SEPALLATA-like genes of *Isatis indigotica* can affect the architecture of the inflorescences and the development of the floral organs

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Background. The architecture of inflorescence and the development of floral organs can influence the yield of seeds and have a significant impact on plant propagation . E-class floral homeotic MADS-box genes exhibit important roles in regulation of floral transition and differentiation of floral organs. Woad (*Isatis indigotica*) possesses unique inflorescence, floral organs and fruit. However, very little research has been carried out to determine the function of MADS-box genes in this medicinal cruciferous plant species.**Results.** *SEPALLATA* orthologs in *I. indigotica* were cloned by degenerate PCR. The sequence possessing the highest identity with *SEP2* and *SEP4* of Arabidopsis were named as *IiSEP2* and *IiSEP4*, respectively. Constitutive expression of *IiSEP2* in Columbia (Col-0) ecotype of Arabidopsis led to early flowering, and the number of the flowers and the lateral branches was reduced, indicating an alteration in architecture of the inflorescences. Moreover, the number of the floral organs was declined, the sepals were turned into carpelloid tissues bearing stigmatic papillae and ovules, and secondary flower could be produced in apetalous terminal flowers. In *35S::IiSEP4-GFP* transgenic Arabidopsis plants in Landsberg erecta (Ler) genetic background, the number of the floral organs was decreased, sepals were converted into curly carpelloid structures, accompanied by generation of ovules. Simultaneously, the size of petals, stamens and siliques was diminished. In *35S::IiSEP4-GFP* transgenic plants of apetalous *ap1 cal* double mutant in Ler genetic background, the cauliflower phenotype was attenuated significantly, and the petal formation could be rescued. Occasionally, chimeric organs composed of petaloid and sepaloid tissues, or petaloid and stamineous tissues, were produced in *IiSEP4* transgenic plants of *apl cal* double mutant. It suggested that overexpression of *IiSEP4* could restore the capacity in petal differentiation. Silencing of *IiSEP4* by Virus-Induced Gene Silencing (VIGS) can delay the flowering time, and reduce the number and size of the floral organs in woad flowers. **Conclusion.** All the results showed that *SEPALLATA*-like genes could

influence the architecture of the inflorescence and the determinacy of the floral meristems, and was also related to development of the floral organs.

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ABSTRACT

Background. The architecture of inflorescence and the development of floral organs can influence the yield of seeds and have a significant impact on plant propagation. E-class floral homeotic MADS-box genes exhibit important roles in regulation of floral transition and differentiation of floral organs. Woad (*Isatis indigotica*) possesses unique inflorescence, floral organs and fruit. However, very little research has been carried out to determine the function of MADS-box genes in this medicinal cruciferous plant species.

Results. *SEPALLATA* orthologs in *I. indigotica* were cloned by degenerate PCR. The sequence possessing the highest identity with *SEP2* and *SEP4* of Arabidopsis were named as *IiSEP2* and *IiSEP4*, respectively. Constitutive expression of *IiSEP2* in *Columbia* (Col-0) ecotype of Arabidopsis led to early flowering, and the number of the flowers and the lateral branches was reduced, indicating an alteration in architecture of the inflorescences. Moreover, the number of the floral organs was declined, the sepals were turned into carpelloid tissues bearing stigmatic papillae and ovules, and secondary flower could be produced in apetalous terminal flowers. In *35S::IiSEP4-GFP* transgenic Arabidopsis plants in Landsberg *erecta* (Ler) genetic background, the number of the floral organs was decreased, sepals were converted into curly carpelloid structures, accompanied by generation of ovules. Simultaneously, the size of petals, stamens and siliques was diminished. In *35S::IiSEP4-GFP* transgenic plants of apetalous *ap1 cal* double mutant in Ler genetic background, the cauliflower phenotype was attenuated significantly, and the petal formation could be rescued. Occasionally, chimeric organs composed of petaloid and sepaloid tissues, or petaloid and stamineous tissues, were produced in *IiSEP4* transgenic plants of *ap1 cal* double mutant. It suggested that overexpression of *IiSEP4* could restore the capacity in petal differentiation. Silencing of *IiSEP4* by Virus-Induced Gene Silencing (VIGS) can delay the flowering time, and reduce the number and size of the floral organs in woad flowers.

Conclusion. All the results showed that *SEPALLATA*-like genes could influence the architecture of the inflorescence and the determinacy of the floral meristems, and was also related to development of the floral organs.

Key words *Isatis indigotica* Fortune, *Arabidopsis thaliana*, *SEPALLATA*-like genes, Inflorescence architecture, Floral transition, Floral organ differentiation, Petal rescue, Floral meristem determinacy, Overexpression, Floral homeotic conversion

INTRODUCTION

The changing process of plant growth from vegetative stage to reproductive stage is known as floral transition and is associated with highly complicated regulatory networks. In the initial period of floral transition, the shoot apical meristem will expand in diameter and become inflorescence meristem gradually. In Arabidopsis, floral meristems will form continuously on the flank of inflorescence meristem and arrange spirally along the stem. In the course of subsequent growth, primordia of four floral organ types will appear in specific areas of floral meristems from outside to inside, and they will differentiate into sepals, petals, stamens and pistil, respectively (*Smyth, Bowman & Meyerowitz, 1990*).

MADS-box family genes play crucial roles in reproduction of flowering plants (*Ma et al., 2020*). In 1990's, Coen and Meyerowitz proposed the "ABC model" for floral organ construction, which provided a theoretical basis for elucidation of Gothe's intuitive idea that all floral organs have arisen from a modified leaf (*Coen & Meyerowitz, 1991*). The flowers in most angiosperms are composed of four whorls of floral organs: sepals, petals, stamens and carpels. The identity of these floral organs is determined by a specific combination of floral homeotic MADS-box transcriptional factors, as suggested in "floral quartet model" and ABCDE model (*Pelaz et al., 2001*; *Honma & Goto, 2001*; *Theissen & Saedler, 2001*; *Shen et al., 2019*). In floral quartet model, complexes of MADS-box proteins are supposed to be involved in specifying the identity of different floral organs (*Theissen & Saedler, 2001*; *Gramzow & Theissen, 2010*).

In Arabidopsis, there are four E-class floral homeotic genes and they are named as *SEPALLATA1* (*SEP1*, previously known as *AGL2*), *SEP2* (*AGL4*), *SEP3* (*AGL9*), and *SEP4* (*AGL3*). Mutation in anyone of these *SEP* genes produces only subtle phenotypic changes, implying their functional redundancy (*Pelaz et al., 2000*). Consistent with this, the four different types of floral organs in *sep1 sep2 sep3* triple mutant are converted into sepals, accompanied by loss of flower determinacy. More seriously, the flowers of *sep1 sep2 sep3 sep4* quadruple mutant are comprised entirely of leaf-like structures (*Pelaz et al., 2000*).

Nonetheless, *SEP1*, *SEP2* and *SEP3* are not completely redundant with *SEP4*, owing to the different phenotypes between the triple mutant and the quadruple mutant. Further investigation revealed that *SEP4* had a more prominent role in maintenance of the determinacy of floral meristems, because *ap1 sep4* double mutants display a cauliflower-like phenotype which was observed neither in the respective single mutants nor in *ap1 sep1 sep2* or *ap1 sep3* mutants (*Ditta et al., 2004*). The single mutants of *SEP3* produced sepaloid petals resemble to those of the intermediate *ap1* mutants (*Pelaz et al., 2001*), indicating the redundancy of *SEP1*, *SEP2* and *SEP3* is not complete either. Besides, the dramatic floral defects of *lfy sep3* double mutants are not found in either of the single mutants (*Liu et al., 2009*). It suggests that the phenotypic variations produced by loss-of-function allele of *SEP3* could not be compensated by other *SEP* genes when *LFY* was mutagenized

98   simultaneously.

99   The functional differences among *SEP* genes are probably associated with their inconsistency in

100   expression. The transcripts of *SEP1*, *SEP2* and *SEP4* are detectable in floral meristems and in sepal primordia.

101   In contrast, *SEP3* is activated at the end of the second stage during flower development (*Flanagan & Ma, 1994*;

102   *Savidge, Rounsley & Yanofsky, 1995*; *Mandel & Yanofsky, 1998*). Unlike other *SEP* genes, the transcription of

103   *SEP4* is not confined to flowers, the mRNA of this gene can also be detected in vegetative tissues, including

104   leaves and stems (*Ma, Yanofsky & Meyerowitz, 1991*). It is very likely that these variations in expression are

105   related to the diversified functions of the *SEP* genes (*Jack, 2001*).

106   A number of *SEP*-like genes have been characterized in other plant species. Down-regulation of *GRCD1*

107   (*Gerbera Regulator of Capitulum Development 1*), a homologous gene of *SEP1* in *Gerbera hybrida*, converted

108   the female florets into petals (*Kotilainen et al., 2000*). Inhibition of *TM29*, a tomato *SEPALLATA* homolog,

109   resulted in aberrant phenotypes in the inner whorls of floral organs, resembling the cosuppression lines of

110   *FBP2* in petunia and *TM5* in tomato, two other *SEP*-like genes (*Ampomah-Dwamena et al., 2002*). In cultivar

111   'Ryokusei' of *Habenaria radiate*, a mutant containing a retrotransposon-like element in the first exon of

112   *HrSEP-1*, the white petals were changed into greenish petals, sepaloid tissues and a ventral column were

113   formed in the inner whorls of flowers. Transcript of *HrSEP-1* was undetectable in 'Ryokusei', namely loss-of-

114   function of *HrSEP-1* could influence the development of the floral organs (*Mitoma & Kanno, 2018*).

115   In five *SEP*-like genes of London plane (*Platanus acerifolia*), a basal eudicot tree, *PlacSEP1.1*,

116   *PlacSEP1.2* and *PlacSEP1.3* belong to the *SEP1/2/4* clade, *PlacSEP3.1* and *PlacSEP3.2* are members of *SEP3*

117   clade. The transcripts of *PlacSEP1.3*, *PlacSEP3.1* and *PlacSEP3.2* were detectable during the initiation stage

118   of male and female inflorescences, and throughout the development of flowers and fruits, whereas *PlacSEP1.2*

119   was expressed only in female inflorescences. In addition, the expression of *PlacSEP1.3* and *PlacSEP3.1* was

120   weak in vegetative buds of adult trees, implying their possible roles in regulation of dormancy. These results

121   also showed that patterning of the inflorescences was associated with *SEP*-like genes. Except early flowering,

122   overexpressing tobacco plants of *PlacSEP1.1* and *PlacSEP3.1* produced more lateral branches (*Zhang et al.,*

123   *2017a*). Knockdown of *SLMBP21*, a tomato *SEPALLATA*-like gene in FBP9/23 subclade, could suppress the

124   development of abscission zone in flower pedicels by affecting a subset of genes related to meristem activity,

125   and overexpression of this gene could produce small cells at the proximal section of pedicels and peduncles

126   (*Liu et al., 2014*).

127   *Thalictrum thalictroides* is a non-core eudicot species whose flowers are apetalous. By Virus-Induced

128   Gene Silencing (VIGS), *SEP*-like genes in *T. thalictroides* were found to be partially redundant in specifying

129   the identity of sepals and stamens, and in promoting petaloidy of sepals. Moreover, the ortholog of *SEP3*

130   showed a pronounced role in determination of carpel identity, and in carpel development. Furthermore,

131   *ThtSEP1* was involved in defining the boundary between sepals and stamens, and *ThtSEP2* functioned in

132   maintaining the boundary between stamens and pistil. In double-knockdown or triple-knockdown plants,

133   partial to complete homeotic conversion of stamens and carpels to sepaloid organs or green sepals was

134   occurred (*Soza et al., 2016*). Silencing of *FaMADS9*, a *SEP1/2*-like gene in strawberry, could inhibit the

135   development of petals, and the ripening of achene and receptacle (*Seymour et al., 2011*).

136   In cucumber (*Cucumis sativus* L.), a mutant showing perturbations in the development of stamens, pistils

137   and fruits was found to be related to *CsSEP2*. In this *SEP*-like gene, the change at the donor splice site in 5'-

138   end of the sixth intron results in skipping of the sixth exon during RNA processing, which leads to loss of the

139   transcriptional activation capacity of the protein encoded by the alternative transcript. The phenotypes of

140   *CsSEP2* mutant include enormously large sepals in female and male flowers, irregular shape or loss of three

141   stigma lobes and three-fold longer style in female flowers, larger male flowers with separate filaments and an

142   inner cavity filled by style-like tissues. Moreover, elongated carpel primordia can be observed before anthesis

143   in female and male flowers of the mutant, accompanied by early shedding of the fruits (*Wang et al., 2016*).

144   These results indicated that *CsSEP2* was a homologous gene of Arabidopsis *SEP2* and was involved in floral

145   organ and fruit development in cucumber.

146   Woad (*Isatis indigotica* Fortune) is one of the traditional Chinese medicinal plants and is a biennial

147   crucifer with bisexual flowers. Woad flower is composed of four whorls of floral organs: sepals, petals,

148   stamens, and carpels. Up to now, a good deal of research has been devoted to examine the medicinal value of

149   woad (*Hu et al., 2011*; *Li et al., 2014*), but the genes involved in regulation of flowering in this plant species

150   are so far very rarely concerned. There are also four *SEP*-like genes in woad, and these genes were named as

151   *IiSEP1*, *IiSEP2*, *IiSEP3* and *IiSEP4* according to the degree of similarity with the corresponding orthologous

152   gene in Arabidopsis (*Ma et al., 2019; Pu et al., 2020*). In the present work, the function of *IiSEP2* and *IiSEP4*

153   was investigated. To gain insight into the conservation of *SEP* activity among different species, transgenic

154   Col-0 (*Columbia*) Arabidopsis plants overexpressing *IiSEP2* were generated and analyzed. *IiSEP4* isolated in

155   previous work of our laboratory (*Pu et al., 2020*) was used in preparation of the ectopic expressing lines of

156   wild-type Arabidopsis or *ap1 cal* double mutant in Ler (Landsberg *erecta*) genetic background. At the same

157   time, silencing by VIGS was carried out to determine the function of *IiSEP4*.

158

159   **MATERIALS AND METHODS**

160   **Plant Materials and Bacterial Strains**

161   Woad plants, wild-type Arabidopsis ecotype Col-0, wild-type Arabidopsis ecotype Ler, and *ap1 cal* double

162   mutant in Ler genetic background (CS67157) were used as materials in the present work, and were grown

163   under normal greenhouse conditions (23±2 °C, 16 h light / 8 h dark). Woad flowers and leaves were used as

164   materials in extraction of RNA and DNA. pMD 18-T was used in cloning of cDNA and genomic DNA

165   fragments. pCAMBIA1302 was used in construction of the binary expression vectors. *Escherichia coli* strain

166   DH5α and *Agrobacterium tumefaciens* strain GV3101 were used as host in isolation of *IiSEP2* and in

167   transformation of Arabidopsis, respectively.

168

169   **Cloning of the Coding Sequence and the Promoter of *IiSEP2***

170   Total RNA was extracted from floral organs of *I. indigotica* with Trizol reagent (Invitrogen). Genomic DNA

171   was eliminated by RNase-free DNase I (Takara). First strand cDNA was synthesized with PrimeScript 1st

172   Strand cDNA Synthesis Kit (Takara). The coding sequence of *IiSEP2* was obtained by PCR and the primers

173   were designed according to the open reading frames of the MADS-box genes in different plant species (Table

174   S1).

175   Promoter sequences were isolated with Universal Genome Walker 2.0 Kit (Clontech). The primers were

176   designed according to the sequence in the first exon of *IiSEP2* (Table S1). Genomic DNA was extracted from

177   leaves of *I. indigotica* by CTAB method (*Stewart & Via, 1993*). Uncloned libraries were created after

178   restriction digestion of the genomic DNA with *Dra* I, *Eco*R V, *Pvu* II or *Stu* I, and ligation of the adaptors

179   provided by Universal Genome Walker 2.0 Kit (Clontech). Upstream sequences were amplified with Adaptor

180   Primer 1 and IiSEP2-Upstream1 with Advantage 2 PCR Kit (Clontech). Nested PCR was carried out with

181   Adaptor Primer 2 and IiSEP2-Upstream2. The PCR products were ligated into pMD 18-T and were sequenced

182   after identification by colony PCR.

183

184   **Analyses of the Expression Pattern of *IiSEP2* in *I. indigotica***

185   Total RNA was extracted from different tissues and floral organs of *I. indigotica* with RNAprep Pure Plant Kit

186   (TIANGEN) and the first strand cDNA was synthesized with PrimeScript 1st Strand cDNA Synthesis Kit

187   (Takara). Quantitative real-time PCR (qRT-PCR) was conducted in BIO-RAD real-time PCR system (BIO-

188   RAD, USA) with the primers shown in Table S1 (*Huang et al., 2008; Ma et al., 2019*). Woad *actin* (GenBank

189   accession No. AY870652.1) was used as a reference gene. Triplicate qRT-PCRs were carried out and relative

190   quantification of the transcript levels was accomplished using the comparative threshold cycle (Ct) method.

191   Relative quantification refers to that the PCR signals of the *IiSEP2* transcript in leaves, stem apexes, early

192   inflorescences (the inflorescence shrinks into a ball), later inflorescences (the inflorescence is completely

193   unfolded), flowers in full bloom, floral organs (include sepals, petals, stamens, and pistils), and young silicles

194   are normalized to the PCR signal in roots. The fold change was calculated using the following formula: fold

195   change = $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{IiSEP2} - Ct_{Iiactin})_{different\ sample} - (Ct_{IiSEP2} - Ct_{Iiactin})_{root}$.

196

197 **Subcellular Localization by Confocal Laser Scanning Microscopy**

198 The coding sequence of *IiSEP2* was inserted into pCAMBIA1302 containing CaMV 35S promoter after high-

199 fidelity amplification with the primers shown in Table S1. The recombinant construct was introduced into

200 competent cells of *A. tumefaciens* GV3101 by freeze-thaw method (*Höfgen & Willmitzer, 1988*). Transformed

201 *A. tumefaciens* cells were selected on YEB medium containing rifampicin (50 μg/L), gentamicin (50 μg/L),

202 and kanamycin sulfate (50 μg/L). Positive colony carrying recombinant plasmid was propagated in liquid

203 medium for 24 h at 30 °C in a shaker incubator (~200 rpm). Precipitated bacterial cells were resuspended with

204 fresh YEB medium containing the same antibiotics and were grown to an OD_{600} value of 0.8. The bacterial

205 cells were collected by centrifugation at 6,000 rpm for 10 min under room temperature. The pellet was

206 resuspended with agroinfiltration buffer [10 mmol/L 2-(*N*-morpholino)ethanesulfonic acid, 200 μmol/L

207 acetosyringone, 10 mmol/L $MgCl_2$] to an OD_{600} value of 0.7. The bacterial suspension was infiltrated into the

208 abaxial leaf epidermis of 6-week-old *Nicotiana benthamiana* plants using a syringe without needle. The

209 quality of transient expression was estimated 60 h after agroinfiltration. Confocal laser scanning microscopy of

210 the living plant tissues was performed using an OLYMPUS FLUOVIEW FV1000 microscope. Green

211 fluorescence of GFP was observed with an exciting light of 488 nm.

212

213 **Identification of *IiSEP2* Transgenic Arabidopsis Plants**

214 The recombinant binary expression vector pCAMBIA1302-*IiSEP2*, in which *IiSEP2* is controlled by CaMV

215 35S promoter, was introduced into *A. tumefaciens* GV3101. *Agrobacterium*-mediated transformation of

216 Arabidopsis was performed essentially according to the protocol of Clough and Bent (1998). Col-0 transgenic

217 seedlings were selected on hygromycin-containing medium, and the expression levels of *IiSEP2* in T2

218 generation were detected by RT-PCR using *IiSEP2* specific primers (Table S1), and by Western blotting using

219 rabbit monoclonal antibody of GFP (Sino Biological Inc.). Transgenic phenotypes were confirmed in T1 and

220 T2 generations. Influence of *IiSEP2* on Arabidopsis MADS-box genes, including *AP3* (*APETALA3*), *PI*

221 (*PISTILLATA*), *SHP1* (*SHATTERPROOF1*) and *SHP2*, was determined by qRT-PCR.

222

223 **Preparation of *IiSEP4* Transgenic Plants in the Genetic Background of Ler**

224 The binary expression vector pCAMBIA1302-*IiSEP4* constructed in our previous work (*Pu et al., 2020*) was

225 introduced into *A. tumefaciens* GV3101 and was used in genetic transformation of wild-type Arabidopsis

226 plants (Ler) or *ap1 cal* double mutant (Ler). The expression of the transgene was analyzed by qRT-PCR with

227 IiSEP4-qRT-F (5'-AGATAGCCGGGATGGGAGTG-3') and IiSEP4-qRT-R (5'-

228 AATCATCGACCGGGCCTTTG-3') (*Pu et al., 2020*). To compare the phenotypic differences, 25 *IiSEP4*

229 transgenic Arabidopsis plants were grown in greenhouse together with 25 wild-type plants every time.

230

Silencing of *IiSEP4* by VIGS

To investigate the function of *IiSEP4* in woad, its expression was downregulated by *Agrobacterium*-mediated

and TRV-based (tobacco rattle virus-based) VIGS (*Liu, Schiff & Dinesh-Kumar, 2002*). *A. tumefaciens*

GV3101 was cultured in LB liquid medium containing 50 mg/L kanamycin, 25 mg/L rifampicin and 25 mg/L

gentamicin to OD_{600}=2.0. After centrifugation, the bacterial cells were resuspended with infiltration solution

[100 mL was composed of 100 µL 0.2 mol/L acetosyringone, 2 mL 0.5 mol/L 2-(*N*-morpholino)ethanesulfonic

acid, 1 mL 1 mol/L $MgCl_2$]. During four-leaf stage of growth, the leaves of woad plants were infiltrated with a

mix of *A. tumefaciens* GV3101 carrying pTRV1 and *A. tumefaciens* GV3101 carrying recombinant pTRV2-

IiSEP4 containing a 300 bp specific fragment coming from 5'-end of *IiSEP4* coding sequence (*Pu et al., 2020*).

The fragment of *IiSEP4* was amplified by high-fidelity PCR with primers VIGS-IiSEP4-F (5'-

CGCGAATTCCGATGATTGATCAACTATCG-3') and VIGS-IiSEP4-R (5'-

CGCGGATCCTCTGGGATGTTGTTGCAGAG-3'). In each VIGS experiment, the phenotypes of 30 woad

plants in treatment group and 30 woad plants in control group were compared.

Data Analysis

SPSS22.0 (SPSS Inc., Chicago, IL) was used for data analysis. The data of rosette leaf number, the angle

between cauline leaf and stem, flower bud number, stamen number in *IiSEP2* overexpressing Arabidopsis

plants, and the data about the expression pattern of *IiSPE2* in woad plants, were analyzed by one-way ANOVA,

followed by Tukey's post-hoc test. The data obtained in qRT-PCR analysis of the MADS-box genes (including

AP3, *PI*, *SHP1* and *SHP2*) in *IiSEP2* overexpressing Arabidopsis plants, *IiSEP4* in 35S::*IiSEP4-GFP*

transgenic plants in wild-type Ler genetic background and in 35S::*IiSEP4-GFP* transgenic plants of *ap1 cal*

double mutant in Ler genetic background, and *IiSEP4* in distal noninfiltrated leaves in woad plants infiltrated

with pTRV1 + pTRV2-IiSEP4, were analyzed by two-sided Student's *t*-test (*Pu et al., 2020*).

RESULTS

Identification of the Coding Sequence and the Promoter of *IiSEP2*

The coding sequence of *IiSEP2* was amplified with high-fidelity DNA polymerase by RT-PCR. Sequencing

result showed that the open reading frame of *IiSEP2* was 753 bp in length and encoded a 250-amino-acid

protein (Fig. S1). IiSEP2 comprises the four regions of typical plant MIKC-type MADS-box proteins, namely

MADS domain (M, 2-60aa), intervening region (I, 61-83aa), keratin-like domain (K, 84-171aa), and C-

terminal region (C, 172-250aa). At the same time, the protein encoded by *IiSEP2* shows high identity in amino

263   acid sequence with the homologous proteins of other plant species. For instance, the identity between IiSEP2

264   and Arabidopsis SEP2 is 95% (Fig. S2A). Eight conserved motifs with a length of 6~50 amino acids could be

265   found in IiSEP1, IiSEP2 and IiSEP2-like proteins by the online tool in MEME website (http://meme-suite.org/)

266   (*Bailey et al., 2009*), and two motifs (Motif 1 and Motif 9) in MADS domain and three motifs (Motif 3, Motif

267   4 and Motif 5) in K-box domain existed in all the analyzed proteins (Fig. S2B). The logo of these motifs

268   discovered by MEME is shown in Figure S2C.

269   To clarify the relationship of IiSEP2 and other plant MADS-box proteins, we analyzed the phyletic

270   evolution of IiSEP2 (Fig. S3). The results showed that IiSEP2 was closely related to Arabidopsis SEP2. These

271   results demonstrated that *IiSEP2* was an E-class floral homeotic gene and further confirmed that *IiSEP2* was

272   the orthologous gene of Arabidopsis *SEP2*.

273   In 1Kb regulatory sequence upstream of the initiation codon characterized by Genome Walking method

274   (Supplementary sequence data 1), there are three CArG-boxes, indicating the expression of *IiSEP2* can be

275   affected by other MADS-box transcriptional factors.

276

277   **Spatial and Temporal Expression of *IiSEP2* in *I. indigotica***

278   The abundances of *IiSEP2* mRNA in various tissues and floral organs of *I. indigotica* were analyzed by qRT-

279   PCR. The transcriptional levels of *IiSEP2* were low in roots and leaves. However, the quantity of *IiSEP2*

280   transcript was significantly increased in stem apex and was accumulated to a high level in flowers and young

281   silicles. Further testing in floral organs showed that *IiSEP2* mRNA was highly expressed in sepals, petals,

282   stamens, and pistil (Fig. 1). Taken together, the qRT-PCR results illustrate that *IiSEP2* expression occurs from

283   the early stages of flower development to young silicles.

284

285   **Subcellular Localization of IiSEP2**

286   To determine the subcellular localization of IiSEP2 *in vivo*, the coding sequence of *IiSEP2* without the stop

287   codon was fused in frame with *GFP* reporter gene in pCAMBIA1302 and was driven by CaMV 35S promoter.

288   *A. tumefaciens* carrying the recombinant expression vector was used to perform a transient expression assay in

289   *N. benthamiana* leaves. The GFP fluorescence of the control experiment using empty vector evenly distributed

290   throughout the cell, whereas the GFP fluorescence of IiSEP2-GFP was observed only in the nucleus (Fig. S4).

291   The result is consistent with the predictions using PSORT II online server (http://psort.hgc.jp/form2.html),

292   namely KRIENKINRQVTFAKRR in N-terminus of IiSEP2 is a bipartite nuclear localization signal (NLS).

293

294   **Overexpression of *IiSEP2* Leads to Morphologic Changes in Transgenic Arabidopsis Plants**

295   To examine the function of *IiSEP2*, transgenic Arabidopsis plants under the control of the constitutive CaMV

296   35S promoter were prepared in this work. Seeds were selected on MS medium containing hygromycin, and the

297   putative transgenic plants were confirmed by PCR and Western blotting (Fig. S5).

298   In 28 *35S::IiSEP2* independent transgenic lines, 9 transgenic lines showed obvious macroscopic

299   differences in comparison with the wild-type Arabidopsis plants. *35S::IiSEP2* transgenic Arabidopsis plants

300   were extremely early in bolting. The flowering time was evaluated by counting the number of the leaves

301   produced when the first flower bloomed. The transgenic lines generated 4-5 leaves prior to the onset of

302   flowering, whereas wild-type plants produced approximately 9 leaves (Fig. 2A).

303   In wild-type Col-0 Arabidopsis plants, cauline leaves are generated at the lower part of the inflorescence

304   stems, and the oval-shaped blades are slightly curved outward. Moreover, the angles between the cauline

305   leaves and the stems in wild-type Col-0 Arabidopsis plants are larger (Fig. 2B, Figs. 3A-3B). Different from

306   wild-type Col-0 Arabidopsis plants, the cauline leaves of *IiSEP2* transgenic Arabidopsis plants are clearly

307   curling inward and the margin is involute. Simultaneously, the angles between the cauline leaves and the stems

308   become smaller in transgenic Arabidopsis plants, and the whole blade looks like an inverted cone (Fig. 2B,

309   Figs. 3C-3G).

310   The process that the plant switches from vegetative growth to reproductive growth is named as floral

311   transition. At the early stage of floral transition, the vegetative shoot apical meristem is transformed into an

312   inflorescence meristem, and floral meristems are formed in the peripheral regions of the inflorescence

313   meristem. Then the primordia of the floral organs will be produced along with the development of floral

314   meristems, which is accompanied by definition of the boundaries between different floral organs. The

315   inflorescence meristem of wild-type Col-0 Arabidopsis plants is highly active during the growth process and

316   can produce a lot of flower buds continuously (Figs. 4A-4B). However, the activity of the inflorescence

317   meristem in *IiSEP2* transgenic Arabidopsis plants is reduced remarkably. In general, only a few flower buds

318   could be generated at the top of the inflorescence stem in *IiSEP2* transgenic Arabidopsis plants (Fig. 2C, Figs.

319   4C-4F), illustrating that constitutive expression of *IiSEP2* could reduce the number of flowers and the number

320   of lateral branches. Namely, IiSEP2 can affect the architecture of the inflorescences.

321   Under normal circumstances, the flowers of the wild-type Col-0 Arabidopsis plants consist of four whorls

322   of floral organs, including four sepals in the outermost whorl, four petals in the second whorl, six stamens in

323   the third whorl, and a gynoecium in the fourth whorl (Figs. 5A-5C). By observation under stereomicroscope, it

324   was found that overexpression of *IiSEP2* in Arabidopsis resulted in seriously anomalous development of the

325   floral organs. A number of flowers were male sterile (Fig. 4D), owing to the abnormality of stamens in

326   development. At the same time, the inflorescence meristems were transformed into defective terminal flowers

327   without sepals and petals, and the number of stamens was reduced (Figs. 4E-4G). In Figure 4E, only three

328   stamens could be observed under the pistil in the middle. In many flowers, the sepals were converted

329 homeotically into carpelloid structures with stigmatic papillae and ovules in transgenic lines 7 and 8 (Figs.

330 5E,5J).

331   In transgenic lines 3 and 4, secondary flower could be generated in terminal flowers. The number of

332 sepals, petals and stamens was decreased in these terminal flowers, whereas the secondary flowers were nearly

333 normal in structure (Fig. 5D,5F). Different from four petals in wild-type flowers, a few apical flowers of the

334 *IiSEP2* overexpressing plants produced five petals or lacked petal, together with anomalous development of the

335 carpels in transgenic lines 5 and 6 (Figs. 5G-5H). Quantitative expression analyses showed that *AP3* and *PI*,

336 two Arabidopsis MADS-box genes related to petal formation, were suppressed by ectopic expression of

337 *IiSEP2* (Figure S6). On the contrary, the transcripts of *SHP1* and *SHP2* were increased in *IiSEP2* transgenic

338 Arabidopsis plants.

339   In order to fully understand the function of *IiSEP2*, transgenic line 8 was genetically crossed with the

340 wild-type Col-0 plants, and the phenotypes of the crossing progenies were compared to that of the *IiSEP2*

341 transgenic line 8. The phenotypes of the *IiSEP2* transgenic lines were maintained in filial generations of the

342 crossing lines. These offsprings exhibited the phenotypic variations of the *IiSEP2* overexpressing plants,

343 including the secondary flowers, the loss of petals, and the reduction of stamens (Figs. 5I,5K,5L). All these

344 phenotypic changes indicated that constitutive expression of *IiSEP2* could affect the morphogenesis of the

345 flowers.

346

347 **Ectopic expression of *IiSEP4* in Ler genetic background can affect the development of floral organs**

348 In order to analyze the regulatory function of *IiSEP4* in floral transition and flower development, transgenic

349 Arabidopsis plants were prepared. The transcriptional levels of *IiSEP4* in transgenic plants were detected by

350 qRT-PCR. The results showed that *IiSEP4* could express effectively in transgenic plants of wild-type

351 Arabidopsis in Ler genetic background, and in transgenic plants of *ap1 cal* double mutant in Ler genetic

352 background (Fig. S7).

353   All the *35S::IiSEP4-GFP* transgenic plants displayed abnormal phenotypes, and the number of the floral

354 organs was reduced. In particular, sepals were curly and were converted into carpelloid structures, and were

355 obviously accompanied by the generation of ovules (Fig. 6B-6C). In addition, dwarf and abnormal petals and

356 stamens were produced (Fig. 6D), and the size of the silique was also decreased significantly in comparison

357 with the wild-type Ler plants (Fig. 6F).

358   On account of the apetalous phenotype of *ap1 cal* double mutant, ectopic expression in this mutational

359 material was conducted to clarify the roles of *IiSEP4* in petal differentiation. In *ap1 cal* double mutant in Ler

360 genetic background, floral meristems were transformed into inflorescence meristems, and new meristems were

361 elaborated continuously. As a result, the inflorescence resembles a cauliflower in *ap1 cal* double mutant. In

362   addition, *ap1 cal* plants produced apetalous flowers, namely only sepals, stamens and pistil could be formed

363   (Figs. 7A-7B). In *35S::IiSEP4-GFP* transgenic plants of *ap1 cal* double mutant, the cauliflower phenotype was

364   attenuated significantly, and the petals could be recovered (Figs. 7C-7H). Occasionally, chimeric organ

365   composed of petaloid and sepaloid tissues (Figs 7F-7G), and chimeric organ composed of petaloid and

366   stamineous tissues (Fig. 7E), could be observed.

367

368   **Phenotypic variations of woad plants after downregulation of *IiSEP4* by VIGS**

369   To analyze the moderating effects on woad flowering and to verify the results obtained in Arabidopsis, the

370   expression level of *IiSEP4* was downregulated by VIGS mediated by *Agrobacterium*. The results of RT-PCR

371   showed that RNA molecules of TRV1 and TRV2 could be detected in distal noninfiltrated leaves of the control

372   woad plants treated with pTRV1 + pTRV2, and in distal noninfiltrated leaves of the woad plants treated with

373   pTRV1 + pTRV2-IiSEP4 (Fig. S8). qRT-PCR data showed that the mRNA abundance of *IiSEP4* was reduced

374   dramatically in woad plants treated with pTRV1 + pTRV2-IiSEP4 (Fig. S9), indicating *IiSEP4* could be

375   silenced effectively by VIGS.

376   Silencing of *IiSEP4* can delay the flowering time. Compared to woad plants infiltrated with a mixture of

377   *A. tumefaciens* GV3101 carrying pTRV1 and *A. tumefaciens* GV3101 carrying the empty pTRV2, the woad

378   plants infiltrated with a mixture of *A. tumefaciens* GV3101 carrying pTRV1 and *A. tumefaciens* GV3101

379   carrying pTRV2-IiSEP4 start bolting nearly half a month later, indicating downregulation of *IiSEP4* can lead

380   to late-flowering phenotype in woad. Specifically, wild-type woad plants begin to bloom in first ten-days of

381   May, whereas *IiSEP4*-silenced lines bear flowers in late May.

382   More than 90 percent of the woad plants in treatment group showed phenotypic variations. Observation

383   of the flowers showed that the floral organs were unchanged after infiltration with pTRV1 + pTRV2 (Fig. 8A),

384   whereas the floral organs in woad plants infiltrated with pTRV1 + pTRV2-IiSEP4 presented pronounced

385   anomalous phenotypes, and the number of sepals, petals and stamens was reduced (Figs. 8B-8E). In

386   comparison with the control group, the size of the floral organs in woad plants infiltrated with pTRV1 +

387   pTRV2-IiSEP4 was much smaller (Figs. 8F-8H). In some woad plants treated with pTRV1 + pTRV2-IiSEP4,

388   the number of petals was increased. For instance, one flower in a woad plant infiltrated with pTRV1 + pTRV2-

389   IiSEP4 produced five petals, but the number of stamens was reduced from six to five. In these plants, except

390   the smaller sepals, no obvious change could be observed in the size of petals and stamens (Figs. 8I-8L).

391

392   **DISCUSSION**

393   Although A-, B- and C-class floral homeotic MADS-box genes are essential to the development of flowers,

394   ectopic expression of these genes is not enough to completely induce the formation of floral organs, indicating

395   other transcriptional factors are involved in floral patterning regulation (*Krizek & Meyerowitz, 1996*).

396   Phenotypic observation of the multiple mutant showed that *SEPALLATA1/2/3/4* (*SEP1/2/3/4*) are also

397   necessary for differentiation of the four types of floral organs, and for initiation of floral meristems (*Pelaz et*

398   *al., 2000*; *Ditta et al., 2004*). Simultaneous mutation of *SEP1/2/3* converts the four types of floral organs into

399   sepaloid tissues (*Pelaz et al., 2000*). If *SEP1/2/3/4* are all knocked out, the four types of floral organs will be

400   reversed as leaf-like tissues bearing epidermal trichomes, representing the functions of the E-class floral

401   homeotic genes are completely destroyed (*Ditta et al., 2004*).

402   Flower development of Arabidopsis from initiation until the opening was divided into 12 stages (*Smyth,*

403   *Bowman & Meyerowitz, 1990*). *SEP2* was expressed in the entire floral meristems at stage 2, slightly earlier

404   than *SEP3*. In subsequent stages, *SEP2* was expressed in all the four types of floral organs (*Pelaz et al., 2000*;

405   *Ditta et al., 2004*). *ThtSEP2*, a *SEP2* homologous gene characterized in *T. thalictroides*, was expressed at low

406   levels in all the floral organs and at all developmental stages of flowers (*Soza et al., 2016*). In the present work,

407   the coding sequence of *IiSEP2* was cloned by degenerate PCR and its length is 753 bp, and the MADS-box

408   protein encoded by *IiSEP2* consists of 250 amino acid residues. Multiple alignments showed that MADS-box

409   proteins were highly conserved and the identity between IiSEP2 and Arabidopsis SEP2 was 95%. According to

410   the results of qRT-PCR, *IiSEP2* was expressed in early stage of flower development, all the four types of floral

411   organs, and silicles. In four types of floral organs, *IiSEP2* was highly expressed in sepals and petals. On the

412   contrary, *IiSEP2* mRNA could not be detected obviously in vegetative tissues. These results illuminate that

413   *IiSEP2* plays an ubiquitous and crucial role in flower development of *I. indigotica*.

414   In Arabidopsis, *SEP1* and *SEP2* are target genes of FAR-RED ELONGATED HYPOCOTYL3 (FHY3).

415   FHY3 is associated with maintenance of shoot apical meristem, determinacy of floral meristem, and

416   differentiation of petals and stamens. *SEP2* can be activated directly by FHY3, and ultimately promote the

417   determinacy of floral meristem (*Li et al. 2016*). In *fhy3* single mutant, the pistil from the indeterminate floral

418   meristem could generate plenty of floral organs. In inflorescences of *fhy3-68 ag-10* double *mutant*, the

419   transcripts of *SEP1* and *SEP2* were decreased. On the contrary, *SEP3* expression was upregulated in *fhy3-68*

420   single mutant and unchanged in *fhy3-68 ag-10* double mutant (*Li et al., 2016*). In consistent with this, ectopic

421   expression of *IiSEP2* in Arabidopsis can also influence the normal development of the floral organs and the

422   siliques. Similar to *35S::IiSEP4-GFP* transgenic plants in wild-type Ler genetic background obtained in the

423   present work (Fig. 6F), *FHY3* mutants in the genetic background of *ag-10* produced very short and bulged

424   siliques with an increased carpel number, accompanied by formation of very small petals and sterile anthers.

425   *IiSEP2* and *IiSEP4* of *I. indigotica* probably also play an important role in determinacy of the floral meristems,

426   and are controlled by the ortholog of FHY3 in *I. indigotica*.

427   Ectopic expression of *SEP2* could rescue the defects of *fhy3-68 ag-10* double mutant in floral meristem

428   determinacy. In *35S:SEP2 fhy3-68 ag-10* transgenic Arabidopsis plants, normal siliques composed of

429   gynophore and two fused carpels could be produced. It suggested that the function of *FHY3* in floral meristem

430   determinacy was mediated by *SEP2*. However, the other phenotypic variations in *fhy3-68 ag-10* double mutant,

431   including small inflorescence, short petal and sterile anther, could not be rescued by *SEP2*. Different from

432   *SEP2*, overexpression of *SEP1* in *fhy3-68 ag-10* double mutant under the control of CaMV 35S promoter

433   could not recover the defective phenotypes of floral meristems, indicating *SEP2* possesses an unique role in

434   pistil development and in determinacy of flowers. When *SEP2* was driven by the flower-specific *SEP3*

435   promoter, its expression in *fhy3-68 ag-10* double mutant could also rescue the floral meristem indeterminacy.

436   Nonetheless, short siliques with multiple carpels and additional organs, and sterile anthers could be produced.

437   When *SEP2* was down-regulated by artificial miRNA in the genetic background of *ag-10*, bulged siliques with

438   additional organs growing inside were generated, similar to the phenotype of *fhy3-68 ag-10* double mutant,

439   indicating suppression of *SEP2* can enhance the indeterminacy of floral meristem (*Li et al., 2016*). *IiSEP2* and

440   *IiSEP4* may also possess divergent or specified functions in comparison with other *SEP*-like genes in *I.*

441   *indigotica*.

442   *SEP*-like genes were found to be involved in regulation of the inflorescence architecture. In *sep1sep2sep3*

443   triple mutant and *sep1sep2sep3sep4* quadruple mutant, the degree of inflorescence branching was increased

444   (*Pelaz et al., 2000*; *Ditta et al., 2004*). The transcript of *SlCMB1*, a *SEP*-like gene of tomato, was mainly

445   accumulated in sepals. In RNA interference lines of *SlCMB1*, longer peduncles and more branches were

446   generated. Moreover, generation of new leaves and apical meristem in RNA interference lines represents a loss

447   of the inflorescence determinacy and a transition from reproductive growth to vegetative growth. In particular,

448   abnormally fused and enlarged sepals could be observed in RNA interference lines (*Zhang et al., 2018*). In

449   *Gerbera hybrida*, *SEPALLATA*-like MADS-box genes were associated with patterning regulation of the

450   pseudanthial inflorescence (*Zhang et al., 2017b*). In monocotyledons, *SEPALLATA*-like genes could also

451   influence the architecture of inflorescences (*Gao et al., 2010*; *Lin et al., 2014*; *Song et al., 2018*). In the present

452   work, it was confirmed that overexpression of *IiSEP2* and *IiSEP4* in Arabidopsis resulted in reduction of the

453   flower number and the lateral branch number.

454   *TM5* is a *SEP*-like gene in tomato and is expressed at high levels in meristematic territories fated to form

455   petals, stamens, and gynoecia. Suppression of *TM5* resulted in morphogenetic alterations in the internal three

456   whorls of floral organs. In antisense transgenic plants of *TM5*, the petals were green throughout the life span of

457   the flowers, the anthers were also green and were converted into sepaloid or petaloid structures. Moreover,

458   incomplete fusion of carpels and failure to form normal style could be observed (*Pnueli et al., 1994*). As a

459   *SEP*-like gene in rice, severe loss-of-function mutations in *OsMADS1* could cause complete homeotic

460 conversion of lodicules, stamens and carpels into lemma-like or palea-like structures (*Agrawal et al., 2005*).

461 *PlacSEP1.1*, *PlacSEP1.2* and *PlacSEP1.3* of London plane have been characterized functionally.

462 Overexpression of *PlacSEP1.1*, *PlacSEP1.2* and *PlacSEP1.3* in *Arabidopsis* resulted in different phenotypic

463 alterations. *35S:PlacSEP1.1* transgenic plants with the small size and curled leaves were early flowering

464 obviously. *35S:PlacSEP1.3* transgenic lines also exhibited early flowering phenotype, and possessed less

465 rosette leaves and more cauline leaves in comparison with the wild-type *Arabidopsis* plants. However, curled

466 leaf could not be found. On the contrary, *35S:PlacSEP1.2* transgenic plants showed no visible phenotypic

467 changes (*Zhang et al., 2017a*). *TaMADS1* is a *SEP*-like gene isolated from wheat (*Triticum aestivum* L.).

468 Compared to wild-type *Arabidopsis*, *35S:TaMADS1* transgenic lines were early flowering, the number of

469 petals and stamens was reduced, some flowers had short filament and sterile petaloid anthers (*Zhao, Cheng &*

470 *Zhang, 2006*). All these reports together with the results in the present work indicated that *SEP*-like genes were

471 conserved in regulation of the development of floral organs in both dicotyledons and monocotyledons.

472   In this work, influence of *IiSEP2* overexpression on MADS-box genes of Arabidopsis was also analyzed,

473 including *AP3*, *PI*, *SHP1* and *SHP2*. *AP3* and *PI* are B-class floral homeotic MADS-box genes and are

474 responsible for specifying the identity of petals. *SHP1* and *SHP2* are associated with formation of the

475 dehiscence zone in siliques and development of ovules (*Pinyopich et al., 2003*). The results showed that *AP3*

476 and *PI* were downregulated in *IiSEP2* transgenic Arabidopsis plants, whereas *SHP1* and *SHP2* were

477 upregulated. Similar to *IiSEP2* and *IiSEP4*, constitutive expression of *SHP* in Arabidopsis also resulted in

478 ectopic formation of carpels and ovules (*Favaro et al., 2003*). In like manner, when *LMADS2*, a carpel-specific

479 MADS-box gene of *Lilium longiflorum*, was constitutively expressed in Arabidopsis, sepals would be

480 converted homeotically into carpelloid structures with stigmatic papillae and ovules (*Tzeng, Chen & Yang,*

481 *2002*).

482   Moreover, the apical flowers in *IiSEP2* transgenic Arabidopsis plants often produced five petals, three

483 pistils, shrunken stamens and secondary flowers, or apetalous. These data showed that the development of

484 petals and stamens in Arabidopsis could be affected by ectopic expression of *SEP*-like genes, and phenotypic

485 changes similar to *ap3-3* and *pi-1* mutants could be produced (*Bowman et al., 1999*). Quantitative real-time

486 PCR showed that the expression of *AP3* and *PI* could be inhibited by IiSEP2. However, ectopic expression of

487 *IiSEP4* could restore the development of petals in *ap1 cal* double mutant.

488   Except for the influences on flower development, *IiSEP2* and *IiSEP4* can affect the growth of leaves. The

489 cauline leaves in *IiSEP2* transgenic Arabidopsis are shaped like inverted cones, and the angles between the

490 cauline leaves and the stems were reduced, which was probably associated with *CURLY LEAF* (*CLF*). The

491 protein encoded by *CLF* was a subunit of polycomb repressive complex 2 (PRC2). CLF could change the

492 transcriptional activity of the target genes by trimethylation of lysine 27 on histone H3 (H3K27me3) with its

493 methyltransferase activity, and resulted in repression of the target genes (*Ré et al., 2020; Singkaravanit-Ogawa*
494 *et al., 2021*). *clf* mutants showed leaf curling and early flowering phenotypes, which could be suppressed by
495 mutation of *SEP3*. It has been reported that derepression of *SEP3* could cause alterations in morphology of
496 leaves (*Mateo-Bonmatí et al. 2018*). In *clf* mutant, *SEP3* was highly expressed in leaves. Consistent with this,
497 ectopic expression of *SEP3* driven by CaMV 35S promoter produced curled rosette leaves (*Pelaz et al. 2001*),
498 and overexpression of *SEP*-like genes can also alter the morphology of leaves (*Adal et al., 2021*).
499

500 **CONCLUSIONS**
501 In Arabidopsis, ectopic expression of *IiSEP2* could lead to early flowering. In woad, downregulation of *IiSEP4*
502 by VIGS could produce late-flowering phenotypes. In comparison with the wild-type plants, transgenic
503 Arabidopsis plants of *IiSEP2* and *IiSEP4* were relatively small and the main stems of these plants were thin.
504 Constitutive expression of *IiSEP2* and *IiSEP4* in Arabidopsis could disturb the indeterminacy of inflorescence
505 meristems, and only a small number of flower buds would be produced, accompanied by generation of
506 terminal flower. Furthermore, *IiSEP2* and *IiSEP4* can influence the development of floral organs. In transgenic
507 plants overexpressing *IiSEP2* and *IiSEP4*, abnormal floral structures without sepals and petals were formed,
508 and the number of stamens was reduced. Meanwhile, carpelloid structures with stigmatic papillae and ovules
509 could be observed. In *IiSEP4* transgenic plants in the genetic background of *ap1 cal* double mutant, petal
510 formation could be rescued.
511

512 **ADDITIONAL INFORMATION AND DECLARATIONS**
513

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525 **Competing Interests**

526   The authors declare there are no competing interests.

527

528   **Author Contributions**

529   • Yan-Qin Ma, Zuo-Qian Pu, Xiao-Min Tan, Qi Meng, Kai-Li Zhang, Liu Yang, Ye-Ye Ma designed and

530   performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

531   • Yan-Qin Ma, Xuan Huang and Zi-Qin Xu conceived the research, wrote the manuscript, authored or

532   reviewed drafts of the paper, and approved the final draft.

533

534

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685   **Figure legends**

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689   **Figure 1 Expression pattern of *IiSEP2* in *I. indigotica* analyzed by qRT-PCR.** Error bars represent the

690   standard deviation. Root was used as normalization sample. Multiple testing correction was conducted with

691   SPSS software, different letters indicate significant difference at 5% level.

692

693   **Figure 2 Effects of *IiSEP2* overexpression on aerial architecture of Arabidopsis plants.** (A) Comparison

694   of rosette leaf number produced by *35S::IiSEP2* transgenic and wild-type Col-0 plants before flowering. (B)

695   Comparison of the angle between the cauline leaves and the stems. (C) Comparison of the flower bud number

696   produced by *35S::IiSEP2* transgenic and wild-type Col-0 plants. Values correspond to mean ± standard error

697   (n = 15). Multiple testing correction was conducted with SPSS software, different letters indicate significant

698   difference at 5% level.

699

700   **Figure 3 Morphological comparison of the cauline leaves between wild-type Col-0 and *IiSEP2* transgenic**

701   **plants.** (A) The rosette leaves and cauline leaves of wild-type Col-0 plants, the angles between the oval-shaped

702   cauline leaves and the stems are larger. (B) The upper part of a wild-type Col-0 plant in the flowering stage. (C)

703   *IiSEP2* transgenic Arabidopsis plants. (D) IiSEP2-OE-6#. (E) IiSEP2-OE-7#. (F) IiSEP2-OE-8#. (G) IiSEP2-

704   OE-9#. *IiSEP2* transgenic Arabidopsis plants in F and G show the inward-curling cauline leaves, and the

705   angles between the cauline leaves and the stems are reduced. In A, B and C, scale bars represent 1 cm. In D, E,

706   F and G, scale bars represent 1 mm.

707

708   **Figure 4 Influence of *IiSEP2* on development of inflorescences.** (A-B) Indefinite inflorescences of wild-type

709   Col-0 plants. The inflorescence meristem could produce new flower buds continuously. (C) Inflorescence of

710   *IiSEP2* overexpressing Arabidopsis plant, only a few flower buds could be observed. (D) Wilted inflorescence

711   with undersized flower buds at early development stage in *IiSEP2* transgenic Arabidopsis plant. (E-F) The

712   inflorescences converted into defective terminal flowers in *IiSEP2* transgenic Arabidopsis plant, arrow indicate

713   stamens. (G) Comparison of the number of stamens per flower. Scale bars represent 1 mm. Multiple testing

714   correction was conducted with SPSS software, different letters indicate significant difference at 5% level.

715

716   **Figure 5 Abnormal phenotypes of various floral organs induced by *IiSEP2* overexpression.** (A-C) The

717   flowers of wild-type Col-0 Arabidopsis plants. The sepals and petals have been removed from Arabidopsis

718   flower in Figure 5C. (D) *IiSEP2* transgenic line 3, red arrow indicate terminal flower containing a secondary

719   flower, blue arrow indicate lateral flower. (E) *IiSEP2* transgenic line 7, the sepals were converted into

720   carpelloid tissues. (F) *IiSEP2* transgenic line 4, red arrow indicate terminal flower containing a secondary

721   flower. (G) *IiSEP2* transgenic line 5, five petals could be observed. (H) *IiSEP2* transgenic line 6, apetalous

722   flower was generated. (I) Terminal flowers and secondary flower in crossing progeny of *IiSEP2* transgenic line

723   8 and wild-type Col-0. (J) *IiSEP2* transgenic line 8, abnormal flowers were produced. (K) Flower lacking petal

724   in crossing progeny of *IiSEP2* transgenic line 8 and wild-type Col-0 plant. (L) Abnormal floral organs in

725   crossing progeny of *IiSEP2* transgenic line 8 and wild-type Col-0 plant. Scale bars represent 1 mm.

726

727   **Figure 6 Phenotypic variations of the floral organs in *IiSEP4* transgenic Arabidopsis (in Ler genetic**

728   **background).** (A) The flower of the wild-type Ler plant. (B-E) The abnormal phenotype of the flowers in

729   *35S::IiSEP4-GFP* transgenic plants. (F) The silique in *35S::IiSEP4-GFP* transgenic plant (left) and wild-type

730   Ler plant (right). Bar = 1 mm.

731

732   **Figure 7 Phenotypic variations of *IiSEP4* transgenic plants of *ap1 cal* double mutant in Ler genetic**

733   **background.** (A-B) The inflorescence of *ap1 cal* double mutant. (C-H) The inflorescence of *IiSEP4* transgenic

734   plants of *ap1 cal* double mutant. C shows an incomplete cauliflower phenotype and petals reappeared. D is an

735   enlarged photo of C, a number of sepals, stamens and pistils can be found in one flower; In comparision with

736   *ap1 cal* double mutant, multiple petals can be produced. E is the photo of the flower in D taken in different

737   direction, arrow shows a chimeric organ composed of petaloid and stamineous tissues, representing fusion of

738   petal and stamen. In F and G, arrows show the chimeric organ composed of petaloid and sepaloid tissues,

739   representing fusion of petal and sepal. G and H are the photos of the flower in F taken in different directions.

740   Bar = 1 mm.

741

742   **Figure 8 Phenotypic observation of the floral organs in woad plants treated with TRV-*IiSEP4*.** (A) Wild-

743   type woad flower. (B-L) Phenotypic variations of the flowers in woad plants infiltrated with TRV-*IiSEP4*. B, a

744   flower constituted by one sepal, one petal, one stamen and one pistil. C and D, anatomy view of the flower in

745   B. E, a flower with three sepals, three petals, 3 stamens and one pistil. F-H, comparison of the size of the floral

746   organs in the flower of E (up) with the floral organs in wild-type flower (down). I, a flower contains four

747   sepals, five petals, five stamens and one pistil. J-H, comparison of the size of the floral organs in the flower of

748   I (up) with the floral organs in wild-type flower (down). Bar = 1 mm.

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Figure 1

Figure 1 Expression pattern of *IiSEP2* in *I. indigotica* analyzed by qRT-PCR.

Error bars represent the standard deviation. Root was used as normalization sample. Multiple testing correction was conducted with SPSS software, different letters indicate significant difference at 5% level.
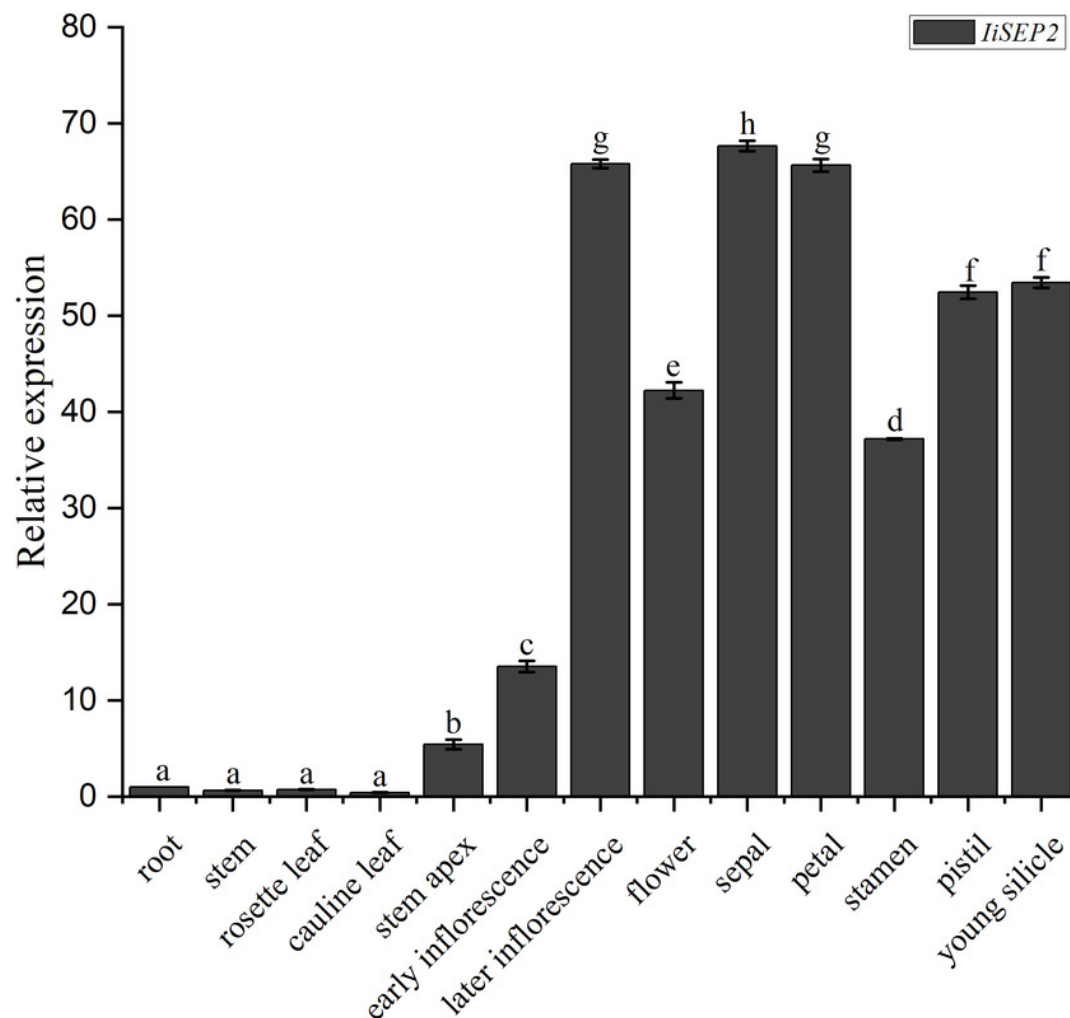
Figure 2

Figure 2 Effects of *IiSEP2* overexpression on aerial architecture of Arabidopsis plants.

(A) Comparison of rosette leaf number produced by *35S::IiSEP2* transgenic and wild-type Col-0 plants before flowering. (B) Comparison of the angle between the cauline leaves and the stems. (C) Comparison of the flower bud number produced by *35S::IiSEP2* transgenic and wild-type Col-0 plants. Values correspond to mean ± standard error (n = 15). Multiple testing correction was conducted with SPSS software, different letters indicate significant difference at 5% level.

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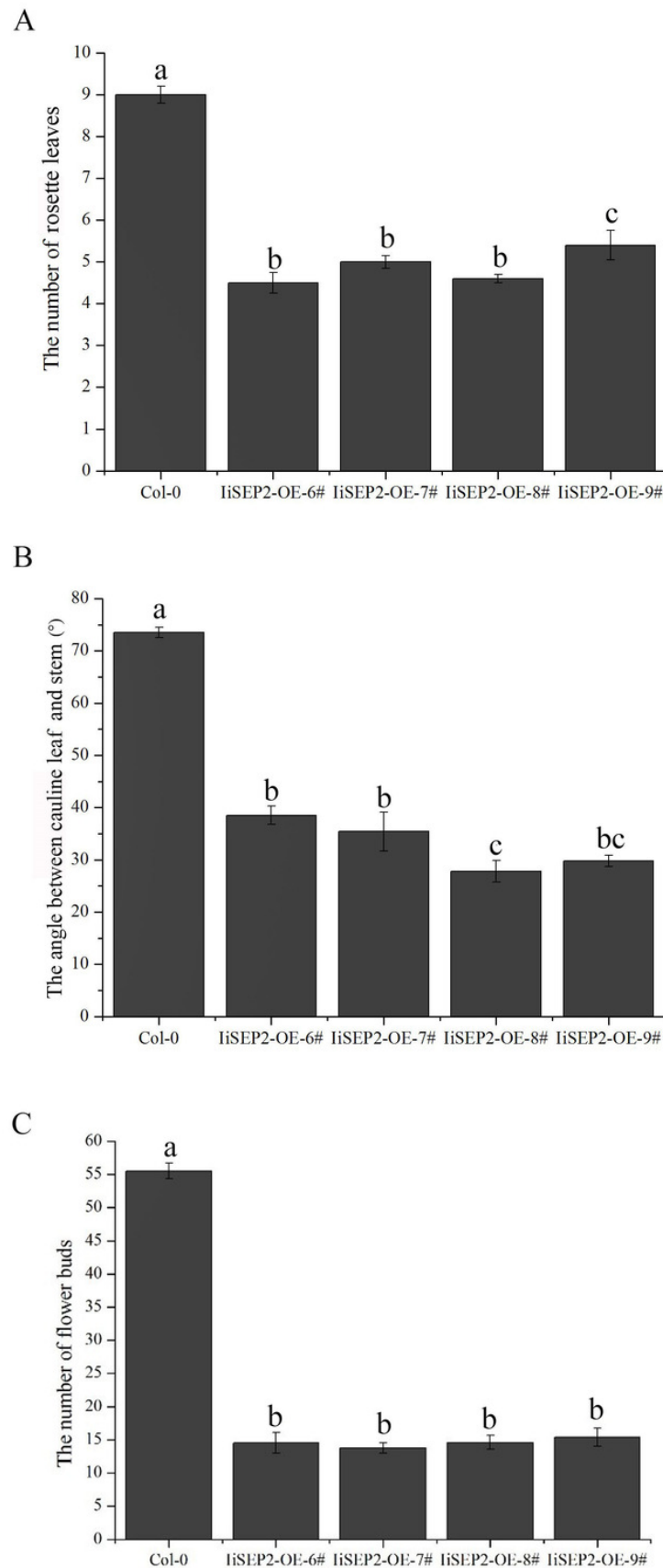
Figure 3

Figure 3 Morphological comparison of the cauline leaves between wild-type Col-0 and *IiSEP2* transgenic plants.

(A) The rosette leaves and cauline leaves of wild-type Col-0 plants, the angles between the oval-shaped cauline leaves and the stems are larger. (B) The upper part of a wild-type Col-0 plant in the flowering stage. (C) *IiSEP2* transgenic Arabidopsis plants. (D) IiSEP2-OE-6#. (E) IiSEP2-OE-7#. (F) IiSEP2-OE-8#. (G) IiSEP2-OE-9#. *IiSEP2* transgenic Arabidopsis plants in F and G show the inward-curling cauline leaves, and the angles between the cauline leaves and the stems are reduced. In A, B and C, scale bars represent 1 cm. In D, E, F and G, scale bars represent 1 mm.
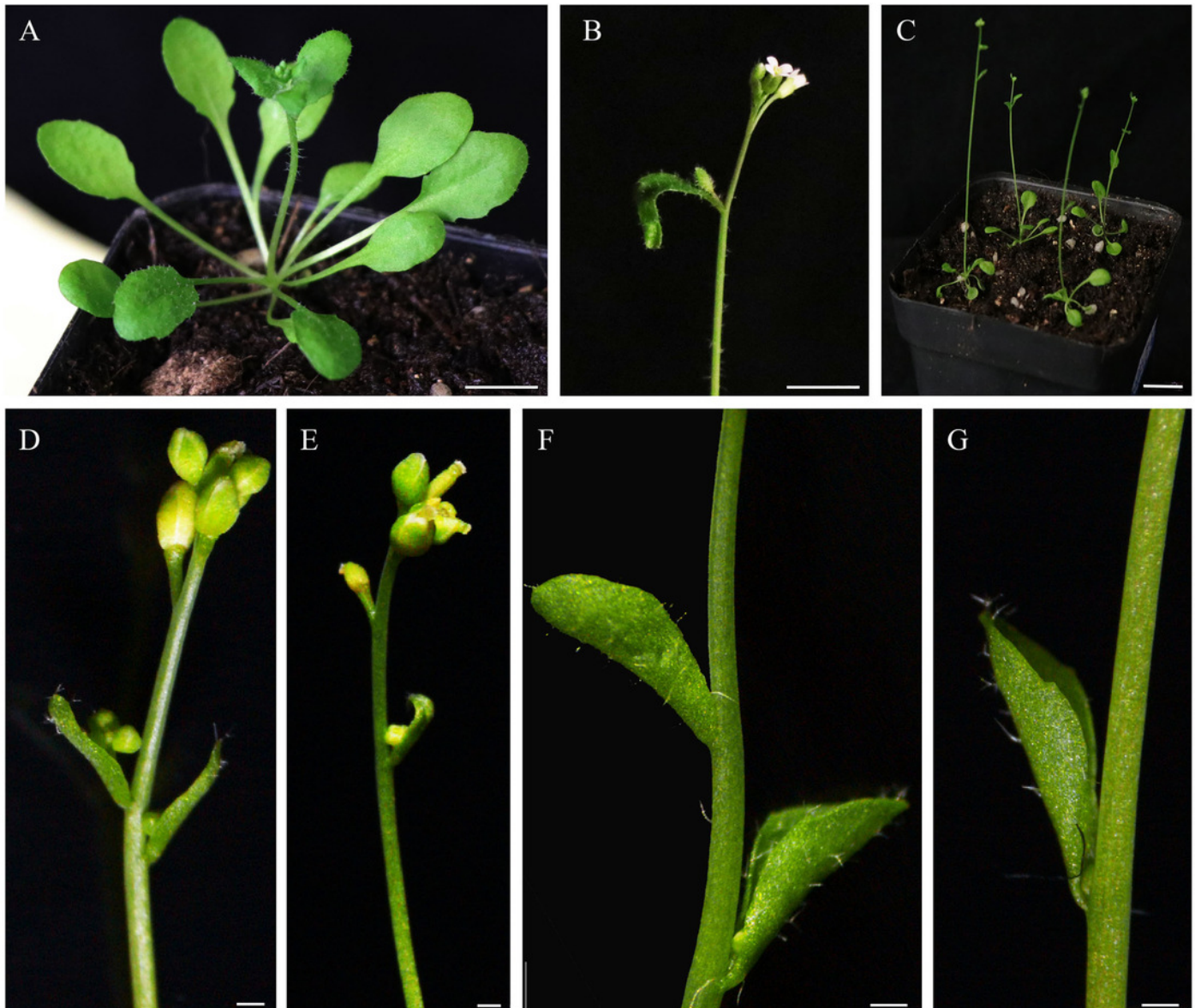
Figure 4

Figure 4 Influence of *IiSEP2* on development of inflorescences.

(A-B) Indefinite inflorescences of wild-type Col-0 plants. The inflorescence meristem could produce new flower buds continuously. (C) Inflorescence of *IiSEP2* overexpressing Arabidopsis plant, only a few flower buds could be observed. (D) Wilted inflorescence with undersized flower buds at early development stage in *IiSEP2* transgenic Arabidopsis plant. (E-F) The inflorescences converted into defective terminal flowers in *IiSEP2* transgenic Arabidopsis plant, arrow indicate stamens. (G) Comparison of the number of stamens per flower. Scale bars represent 1 mm. Multiple testing correction was conducted with SPSS software, different letters indicate significant difference at 5% level.
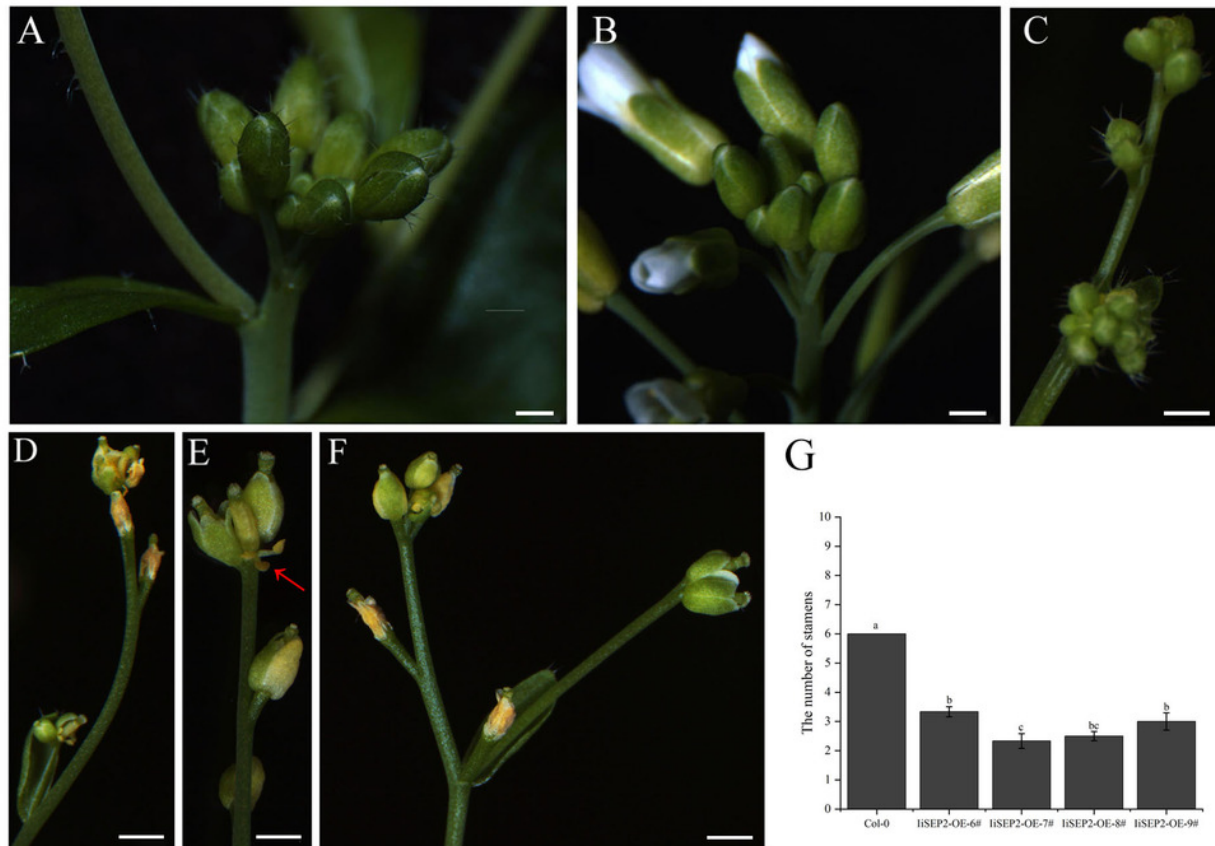
Figure 5

Figure 5 Abnormal phenotypes of various floral organs induced by *IiSEP2* overexpression.

(A-C) The flowers of wild-type Col-0 Arabidopsis plants. The sepals and petals have been removed from Arabidopsis flower in Figure 5C. (D) *IiSEP2* transgenic line 3, red arrow indicate terminal flower containing a secondary flower, blue arrow indicate lateral flower. (E) *IiSEP2* transgenic line 7, the sepals were converted into carpelloid tissues. (F) *IiSEP2* transgenic line 4, red arrow indicate terminal flower containing a secondary flower. (G) *IiSEP2* transgenic line 5, five petals could be observed. (H) *IiSEP2* transgenic line 6, apetalous flower was generated. (I) Terminal flowers and secondary flower in crossing progeny of *IiSEP2* transgenic line 8 and wild-type Col-0. (J) *IiSEP2* transgenic line 8, abnormal flowers were produced. (K) Flower lacking petal in crossing progeny of *IiSEP2* transgenic line 8 and wild-type Col-0 plant. (L) Abnormal floral organs in crossing progeny of *IiSEP2* transgenic line 8 and wild-type Col-0 plant. Scale bars represent 1 mm.

Figure 6

Figure 6 Phenotypic variations of the floral organs in *IiSEP4* transgenic Arabidopsis (in Ler genetic background).

(A) The flower of the wild-type Ler plant. (B-E) The abnormal phenotype of the flowers in *35S::IiSEP4-GFP* transgenic plants. (F) The silique in *35S::IiSEP4-GFP* transgenic plant (left) and wild-type Ler plant (right). Bar = 1 mm.

Figure 7

Figure 7 Phenotypic variations of *liSEP4* transgenic plants of *ap1 cal* double mutant in Ler genetic background.

(A-B) The inflorescence of *ap1 cal* double mutant. (C-H) The inflorescence of *liSEP4* transgenic plants of *ap1 cal* double mutant. C shows an incomplete cauliflower phenotype and petals reappeared. D is an enlarged photo of C, a number of sepals, stamens and pistils can be found in one flower; In comparision with *ap1 cal* double mutant, multiple petals can be produced. E is the photo of the flower in D taken in different direction, arrow shows a chimeric organ composed of petaloid and stamineous tissues, representing fusion of petal and stamen. In F and G, arrows show the chimeric organ composed of petaloid and sepaloid tissues, representing fusion of petal and sepal. G and H are the photos of the flower in F taken in different directions. Bar = 1 mm.
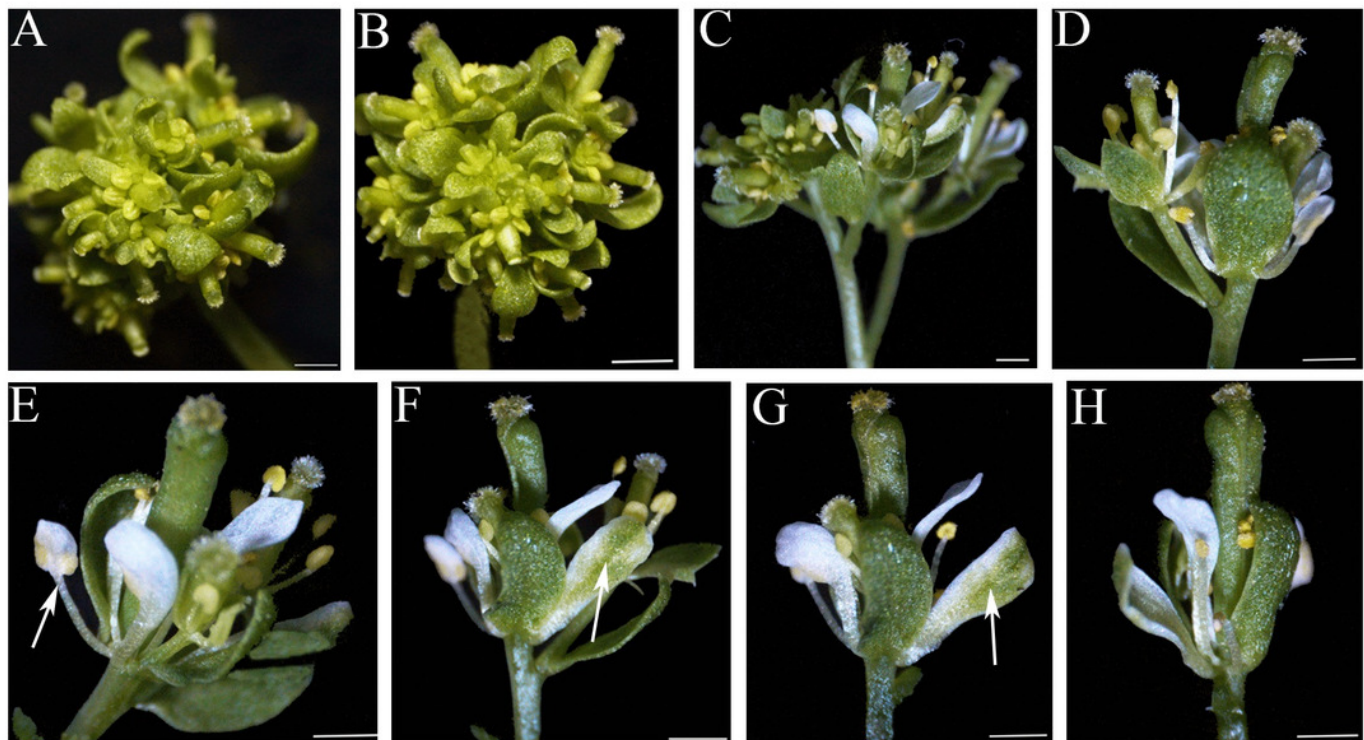
Figure 8

Figure 8 Phenotypic observation of the floral organs in woad plants treated with TRV-*liSEP4*.

(A) Wild-type woad flower. (B-L) Phenotypic variations of the flowers in woad plants infiltrated with TRV-*liSEP4*. B, a flower constituted by one sepal, one petal, one stamen and one pistil. C and D, anatomy view of the flower in B. E, a flower with three sepals, three petals, 3 stamens and one pistil. F-H, comparison of the size of the floral organs in the flower of E (up) with the floral organs in wild-type flower (down). I, a flower contains four sepals, five petals, five stamens and one pistil. J-H, comparison of the size of the floral organs in the flower of I (up) with the floral organs in wild-type flower (down). Bar = 1 mm.