Characteristics of a Radish Mutant with Longer Siliques

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Short Report

Keywords: Silique, mutant, overexpression, phenotype, radish

Posted Date: March 1st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1354810/v1

Abstract

The silique is an important organ in plant reproduction and maintains biodiversity among species; however, little is known about the regulation of radish silique development. In this study, we conducted research on the radish long silique (*ls*) mutant and wild-type (WT) radish and compared their morphological and molecular markers. The results showed that the mutant obtained by ethyl methane sulfonate treatment had the following stable characteristics: the lengths of mutant and WT siliques were 20.50 and 9.10 cm, respectively; and the ovule number per silique of the mutant and WT was 9.5 and 4.5, respectively. Polymerase chain reaction (PCR) analysis revealed abundant polymorphisms between the *ls* mutant and WT in HZ001, SRC9-022, and OIL2F11 simple-sequence repeat molecular markers, and the expression of *LS1* and *LS2* (*RsNAC66*). *Arabidopsis thaliana*-transformed plants with *RsNAC66* overexpression were obtained by the floral dip method. Quantitative PCR showed that *LS2* (*RsNAC66*) was more highly expressed in transformed lines than in WT, and the expression of *LS2* (*RsNAC66*) in the transformed lines was higher in siliques than leaves. Phenotypic analysis revealed abnormal ovule numbers and shoots, and altered plant height in the transformed plant. Phenotypic and gene expression analyses showed that *LS2* (*RsNAC66*) had a positive effect on silique length and the number of seeds per silique. Together, the results showed that the radish *ls* mutant is specific and stable, and thus constitutes an excellent research resource for improving the seed yield of radishes.

Introduction

Radish (*Raphanus sativus* L.) is one of the most important and widely cultivated vegetables worldwide. The fleshy root is one of its edible parts, and the Chinese radish is highly productive and resistant to storage. The radish cultivation area in China is approximately 1.2×10^6 m^2, accounting for about 40% of the world's cultivated area and 47% of the world's total production (Wang 2016). In the process of long-term evolution, cultivation, and breeding, new uses for radishes have gradually emerged, including as a food source (where the leaves and siliques are edible) and cover crop (oilseed radish); radish has thus received increasing attention from researchers. Radish seeds contain high amounts of lipids and active substances (Kim et al., 2014), among which sulforaphane is a natural active substance with antitumor activity (Zhao et al., 2011). However, the low yield of radish seeds limits their versatility and value. There have been few studies on the mechanisms of radish seed yield and silique development. Zhai et al. (2016) showed that 5,777 differentially expressed genes appeared in radish ovules within 15 days before and after artificial pollination, including *auxin-response factor*, *Leafy cotyledon 1*, *somatic embryogenesis receptor kinase*, and other important regulatory genes involved in embryogenesis. These results provide an important reference for understanding the early embryonic development process of radish.

Our group previously revealed the developmental process of radish siliques and ovules through morphological analysis (Li et al., 2018). The ovule development of radishes is similar to that of oilseed rape (*Brassica napus*) (Uyttewaal et al., 2008; Li et al., 2018). Under normal conditions, the average seed yield of radish per mu (0.067 hectare) is 50 kg (Li 1992), which is significantly lower than that of similar economic crops such as oilseed rape. The low seed yield severely affects the ability to multi-purpose the radish.

In this study, we identified mutation traits in a radish mutant with a long silique (*ls*), based on a mutant pool constructed by ethyl methanesulfonate (EMS) mutagenesis (Li et al., 2019). The results showed that the number of seeds per silique in the mutant was significantly increased compared to wild-type (WT) radish, and there were significant differences in molecular markers and gene expression related to silique development between the radish *ls* mutant and WT. In addition, the radish silique-related gene *RsNAC66* was transformed into *Arabidopsis*, and phenotypic analysis showed that it may increase silique length and ovule number per silique.

Results

Phenotypic traits of the radish ls mutant

Our research group previously established a radish mutagenesis system and constructed a radish mutant pool (Li et al., 2019); after manyyears of self-fertilization, they were used to screen the radish *ls* mutant with stable silique and ovule phenotypes (Fig.1). There were significant differences between the *ls* mutant and WT radish in silique length and number of seeds per silique (Fig.2A–F). Specifically, the average length of silique in the *ls* mutant was 20.50 cm, compared to 9.10 cm in the WT radish (Fig.2G), and the average number of seeds per silique in the radish *ls* mutant was 9.5, whereas the average number of ovules of WT radish was 4.5 cm (Fig.2H). The statistical results showed that the siliques in the radish *ls* mutant were 2.3 times longer than those of WT radish, and there was a greater number of seeds per silique in the *ls* mutant than WT, indicating that the *ls* mutant not only has a longer silique than WT radishes but also a significantly higher number of ovules per silique. The radish *ls* mutant, with its significantly increased number of seeds per silique, has great research value and is an important experimental material for studying radish ovule formation and improving radish seed yield.

Detection of silique-related molecular markers and gene expression in the radish ls mutant

The results of screening of 42 simple-sequence repeat (SSR) molecular markers related to crop traits have been published (see **Materials and Methods**), and showed that the amplification products of the SSR molecular marker primers HZ001, SRC9-022, and OI12F11 were different between WT and *ls* mutant radishes. There were also abundant polymorphisms in the *ls* mutant and WT radish molecular markers (Fig. 3A). The bands of WT radish amplified by molecular markers HZ001 and SRC9-022 primers were faint (Fig. 3A). By contrast, amplification of the radish *ls* mutant by the molecular marker OI12F11 did not produce bands (Fig. 3A). The *LS1* gene was derived from the BnaA09g39480D sequence of the *B. napus* gene (Shen et al., 2019) through sequence alignment by Blast (LR778317), and the *LS2* gene was designed from the radish gene (XM_018634088) sequence obtained by homologous alignment in the National Center for Biotechnology Information database. Semi-quantitative polymerase chain reaction (PCR) results showed that the expression of *LS1* and *LS2* in the silique of the radish *ls* mutant was lower than that in WT radish. In addition, there were clear differences in the expression of *LS1* and *LS2* between mutant and WT radish cotyledons.

Verification and qualification of Arabidopsis-transformed plants overexpressing LS2 (RsNAC66)

Transformed *Arabidopsis* lines overexpressing *LS2 (RsNAC66)* were screened using amplification fragments of the kanamycin gene by PCR (Fig. 4A). The semi-quantitative PCR results showed that the expression of *LS2 (RsNAC66*) was significantly enhanced in the transgenic plants (Fig. 4B, C). Relative expression of the *LS2 (RsNAC66)* gene in the transgenic lines (lines 3, 5, 7, 9) was upregulated to different degrees compared to WT, and increased 6,054-, 2,427-, 2-, and 99-fold in the siliques of lines 3, 5, 7, and 9, respectively (Fig. 4C). These results showed that the expression of *LS2 (RsNAC66)* was significantly increased in the overexpressed lines.

Statistical analyses of the phenotypic characteristics of A. thaliana**-transformed plants overexpressing** LS2 **(**RsNAC66**)**

Statistical analyses showed that the average plant height of *A. thaliana* transgenic lines 3 and 7 was higher than that of WT, and the plant height of transformed line 7 was significantly different from that of WT ($P < 0.01$; Fig. 5A). There was no significant difference in plant height between transformed lines 3, 5, and 9 and WT ($P > 0.05$). *A. thaliana* transgenic line 5 showed abnormal bolting (Fig. 5B), and early bolting and flowering were observed during week 6 of *A. thaliana* growth. The average silique length and number of ovule per silique of transgenic *A. thaliana* were measured and analyzed (Fig. 5C–F). Among them, the silique length of transgenic lines 5 and 9 was longer than that of WT ($P < 0.05$). There were more ovules per silique in lines 3, 7, and 9 than in WT ($P < 0.01$).

Detection and analyses of the expression of silique development-related genes

AP2, OPT4, and Atsus2 are silique development regulatory genes (Kunst et al., 1989; Bowman et al., 1989; Chai et al., 2011; Huang et al., 2013; Niuetal., 2002; Ohto et al., 2005). The heterologous expression of *LS2* (*RsNAC66*) affects the development of *Arabidopsis* siliques. We further examined the correlation between the two genes in siliques. As shown in Fig. 11, the relative expression of *AP2* in the transgenic lines was upregulated in siliques, as well as 6-, 17-, 1- and 2-fold in the four tested transgenic lines (3, 5, 7, and 9), respectively (Fig. 5G). *OPT4* and *Atsus2* were both expressed at high levels in the four tested transgenic lines (3, 5, 7, and 9). The high expression and function of *LS2* (*RsNAC66*) in siliques suggest that the *AP2* gene coordinated with *LS2* (*RsNAC66*) is involved in the regulation of silique development.

Discussion

Comparison of morphology and detection of molecular markers and gene expression showed that the radish *ls* mutant induced by EMS was stable, and the number of ovules per silique was significantly higher than that of WT (Fig. 2B, C, D, G). The radish *ls* mutant is an important resource to improve the seed yield of radish. Studies on the variation and regulation of silique length in radish have not been conducted. At present, applied research on the development of molecular markers in radish is mainly focused on purity and germplasm identification (Qiu et al., 2014; Li et al., 2018; Wang et al., 20119; Yang et al., 2019). Therefore, this study can only refer to other morphology-related molecular markers of radish and genes, and to markers related to the numbers of silique and ovules in other crops, to detect this radish *ls* mutant.

Through polymorphism screening of the above 42 SSR molecular marker primers, three pairs of primers with clear amplification conditions and polymorphism were screened, indicating that the above three pairs of SSR molecular markers were universal in radish, turnip rape, and oilseed rape. Based on the sequence information of silique length regulatory genes in oilseed rape (Zhou et al., 2017; Sheng et al., 2019), we obtained the homologous sequence of silique length regulatory genes in radish through homology comparison, and found that the expression of these genes was significantly different between the *ls* mutant and WT radish.

NAC (NAM, ATAF, CUP) is one of the largest families of transcription factors in plants. The N-terminal has a highly conserved NAC domain, while the C-terminal has high diversity in the structural characteristics of NAC transcription factors (Munir et al., 2020). After constructing *RsNAC66* overexpression transgenic lines in *A. thaliana*, the phenotypes of the transformants were analyzed by morphological and statistical methods. Among the 11 *Arabidopsis* transgenic lines, abnormalities in bolting, silique length ovule number per silique, and plant height were observed. Quantitative analysis of *RsNAC66* expression confirmed high expression of the *RsNAC66* gene in transgenic *Arabidopsis* siliques. The expression of the *AP2* gene, which is related to ovule development in *A. thaliana*, was quantitatively analyzed, and found to be significantly upregulated in transgenic lines. These results showed that *RsNAC66* had an effect on silique and ovule development, and increased silique length and ovule number per silique were observed in transgenic lines with high *RsNAC66* expression compared to WT. The expression of *AP2* in silique was also upregulated in transgenic lines with high *RsNAC66* expression, indicating that they both participated in the regulation of silique and ovule development; however, the mechanisms by which they exert their synergistic roles merit further study.

Materials And Methods

Experimental materials and primers

The radish *ls* mutant with a longer silique based on EMS mutagenesis, as reported by Li et al. (2019), and the WT radish with a normal silique and *A. thaliana* (Columbia-0), were used in this study. The above plant materials were obtained from the germplasm resource bank of Hangzhou Radish Research and Development Center of Huanggang Normal University. The primers for molecular markers and related genes were as follows: primers 1–9 were selected according to the primer sequence information related to the bolting of radish published by Yang *et al*. (2019) and the molecular markers related to the number of seeds per silique of *B. napus* published by Zhang et al. (2012). Thirty-three pairs of primers corresponding to SSR molecular markers distributed in A01, A03, and A09 linkage groups, which are associated with silique length quantitative trait loci, were screened with primers 10–42 (Wang et al., 2011; Zhang et al., 2012; Yin et al., 2015; Yang et al., 2019). Through homologous comparison of the length-related gene sequence of silique in *B. napus* published by Shen et al. (2019), the radish gene sequence was obtained, and primers 43 and 44 were designed from this sequence. The primer sequence used in this experiment is shown in Table 1. The primers were synthesized by GenScript Biotech Corporation (Piscataway, NJ, USA).

Table 1
Primer sequences of silique-related molecular markers and genes

Serial number	Name Of Primer	Forward primer (5'→3')	Reverse primer (5'→3')
1	HZ001	CAGATCCGAGGGAGTTTGAA	TTCTCTCATCCTCGGTTTCC
2	HZ002	ACTTCCCCGGACCTTAAAGA	GCATCGCAAAGAACAGACAA
3	HZ003	GTGGCCGAGTAGATGGTTGT	TGGCAATAGCTTTCCTTGCT
4	HZ004	CGAAGTTTGCGTTTTCGATT	AGTGTCTTGCAAGCTCGGAT
5	HZ005	GCTCTTTCTAGCCCGAGGAT	CCGATGGTCATGGGTAGTTT
6	BoGMS1413	TGGGCTTCTTCTTACTTACCT	TGCTCTGTCTATTATTCGTTTG
7	SRC9-022	TTGTGGTTTTTCTCTCCGTGATGCT	TCGAGGCAGAACCATCGGGGT
8	SCC9-005	GCAGCAACTTGGTTTCAGGT	CAGCCCTTGGCTTAGACACT
9	Ss2066	AATTAAGGGACCACGCAACA	CCAGAACCCTCTTGATTCACTT
10	BoE667	TCACGCTTTTCTCGATCTCACTGT	ATCTTCCTCTTCCCGCCTCTAAAT
11	KBRH139B23	ATCTCATGGTTGGTTCACCG	ATTTCCAAAACACACACGCA
12	Ra2G09	ACAGCAAGGATGTGTTGACG	GATGAGCCTCTGGTTCAAGC
13	BRMS056	GATCAAGGCTACGGAGAGAGAG	CGTGACGCTAGAGTAATCGAGT
14	OI12F11	AAGGACTCATCGTGCAATCC	GTGTCAGTGGCTACAGAGAC
15	BC46	CTTTTGCTGCCCGACGAGA	AAGGAAGCAGGAAAGAGATAAAAG
16	ENA28	GGAGTCCGAGCGTTATGAAT	CTTCATCGACCCACCTTGTT
17	BRMS303	ACTCAACAACCGAACAAGAAAAACA	CGGTAGAGAACAGAGGAAGCCTAAG
18	BoE368	GGAGGTGGAGGTCAGTG	GGCTTTCCATTCATATCTTTCTCC
19	Ra2E11	GGAGCCAGGAGAGAAGAAGG	CCCAAAACTTCCAAGAAAAGC
20	BRMS269-2	AATTTTCAACGCCTTCATCCTGT	GGTGAATGCATCAGTCGACATAACT
21	BRMS043	GCGATGTTTTTTCTTCAGTGTC	TTAATCCCTACCCACAATTTCC
22	BoE76	GCCCATCTCAGTTTTTCAA	AGCGCGGCGTGGTTCAGT
23	BoE320	CACGACACGAAACCCTAAACT	CATCCGCTGAGAACTGTCG
24	BRMS042	TCGGAATTGGATAAGAATTCAA	GGATCAGTTATCTGCACCACAA
25	B126.3	GGAAGATTCAAAGTCGAGTG	GTTGGGTTCGTTGTATCATC
26	BRMS042-2	AGCTCCCGACAGCAACAAAAGA	TTCGCTTCCTTTTCTGGGAATG
27	BRMS050	AACTTTGCTTCCACTGATTTTT	TTGCTTAACGCTAAATCCATAT

Serial number	Name Of Primer	Forward primer (5’→3’)	Reverse primer (5’→3’)
28	BoE887	CCTCCACGCGCACAGAAG	TCGAAAGAAAGAAAAAGACAGGAA
29	BoE073	CGTCGCTGATTCACCAACCAA	GAAGGACGGGGAGAAGTGAAAGAA
30	OI10D08	TCCGAACACTCTAAGTTAGCTCC	GAGCTGTATGTCTCCCGTGC
31	KBRH143K20	CAAATGTCTCAAGACACATAAACCA	CTAAAGCAGCAATTGGGTGTTC
32	BoE828	AATGCGGCTTCTAATCCTTTTG	TGTCTGGCCAGCCGTAGCAC
33	BoE338	AGGAAAGCAAAGAAGCCAAGAC	TACAAGCCCTAAAGGAGACACA
34	BoE032	CTCTCACCGAATCGAATAAAAC	GTAGAGAATGGGGACGAGACCT
35	BoE334	GAGAAGGCGGTGATGACGA	TAAAACGCTAAAACCACAG
36	BoE075	GGTGCTGCCAAGGAGGAAATC	AAGGCTTGTAGGCACCACCAC
37	nia-ssr039	GCATATGACCAAAGCAAGAGAA	GTGTGCCCTGATATGTGCAA
38	BoE851	TCATTTTCAACGACTCTCCACTCT	AGGGGGTTTGATTCTCTGA
39	BoE390	GGTGGCGTATCCGTAAAGGTAGA	CGCCGTCGCTGCTCCACT
40	BRMS016	TCCCGTATCAATGGCGTAACAG	CGATGGTGACATTATTGTGGCG
41	ENA27	AAAGGACAAAGAGGAAGGGC	TTGAAATCAAATGAGAGTGACG
42	BoE974	TAGATCGGACGGAGGAAGGAAGA	TTGGGCTGGAGGTAGACGAAGAG
43	*LS1*	GATCGATCAAAACGCCGTCA	AAGAGGCCCAGCTAGTGATG
44	*LS2*	TGGAGGCCGGAGAAAAGAAG	TCACACTGTTGAGGCGAAAC

Phenotypic statistical analyses of radish and Arabidopsis

After the bolting and flowering of plants, abnormal traits of silique were observed, and the silique length of radish was measured by tape measure and ImageJ software (NIH, Bethesda, MD, USA). Siliques were treated with 8 mol/L sodium hydroxide solution to observe the distribution and number of ovules in siliques. *Arabidopsis* material was taken at week 14 and three phenotypic traits were recorded: plant height, silique length, and ovule number per silique.

Extraction, amplification, and detection of DNA

Genomic DNA was extracted from the samples using the Plant Genomic DNA Kit (DP305-02; Tiangen Biotech, Beijing, China), and the quality of DNA was analyzed by agarose gel electrophoresis. The PCR amplification system included 2× Taq PCR Master Mix (12.5 μL), DNA (0.5 μL), 10 μmol/L primers (1 μL), and ddH_2O (10 μL). The amplification procedure was as follows: pre-denaturation at 94℃ (3 min), 35 cycles of denaturation at 56℃ (30 s), annealing at 56 ℃ for 30s, extension at 72℃ (1 min), final extension at

72℃ (5 min), and maintenance at 4℃. The PCR products were separated by 1% agarose gel electrophoresis.

Construction of the RsNAC66 overexpression vector

Amplification and recovery of the target gene

The cDNA sequence of *RsNAC66* was used, along with the specific primers LSS-F(ActagggtctcGcaccATGAACATATCAGTAAACGGA) and LSS-R(ActagggtctcTACCGCTAGTGTGACGATGGCGTCGT) for PCR amplification. The PCR product was subjected to agarose gel electrophoresis, the 978 bp electrophoretic fragment was cut out, and the target fragment was recovered using the TIANgel Midi Purification Kit (DP209-03; Tiangen).

Transformation and identification of Escherichia coli

The empty plasmid was digested with *BsaI*-HF and ligated to the target fragment with T4 DNA ligase, and transformed into *E. coli* DH5α competent cells. After culture, it was coated on LB solid medium plates with kanamycin and cultured overnight in a constant temperature incubator at 37℃. The primers LSS (Test +)(AAGAGGAGACCATTCCAGAC) and Nos-R (ATCATCGCAAGACCGGCAAC) were used for colony PCR detection, and the fragment size was about 416 bp. Positive plaque was confirmed by sequencing, and single bacterial colonies were selected for shaking culture.

Plasmid sequencing and digestion validation

The plasmid was extracted using a plasmid extraction kit, and Sanger sequencing was performed. The sequencing primer was NOS-R(ATCATCGCAAGACCGGCAAC), and the sequence was consistent with the target fragment sequence; thus, the overexpression vector was successfully constructed. The extracted plasmids were confirmed by restriction endonuclease digestion with *EcoRI*/ *HindIII*.

Genetic transformation and resistance screening in A. thaliana

The plasmid with the target fragment was transformed into competent cells of *Agrobacterium tumefacien*, and the flowering *A. thaliana* WT was transformed by the floral dip method. The seeds were collected and seeded on MS solid medium containing kanamycin, and positive plants were identified by PCR; positive plants with *RsNAC66* overexpression were screened.

Detection of RsNAC66 expression

Extraction and reverse transcription of RNA from plants

Using the HiPure Plant RNA Mini Kit (R4151-02; Magen Biotechnology, Guangzhou, China), the was extracted from the radish mutant, WT radish, *Arabidopsis*, and transgenic *A. thaliana*. The extracted RNA was reverse-transcribed into cDNA using the PrimeScript RT Reagent Kit (RR047A; Takara Bio, Shiga, Japan) as a template for PCR.

Semi-quantitative PCR

The expression of *RsNAC66* in plants was first detected by semi-quantitative PCR. The reaction system consisted of 2× Taq PCR MasterMix (12.5 μL), cDNA (0.5 μL), forward primer (1 μL), reverse primer (1 μL), and ddH_2O (10 μL) in a total volume of 25 μL. The PCR amplification procedure was as follows: 95℃ for 3 min; 35 cycles of 95℃ for 30 s, 55℃ for 30 s, and 72℃ for 1 min; and 72℃ for 5 min. After semi-quantitative PCR amplification, 1% agarose gel electrophoresis was used for detection. The reference gene primer sequences used were Atactin-F: GGAGCTGAGAGAGATTCCGTTG and Atactin-R: GGTGCAACCACCTTGATCTT; the specific primer sequences for *RsNAC66* were Rsls376-F: CAAGTGCCTCCTGGCTTTAG and Rsls376-R: GCATGATCCAGTCGGATTTT.

Real-time quantitative PCR

Quantitative fluorescence PCR was performed using the 7500 instrument (Applied Biosystems, Foster City, CA, USA). TB Green Premix Ex Taq (Tli RNase H Plus) (RR420A; Takara) was used as the fluorescence quantification kit. The reaction system of 10 μL consisted of TB Green Premix Ex Taq (5 μL), forward primer (0.2 μL), reverse primer (0.2 μL), Rox Reference Dye □ (0.2 μL), cDNA (1 μL), and ddH_2O (3.4 μL). The reaction procedure for real-time fluorescence quantification was as follows: holding stage: 50℃ for 2 min and 95℃ for 10 min; cycling stage (40 cycles): 95℃ for 15 s and 56℃ for 1 min; and melting curve stage: 95℃ for 15 s, 56℃ for 1 min, 95℃ for 15 s and 56℃ for 15 s. The data were processed by the $2^{-\Delta\Delta C_T}$ method and plotted using GraphPad Prism 9 (GraphPad Software Inc., La Jolla, CA, USA). The specific primer sequences for *AP2, OPT4*, and *Atsus2* were AP2- F: AGCAGCACAACCCTCAACAG and AP2-R: CCAGATGTGCTAAAGACGGAG; AtOPT4 F: CTATGGATACATGAGTATGGCACAAG and AtOPT4 R: GATAGAGTTTAACTGCCACCATGC; Atsus2 F: TAGTGGTACAGAACACGCACACATTCTG and Atsus2 R: GTCTCAAAGAAGCTGACCAAGGTAGCTG.

Declarations

Author contributions

Conceived and designed the experiments: Fu Jun, Yu Xiaomin and Li Shisheng. Performed the experiments: Fu Jun, Li Zhengrong, Jin Die, Zhu Jinjin and Yin Yanni. Analyzed the data: Yu Xiaomin, Li Zhengrong, Jin Die and Zhu Jinjin. Original Draft Preparation: Fu Jun, Li Zhengrong, Jin Die and Li Shisheng. All authors read and approved the final manuscript.

Acknowledgements

This work supported Special Project for Development of Science and Technology in Local by Central Guidance (2018ZYYD019). Young outstanding talent project of Huanggang City.

Conflicts of interest

The authors declare no conflicts of interest.

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Figures

Figure 1

Comparison between radish *ls* mutants and WT radish. Radish *ls* mutants (B, mature; C, young) with longer siliques and WT radish (A, mature; D, young) with normal siliques.

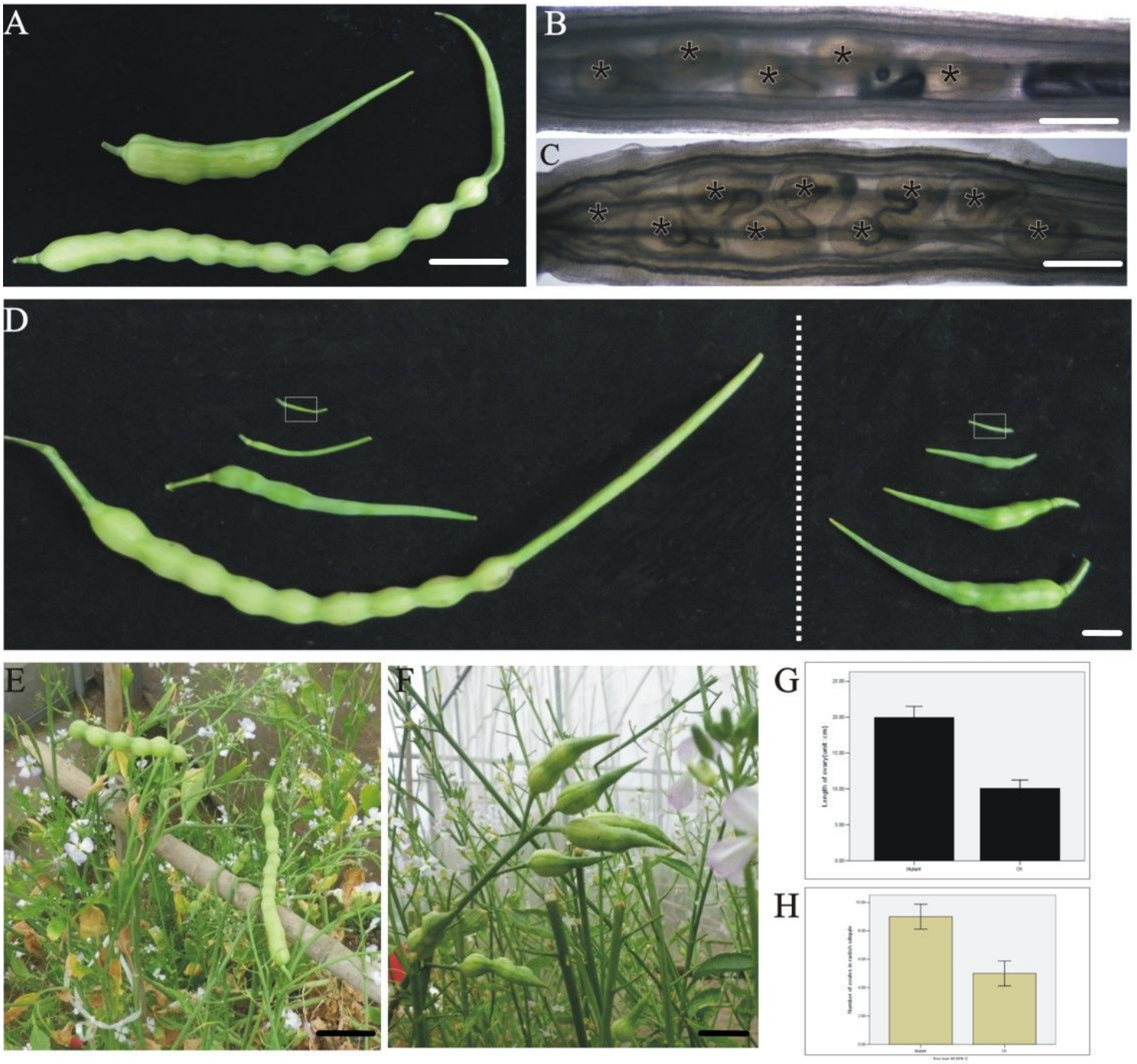

Figure 2

Comparison of silique length and number of ovules in one silique between radish *ls* mutants and WT radish. A: Siliques of radish *ls* mutant (upper) and WT radish (bottom); B, C: Ovule distribution in the silique is shown for the radish *ls* mutant and WT radish (lower and upper panels, respectively); D: The process of silique development in radish *ls* mutant (left) and WT radish (right); E, F: siliques of radish *ls* mutant (E) and WT radish (F); G, H: Statistical results of the comparison of silique length (G) and number of ovules (H) between radish *ls* mutants with longer siliques and WT radish. Bar = 2.5 cm in A, D, E and F; Bar = 0.15 cm in B and C.

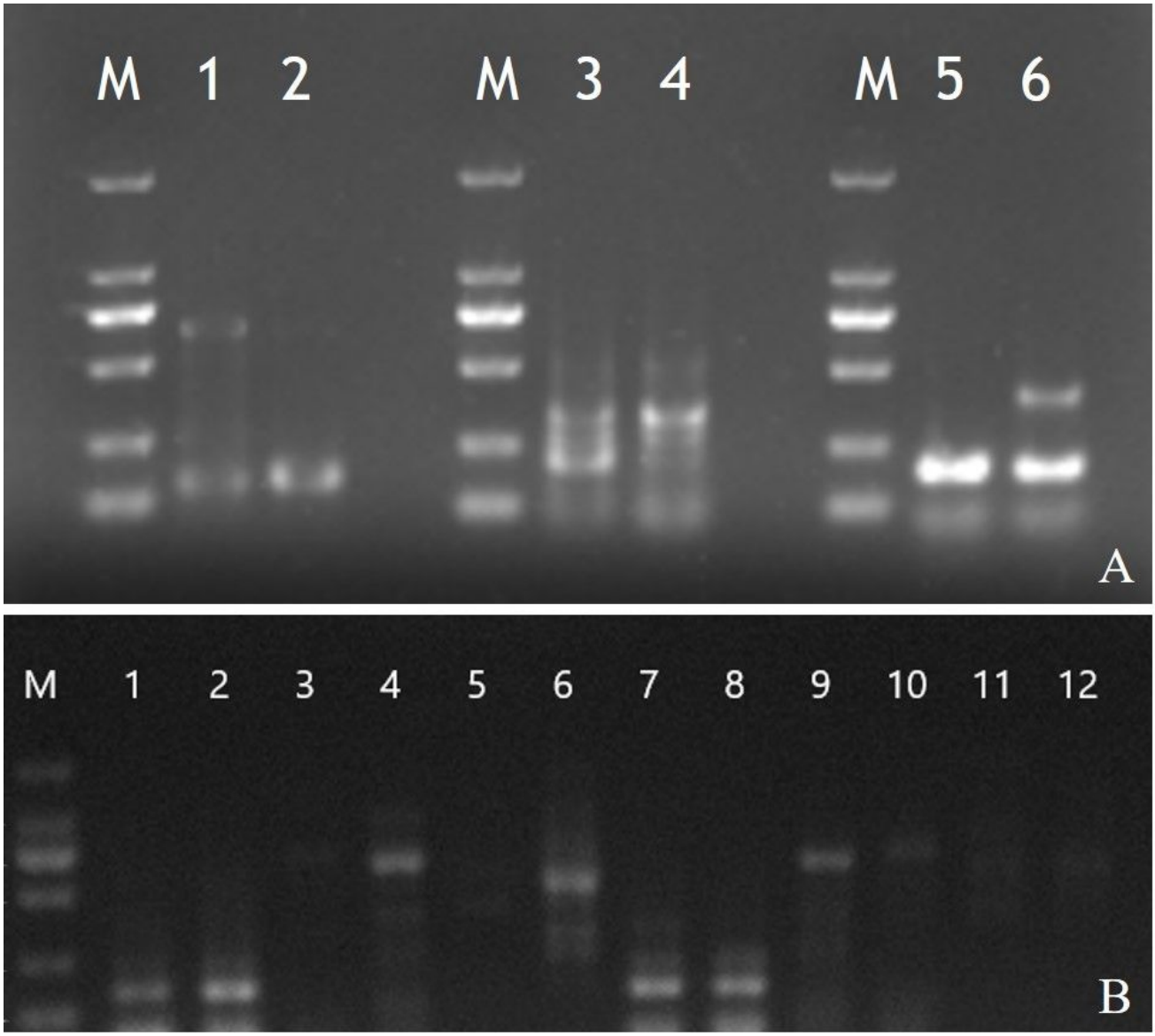

Figure 3

PCR amplification results of molecular markers and semi-quantitative PCR detection of silique-related genes. A. M: marker, lanes 1 and 2: The amplification products of HZ001 for radish *ls* mutant (1) and WT radish (2); lanes 3 and 4: The amplification products of SRC9-022 for radish *ls* mutant (3) and WT radish (4); lanes 5 and 6: The amplification products of OI12F11for radish *ls* mutant (5) and WT radish (6); B. M: marker, lanes 1 and 2: Amplification results of *actin* in early siliques from radish *ls* mutant (1) and WT radish (2); lanes 3 and 4: Amplification results of *LS1* in early siliques from radish *ls* mutant (3) and WT radish (4); lanes 5 and 6: Amplification results of *LS2* in early silique from radish *ls* mutant (5) and WT radish (6); lanes 7 and 8: Amplification results of *actin* in cotyledons from radish *ls* mutant (7) and WT radish (8); lanes 9 and 10: Amplification results of *LS1* in cotyledons from radish *ls* mutant (9) and WT radish (10); lanes 11 and 12: Amplification results of *LS2* in cotyledons from radish *ls* mutant (11) and WT radish (12).

Figure 4

Qualification of *Arabidopsis*-transformed plants overexpressing *LS2(RsNAC66).* A. Electrophoretic assay of overexpression screening. M, DL2000 marker; lanes 1–6 and 8–13 are amplification products of a KANA gene fragment from 1–12 transgenic lines; lane 7 (with bands) corresponds to amplification products of a KANA gene fragment from WT lines; B. *LS2* (*RsNAC66)* genes were detected by semi-quantitative PCR. M, DL2000 DNA marker (2,000, 1,000, 750, 500,or 250 bp); lanes 1–5 show amplification results for WT (lane 1) Arabidopsis leaves and leaves of transgenic lines 3, 5, 7, and 9, respectively; lanes 6–10 show amplification results for WT (lane 9). *Arabidopsis* siliques and siliques of transgenic lines 3, 5, 7, and 9, respectively; C. Relative expression of *RsNAC66.*

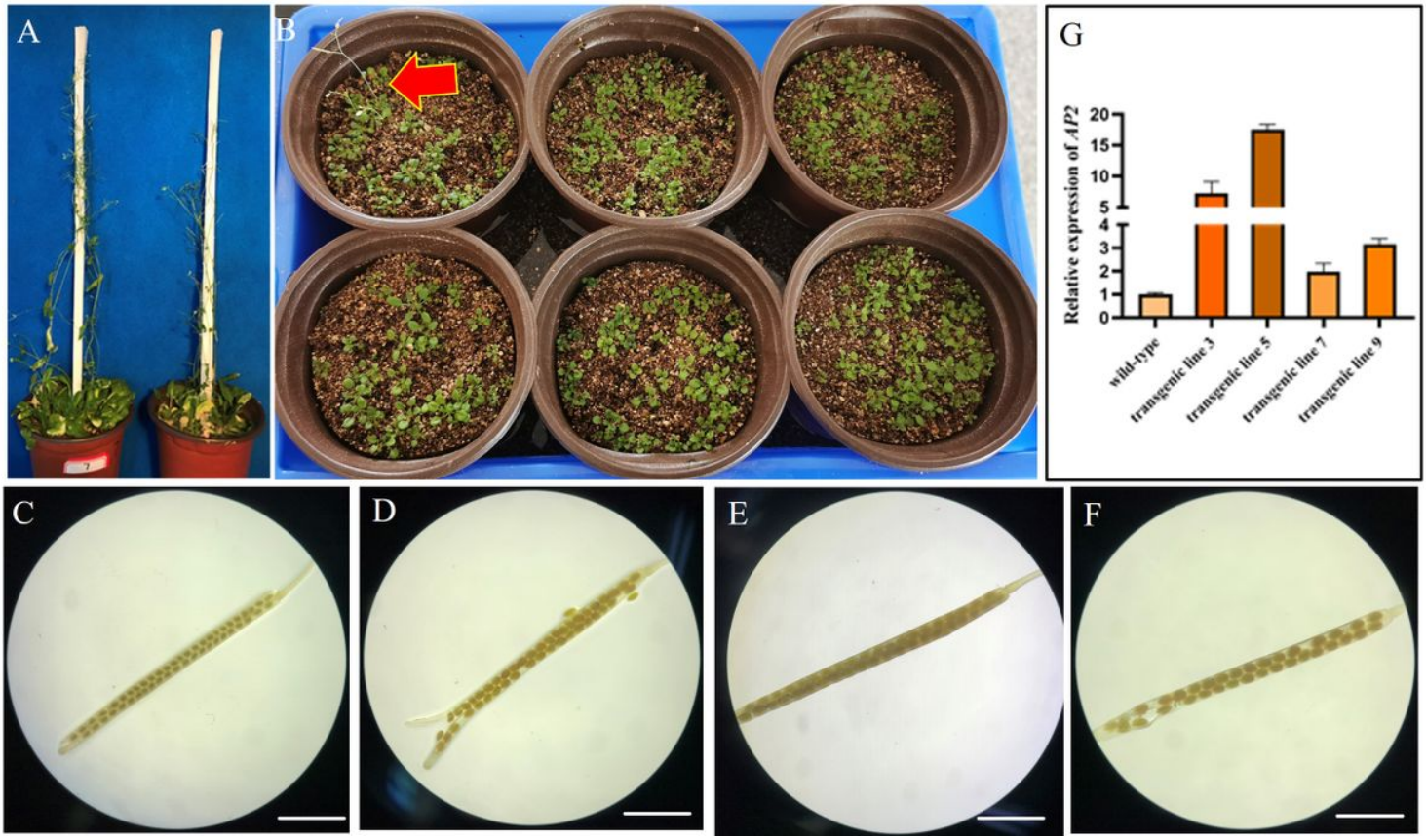

Figure 5

Phenotype analysis of *Arabidopsis thaliana*-transformed plants overexpressing *LS2 (RsNAC66).* A. Height of transgenic lines compared to WT lines. Left, *Arabidopsis*-transformed line 7 (average plant height = 37.9 cm). Right, WT line.(average plant height = 25.2 cm); B. Transformed *Arabidopsis thaliana* showing an early flowering phenotype (denoted by the red arrow); C–F. Microscopic view of siliques from transformed and WT plants, C: Silique of transformation line 3 (49 ovules), D: Silique of transformation line 7 (46 ovules), E: Silique of transformation line 9 (41 ovules), F: WT silique (33 ovules), G. Relative expression of *AP2*. Bar = 0.2 cm.

Supplementary Files

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- TableS1.docx