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Mutation in *LSU4* Gene Affects Flower Development in *Arabidopsis thaliana*

Yu. A. Myakushina[a], E. L. Milyaeva[b], G. A. Romanov[b], and V. Yu. Nikiforova[a, b]

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Flower development is determined by expression of a large number of genes. According to the commonly accepted ABCE model [1–3], genes regulating the development of flower organs in *Arabidopsis thaliana* are divided into several classes—A, B, C, and E. The development of sepals is regulated by class A genes *APETALA1* (*AP1*) and *APETALA2* (*AP2*). Their combined expression with class B genes *APETALA3* (*AP3*) and *PISTILLATA* (*PI*) leads to the formation of sepals. The development of stamens is regulated by simultaneous function of class B and C genes. The activity of only class C genes (*AGAMOUS* (*AG*)) is required for the initiation of carpel primordia. Class E genes (*SEPALLATA 1–4*) together with genes of classes A, B, and C are involved in the development of petals, stamens, and carpels. All genes of the ABCE model encode the proteins that are transcription factors.

Knockout mutations of genes of the ABCE model cause transformations of flower organs. For instance, mutation in the *AP1* gene results in the absence of petals and leads to the formation of leaflike sepals, with secondary flowers developing in their axils. As a result of mutation in the *AP2* gene, sepals are replaced by carpels and petals are replaced by stamens. Mutations in *AP3* and *PI* genes are manifested in the transformation of stamens into carpels and petals into sepals. A characteristic trait of mutation in the *AG* gene is multiple initiation of secondary flower primordia in the central part of the flower. Mutations in certain genes of the *SEPALLATA* family cause no visible changes in flower development. However, flowers of the triple mutant (*sep1 sep2 sep3*) consist of only sepals, whereas mutations in four genes (*sep1 sep2 sep3 sep4*) results in the transformation of all flower organs into leaflike structures [4, 5].

The *LEAFY* (*LFY*) gene plays a key role in the regulation of the function of genes involved in the formation of flower organs [6, 7]. This gene is involved in the initiation of *AP1* gene expression [8]; together with the *UNUSUAL FLORAL ORGANS* (*UFO*) gene, its stimulates the *AP3* gene functioning [9]; and, together with the *WUSCHEL* (*WUS*) gene, it controls the *AG* gene transcription [10].

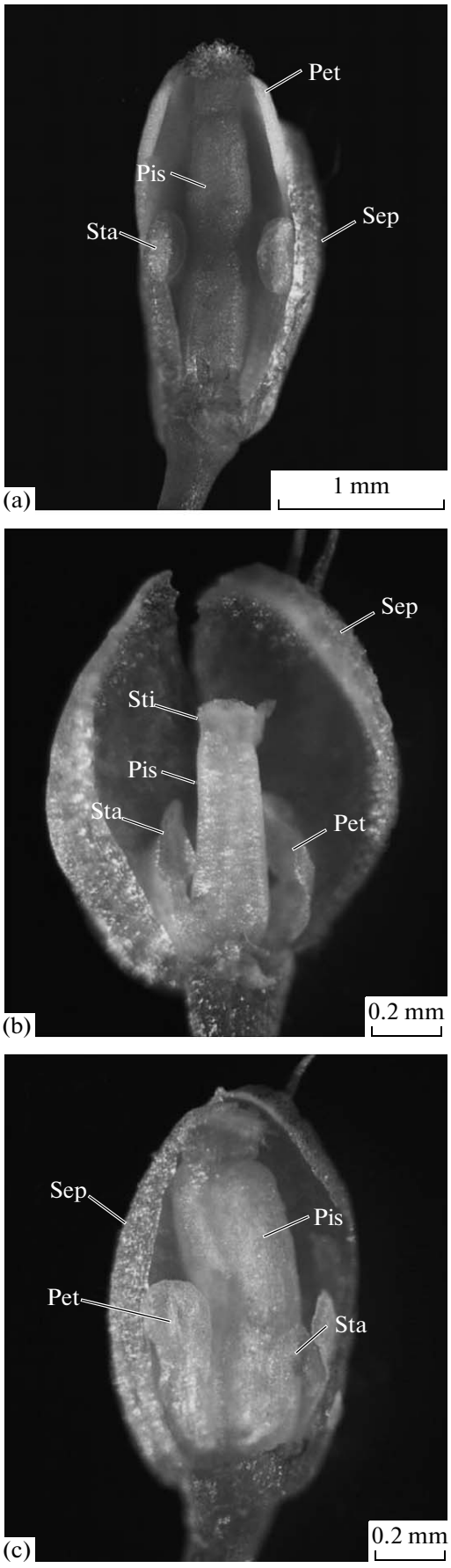
While studying the phenotype of an as yet unstudied insertion mutant *lsu4* of *A. thaliana* with a suppressed expression of the *LSU4* gene (At5g24655), we discovered disturbances in flower development. *LSU4*, a small gene 525 bp long, encodes a protein with an unknown function. On the basis of nucleotide sequence similarity, *LSU4* is classified into the *RESPONSE TO LOW SULFUR* (*LSU*) gene family, some members of which enhance expression in response to sulfur deficiency in the environment.

The goal of this work was to establish the function of the *LSU4* gene by studying the development of flower organs and the function of the ABCE model genes in the *lsu4* insertion mutant in comparison to the wild-type *A. thaliana* plants. This is the first study to demonstrate the pleiotropic effect of the *LSU4* gene on the flower and inflorescence formation, which strongly depended on the photoperiod and was phenotypically expressed under short- but not long-day conditions.

The insertion mutant *lsu4* (*A. thaliana* ecotype Columbia-0) seeds were obtained from the Nottingham *Arabidopsis* Stock Center. The mutation was induced by the integration of plasmid DNA into the exon part of the *LSU4* gene (SALK_069114). To eliminate possible insertions in other parts of the genome, we performed two reciprocal crosses of the mutant plants with the wild-type plants of the same ecotype. For further study, we selected the lines homozygous for the mutant *lsu4* gene using PCR with three primers performed by the standard technique. The plants were grown in soil at 22°C under long-day (16 h/8 h

[a] *Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, Potsdam-Golm, 14476 Germany*

[b] *Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, ul. Botanicheskaya 35, Moscow, 127276 Russia*



light/dark cycle) or short-day (8 h/16 h light/dark cycle) conditions. Plants were illuminated with fluorescent lamps at a light intensity of 120 μmol m^{-2} s^{-1}. Flower morphology was studied under a Leica MZ12.5 binocular stereomicroscope (Germany). Total RNA was isolated from plants at the flowering stage using the Trizol reagent kit (Invitrogen, United States) according to the protocol provided by the manufacturer. cDNA was synthesized using the SuperScript III Reverse Transcriptase reagent kit (Invitrogen, United States) as recommended by the manufacturer. Quantitative real-time PCR was performed using the SYBR Green PCR Master Mix kit (Applied Biosystems). The content of individual mRNAs was normalized relative to the *UBIQUITIN 10* (*UBQ10*) gene transcription level.

The study of growth and development of plants revealed no differences between the *lsu4* mutants and wild-type plants grown under the long-day conditions. However, the mutant plants grown under the short-day conditions started flowering 37 days later than the wild-type plants. Approximately one-third of flowers in the mutant plants, especially those located along the central axis of the inflorescence, had developmental disturbances. In contrast to the flowers of the wild-type plants (Fig. 1a), the flowers of the mutant plants formed in the first flowering phase did not open and remained bud-like (Figs. 1b, 1c). The primary flowers had normal sepals but reduced undeveloped petals, stamens, and pistil (Fig. 1b). Such unopened flowers formed no siliques and later dropped. The flowers of mutant plants formed later, along with normal sepals but undeveloped petals and stamens, had also a bulky pistil occupying nearly the entire internal space (Fig. 1c). Such aberrant flowers formed siliques but did not form viable seeds. At the same time, the flowers formed at later flowering stage developed normally.

The study of expression of the main flower meristem identity genes revealed significant changes in their function in the insertion mutant compared to the wild-type plant. In particular, the level of transcription of *LFY, AP1, AP3, PI*, and *SEP3* genes decreased nearly twice. On the other hand, as can be seen in Fig. 2, the expression of *AP2, AG*, and *SEP2* genes significantly increased (9.6, 7.5, and 3.6 times, respectively). These changes in gene expression were observed in the plants grown under short-day, but not long-day, conditions. Note that the *LSU4* gene expres-

Fig. 1. Flowers of (a) wild-type and (b, c) *lsu4* mutant *A. thaliana* plants. Part of sepals, petals, and stamens was removed. (a) Sepals (Sep), petals (Pet), stamens (Sta), and pistil (Pis) of a normal flower of the wild-type plant. (b) One of the primary flowers of the *lsu4* mutant formed at the initial flowering stage. Reduced petals, stamens, pistil, and stigma (Sti) can be seen. (c) Unopened flower of the *lsu4* mutant developed after formation of several primary flowers. Reduced petals and stamens and a bulky pistil are seen.

sion detected in the mutants decreased by more than an order of magnitude under all illumination conditions. Therefore, it can be assumed that the *LSU4* gene knockout changed the function of flower meristem identity genes, leading to disturbances in the development of flower organs and seeds.

Similarly to the *lfy* mutants described in [7, 11], the most pronounced disturbances in the development of flowers of the plants mutant for the *LSU4* gene were observed in the lower part of the inflorescence, which may be related to the suppression of the *LFY* gene expression and a decrease in the level of transcription of *AP1, AP3*, and *PI* genes.

The changes in the level of transcription of ABCE model genes, shown in Fig. 2, allowed us to explain the discovered disturbances in the development of flower organs as follows. (1) The normal sepals in the flowers of *lsu4* mutants continued to form owing to the high level of *AP2* transcription. (2) A decrease in the expression of class A and B genes (*AP1, AP3*, and *PI*) caused formation of abnormal petals. (3) A decrease in the transcription level of class B genes (*AP3* and *PI*) resulted in the formation of aberrant stamens. (4) Apparently, the suppression of *LFY* expression caused a delayed activation of class C gene (*AG*), as a result of which the primary flowers had an undeveloped pistil. (5) A decrease in the *SEP3* gene expression had a detrimental effect on the development of petals, stamens, and carpels. (6) An increase in the *SEP2* gene transcription, apparently, compensated for the insufficient expression of the *SEP3* gene. (7) The unopened flowers of the *lsu4* mutant, formed at later flowering stages, formed a bulky pistil owing to suppression of the *PI* gene expression and activation of the *AG* gene, as reported in [12].

Thus, in this study we have established that mutation in the *LSU4* gene caused a delayed flowering and various disturbances in the formation of flower organs in *A. thaliana* plants grown under short-day conditions. Quantitative real-time PCR data demonstrated significant changes in the expression of the *LFY* gene and ABCE model genes.

The *LSU4* gene is homologous to other genes of the *LSU* family. One of its homologues, *LSU1* (At3g49580), is characterized by the central position with a large number of bonds in the correlation network of sulfur deficiency-response genes [13]. Transcriptional data deposited in the Genevestigator repositorium [14] show that the *LSU4* gene expression increased two to three times under conditions of deficiency of P, NO_3, K, and Fe ions in the ambient medium, despite the fact that, in a sulfur-free medium, the activity of this gene, unlike the *LSU1* gene, does not change. Possibly, the *LSU4* gene encodes a stress protein and regulates plant development under nutrient deficiency conditions. In addition, the *LSU4* gene expression increases during flowering and fruit formation (i.e., the processes that begin earlier under nutrient deficiency conditions). Our data are suggestive of the involvement of the *LSU4* gene in the regulation of function of genes that control the development of flower organs in *A. thaliana*. It cannot be ruled out that this involvement is associated with high demands of flowering shoots for nutrients, which is especially pronounced under short-day and/or nutrient deficiency conditions.

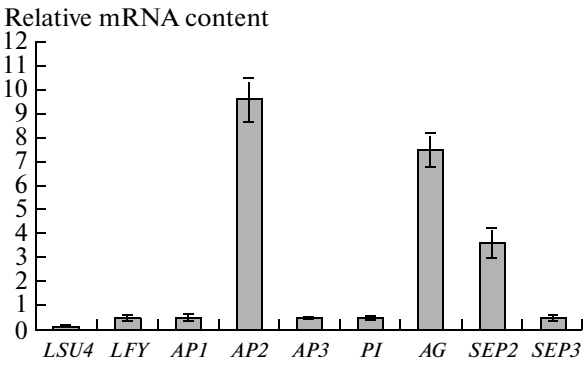


Fig. 2. Effect of *lsu4* mutation on the expression of the genes regulating *A. thaliana* flower development. The ordinate axis shows the content of mRNA of *LSU4* and *LFY* genes and *ABCE* model genes in the *lsu4* mutant compared to the content of mRNA of the same genes of the control plants grown under short-day conditions. The experiment was performed in triplicate; data are expressed as the mean value and the standard deviation.

Thus, we discovered a new gene that is important for coordinated function of regulatory genes responsible for flower and inflorescence development in *A. thaliana* plants grown under short-day conditions. Further studies, including the studies of plants of other species, will show whether or not the function of this gene in plants is universal.

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