Molecular Cloning and Characterization of SQUAMOSA-Promoter Binding Protein-Like Gene FvSPL10 from Woodland Strawberry (Fragaria vesca)

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Received: 14 July 2019; Accepted: 3 September 2019; Published: 11 September 2019

Abstract: SQUAMOSA-promoter binding protein-like (SPL) proteins are plant-specific transcript factors that play essential roles in plant growth and development. Although many SPL genes have been well characterized in model plants like Arabidopsis, rice and tomato, the functions of SPLs in strawberry are still largely elusive. In the present study, we cloned and characterized FvSPL10, the ortholog of AtSPL9, from woodland strawberry. Subcellular localization shows FvSPL10 localizes in the cell nucleus. The luciferase system assay indicates FvSPL10 is a transcriptional activator, and both in vitro and in vivo assays indicate FvSPL10 could bind to the promoter of FvAP1 and activate its expression. Ectopic expression of FvSPL10 in Arabidopsis promotes early flowering and increases organs size. These results demonstrate the multiple regulatory roles of FvSPL10 in plant growth and development and lay a foundation for investigating the biological functions of FvSPL10 in strawberry.

Keywords: molecular cloning; characterization; FvSPL10; woodland strawberry

1. Introduction

As a class of development-related plant-specific transcription factor (TF), the most common feature of SQUAMOSA-promoter binding protein-like (SPL) TFs is that they all contain a highly conserved DNA binding domain. This is known as the SQUAMOSA-promoter-binding (SBP) domain. The SBP consists of two Zn-finger-like structures and one nuclear localization signal motif, which is approximately 76 amino acids in length [1]. Since the first identification in Antirrhinum majus [2], SPL genes have been discovered and cloned in almost all green plant species [3]. However, different from other plant-specific TFs, such as AP2/ERE (Apetala2/ethylene response factor) and NAC (NAM, ATAF and CUC) which contain more than one hundred members in their families, the SPL gene family in plant is relatively smaller. For example, the SPL family in maize, rice, Arabidopsis, tomato, grape and woodland strawberry contains only 31, 19, 16, 15, 18 and 14 members, respectively [4–8].

Though SPLs have been identified in various plants, only a few have been characterized. Additionally, of the SPLs that have been characterized, most are limited to model plants such as Arabidopsis, rice and tomato. In Arabidopsis, the 16 SPL TFs could be grouped into two classes according to their protein size. Class I includes five members: AtSPL1, AtSPL7, AtSPL12, AtSPL14 and AtSPL16. These are all large proteins that contain more than 800 amino acids. In contrast, the remaining 11 SPL TFs in class II are small proteins that contain less than 400 residues [1,9]. Studies have indicated that SPL TFs play important roles in plant growth and development. AtSPL1 and
AtSPL12 regulate plant thermotolerance at the reproductive stage [10]. AtSPL2, AtSPL10 and AtSPL11 are functionally redundant genes that play roles in controlling the development of lateral organs at the reproductive phase [11]. AtSPL3, AtSPL4 and AtSPL5 are also functionally redundant genes that regulate floral meristem identity [12–14]. Interestingly, and in contrast to most of the other SPLs in Arabidopsis that mainly play roles in plant growth and development, AtSPL6 was found to positively regulate plant innate immunity in defense against pathogens [15]. AtSPL7 was found to help regulate Cu homeostasis [16,17], while AtSPL8 helps to regulate pollen sac development [18]. AtSPL9 and AtSPL15 are a pair of functionally redundant genes that control plant juvenile-to-adult growth phase transition [19]. Moreover, AtSPL9 was found to play roles in negatively regulating anthocyanin biosynthesis [20] and axillary bud formation [21], and AtSPL9 and AtSPL13 regulate trichome distribution [22]. Finally, AtSPL14 functions in plant development and sensitivity to fungal toxin fumonisin B1 [23], while AtSPL16 is a non-functional duplication of AtSPL14 [3]. OsSPL14 and OsSPL16 are two well-characterized SPL genes in rice, the former was found to regulate plant architecture [24,25] and the latter regulate grain size [26]. In tomato, the most functional characterized SPL gene was CNR (colorless non-ripening), which has been identified as a key regulator involved in tomato fruit ripening [27]. In summary, as a class of plant-specific TF, most of the SPLs play critical roles in plant growth and development, especially in juvenile-to-adult transition through regulating flowering related genes.

Strawberry is an economically important and nutritionally rich fruit crop. The market value of strawberry is not only determined by its quality, but also by its launch time. To fruit, the earlier it is put on the market, the more expensive it will be to purchase. However, fruit maturation is closely related to the flowering time, promoting fruit plant flowering could consequently lead to earlier fruit maturation. Therefore, promoting flowering is an important breeding goal for strawberry and other fruit crops. Although numerous studies in other plants have elucidated the important regulating roles of SPL genes in the plant phase transition, the function of strawberry SPL genes remains elusive.

In a previous study, we systematically identified 14 SPLs in woodland strawberry [8]. Further tissue expression analysis indicated that one FvSPL gene, FvSPL10 (mrna22660.1), was mainly expressed in vegetative tissues, especially in the runner [8]. Because the expression pattern of FvSPL10 is quite different from its Arabidopsis ortholog AtSPL9, and considering the important roles of AtSPL9 during plant development in Arabidopsis, we decided to perform a detailed investigation about FvSPL10 in this study. The results indicate that FvSPL10 is a transcriptional activator that could specifically recognize and bind to the GTAC motif in the promoter of FvAP1 and activate its expression. Ectopic expression of FvSPL10 in Arabidopsis induces the expression of several flowering related genes, and therefore promotes early plant flowering (3–5 days). In addition, overexpression of FvSPL10 in Arabidopsis increases flower and seed size. These results demonstrate the multiple regulatory roles of FvSPL10 in plant growth and development, which provides valuable information for further investigating the function of FvSPL10 on plant architecture and flowering-related traits in strawberry.

2. Results

2.1. Cloning and Characterization of FvSPL10 in Woodland Strawberry

In our previous study, we identified 14 SPL genes in woodland strawberry and named FvSPL1 to FvSPL14 based on their chromosome location. Tissue expression analysis indicated FvSPL10 was highly expressed in runners. The coding sequence (CDS) of FvSPL10 is 1143 bp in length, which encodes 380 amino acids, with a predicted molecular mass of 40.04 kDa and an isoelectric point (pI) of 9.38. Phylogenetic analysis with AtSPLs indicated that FvSPL10 was closely related to AtSPL9 (Figure 1A). Further amino acid sequence alignments indicated that two SPLs shared 36% similarity, and the most conserved region was the N-terminus, which contains the SBP domain (Figure 1B).
As a class of transcriptional regulators, TFs usually activate or repress the expression of their target genes. Therefore, we further employed a dual-luciferase assay system to investigate which kind of transcriptional activity that FvSPL10 possesses (Figure 2B). The results showed that when co-transformation occurs with the reporter vector, the value of LUC/REN ratio with FvSPL10 is 2.5 folds higher than the negative control (Figure 2C). This indicates that FvSPL10 possesses transcriptional activation activity. In short, the subcellular localization and transcriptional activity assay results confirmed that FvSPL10, similar to its homologs, is a transcriptional activator in plant.

Figure 1. Bioinformatic analysis of FvSPL10. (A) The amino acid sequences of FvSP10 and 16 AtSPLs were aligned using the Clustal W algorithm, and the neighbor-joining tree was constructed by MEGA6.0 software with 1000 bootstrap replicates. The scale bar represents a distance of 0.1 substitutions per site. FvSPL10 is marked with a diamond. (B) Amino acid sequences alignment of FvSPL10 and AtSPL9. Identical amino acids were highlighted in black and marked by star, and similar amino acids were marked by double dots. In addition, the conserved SQUAMOSA-promoter-binding (SBP) domain in their sequences is underlined.

2.2. Subcellular Localization and Transcriptional Activity of FvSPL10

Usually, TFs are located in the nucleus to regulate the expression of their target genes. To validate the subcellular localization of FvSPL10, we transiently expressed GFP-tagged FvSPL10 fusion protein in tobacco leaves with GFP alone as a control. As expected, the GFP-FvSPL10 fusion protein exclusively localized in the cell nucleus, and GFP was distributed both at the cytoplasm and nucleus (Figure 2A). As a class of transcriptional regulators, TFs usually activate or repress the expression of their target genes. Therefore, we further employed a dual-luciferase assay system to investigate which kind of transcriptional activity that FvSPL10 possesses (Figure 2B). The results showed that when co-transformation occurs with the reporter vector, the value of LUC/REN ratio with FvSPL10 is 2.5 folds higher than the negative control (Figure 2C). This indicates that FvSPL10 possesses transcriptional activation activity. In short, the subcellular localization and transcriptional activity assay results confirmed that FvSPL10, similar to its homologs, is a transcriptional activator in plant.

Figure 2. Cont.
when incubated with biotin-labeled probes that contain a fragment of \textit{FvAP1} to confirm the interaction between

The results indicated that the recombinant \textit{GST-FvSPL10} all contain at least one GTAC motif in their promoter, among them, there are five GTAC motifs in the promoter of \textit{FvAP1} in the woodland strawberry. Many flowering-related genes such as \textit{FvAGL24}, \textit{FvSOC1}, \textit{FvSOC1}, \textit{FvTFL}, and \textit{FvAPI} all contain at least one GTAC motif in their promoter, among them, there are five GTAC motifs in the promoter of \textit{FvAP1} (Figure 3A). Therefore, we applied electrophoretic mobility shift assay (EMSA) to confirm the interaction between \textit{FvSPL10} and the GTAC motifs in the \textit{FvAP1} promoter. The results indicated that the recombinant \textit{GST-FvSPL10} protein could form DNA-protein complexes when incubated with biotin-labeled probes that contain a fragment of \textit{FvAP1} promoter including one GTAC motif. However, the DNA-protein complexes were gradually abolished with the increasing concentrations of competitors, but not abolished when incubated with mutated probes (Figure 3B). In summary, these results showed that \textit{FvSPL10} could specifically recognize and bind to the GTAC motifs in the promoter of \textit{FvAPI}.

**Figure 2.** Subcellular localization and transcriptional activity analysis of \textit{FvSPL10}. (A) Subcellular localization of \textit{FvSPL10}. Binary vectors containing \textit{GFP-FvSPL10} or \textit{GFP} were transiently expressed in tobacco leaves through the \textit{Agrobacterium} infiltration approach. The fluorescence was detected 48 h after infiltration, and the nuclei were stained by 4′,6-Diamidino-2-phenylindole (DAPI). Bar, 20 μm. (B) Schematic representation of the reporter and effector constructs for transcriptional activity analysis. LUC, firefly luciferase; REN, renilla luciferase. (C) Transcriptional activation ability of \textit{FvSPL10} in tobacco leaves. Reporter vector was co-transformed into tobacco leaves by \textit{Agrobacterium} empty vector control or \textit{FvSPL10}, respectively. Each value represents the means of six biological replicates. The ratio of LUC/REN of the pBD control was used as a calibrator (set to 1). Double stars represent a significant difference between the sample and control at \(p < 0.01\), based on the Student’s \(t\)-test.

2.3. Interaction of \textit{FvSPL10} with the GTAC Motif in \textit{FvAP1} Promoter in Vitro

Numerous studies have confirmed that SPL TFs could bind to the GTAC motif [16,28]. For example, the first two SPL genes in \textit{A. majus} could be identified because they could bind to the GTAC motif in the promoter of the floral meristem identify gene \textit{SQUAMOSA} [2]. In this study, we observed that in woodland strawberry many flowering-related genes such as \textit{FvAGL24}, \textit{FvSOC1}, \textit{FvTFL} and \textit{FvAPI} all contain at least one GTAC motif in their promoter, among them, there are five GTAC motifs in the promoter of \textit{FvAP1} (Figure 3A). Therefore, we applied electrophoretic mobility shift assay (EMSA) to confirm the interaction between \textit{FvSPL10} and the GTAC motifs in the \textit{FvAP1} promoter. The results indicated that the recombinant \textit{GST-FvSPL10} protein could form DNA-protein complexes when incubated with biotin-labeled probes that contain a fragment of \textit{FvAP1} promoter including one GTAC motif. However, the DNA-protein complexes were gradually abolished with the increasing concentrations of competitors, but not abolished when incubated with mutated probes (Figure 3B). In summary, these results showed that \textit{FvSPL10} could specifically recognize and bind to the GTAC motifs in the promoter of \textit{FvAPI}.
Figure 3. Electrophoretic mobility shift assay (EMSA) verification the binding capability of FvSPL10. (A) Schematic diagram of the FvAP1 promoter (−1000 bp upstream from the start codon) and probes used for EMSA. The five red bars represent the five GTAC motifs in the promoter and the number represents the position of these motifs in the promoter. A total of 47 bp oligonucleotides containing the second GTAC motif was selected as the probe. The mutant probe was the same as the wild-type probe except G substituted by A in the GTAC motif. (B) Binding of FvSPL10 to the promoter of FvAP1 in EMSA. The biotin-labeled probes were incubated with purified GST or GST-FvSPL10 fusion protein to detect the binding ability, and the unlabeled or mutant probes were used as competitors to test the binding specificity of FvSPL10. Formed DNA-protein complexes were separated by native polyacrylamide gel. Arrows indicate the position of the shifted bands and free probes, respectively.

2.4. FvSPL10 Activates the Transcription of FvAP1 in Vivo

As luciferase assay and EMSA have respectively confirmed, FvSPL10 is a transcriptional activator and FvSPL10 has binding ability to the promoter of FvAP1. We further applied a dual-luciferase transient expression system to confirm whether FvSPL10 could activate the transcription of FvAP1 in vivo. As illustrated in Figure 4, when the transient expression effector vector containing FvSPL10 co-transformed with the reporter vector containing the promoter of FvAP1 in tobacco leaves, the value of LUC/REN ratio was significantly higher than those of empty effector vector with the reporter (Figure 4). These results confirmed that FvSPL10 could activate the transcription of FvAP in vivo.
Figure 4. *FvSPL10* directly activates the expression of *FvAP1*. (A) Schematic representation of the reporter and effector constructs used in this study. LUC, firefly luciferase; REN, renilla luciferase. (B) *FvSPL10* activates the promoter of *FvAP1*. The activation was calculated by the ratio of LUC/REN between sample and control. The ratio of LUC/REN of empty vector together with *FvAP1* promoter was used as a calibrator (set to 1). Each value represents the means of six biological replicates. Double stars represent a significant difference between the sample and control at p < 0.01, based on the Student’s t-test.

2.5. Ectopic Expression of *FvSPL10* in *Arabidopsis* Promotes Flowering

In *Arabidopsis*, AtSPL9 has multiple functions. These functions include controlling vegetative phase change [19], anthocyanin biosynthesis [20] etc. To analyze the functions of *FvSPL10*, we fused *FvSPL10* downstream of the CaMV35S promoter in the binary expression vector pJX001 to generate *FvSPL10* overexpression construct (*FvSPL10-OE*). We then transformed it into *Arabidopsis* through Agrobacterium-mediated transformation approach. Nine independent transgenic lines were obtained. Compared with wild-type plants, *FvSPL10-OE* plants significantly promoted flowering under long-day conditions (3–5 days early) (Figure 5A,B).
wild-type plants. At the seedling stage, plant phase transition, but it also has other phenotypic consequences, such as regulating organ size in Arabidopsis. In Arabidopsis, many SPL genes, including AtSPL9, can directly target and activate the expression of several MADS-box genes [12]. To analyzed whether FvSPL10 possesses similar functions as AtSPL9, we further detected the expression levels of several known MADS-box and other flowering-related genes such as AtFUL, AtCO, AtAGL42, AtLFY and AtAPI by quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR). The results showed that all these flowering-related genes were significantly up-regulated in FvSPL10-OE lines. Two transgenic lines, L21 and L22 were selected as representatives. Single or double stars at the top of each column in the figure represent a significant difference among different lines at 0.01 < p ≤ 0.05 and p < 0.01, respectively, based on the Student’s t-test.

2.6. Ectopic Expression of FvSPL10 in Arabidopsis Enlarges Organs Size

Apparent difference was observed in some organs between FvSPL10-OE transgenic lines with wild-type plants. At the seedling stage, FvSPL10-OE lines exhibited longer root than the wild-type plants (Figure 6A). At the reproductive stage, the floral organs of FvSPL10-OE lines were larger than the wild-type plants (Figure 6B). In addition, FvSPL10-OEs produced longer and larger siliques than the wild-type plants (Figure 6C). Finally, we observed that the seeds of FvSPL10-OEs were larger than the wild-type plant seeds (Figure 6D,E). These findings indicate that not only does FvSPL10 regulate plant phase transition, but it also has other phenotypic consequences, such as regulating organ size in Arabidopsis.

Figure 5. Overexpression of FvSPL10 in Arabidopsis promotes early flowering. (A) Phenotypes of 3-week-old plants under long-day conditions. Wild-type (WT) Arabidopsis; L21, L22, homozygous transgenic lines with overexpression of FvSPL10. (B) Comparison of flowering time between WT and transgenic plants. The value of each column represents mean SD (n = 12). (C) Relative transcript levels of flowering-related genes in WT and transgenic lines. Two transgenic lines, L21 and L22 were selected as representatives. Single or double stars at the top of each column in the figure represent a significant difference among different lines at 0.01 < p ≤ 0.05 and p < 0.01, respectively, based on the Student’s t-test.
3. Discussion

SPLs are plant-specific TFs that play important roles in plant growth and development. In model plants like Arabidopsis and rice, nearly all the members of SPLs have been identified and characterized. However, few research studies have been performed on strawberry SPL genes. In this study, one SPL gene, FvSPL10, was cloned from woodland strawberry on the basis of our previous study [8]. Phylogenetic analysis indicated that FvSPL10 was the ortholog of AtSPL9 (Figure 1A). Further sequence alignment indicated the two SPLs only shared 36% amino acid identity, and the highest similarity region was the SBP domain (Figure 1B). However, to most SPLs, the functional regions are the variant parts, not the highly conserved SBP domain that is required for DNA binding ability. For example, in bread wheat, Liu et al. identified that the N-terminal of the TaSPL3/17 was required for interacting with TaD53 (DWARF53) and TOPLESS proteins to regulate the strigolactone signaling pathway [29]. In addition, Yu et al. found the C-terminal of AtSPL9 was required for physical interaction with DELLA proteins to regulate plant flowering [30]. Considering the low sequence similarity, except the SBP domain between FvSPL10 and AtSPL9, it is reasonable to speculate that although FvSPL10 might share similar functions to AtSPL9, it might have different functions as well.

To explore the biological functions of FvSPL10, we first analyzed its subcellular localization and transcriptional activity in tobacco leaves through agroinfiltration-based transient gene expression approach, because it is difficult to carry out the same study in strawberry leaves. As expected, FvSPL10 was a typical TF that localized in the nucleus with transcriptional activator activity (Figure 2). SPL genes were first identified from A. majus because they were found binding to the GTAC motif in the promoter of the floral identity gene SQUAMOSA [2]. In this study, we also confirmed that...
FvSPL10 could recognize and bind to the same motifs in the promotor of FvAP1 (Figure 3). Further transcriptional activity assay indicated that FvSPL10 can promote the expression of FvAP1 (Figure 4). In Arabidopsis, AtSPL9 directly activate flowering-promoting MADS-box genes to promote plant phase transition [12]. To verify the functions of FvSPL10, we first tried to transform FvSPL10 into woodland strawberry. However, due to the low transformation efficiency and time-consuming process of strawberry genetic transformation, we have not obtained genetically transformed strawberry plants. We therefore ectopically expressed it in Arabidopsis. As expected, compared to the wild-type plants, FvSPL10-OE plants exhibited early flowering (3–5 days earlier) under long-day conditions (Figure 5A,B). Several flowering related genes, such as AtAP1, AtFUL, AtCO, AtAGL42 and AtLFY were significantly up-regulated (Figure 5C). More interestingly, apart from leading to early flowering, ectopic expression of FvSPL10 in Arabidopsis resulted in other phenotypic changes, such as elongated roots and enlarged siliques and seeds (Figure 6). It was interesting to observe that there was only partial phenotypic similarity between transgenic Arabidopsis that overexpression AtSPL9 and FvSPL10. Except significantly promoted flowering in both transgenic lines, the former was found reduced the content of anthocyanin [20], while the latter was found increased organs size in this study. Interestingly, the phenomena of increasing seed size were observed when two grain-development-related SPL genes, OsSPL16 and TaSPL16, were overexpressed in Arabidopsis [27,31]. In strawberry, seed-derived auxin plays a critical role in fruit growth and development, and removing the seeds from strawberry fruit resulted fruit growth ceasing [32]. Therefore, in the future, besides investigating the roles of FvSPL10 in plant phase transition and its relationship to flowering, it is also worth investigating whether FvSPL10 has functions like OsSPL16 and TaSPL16, which could regulate strawberry seed development and eventually influence fruit development through strawberry transformation.

4. Materials and Methods

4.1. Plant Materials and Growth Conditions

Seeds of Woodland strawberry (Fragaria vesca accession Hawaii 4) were gifted by Professor Janet P. Slovin (USDA). Plants were grown in the growth chamber with 16 h light at 25 °C and 8 h dark at 20 °C. Plant tissues were collected and immediately frozen in liquid nitrogen then stored at −80 °C for future analysis. Arabidopsis thaliana accession Columbia-0 was used as wild-type, plants were grown at 22 °C under long-day conditions (16 h light/8 h dark) in a greenhouse.

4.2. Nucleic Acid Isolation

Genomic DNA was isolated from young leaves of woodland strawberry according to the cetyltrimethylammonium bromide (CTAB) method [33]. Total RNA of woodland strawberry was extracted from runners by using a Plant Total RNA Isolation Kit Plus (Foregene, Chengdu, China) according to the manufacturer’s protocol. First-strand cDNA was synthesized by using PrimeScript™ II 1st Strand cDNA Synthesis Kit (TAKARA, Dalian, China) according to the user manual instructions.

4.3. Bioinformatic Analysis

For phylogenetic analysis, the amino acid sequences of AtSPLs and FvSPL10 were aligned with the Clustal W algorithm and the unrooted radial tree was generated by MEGA 6.0 software using neighbor-joining (NJ) method with 1000 bootstrap replicates [34].

4.4. Subcellular Localization Analysis of FvSPL10

The cDNA of FvSPL10 was inserted downstream of the GFP reporter of the binary vector pJX002 (primers are listed in Supplementary Table S1). The binary vector pJX002 harboring 35S: GFP-FvSPL10 expression cassette or the empty pJX002 vector were separately transformed into A. tumefaciens strain GV3101 and then infiltrated into tobacco (Nicotiana benthamiana) leaves as described by Cheng et al. [35].
Transient expressed GFP-\textit{FvSPL10} or GFP was detected by using a fluorescence microscope (FV3000, Olympus, Tokyo, Japan) two days after infiltration.

4.5. EMSA

The cDNA of \textit{FvSPL10} was cloned into the prokaryotic expression vector pGEX-6P-1 (GE Healthcare) to generate a GST-\textit{FvSPL10} expression construct, which was further transformed into \textit{Escherichia coli} strain BL21 (DE3). The GST-\textit{FvSPL10} fusion protein was induced by 0.3 mM isopropyl thio-\textbeta-\textgamma-d-galactoside (IPTG) at 16 °C overnight. The GST-\textit{FvSPL10} fusion protein was lysed from bacteria by sonication and further purified by glutathione-superflow resin.

A 47 bp fragment of \textit{FvAP1} promoter containing the second GTAC motif was selected as a probe. The probe was labeled by biotin at the 3' end, and the GTAC motif mutated or non-mutated probes without biotin labeling were used as competitors. Chemiluminescent EMSA Kit (Beyotime Biotechnology, Shanghai, China) was used to perform EMSA following the user manual instructions. The protein-DNA complexes were separated by native polyacrylamide gel electrophoresis and detected by chemiluminescence method. All primers are listed in the Supplementary Materials, Table S1.

4.6. Transcriptional Activity Analysis of \textit{FvSPL10}

Dual-luciferase assay system was employed to analyze the transcriptional activity of \textit{FvSPL10} in tobacco leaves. The reporter was adopted based on the pGreenII 0800-LUC vector, and the effector construction was adopted based on a CaMV35S promoter-driven pBD vector [36]. The activity was measured as described by Cheng et al. [35], in order to study the trans-activation of \textit{FvSPL10} to \textit{FvAP1}. The promoter of \textit{FvAP1} (1000 bp upstream from the start codon, the nucleotide sequence was listed in Supplementary Data S2) was cloned into the pGreenII 0800-LUC vector as reporter, and the \textit{FvSPL10} was inserted into the pEAQ vector as effector. The reporter and effector vectors were transiently expressed in tobacco leaves through \textit{Agrobacterium} infiltration. The LUC/REN activity was detected by using a DLR assay system kit (Promega, Madison, WI, USA) two days after infiltration. The trans-activity of \textit{FvSPL10} to the promoter of \textit{FvAP1} was calculated as described by Cheng et al. [35]. Primers are listed in the Supplementary Materials, Table S1.

4.7. Construction of \textit{FvSPL10} Overexpression Vector and \textit{Arabidopsis} Transformation

The full-length of \textit{FvSPL10} was cloned into the binary vector pJX001 downstream of the CaMV 35S promoter and transformed into \textit{Arabidopsis} wild-type through \textit{A. tumefaciens}-mediated floral-dip method [37]. Transgenic plants were screened on MS plates supplied with 30 mg/L Hygromycin and further verified by genomic PCR. Plants were grown in greenhouse conditions described above, and T3 generation transgenic lines were used for analysis.

4.8. Gene Expression Analysis

QRT-PCR analysis was performed to detect the transcription levels of flowering genes. Total RNA was isolated from 2-weeks old \textit{Arabidopsis} plants by TRizol reagent (Invitrogen). First-strand cDNA was synthesized by using the Prime Script RT reagent kit according to the manufacturer’s instruction (TAKARA, Dalian, China).

QRT-PCR was conducted on the CFX96 touch real-tim tem (Bio-Rad, Hercules, CA, USA). The amplification parameters were 95 °C for 2 min, 40 cycles at 94 °C for 5 s then 60 °C for 30 s. The relative expression levels of flowering related genes were calculated using the $2^{-\Delta\Delta CT}$ method [38], and \textit{At\beta-tubulin2} was used as an internal reference. Three biological replicates and three technical replicates were conducted. All primers used were listed in the Supplementary Materials, Table S1.
4.9. Phenotypic Measurement and Statistical Analysis

The flowering time of *Arabidopsis* plants was counted from the day of germination to the day of first bud emergence. At least 10 plants were counted for each genotype. Seven-day-old seedlings of wild-type or transgenic lines growing on MS plates were used to measure the root length. For measurement the size of seeds. Dry seeds of the wild-type or transgenic plants were randomly selected and photographed by Stereo microscope (SZ61, Olympus, Tokyo, Japan). The seeds size were imaged by Image J software [39]. At least 30 seeds were measured for each genome type.

5. Conclusions

In conclusion, *FvSPL10*, one of the SPL genes from woodland strawberry, was cloned and characterized in this study. Transgenic experiments demonstrated that *FvSPL10* has multiple functions in regulating plant growth and development. These findings paved the foundation for further investigation of the function of *FvSPL10* in plant architecture and flowering-related traits of strawberry.

Supplementary Materials: The following are available online at http://www.mdpi.com/2223-7747/8/9/342/s1, Supplementary Table S1: Primers used in this study; Supplementary Data S2: Nucleotide sequence of *FvAP1* promoter.

Author Contributions: J.X. and Z.C. designed the research, J.X., Y.B., C.M., H.Z. and D.Z. performed the experiments. J.X. and Z.C. analyzed the data and wrote the paper.

Funding: This study was supported by the National Natural Science Foundation of China (31401849).

Conflicts of Interest: The authors declare no conflicts of interest.

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