stamen

261   petaloid, stamen, carpel, and carpel petaloid were collected when the flowers bloomed (**Figure 1**).

262   After sampling, the floral organs were snap frozen in liquid nitrogen and stored at -80 ℃ until RNA

263   and protein extraction.

264

265   **RNA isolation and qRT-PCR gene expression analysis**

266   Total RNAs were isolated using an RNA reagent (OminiPlant RNA Kit, CWBIO, China), and remove

267   genomic DNA contamination was removed by treating with RNase-free DNaseI (Thermo, Shanghai,

268   China). Primers used for qRT-PCR were listed in **Table S3**. The qRT-PCR reactions were performed

269   as described by Lin et al. [15].

270

271   **Sequencing and data processing**

272   The RNA integrity number (RIN) of each sample is at least above 6.5. Per sample should prepare more

273   than 1.5 μg total RNA. Fifteen cDNA libraries were constructed, and Illumina sequenced by Beijing

274   Novogene Bioinformatics Technology Co., Ltd. using the Illumina HiSeq 2500 high throughput

275   sequencing platform. The transcriptome sequencing data were deposited in PRJNA524054.

276   After quality control, clean data was deposited in BMKCloud (https://www.biocloud.net/) for

277   analysis. We mapped the data to the reference genome of *Nelumbo nucifera* (China lotus 1.1) [18]

278   using TopHat2 Software v2.0.9 [43]. Transcript assembly, differential expression, and divergent

279   regulation were performed using Cufflinks v2.1.1 [44]. The differentially expressed genes (DEGs)

280   were   carried   out   by   using   DESeq   package

281   (http://bioconductor.org/packages/release/bioc/html/DESeq.html). The expression (read counts) was

282   calculated by RSEM v1.2.15 [45]. The DEGs were identified by false discovery rate (FDR) < 0.01 and

283   a fold change ≥ 2. Any genes with an adjusted P-value < 0.05 were assigned as differentially

284   expressed. The heatmap was constructed by using Multiple Experiment Viewer software (MeV 4.9.0,

285   https://sourceforge.net/projects/mev-tm4/files/mev-tm4/MeV%204.9.0/).

286

287   **Scanning electron microscope (SEM) observation**

288   Petal, stamen petaloid, and carpel petaloid were collected from cultivar lotus 'Sleeping Beauty'. SEM

289   was used to view epicuticular cells. Samples were prepared and imaged by SEM as described by Lü et

290   al. [46].

291

292   **Weighted gene co-expression network analysis (WGCNA**)

293   Weighted gene co-expression network analysis was applied for identification of co-expressed genes by

294   WGCNA package in the R software. The hub genes were further grouped into six modules using

295   WGCNA.

296

297   **Declarations**

298

299   **Funding**

302

303   **Author contributions**

304   ZL and FY designed the experiments. ZL contributed to data analysis and wrote the manuscript. ZL,

305   DC, and ND performed the experiments. DC, ND and FY revised the manuscript. All authors

306   commented on the manuscript.

307

308   **Availability of data and materials**

309   The RNA-seq data generated in this study are available in the NCBI using accession numbers

310   PRJNA524054.

311

312 **Competing interests**

313 The authors declare that they have no competing interests.

314

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316 Not Applicable

317

318 **Consent for publication**

319 Not applicable

320

321 **Ethics approval and consent to participate**

322 Not applicable.

323

324 **Abbreviations**

325 AG: AGAMOUS; AP: APETALA; C: carpel; Cp: carpel petaloid; DEGs: Differentially Expressed

326 Genes; FDR: False discovery rate; FPKM: Fragments per kilo base of transcript per million base pairs

327 sequenced; LFY: LEAFY; MADS: MCM1, AG, DEFA and SRF; P: petal; PI: PISITTALA; qRT-PCR:

328 Quantitative real-time PCR; RIN: RNA integrity number; SEM: Scanning electron microscope; Sp:

329 Stamen petaloid; St: Stamen; WGCNA: Weighted gene co-expression network analysis; WUS:

330 WUSCHEL;

331

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463

464 **Legends of Figure**

465

466 **Figure 1 The flower of sacred lotus 'Sleeping Beauty'**

467 (**A-C**) The different flower morphology of 'Sleeping Beauty' was imaged in different time stages. (**D**)

468 Five floral organs, including petal (P), stamen petaloid (Sp), stamen (St), carpel petaloid (Cp), and

469 carpel (C). Bars are all 1 cm..

470

471 **Figure 2 Scanning electron micrographs (SEM) observation of epidermal structure of the**
472 **petal-like organ in lotus**

473 (**A**) Epidermal cells are in upper and lower of petal (P). (**B**) Epidermal cells are in upper and lower of

474 stamen petaloid (Sp). (**C**) Epidermal cells are in upper and lower of carpel petaloid (Cp). Bars of SEM

475   are all 50 μm. Mastoid cell was marked by arrow and wax crystal was marked in green circle.

476

477   **Figure 3 Overview of differentially expressed genes (DEGs）**

478   (**A**) Venn diagram of the number of unique and common DEGs in the two comparisons (P vs Sp,

479   St vs P, and St vs Sp; C vs P, C vs Cp, and P vs Cp). (**B**) The number of up-regulated and

480   down-regulated DEGs in the two comparisons (P vs Sp, St vs P, and St vs Sp; C vs P, C vs Cp, and

481   P vs Cp). The y-axis represents the number of genes and the x-axis represents the different

482   comparison.

483

484   **Figure 4 Validation of RNA-seq data using qRT-PCR.**

485

486   **Figure 5 WGNCA of the lotus tissues (A)** Module trait relationships. (B) Gene trait significance. **(C)**

487   Venn of hub genes and DEGs. **(D)** Heat map of 37 hub DEGs (log2FC > 5 or < -5).

488

489   **Figure 6 The expression pattern of ABC model genes.**

490

491   **Figure 7 The proposed model in flower pattern of lotus. (A)** Normal flower pattern in lotus. **(B)**

492   Aberrant flower pattern in lotus. The petals/stamens/carpels boundaries are slide in the flowers.

493

494   **Supplementary data**

495   **Figure S1 Correlation of FPKMs of all DEGs in pair-wise comparison among C, Cp, P, Sp, and**

496   **St.**

497   **Figure S2 Correlation relationship of the common DEGs in Fenhonglingxiao and Sleeping**

498   **Beauty.**

499   **Figure S3 Venn diagram of the number of unique and common DEGs among P vs Cp, P vs Sp,**

500   **and Cp vs Sp.**

501

502   **Table S1 Summary of RNA sequencing and assembly.**

503   **Table S2 FPKMs and functional categories of genes significantly expressed in stamen petaloid or**

504   **carpel petaloid.**

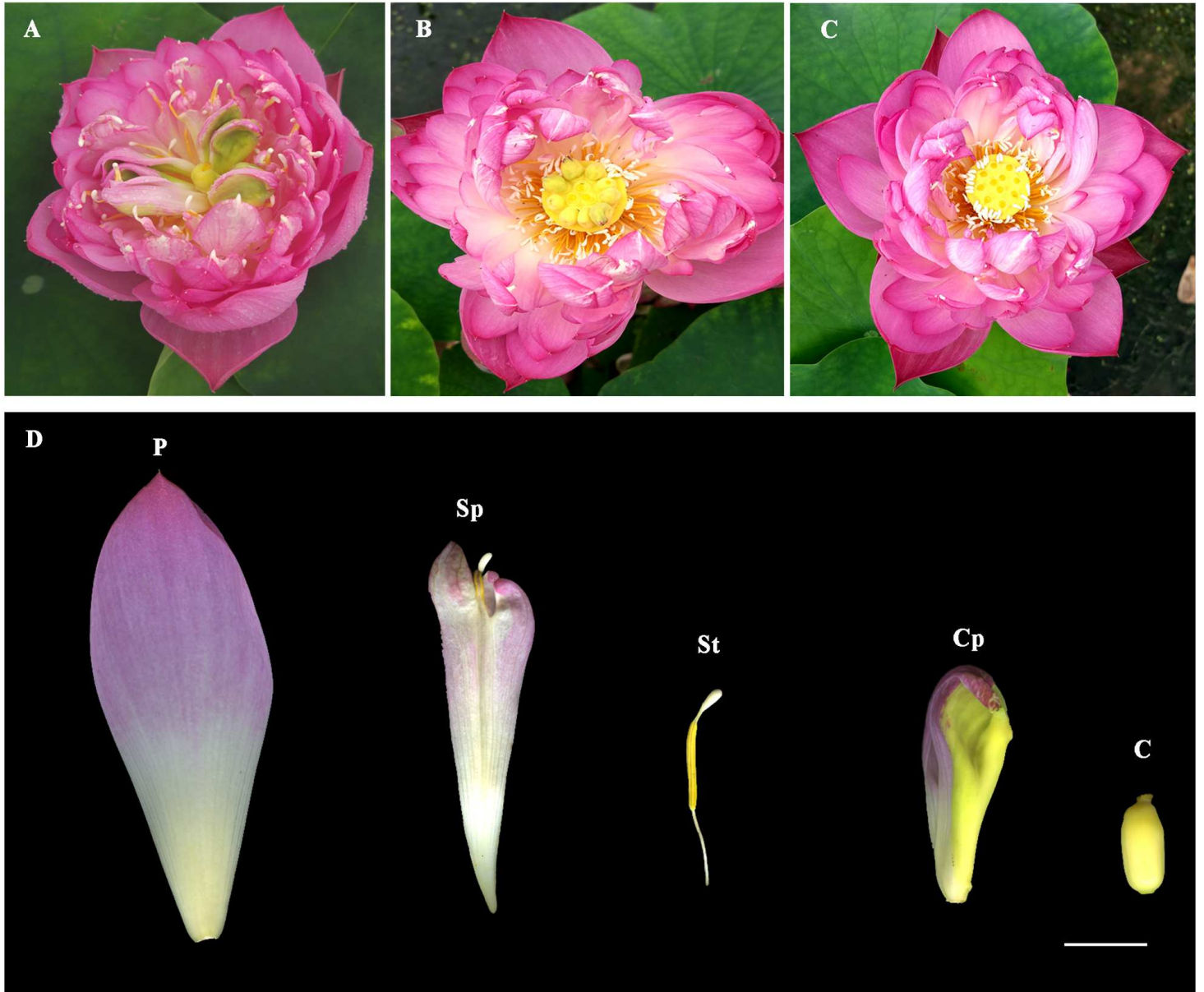505   **Table S3 Primers used in this study.**

Figures

Figure 1

The flower of sacred lotus 'Sleeping Beauty' (A-C) The different flower morphology of 'Sleeping Beauty' was imaged in different time stages. (D) Five floral organs, including petal (P), stamen petaloid (Sp), stamen (St), carpel petaloid (Cp), and carpel (C). Bars are all 1 cm.
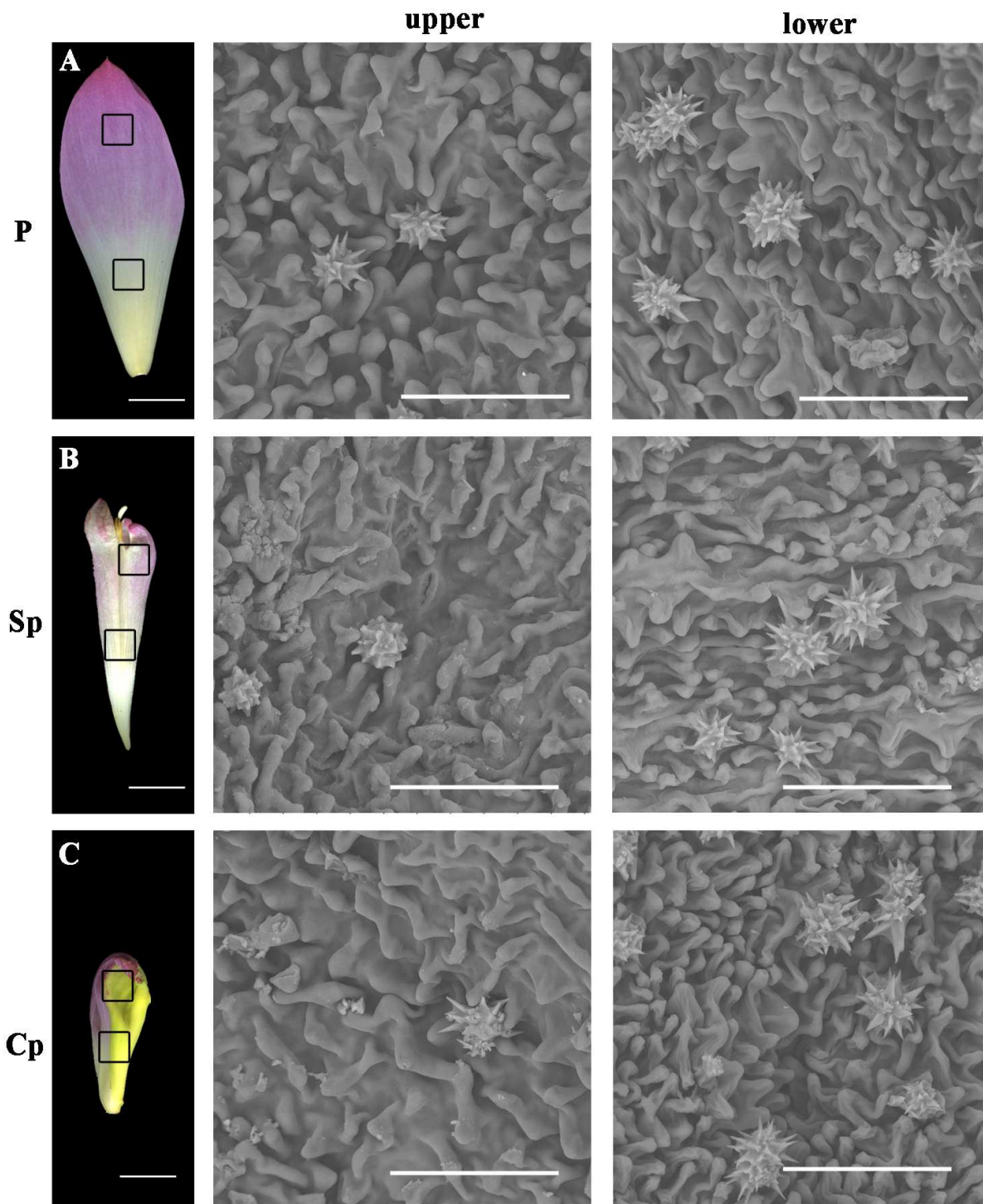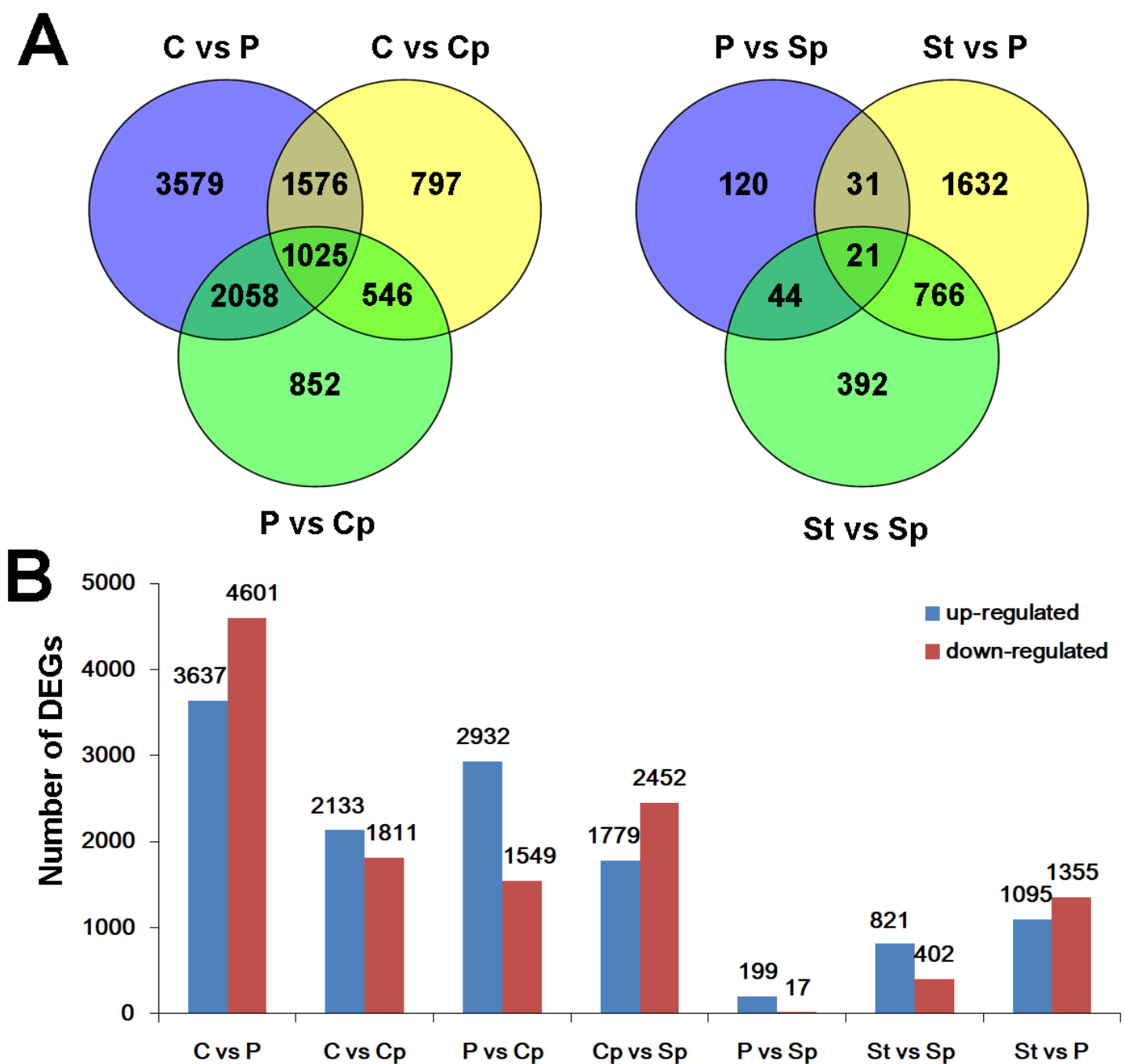
Figure 2

Scanning electron micrographs (SEM) observation of epidermal structure of the petal-like organ in lotus (A) Epidermal cells are in upper and lower of petal (P). (B) Epidermal cells are in upper and lower of stamen petaloid (Sp). (C) Epidermal cells are in upper and lower of carpel petaloid (Cp). Bars of SEM are all 50 μm. Mastoid cell was marked by arrow and wax crystal was marked in green circle.

Figure 3

Overview of differentially expressed genes (DEGs。(A) Venn diagram of the number of unique and common DEGs in the two comparisons (P vs Sp, St vs P, and St vs Sp; C vs P, C vs Cp, and P vs Cp). (B) The number of up-regulated and down-regulated DEGs in the two comparisons (P vs Sp, St vs P, and St vs Sp; C vs P, C vs Cp, and P vs Cp). The y-axis represents the number of genes and the x-axis represents the different comparison.
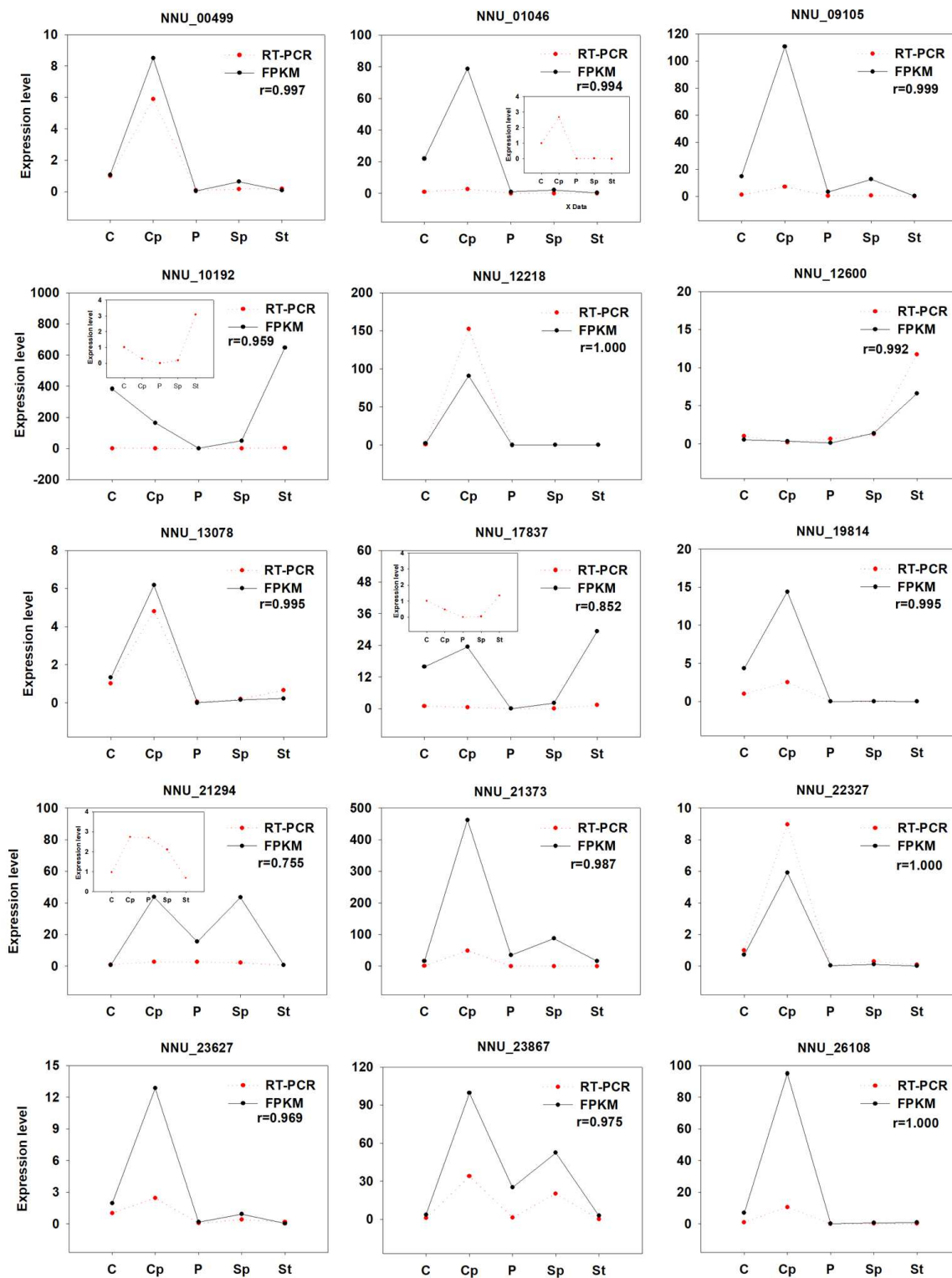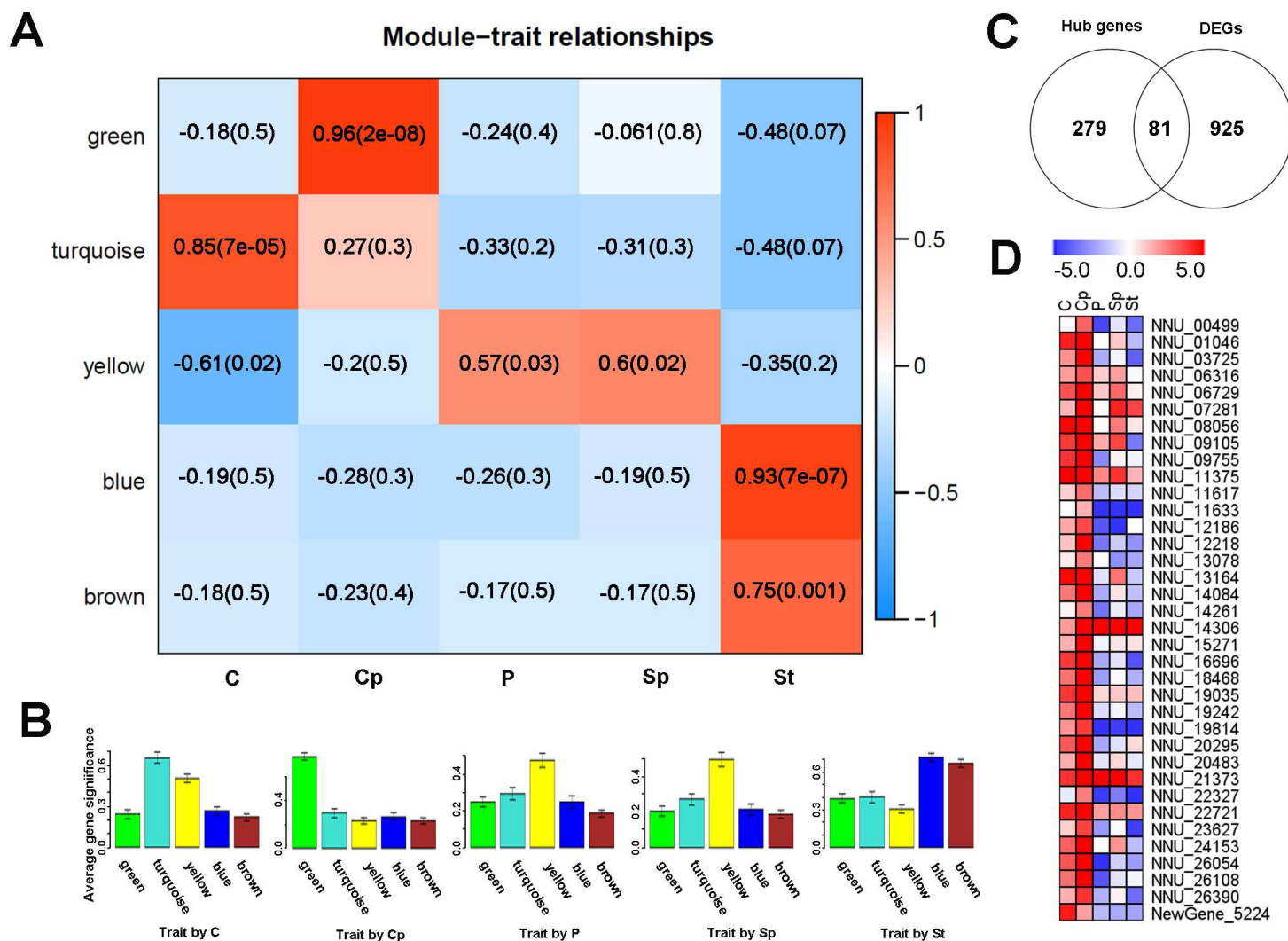
Figure 4

Validation of RNA-seq data using qRT-PCR.

Figure 5

WGNCA of the lotus tissues (A) Module trait relationships. (B) Gene trait significance. (C) Venn of hub genes and DEGs. (D) Heat map of 37 hub DEGs (log2FC > 5 or < -5).
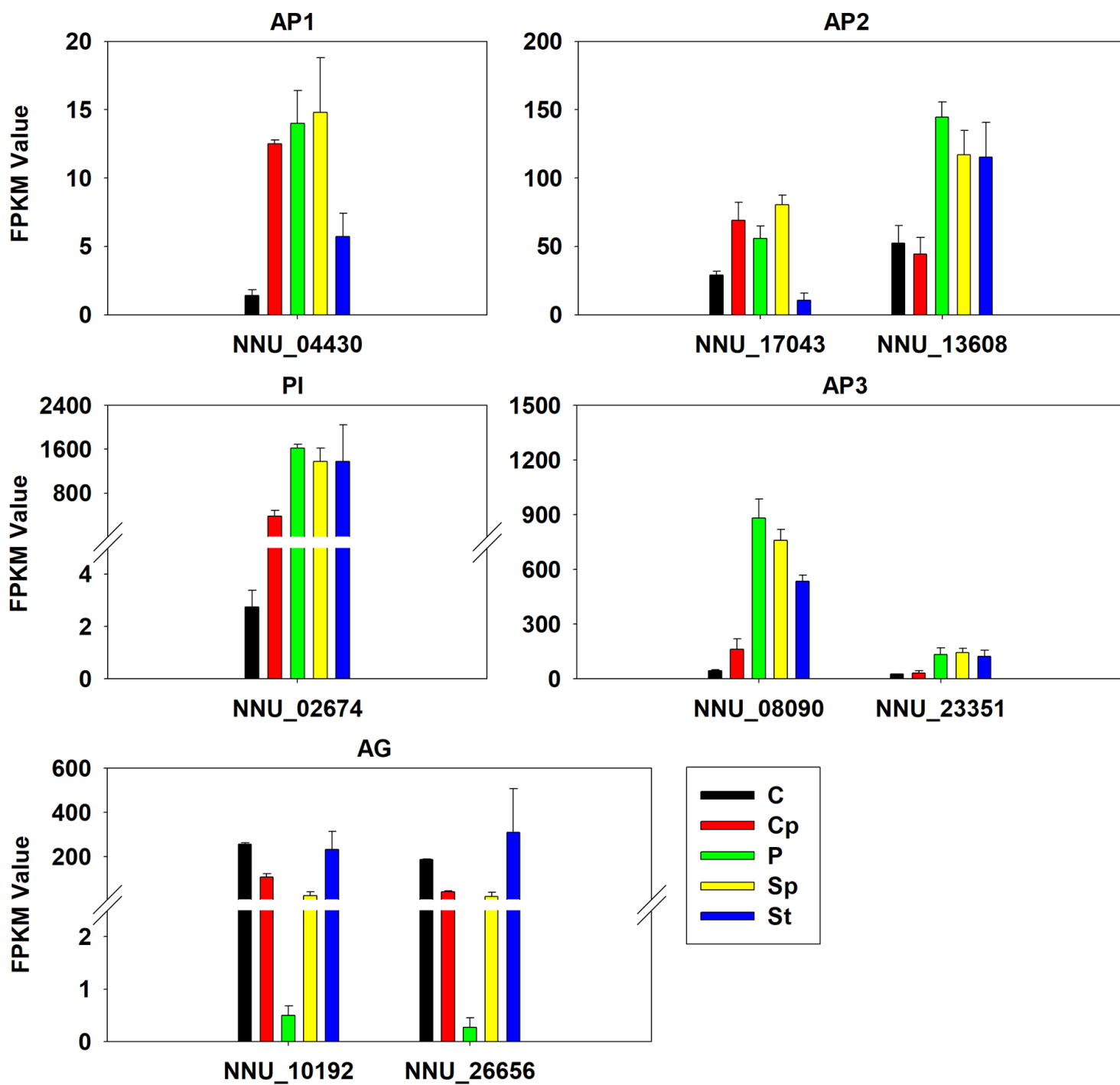
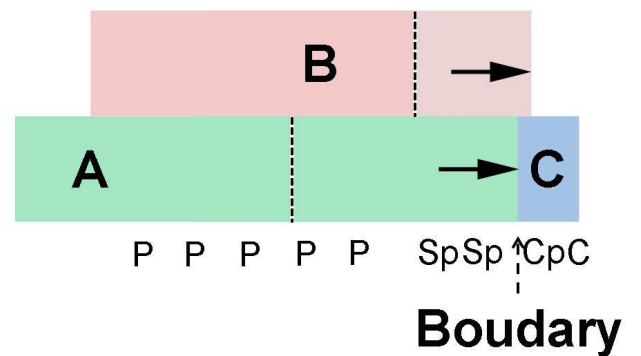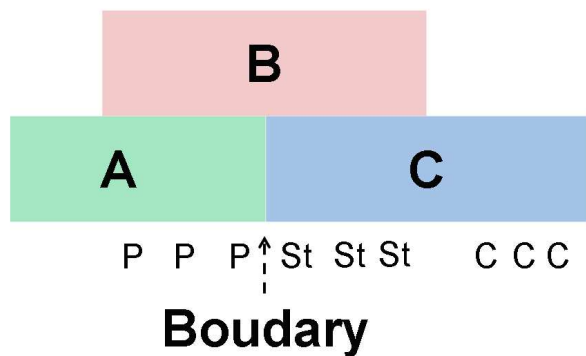**Figure 6**

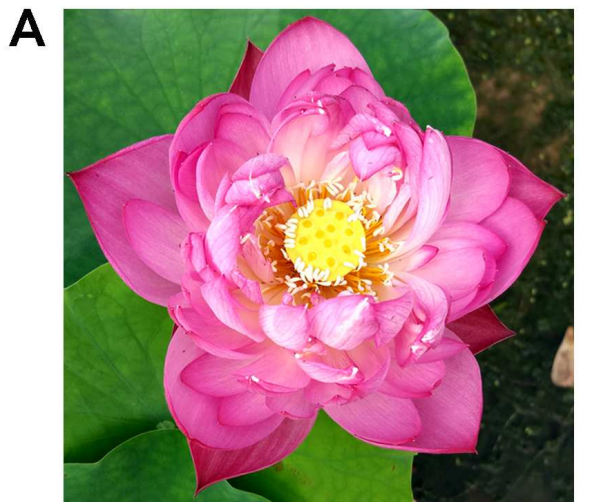The expression pattern of ABC model genes.

Figure 7

The proposed model in flower pattern of lotus. (A) Normal flower pattern in lotus. (B) Aberrant flower pattern in lotus. The petals/stamens/carpels boundaries are slide in the flowers.

Supplementary Files

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- FigureS2.TIF
- FigureS3.png
- TableS1SummaryofRNAsequencingandassembly.docx
- TableS2.docx
- TableS3Primersusedinthisstudy.docx