Genetic control of floral zygomorphy in pea (Pisum sativum L.)


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Edited by Enrico Coen, John Innes Centre, Norwich, United Kingdom, and approved May 13, 2008 (received for review April 4, 2008)

Floral zygomorphy (flowers with bilateral symmetry) has multiple origins and typically manifests two kinds of asymmetries, dorsalventral (DV) and organ internal (IN) asymmetries in floral and organ planes, respectively, revealing the underlying key regulators in plant genomes that generate and superimpose various mechanisms to build up complexity and different floral forms during plant development. In this study, we investigate the loci affecting these asymmetries during the development of floral zygomorphy in pea (Pisum sativum L.). Two genes, LOBED STANDARD 1 (LST1) and KEELED WINGS (K), were cloned that encode TCP transcription factors and have divergent functions to constitute the DV asymmetry. A previously undescribed regulator, SYMMETRIC PETALS 1 (SYPI), has been isolated as controlling IN asymmetry. Genetic analysis demonstrates that DV and IN asymmetries could be controlled independently by the two kinds of regulators in pea, and their interactions help to specify the type of zygomorphy. Based on the genetic analysis in pea, we suggest that variation in both the functions and interactions of these regulators could give rise to the wide spectrum of floral symmetries among legume species and other flowering plants.

Flower development in higher plants gives rise to an enormous variation of flower morphology and immense aesthetic diversification in nature. An important aspect for divergent floral developments is the establishment of floral symmetries, where a few distinct basic forms could be distinguished (1, 2): the monosymmetry (zygomorphy, with one symmetric plane), polysymmetry (actinomorphy, with several symmetric planes), and left-right asymmetry (with no symmetric plane). Among these, zygomorphy is considered the more specialized form and has been the most under investigation for its origin and underlying mechanisms.

Fabaceae (legumes) is one of the largest families in angiosperm, with a range of floral symmetric forms, and its success is thought to be coupled with its predominant zygomorphic flowers (3, 4). Most zygomorphic flowers are found in the subfamily Papilionoideae (5, 6), which attracted the attention of researchers since the end of the 18th century (7). Darwin (8) demonstrated the role of this type of zygomorphy in pollination biology, and the special floral shape of papilionoid legumes was an important factor in Mendel’s groundbreaking work on the laws of genetic inheritance in the 1850s. Pea flowers, like most zygomorphic flowers, possess prominent corolla with three petal types, which are arranged along a dorsalventral (DV) axis, and manifest two types of asymmetries: DV asymmetry in the floral plane and organ internal (IN) asymmetry in the floral organ plane (Fig. 1a). It is well documented that DV asymmetry in papilionoid legumes occurs in the floral meristem when the asymmetric development of floral organ primordia occurs (Fig. 2a) (5, 6). However, IN asymmetry is variable among petals: one dorsal petal (the standard) is IN symmetric, and two lateral (the wing) and two ventral petals are IN asymmetric (the two ventral petals are united on the lower edge and form a keel). This raises the question of how key regulators generate DV and IN asymmetries and superimpose them during zygomorphic development.

The conspicuous zygomorphic flower of pea, for which there is a large collection of mutants, makes it a good model for exploring the key regulators to determine floral symmetry. For example, two loci, KEELED WINGS (K) and LOBED STANDARD 1 (LST1), were identified, respectively, in 1919 and 1985 (9, 10). The mutants at the LST1 locus give rise to the abnormal shape in the dorsal petals (Fig. 1c), whereas the k-1 mutant has been used as a morphological marker in genetic analysis for its homeotic transformation phenotype, i.e., the lateral petals mimic the ventral in size, shape, and color (Fig. 1b). K in pea could be an ortholog of KEELED WINGS in Lotus 1 (KEW1), because their mutants share a similar phenotype by bearing ventralized lateral petals (Fig. 1b and f), and both are located in the syntenic regions in the pea and Lotus genomes, respectively (12). However, neither LST1 nor K has been cloned, partly because of the difficulty caused by the large genome size of pea.

It has been shown that zygomorphy has multiple origins and plays a key role in the speciation and diversification in flowering plants (3, 13). Concomitant with their independent origins, variations in petal arrangement manifest in various superimpositions of the DV and IN asymmetries in distinct species. The flower in snapdragon (Antirrhinum majus) of the Asterid family represents another type of zygomorphy: an IN symmetric petal is positioned in the ventral, and two dorsal and two lateral petals with IN asymmetry are in pairs along the DV axis, in contrast to the arrangement in pea flower (12). Thus, the constitution of the two asymmetries may establish the developmental framework for different floral zygomorphies and give a clue as to the divergent actions of the underlying regulators among plant genomes. It has been shown that, in Antirrhimum, two closely related genes, CYCLOIDEA (CYC) and DICHOSTOMA (DICH) (14, 15), are the key regulators that establish DV asymmetry, encode TCP transcription factors, and are expressed in the dorsal region of the floral meristem (16). These two regulators are also responsible for the elaboration of organ IN asymmetry and regulate and/or interact with two distinct MYB proteins, LST1 and BIC, respectively (15). The BIC-LST1 interaction is necessary for the maintenance of DV asymmetry (17).


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0803291105/DCSupplemental.

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DIVARICATA (DIV) and RADIALIS (RAD), respectively, to determine lateral and ventral identities (17–19). Despite the prominent difference in the zygomorphies between pea and Antirrhinum, recent studies in papilionoid legumes show that CYC orthologs play a key role in determining dorsal identity in two legume species, Lotus japonicus and Cadia (12, 20). Nevertheless, the CYC-like TCP genes, like DV regulators in distinct species, could have divergent functions apart from their common one in the control of DV identity: abolishing the activity of DV regulators in Antirrhinum gives rise to a default ventralized floral form, with all petals manifesting bilateral symmetry (the cyc dich flower, ref. 14), demonstrating that both CYC and DICH work together to determine IN asymmetry (15). However, a default ventralized form in papilionoid legumes should have all petals, which mimic the asymmetric shape of the ventral petal in wild type (12), suggesting that the CYC-like TCP genes might not be a prerequisite in the control of IN asymmetry in legumes. Therefore, different types of zygomorphies could be generated either by the divergent functions of the common key regulators and/or by the evolution of other distinct regulators.

In this study, we cloned K and LST1 loci and demonstrated that they are DV regulators in the control of lateral and dorsal identities, respectively, and that they originate from the duplication of an ancestral TCP gene during the speciation of papilionoid legumes. A locus, SYMP (SYMMETRIC PETALS 1), named for its mutant flowers bearing all symmetric petals without normal IN asymmetry, was isolated, whose function is to establish IN asymmetry of petals. In the genetic analysis, three default floral forms with absent DV or and IN asymmetries were identified in pea, demonstrating that DV and IN asymmetries can be independently controlled by distinct regulators, and that their interaction is important for the zygomorphic development. Taking these together, we propose that variation in both the entities and interactions of these regulators in the control of DV and IN asymmetries could give rise to the wide spectrum of floral symmetries among flowering plants.

Results

k and lst1 Display Deficiency in the Development of DV Asymmetry. The floral morphogenesis of pea mutants at two loci, K and LST1, was characterized by using SEM in the eight developmental stages according to previous studies (20, 21). k-1, k-2, and k-3 display the same phenotype, and no other detectable phenotype was observed apart from the ventralized petals at the lateral position (Fig. 1b). In lst1-1, lst1-2, and lst1-3, flowers bear abnormal standard petals with lobes whose sizes could be sensitive to growth conditions (Fig. 1c). The visible differences between wild type and k or lst1 commence at the same stage (stage 6), when the vascular tissues are developed in petals, and the asymmetric shape of both lateral and ventral petals becomes obvious (Fig. 2d). At this stage, the lateral petals in k mimic the ventral in shape, whereas the dorsal petals in lst1 are retarded in comparison with their wild-type counterparts (Fig. 2d). In mature flowers, alteration of lateral and dorsal identities in both k and lst1 is evident, as judged by the abnormal appearance of the lateralized and ventralized epidermal cell types of the lobed dorsal petals of lst1 and the lateral petals of k, respectively (Fig. 2c).

When lst1-1 was introduced into k-1 backgrounds, a high percentage of flowers displayed variable organ numbers in the dorsal region (Fig. 2b), with absent dorsal petal or more sepals being the most common (data not shown). In a comparison, ≈5% lst1-1 flowers displayed a similar phenotype. SEM analysis showed that primordium initiation was affected in the dorsal region (Fig. 2a), and the ventralized epidermal cell could be found in all petals (Fig. 2c). However, the asymmetric development of the early floral meristem was not altered in the double mutant (Fig. 2c). In the double mutants, not only were the numbers of stamen and sepal at the dorsal region affected, but also the length of the dorsal stamen could be longer than that of wild type (data not shown). Although most lateral petals displayed perfect ventralized forms, occasionally some lateral petals with conspicuous shape could be found (Fig. 1d). In Lotus, ≈3–5% flowers (often higher under stressed conditions) bear repetitive morphs of the lateral petal, rather than the most mirror-image forms in kew1, the putative ortholog of k (Fig. 1g), which manifest an abnormal left–right asymmetry. However, the majority of dorsal petals of k lst1 in pea failed to fully expand and therefore were smaller in size, displaying IN asymmetry to varying degrees [Fig. 1d; supporting information (SI) Fig. S1]. Nevertheless, a small portion of flowers bore the dorsal petals, which mimicked the shape of the ventral petal (Fig. 1d), representing a default state without DV asymmetry. Thus, during
zygomorphic development, K and LST1 play a key role in establishing lateral and dorsal identities, respectively, and their interaction for the development of dorsal organs is revealed in the double mutant.

**Both K and LST1 Encode CYC-Like TCP Proteins.** The comparative genomics approach was conducted to clone K and LST1 in pea. Previous genetic analysis has mapped the K locus in linkage group II (23). In a parallel mapping experiment in *Lotus*, kewl was located in a 295-kb region, where a complete contig and DNA sequence were subsequently obtained (Fig. 3a). However, no recombination haplotype was found in the large mapping population within the 200-kb region containing KEWL, and sequence analysis revealed a candidate gene within the contig, which is predicted to encode a CYC-like TCP protein with 370 aa and has been reported as *LjCYC3* (12). However, there is no sequence alteration in a 4-kb region of *kewl* containing *LjCYC3*, but whose expression level was found to be decreased (data not shown). Thus, three CYC homologs, designated *PsCYC1*, *PsCYC2*, and *PsCYC3*, were cloned in pea (GenBank accession nos. EU574913, EU574914, and EU574915). Phylogenetic analysis indicated *PsCYC3* is the ortholog of *LjCYC3* (Fig. 3b). When the gene structure of *PsCYC3* was analyzed, deletions were found in both *k* and *k-3* (Fig. 3e), and a single base deletion was detected at position 350 of *PsCYC3* ORF in *k-2*, which should cause a frame-shift and disrupt the conserved TCP domain (Fig. S2a). However, *LST1* was mapped and located in the linkage group VI of pea between two SSR markers, AD51 and AA200 (24). We noticed that *lst1* exhibits a similar phenotype to the *LjCYC2* mutant, *squared standard 1* (*squ1*) in *Lotus* (12); both mutants have the dorsal petal phenotype and can interact with *k* or *kewl*, respectively, by giving rise to the ventralized character in all petals. Thus, the ortholog of *LjCYC2*, *PsCYC2*, was subject to detailed analysis, and an STS marker for *PsCYC2* was found to cosegregate with *lst1* in our mapping population (data not shown). When the sequence of *PsCYC2* was analyzed, a single base substitution (C-437-T) was found in *lst1-1*, which would cause an amino acid substitution (S-146-L) in the conserved TCP domain (Fig. S2a), and deletion of *PsCYC2* was found in *lst1-3* (data not shown). Thus, the conserved site of 146S in the TCP domain could be important for protein function. To verify *PsCYC*’s function, a virus-inducible gene silencing assay, based on *Pea early browning* (PEVB-VIGS), was conducted (25). VIGS-*PsCYC* silencing constructs containing different fragments of *PsCYC* genes (Fig. S2a) were applied to wild-type and different mutant plants, respectively, and semiquantitative RT-PCR was conducted to confirm the reduced expression of target genes (data not shown). In VIGS-*PsCYC3* silenced wild-type plants, ≈36.5% and 57.1% of flowers displayed partial and complete conversion of wings into the shape of keels, respectively (Fig. 3h and i). In VIGS-*PsCYC2* silenced plants, ≈30% of flowers would fail to differentiate in the dorsal region.
expressed in standards, whereas the transcripts of K can be found in both dorsal and lateral petals (Fig. 3d). As expected, no transcript of K and LST1 was detected in the deletion mutants of k or lst1, respectively; however, the transcription levels of K and LST1 bearing the point mutation were not down-regulated (Fig. 3e and f). In RNA in situ hybridization experiments, it was found that both PsCYC1 and LST1 are expressed first in the dorsal region of the floral meristem before floral organ initiation and then inside dorsal petals (Fig. S2 b–e). We failed to detect the transcripts of K by RNA in situ hybridization, presumably because of its low expression level. CYC homologs in legumes have been characterized into two major groups, LEGYC group I and II (26, 27), and phylogenetic analysis placed PsCYC1 and LST1 into LEGYC group I and K into group II (Fig. 3b). Thus, the overlapped expression domains and different expression levels are consistent with the functional divergence and phylogenetic relations of these DV regulators.

**Mutant syp1 Gives Rise to a Defect in IN Asymmetry in Petals.** The default state without DV asymmetry in the k lst1, in which all petals are ventralized and manifest IN asymmetry (Fig. 1d), suggests other regulators exist, which should control IN asymmetry independent of K/LST1 function in pea. To target the hypothetical IN regulators, we conducted the screen for mutants bearing petals with altered IN asymmetry. In two large-scale mutagenesis experiments, we identified the SYP1 locus with two mutated alleles (Fig. 4 b and c). In syp1-1, nearly all petals are bilaterally symmetrical but maintain their DV identities (Fig. 4b), and the most conspicuous characteristic is that the symmetric ventral petals possess a keel structure, in contrast to the wild-type flower, whose two ventral petals form a keel (Fig. 4 a and b). However, syp1-2, presumably a weaker allele, has a highly variable effect on IN asymmetry of the lateral but not the ventral petals (Fig. 4c). The SYP1 locus has been located in linkage group II of pea, and its syntenic region is anchored in both Lotus and Medicago genomes, to conduct the cloning work (data not shown).

In syp1-1, approximately one-third of the flowers have increased organ numbers in the ventral region (Fig. 2b), where abnormal primordium initiation was found during early development of the floral meristem (Fig. 2a). In syp1-1, the abnormal bilaterally symmetric shapes of the lateral and ventral petals can be observed at petal developmental stage 6, when the malfunction of petal development also occurs in k and lst1 (Fig. 2d). However, the whole floral meristem became more symmetrical than in wild type from the beginning of floral organ primordium development (Fig. 2a). When the epidermal cells of the syp1-1 petals were analyzed, both cell size and type were found to be the same as their wild-type counterparts (Fig. 2c). Therefore, the syp1-1 flower represents a default state without IN asymmetry in all petals, in contrast to the one without DV asymmetry in the k lst1.

**SYP1 and K/LST1 Are Antagonistic at the Early Stage of Floral Development.** To investigate how DV and IN regulators interact with each other during zygomorphic development, syp1-1 was introduced into k, lst1, and k lst1 genetic backgrounds, respectively. The lst1 syp1-1 double mutant displayed an additive phenotype: lobed standards and petals in the lateral and ventral positions without IN asymmetry (Fig. 4d). However, k syp1-1 flowers possess abnormal standards with an altered shape apart from the expected keels in both lateral and ventral regions (Fig. 4e), revealing a hidden function of SYP1 during the development of the dorsal petal. In the k lst1 syp1-1 triple mutant, the flowers display a radial symmetry (Fig. 4f), and all petals possess ventralized identity with a keel structure. Thus, a default form without DV and IN asymmetries was identified.

In the triple mutant, no other detectable phenotype was found.
apart from floral symmetry. However, there was little variation in floral organ numbers, in contrast to a high portion of k lst1 flowers with variable organ numbers, in the dorsal region, whereas in the ventral region is notably reduced in comparison with the one in syp1-1, indicating that the malfunction of organ primordium initiation in k/lst1 and syp1-1 is completely or partially suppressed in the triple mutant. The expression patterns of K, LST1, and PsCYC1 were analyzed in syp1-1 by RT-PCR, and no detectable alteration was found (data not shown). Thus, the antagonistic interaction of DV and IN regulators is found at the early stage during floral development when floral organ primordia initiate.

Discussion

Zygomorphic development, like other pattern formations in plants and animals, involves the establishment of different body planes with distinct developmental axes, where various asymmetries are generated and superimposed under the control of different regulators. The pea possesses a conspicuous zygomatic flower with an abundant mutant collection and provides an ideal experimental system to analyze the key regulators in control of floral symmetry. However, its complex large genome (5,000 Mb, 10× the size of either Lotus or Medicago, with >90% repetitive sequence) had obscured progress in molecular analysis (28). By adopting a comparative genomics approach and other molecular genetic tools, we successfully cloned two genes in pea, K and LST1, demonstrating that the limited sequence information, large size, and complex genome are no longer impassable barriers for the molecular study of pea.

In this work, the functions of K and LST1 and PsCYC1 are characterized, which comprise a small CYC-like TCP gene cluster and originate from two duplication events of an ancient TCP gene (26, 27). Because the deletion alleles at both K and LST1 loci show no other detectable malfunction apart from the floral symmetry, it is evident that these TCP genes have been destined for the control of zygomatic development. Their expression patterns were found to be overlapped but differ spatially and quantitatively, which is consistent with the divergent functions of LST1 and K to determine the dorsal and lateral identities of petals, respectively. It is also consistent with previous reports that alteration of expression pattern and level of TB1, a CYC-like TCP gene in maize, could lead to novel morphogenesis (29, 30). In a comparison, CYC and its close duplicate DICHE in Antirrhinum determine DV asymmetry and regulate two distinct small MYB proteins, which are involved in the control of lateral and ventral identities (14, 15, 17–19). These indicate that increasing the copy number of TCP genes should be a key step for elaborating their roles in the control of floral DV asymmetry, and then different copies acquire their divergent functions as different DV regulators during zygomatic evolution. Thus, the independent duplication events giving rise to divergent DV regulators in different species could account for the molecular basis on the development of different types of floral zygomorphies.

The conspicuously different types of zygomatic flowers in Antirrhinum and pea demonstrate how different origins of regulators could have resulted in distinct flower forms. When DV regulator function is abolished, the default state without DV asymmetry in both species appears to be a ventralized form, and all petals acquire ventral identity (Fig. 4g, h). However, ventral identities in the two types of zygomatic flowers have distinct properties: the ventral petal in Antirrhinum displays a bilaterally symmetric shape, whereas that in pea possesses IN asymmetry (Fig. 4g). An IN regulator, SYP1, should be responsible for IN asymmetry of petals when DV regulators are mutated in pea. In syp1-1, where IN asymmetry is abolished, all petals are bilaterally symmetric, but their DV identities are maintained (Fig. 4b), revealing another default state without IN asymmetry. Therefore, DV and IN regulators can separately generate different asymmetries in pea (Fig. 4e). In contrast to Antirrhinum, two DV regulators, CYC and DICHE, interplay to modify IN asymmetry of the dorsal petal. Thus, our findings offer evidence to support the notion that organ IN asymmetry also has multiple origins. It is likely that the independent genetic control of DV and IN asymmetry may not be unique in pea, and two relevant pathways could have evolved independently in other species as well. Therefore, recruiting only one or both two kinds of regulators in the control of DV and IN, respectively, could give rise to various floral forms (Fig. 4g). Furthermore, variation or modification of the interaction between DV and IN regulators could generate floral forms with different symmetries, such as the left-right asymmetric flowers in k/lst1 in pea or kew1 in Lotus (Fig. 1d and g) and the radial symmetrical flower in Cadia, where the expanding expression pattern of LegCYC was found (20).

The existence of the two reciprocal default forms in pea with only one DV or IN asymmetry raises the question of how the two asymmetries are superimposed and coordinated during zygomatic development. In this study, the dorsal petal morphology of pea provides a unique example to examine the interaction between IN and DV factors, because it possesses bilateral symmetry. It has been shown that ectopic expression or the altered expression domain of CYC-like TCP genes in papilionoid legumes can suppress the manifestation of IN asymmetry in lateral and ventral petals by altering their identity (12, 20). Consistently, the bilaterally symmetric shape of dorsal petal in pea can become asymmetric when both K and LST1 are mutated (Fig. 1d), indicating that SYPL action is suppressed in the dorsal petal, where DV regulators are expressed. Other data also support this interaction: The abnormality of dorsal primordium initiation in the k lst1 is suppressed by introducing syp1-1 (Fig. 2a), and the morphology of the dorsal petal is altered in the k syp1, whereas its symmetric shape is otherwise maintained (Fig. 4e). The interaction is not limited to the dorsal petal. For example, in syp1-1, floral organ initiation in the ventral region is affected and can be partially suppressed in the k lst1 syp1 triple mutants, suggesting that both DV and IN regulators participate in organ initiation in the two polar regions of the floral meristem. Previous studies show that a TCP protein in Arabidopsis should be involved in the regulation of cell division (31). It is most likely that both DV and IN regulators could participate in similar biological function to regulate cell division and differentiation in floral meristem and floral organ primordia. Thus, both the divergent functions and the delicate interaction between the two kinds of regulators are essential to regulate zygomatic developmental in pea (Fig. 4h).

Using examples from a range of pea floral mutants (Fig. 4g), we suggest that both DV and IN regulators could have arisen through independent evolutionary routes in Papilionoideae and even in other species. Consequently, possession of only one or two types of regulators involved in the control of floral symmetry and the variation or modification of the interaction between them have the potential to generate the wide spectrum of floral forms with varied symmetries found in Fabaceae and among other flowering plants as well.

Materials and Methods

Plant Material and Growth Conditions. All pea lines used in this study were obtained from the John Innes Pismum Germplasm Collection, except syp1-1, k-2 arose by x-ray mutagenesis, and k-3 was derived from fast neutron mutagenesis in J116 and J1226, respectively. lst1-2, lst1-3, and syp1-2 were obtained by fast neutron mutagenesis in J2922. syp1-1 was identified from fast neutron mutagenesis in Térese. Alleles were confirmed by crosses between k-1 and k-2, k-1 and k-3, lst1-1 and lst1-2, lst1-1 and lst3-1, and syp1-1 and syp1-2, respectively. All plants were grown at 18–20°C with a 16-h light/8-h dark photoperiod at 150 μE⋅m−2⋅s−1.
Microscopy. Nonradioactive in situ hybridization was performed essentially as described (32). SEM for mature petals was performed on plastic replicas as described (33). SEM for floral buds was prepared as described (34). Samples were examined in JEOL JSM-6360LV (JEOL).

Gene Cloning and RT-PCR. Primer sets used for amplifying PsCYC1 (SL0842/G3848), LST7 (SL0773/SL0970), and K (SL1254/SL1103) genes were designed by sequence analysis of ESTs from Medicago truncatula and CYC homologs in Lotus. Homologous alignments were performed by using the ClustalX program (version 1.83), and phylogenetic trees were computed by using the Phylip program (version 3.6). Primer pairs SL1098/SL1099, SL1100/SL1101, and SL1102/SL1103 were used for amplification of the PsCYC7, LST7, and K transcripts, respectively. Histone H4 (GenBank accession no. U10042) was amplified with primers SL1815/SL0363 as an internal control. For in situ probes, PsCYC1 and LST7 transcripts were confirmed with primer sets SL0932/SL0933 and SL0868/SL0970, respectively, from cDNA fragments. Semiquantitative RT-PCR was performed as described (12). Genomic DNA was digested with HindIII before the genomic Southern blot analysis.

Mapping and Co-segregation Analysis. kewl was mapped as described (12). Two SSR markers, ADS1 and AA200 (24), were used to locate LST1 in linkage group VI of pea. A STS marker (LJSR1084 and LJSR1085) for PsCYC2 was used to conduct the cosegregation test for lst1 in a lst1− × JI992 population (n = 280). The cosegregation of PsCYC3 with K was performed in a 140 k− × JI992 population by using primers SL1102/SL1103.

Virus-Induced Gene Silencing (VIGS) Assay. The VIGS assay in pea was carried out as described (25). The fragments being used for the constructs, VIGS-PsCYC1, -2, and -3, are marked in Fig. S2A. Fifteen 2-week-old plants for each construct were agroinoculated and repeated three times independently.

ACKNOWLEDGMENTS. We thank Dr. Ida Elisabeth Johansen (Danish Institute of Agricultural Sciences, Frederiksberg, Denmark) for providing the pCAPE1, pCAPE2-PDS, and pCAPE2-GFP constructs for VIGS. We acknowledge H. Xia, H. Yin, S. Hao, Z. Xu, J. Yan, and C. Li for critical comments on the manuscript. We also thank Z. Xu, J. Li, Y. Xue, G. Wang, Y. Tian, H. Lin, L. Zhuang, and Y. Liu for encouragement and support for this experiment. This work was supported by the National High Technology Research and Development Program of China (Grant nos. 2006AA10A110 and 2007AA102113), the National Nature Science Foundation of China (Grant nos. 30430330 and 30528016), and FP6-2002-FOOD-1-506223 (Gran Legumes) from the European Commission.

Fig. S1. Dorsal petals in k lst1 double mutant. In k-lst1−1 double mutant, the dorsal petals displayed asymmetrical shape in various extents. White triangle: the most common ones. All petals are without flattening.
Fig. S2. (a) Sequence alignment of PsCYC homologs. Black line marks the conserved TCP domain. Dashed line indicates R domain. Red panes indicate the regions where the relevant DNA sequence was used to construct VIGS-PsCYCs. Red pentacle: mutation site in k-2 alleles. Red triangle: mutation site in lst1–1 alleles. (b–i) Expression patterns of PsCYC1 and LST1. These two genes have overlapped expression pattern with slightly difference. PsCYC1 is firstly detected in the regions between I1 and I2 (b), whereas LST1 dispersed in I2 (f). With floral development, both genes were expressed in the dorsal region of flower primordial (c–d and g–h) and finally restricted to the dorsal petals (e and i). I1, primary inflorescence; I2, secondary inflorescence; F, floral meristem; dSe, dorsal sepal; dPe, dorsal petal. (Scale bar, 50 μm.) Primer sequences: LjSSR1084: 5′-ACAATGCATAATAGGTTAGTG-3′ LjSSR1085: 5′-AGTTGGCTTTAAAATCTTACATCTT-3′ G3848: 5′-CCACACTTTAAATATTGGTC-3′ SL0363: 5′-GCCAAATCGTAAAGAGTTC-3′ SL0770: 5′-AACATACTAATGTAGACGTGGATTG-3′ SL1254: 5′-TTTACACACCCGAGATCGAGCGAAGAAGAT-3′ SL1098: 5′-TGGTGCATTCTTCCCATGATTTTC-3′ SL1102: 5′-CAAGATGTGTAGCAGCGGAAGAAT-3′ SL1103: 5′-CAAGACTTTCCCATTTTCAACC-3′ SL1254: 5′-ATATTCGCTCGCTCCAGATTTCTC-3′ SL1815: 5′-AAGAACACTACCAAAACCATAC-3′.