

***BnERF114.A1*, a Gene Encoding an APETALA2/ETHYLENE RESPONSE FACTOR, Regulates Plant Architecture Through Blocking Auxin Efflux in Apex of Rapeseed Plant**

Jinyang Lyu

Northwest A&F University <https://orcid.org/0000-0003-1752-4399>

Yuan Guo

Northwest A&F University

Chunlei Du

Northwest A&F University

Haibo Yu

Northwest A&F University

Lijian Guo

Northwest A&F University

Li Liu

Northwest A&F University

Xinfa Wang (✉ zyzy12@126.com)

Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences/Key Laboratory of Biology and Genetic Improvement of Oil Crops

Huixian Zhao

Northwest A&F University

Shengwu Hu

Northwest A&F University

Research Article

Keywords: Rapeseed (*Brassica napus* L.), *BnERF114.A1*, Plant architecture, Shoot branching, Apical dominance, Seed yield

Posted Date: May 6th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-476035/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Plant architecture is very important for rapeseed breeding. Here, we reported an ETHYLENE RESPONSE FACTOR (ERF) transcription factor *BnERF114.A1* of *Brassica napus* participating in plant architecture regulation. *BnERF114.A1* is a member of ERF family group x-a, encoding a putative protein of 252 aa which consisting of an AP2/ERF domain and a conserved CMX-1 motif. *BnERF114.A1* located in nucleus and had transcriptional activity with its functional region located in 142 aa ~ 252 aa of its C-terminus. The GUS staining analysis revealed that *BnERF114.A1* highly expressed in leaf primordia, shoot apical meristem, leaf marginal meristem, and reproductive organs. Ectopic expression of *BnERF114.A1* in *Arabidopsis* reduced plant height, increased branch numbers and silique numbers per plants, and finally increased seed yield per plant. Further investigation demonstrated that overexpression of *BnERF114.A1* can inhibit IAA efflux and cause accumulation of auxin in apex, and arrest apical dominance in *Arabidopsis*. The findings suggested *BnERF114.A1* could provide a candidate gene for rapeseed plant architecture molecular breeding.

Key Message

we reported an ETHYLENE RESPONSE FACTOR (ERF) transcription factor *BnERF114.A1* of *Brassica napus* L. participating in plant architecture regulation through blocking auxin efflux in apex of plant.

Introduction

Since “Green revolution” occurred in wheat and rice which greatly increased crop yield since 1960s, much more attention has been paid to improve plant architecture to increase seed yield in rapeseed (canola, *Brassica napus* L.). Plant architecture is the three-dimensional organization of plant organs, adapting the plant for survival in different environments as well as affecting crop yield (Reinhardt and Kuhlemeier 2002). Crop breeders have focused on aerial architecture, including plant height; branch or tiller number and angle; leaf shape, size and angle; and inflorescence morphology (Wang and Li 2008; Wang et al. 2018; Li et al. 2019). Rapeseed is the most widely distributed and the largest sown edible oil crops in China, which provided more than 55% of edible oil in daily life. With the rapid development of national economy and the transfer of large number of rural labor force to the cities, agricultural farming methods are in urgent need of change, and simplification and mechanization has become the main direction of rapeseed breeding and industrial development in China (Luo et al. 2015). The ideal rapeseed plant architecture for mechanical harvesting is of dwarf, lodging resistance, tight plant (branch habit) suitable for dense planting, uniform ripening, cracking resistance and early maturing (Fu 2008; Fu and Zhou 2013). The branch habit of rapeseed plants includes branch position, branch angle and branch numbers, which are important in breeding of mechanized rapeseed varieties (Duan 2015). Therefore, investigation on branch characters and its genetic control mechanism of rapeseed is an important basis for genetic improvement of rapeseed suitable for mechanical harvesting.

In higher plants, the main stem shoot is derived from the primary shoot apical meristem (SAM), and the branches are differentiated from the meristem of axillary buds (AM) (McSteen and Leyser 2005). Branching occurs via a two-step process: initiation of an axillary meristem in each leaf axil to form a bud and subsequent bud outgrowth (Shimizu-Sato and Mori 2001). In some cases, the axillary bud is dormant as a result of complex interactions between endogenous developmental signals, including auxin, cytokinin and strigolactones, and environmental factors such as shade (Kebrom et al. 2010; Domagalska and Leyser 2011; Wang and Li 2011).

It has been demonstrated that plant architecture, a collection of genetically controlled agronomic traits, is mainly associated with the hormones and their signal transduction in plant. "Green revolution" occurred in rice and wheat mainly attributed to the reduced plant height that was caused by the deficiency of Gibberellin synthesis (*sd1*) and blocked degradation of DELLA proteins (*Rht-B1b* and *Rht-D1b*) (Peng et al. 1999; Sasaki et al. 2002). Indole-3-acetic acid (IAA) likewise, as the first discovered plant hormone, plays an important role in regulating plant architecture. It is well known that apical dominance mainly depends on IAA content in apex. IAA synthesis-deficient double mutants *yuc1yuc4* and *yuc2yuc6*, triple mutants *yuc1yuc2yuc6*, *yuc1yuc2yuc4* and *yuc1yuc4yuc6*, and quadruple mutant *yuc1yuc2yuc4yuc6* displayed noticeable reduced plant height and loss of apical dominance (Cheng et al. 2006). Cytokinin (CTK) acts as an antagonistic hormone of apical dominance determined by IAA. *IPT*, cytokinin biosynthesis gene, promotes the outgrowth of axillary buds and inhibits auxin-dependent apical dominance (Shimizu-Sato et al. 2009).

APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) is one of the largest and the most important plant specific transcription factor superfamilies that encompasses 147 members in Arabidopsis (Nakano et al. 2006). All the members of this superfamily are characterized by harboring one or two essential 68-amino-acid repeat motif that was designated the AP2 domain and having DNA-binding activity (Jofuku et al. 1994; Ohme-Takagi and Shinshi 1995; Heyman et al. 2018). According to their structural features, AP2/ERF family members in Arabidopsis can be classified into four subfamilies: AP2 (18 members), RAV (six members), EREB-DREB (122 members), and AP2P-ISAMDD1 (a single member) (Nakano et al. 2006). The AP2 subfamily harboring two PA2 domains contains several key regulators that control different developmental processes during plant growth. RAV-type transcription factors contain a single AP2 domain and a second conserved DNA-binding domain designated B3 located at their C-terminus transcriptionally, and respond to touch-related stimuli (Kagaya et al. 1999; Kagaya and Hattori 2009). The largest subfamily EREB-DREB can be subdivided into 12 subgroups, namely I to X, VI-L and Xb-L, while the members of the X subgroup (ERF108 to ERF115) possess a hallmark of a conserved N-terminal sequence and participate in wound signaling and tissue repair (Nakano et al. 2006; Heyman et al. 2018).

It has been demonstrated that Arabidopsis AP2/ERF transcription factor gene *EBE* (At5g61890) belonging to the X subgroup and highly expressing in proliferating cells affects cell proliferation and axillary bud outgrowth as well as shoot branching (Mehrnia et al. 2013). A recent study revealed that *ERF115*, an X subgroup member of AP2/ERF transcript factors, functions in wound-induced stem cell division and sensitizes cell to auxin (Canher et al. 2020). In additions, *ERF115* also functions as a

repressor of adventitious root initiation by integrating the crosstalk of JA and CK (Lakehal et al. 2020). *ERF109*, another X subgroup member, has been confirmed that could be induced by wounding, and promoted auxin biosynthesis (Ye et al. 2020). Our previous investigation discovered that monosulfuron ester sodium (MES) could act as a new chemical hybridization agent for rapeseed, and low concentration of MES (10 ml per plant of 0.1 µg/ml) treatment could induce male sterility and increase branch numbers of rapeseed (Cheng et al. 2013; Li et al. 2015). Comparative transcriptome analysis between MES-treated and the control plants revealed that about 36% (542/1501) of differential expression transcripts associated with anther development of rapeseed were significantly up-regulated including some kinases and various transcript factors. One of such transcript factors is a integrase-type DNA-binding superfamily protein that is highly homologous with the *EBE* (At5g61890, also known as *AtERF114*) in Arabidopsis (Li et al. 2015), and therefore named as *BnERF114*. It was demonstrated that *EBE* (*AtERF114*) gene belongs to the X subgroup of ERF transcript factor family and contains AP2/ERF domain, which may affect cell proliferation, promote axillary bud generation and branch grow (Mehrnia et al. 2013). However, the characterization and biological function of *BnERF114* in rapeseed (*B. napus*) remains unclear, and whether the phenotype of branch-increasing is related to the up-regulation of *BnERF114* in rapeseed plants treated with low concentration of MES need to be further investigated.

To well understand the biological role of *BnERF114* in growth and development of rapeseed, the homeologs of *BnERF114* were isolated and their phylogenetic analysis was conducted in the present study. The molecular characterization and functional identification of *BnERF114.A1* in growth and development of rapeseed were explored. Our results suggested *BnERF114.A1* could regulate the property of plant shoot branching and probably serve as a candidate gene in rapeseed plant type breeding.

Materials And Methods

Plant materials and growth condition

B. napus cultivar *Zhongshuang No.9* (ZS9) was planted in Yangling Regional Test Station of Crop Varieties, Shaanxi, China (N34.29°, E108.06°) at growing season 2017-2018. It was introduced from Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan, China, and selfed for at least 10 generations prior to being used in the present investigation. *Arabidopsis thaliana* (Col-0) and transgenic plants were grown at 22°C under a 16-h light/8-h dark cycle (light intensity 6000 ~ 9000 lux) and with the humidity of approximate 60% in phytotron.

Gene characterization and evolution analysis

The protein sequence of Arabidopsis AtERF114 (AT5G61890.1) was obtained from TAIR (<http://www.arabidopsis.org/>) website, and it was used as a query sequence to search for homologous genes in *Brassica* with PSI-Blast tool in NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). To confirm the genetic relationship of ERF114s from Brassica and Arabidopsis, evolution analysis was conducted by Mega 5 using conserved domain of AP2 (SMART accession number: SM00380). One hundred and

twenty-two AP2/ERF family proteins from Arabidopsis were downloaded from TAIR web site and employed for constructing neighbor-joining (NJ) tree (Nakano et al. 2006).

Nucleic acid isolation

Total RNA of different rapeseed tissues and transgenic Arabidopsis was extracted using RNA extraction kit (E.Z.N.A. R Plant RNA Kit, OMEGA) according to the manufacturer's protocol, and digested with RNA-free DNase I (Invitrogen) to remove DNA contamination. Genomic DNA was extracted from the young leaf samples of rapeseed or Arabidopsis plants according to the cetyltrimethylammonium bromide (CTAB) method (Porebski et al. 1997). The quality of the RNA and the DNA samples isolated were assessed by 2.0% and 0.8% agarose gel electrophoresis, respectively.

Cloning of the coding sequence and the promoter of *BnERF114.A1* in rapeseed

For cDNA cloning, the first strand cDNA was synthesized using total RNA isolated from young pods of rapeseed *ZS9* by GoScript™ Reverse Transcription System (Promega) according to the manufacturer's protocol. The coding sequence (CDS) of *BnERF114.A1* was isolated from the cDNA by polymerase chain reaction (PCR) using high-fidelity thermostable DNA polymerase KOD-FX-NEO (TOYOBO) and a primer pair BnERF114.A1-F and BnERF114.A1-R (Table S1). The PCR program was performed as pre-denaturation at 98°C for 2 min followed by 40 cycles of 98°C for 10 sec, 58°C for 30 sec, 68°C for 1 min, and final extension at 68°C for 7 min. The PCR products were cloned into the pMD19-T vector (TakaRa, Dalian, China), and five clones randomly chosen were sequenced.

The promoter of *BnERF114.A1* was cloned from genomic DNA of *ZS9* using primer pair 114pro-F and 114pro-R (Table S1). The PCR program was performed as pre-denaturation at 98°C for 2 min followed by 40 cycles at 98°C for 10 sec, 60°C for 30 sec, 68°C for 1 min and final extension at 68°C for 7 min. The products were cloned into the pMD19-T vector (TakaRa, Dalian, China), and five clones randomly selected were sequenced.

Subcellular localization of BnERF114.A1

To investigate the subcellular localization of BnERF114.A1, a primer pair 114SL-F and 114SL-R (Table S1) was used to clone the CDS region of *BnERF114.A1*, and a pGreen-35S::*BnERF114.A1-eGFP* expression vector was constructed using T4 DNA ligase (Takara) at 16°C for 1h after EcoRI and SpeI double digestion. The constructed vector was confirmed by restriction analysis and sequencing. The recombinant vector was precipitated onto 1 µm gold particles (0.85 mg) and then bombarded into onion epidermal cells via a PDS21000/He particle gun (Bio-Rad) as described previously (Christou et al. 1988) with pGreen-35S::*eGFP* as a control. Onion epidermis transient transformation was performed under 1100 psi rupture pressure with 9 cm target tissue distance. After cultured in darkness for 24h, onion epidermis was teared for observation by laser confocal microscope (Zeiss, Germany).

Transcription activity analysis of *BnERF114.A1*

To identify the transcriptional activity of *BnERF114.A1* and its exact region, the full length cDNA sequence of *BnERF114.A1*, the 246 bp in the 3'-end of *BnERF114.A1*, the 177 bp encoding AP2 domain, and the 333 bp in the 5'-end of *BnERF114.A1* were cloned by PCR, with primer pairs E756-F/E756-R, E246-F/E246-R, E177-F/E177-R, and E333-F/E333-R, respectively (Table S1). These target segments were separately introduced into pGBKT7 (Clontech) vector by T4 DNA ligase (Takara) at 16°C for 1h after NdeI and EcoRI double digestion (Takara). The recombinant constructs were transformed into yeast strain *AH109* using PEG-LiCl method (Gietz and Schiestl 2007), with empty pGBKT7 and the modified pGBKT7-p53 (only containing the DNA binding region) as negative controls, respectively. These colonies were screened on the SD/-Trp and SD/-Trp/-Ade/-His medium.

Construction of *BnERF114.A1* overexpression vector and Arabidopsis transformation

CDS of *BnERF114.A1* was introduced into a binary expression vector pCAMBIA3301 driven by *CAMV 35S* promoter (*35S*) by BamHI and SpeI double digestion and T4 DNA ligation (Takara), to generate *35S::BnERF114.A1-GUS* vector. The expression cassette *114pro::BnERF114.A1-GUS* was constructed by substituting the *35S* promoter of *35S::BnERF114.A1-GUS* vector for native promoter of *BnERF114.A1* (*114pro*) through EcoRI and BamHI double digestion and T4 ligation (Takara). The resulted vectors *35S::BnERF114.A1-GUS* and *114pro::BnERF114.A1-GUS* were confirmed by sequencing, and transformed into *A. thaliana* (Col-0) by *Agrobacterium tumefaciens*-mediated floral-dip method (Zhang et al. 2006). Transgenic plants were screened by spraying 0.1% glufosinate (BASTA) on seedling leaves, and independent transgenic homozygous lines were obtained by self-breeding and spraying 0.1% glufosinate (BASTA) on seedling leaves. To assess the effects of *BnERF114.A1* on transgenic plants, wild-type Arabidopsis and the obtained homozygous transgenic lines were grown at green houses as described above.

GUS staining analysis of *BnERF114.A1* promoter activity

GUS activity was determined as described previously (Plesch et al. 2001). T₃ homozygous transgenic plants of *114pro::BnERF114.A1-GUS* were used for GUS activity analysis. Different tissues were incubated in GUS staining solution (50mM Phosphate buffer (pH=7.2), 0.01% Triton X-100, 2mM K₃Fe(CN)₆, 2mM K₄[Fe(CN)₆]·3H₂O, 10mM EDTA, 0.2mM X-Gluc) for 16 hours at 37°C. Wounded leaves were infiltrated in staining solution after injured with a knife and incubated at 37°C for 45 min. After treated with incremental concentration of ethyl alcohol, plant tissues were observed with stereomicroscope (Olympus SZ61) and camera (Canon 3000D).

Quantitative real-time reverse transcriptase-polymerase chain reaction

Quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) was conducted to analyze the spatio-temporal expression patterns of *BnERF114s* in *B. napus*. The cDNA products of different tissues were normalized with housekeeping gene *Ubiquitin-conjugating enzyme 21* of *B. napus* (*BnUBC21* Gene ID: 106348550) as an internal reference gene. Three biological and three technical replicates were included for each RNA sample/primer combination for the qRT-PCR. Four primer pairs

BnERF114.C2-qF/BnERF114.C2-qR, BnERF114.A1-qF/BnERF114.A1-qR, BnERF114.A6-qF/BnERF114.A6-qR, and BnERF114.C3-qF/BnERF114.C3-qR were used for analyzing expression levels of *BnERF114.A1*, *BnERF114.C2*, *BnERF114.A6*, and *BnERF114.C3*, respectively (Table S1).

The cDNA products of wild-type plants and transgenic lines were normalized using *AtUBC21* (TAIR ID: At5g25760) as a reference gene to determine the expression level of auxin polar transport related genes and IAA synthesis genes. Three biological replicates were included. The qRT-PCR was performed in triplicate for each RNA sample/primer combination. The primer pairs used for qRT-PCR of *AtPIN1~AtPIN8*, *AtAUX1*, *AtLAX1~AtLAX3*, *AtPGP1*, *AtPGP2*, *AtPGP4*, *AtPGP19*, *AtYUCCA1*, *AtYUCCA2*, *AtYUCCA4*, and *AtYUCCA6* were shown in Table S1. The program of qRT-PCR was as follows: pre-denaturation at 94°C for 4 min, followed by 40 cycles at 94°C for 20 sec, 60°C for 20 sec, 72°C for 20 sec and finally determined melting curve from 70°C to 95°C for 5 sec. The qRT-PCR was performed with GoTaq®qPCR master mix (A6001, Promega) on a QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems). For each PCR, the specificity of the amplification was validated and the threshold cycle above background was calculated using Bio-Rad iCycler software, and PCR efficiency close to 100%.

The relative expression levels of the individual target genes were calculated by an improved double delta method (Pfaffl et al., 2002). Error bars of qRT-PCR data in all figures represented standard deviations, and significant differences were estimated at P=0.05 level using Student's T test.

Endogenous IAA content analysis

The main inflorescences from 10 forty-day-old transgenic plants and wild type *Arabidopsis* were collected for IAA measurement, three biological replicates were included. IAA content was determined using Agilent 160 Infinity-6420 liquid chromatograph-mass spectrometer (Agilent Technologies) by Tsingtao Sci-tech innovation limited company.

Phenotype measurement and statistical analysis

To evaluate the effect of *BnERF114.A1* on transgenic plant, plant height, the length of main inflorescence, numbers of the primary branches of transgenic plants and wild type plants were determined at 30, 37, 44, and 51 days after planting. Numbers of siliques per plant, number of seeds per silique, seed yield per plant and biomass per plant were evaluated at maturity. At least 10 plants of each independent transgenic lines or wild type were randomly selected for assessing these traits above mentioned. Statistical analysis was performed with unpaired t-test to evaluate significant difference.

Results

Homology-based cloning and molecular characterization of *BnERF114*

To clone the orthologs of *EBE* (*AtERF114*) in *B. napus*, we blasted the full length amino acids of *AtERF114* (AT5G61890.1) against *Brassica* Non-redundant protein sequences database of NCBI. As a result, eight highly homologous genes were obtained, with four genes from *B. napus*, two from *B. rapa*,

and two from *B. oleracea* (Table S2). To identify the phylogenetic relationship of *BnERF114s* in *B. napus*, phylogenetic analysis and homologous analysis were conducted using AP2/ERF conserved domain of BnERF114s, BrERF114s, BoERF114s and Arabidopsis AP2/ERF superfamily genes. The result suggested that BnERF114.C2 derived from BoERF114.C2 of *B. oleracea*, BnERF114.A6 from BrERF114.A6 of *B. rapa*, BnERF114.C3 from BoERF114.C3 of *B. oleracea*. However, BnERF114.A1 may be derived from BrERF114.A2 (Figure S1, A). Phylogenetic analysis of *BnERF114s* and all *ERF* family members of Arabidopsis showed that *BnERF114s* were orthologs of *AtERF114* (Figure S1, B) and belonged to the group X-a of AP2/ERF superfamily (Nakano et al. 2006). Consistent with *AtERF114*, all four *BnERF114s* contained two exons and one intron (Figure 1, A), and the encoding proteins contained an AP2/ERF domain (Figure 1, A & C) and a conserved motif CMX-1 (Figure 1, A & B). These results indicated that *BnERF114s* from *B. napus* were orthologs of *AtERF114*.

***BnERF114.A1* expresses in proliferating tissue**

To explore the spatio-temporal expression profile of *BnERF114s*, we measured their transcriptional levels in 13 different tissues of rapeseed cultivar ZS9. The results showed that the expression level of *BnERF114.A1* was particularly higher than other three copies (*BnERF114.C2*, *BnERF114.A6*, and *BnERF114.C3*) in all tissues, indicating that *BnERF114.A1* probably worked as a major gene among four *BnERF114s*. *BnERF114.A1* highly expressed in root, cotyledon, flower, sepal, petal and pod, and had lower expression levels in other tissues such as rosette leaf, stem, small and middle bud, stamen and pistil (Figure 2, A). Considering the expression profiles of the *BnERF114s* during rapeseed development described above, *BnERF114.A1* was selected for subsequent functional studies.

To further detect the expression position of *BnERF114.A1*, we fused the -1836 bp ~ 0 bp of *BnERF114.A1* promoter sequence (114pro) and the CDS of *BnERF114.A1* with *GUS*. The recombinant construct 114pro::BnERF114.A1-GUS was transformed into Arabidopsis by *Agrobacterium tumefaciens*-mediated method. The GUS activity was analyzed in different tissues of transgenic Arabidopsis plants. The results exhibited that *BnERF114.A1* remarkably expressed in leaf primordia, shoot apical meristem, leaf marginal meristem, tender cauline leaves (Figure 2, B~I), senescent leaves and cutting positions (Figure 2, J), and reproductive organs which including pistils and anthers (Figure 2, K~Q). In view of this, mechanical injury was performed on middle-aged leaves at 45 minutes before GUS staining. The result showed that mechanical injury could strongly and rapidly induce the expression of *BnERF114.A1* (Figure 2, R). These results suggested that *BnERF114.A1* may be involved in biological process of cell proliferation and damage signal response.

BnERF114.A1 mainly located in nucleus and had transcriptional activity

To confirm the subcellular localization of BnERF114.A1, the full length CDS of *BnERF114.A1* from rapeseed cultivar ZS9 was isolated and fused with *eGFP* in frame to construct transient expression vector p35S::BnERF114.A1-eGFP. This transient expression vector was introduced into onion epidermal cells to express BnERF114.A1-eGFP fusion protein, with p35S::eGFP as a control. The result indicated that BnERF114.A1 mainly located in nucleus (Figure 3).

AP2 domain of *BnERF114.A1* was predicted to be 83~141 amino acid. According to previous research (Nakano et al. 2006), this domain should be the DNA binding domain. To identify the transcription activity of *BnERF114.A1* and active domain position, we introduced different fragments of the *BnERF114.A1* coding region, including full length CDS, N-terminus (246-), AP2 domain (-177-) and C-terminus of *BnERF114.A1*(-333) into pGBKT7 vector, respectively. The recombinant constructs were transformed into the yeast *AH109* and screened on the yeast medium SD/-Trp and SD/-Trp/-Ade/-His. Yeast self-activation experiments proved that *BnERF114.A1* had transcriptional activity and its active region was located in 142 aa ~ 252 aa of its C-terminus (Figure 4).

Ectopic expression of *BnERF114.A1* in Arabidopsis reduced plant height and increased branch numbers

To explore the effects of *BnERF114.A1* on plant architecture, we developed 35S::*BnERF114.A1* (named OE₃₅ for short) and 114pro::*BnERF114.A1* (named OE₁₁₄ for short) transgenic Arabidopsis. At the beginning of bolting stage (about 30 days post planting), no differences of phenotype could be detected between wild type (Col-0) and transgenic Arabidopsis lines ectopically expressing *BnERF114.A1*. After 30 days post planting, it was observed that ectopic expression of *BnERF114.A1* significantly inhibited the elongation of main inflorescence, which led to reduce plant height, with OE₃₅-18-1 as a representative of OE₃₅ lines and OE₁₁₄-46-3 as a representative of OE₁₁₄ lines shown in Figure 5 A~C because of their similar phenotypes among the same genotype. In addition, ectopic expression of *BnERF114.A1* accelerated the emergence and outgrowth of lateral branches and first-order rosette branches of transgenic lines (Figure 5, D & E). OE₃₅ and OE₁₁₄ transgenic lines produced more rosette branches after 51 days of growing, compared with the wild type plants (Figure 5, E). The expression level of *BnERF114.A1* in transgenic lines and wild type plants was determined, and the result showed that transgenic lines (OE₃₅ and OE₁₁₄) had significantly higher expression levels of *BnERF114.A1* in the main inflorescences, rosette branch inflorescences and tender cauline leaves, compared to the wild type plants (Figure 5, F). These results suggested that *BnERF114.A1* participated in regulating plant branching habit and inhibiting apical dominance of plants.

Ectopic expression of *BnERF114.A1* in Arabidopsis increased seed yield

To evaluate the effect of *BnERF114.A1* on seed yield, we investigated yield-related traits of wild type Arabidopsis and transgenic lines overexpressing *BnERF114.A1*. Considering OE₃₅ lines with significant higher expression level of *BnERF114.A1* than OE₁₁₄ lines, we compared the yield-related traits of OE₃₅ transgenic lines with those of the wild type plants. The OE₃₅ transgenic lines had a greater number of siliques per plant (270-320) than wild type Arabidopsis (170) (Figure 6, A), and had similar number of seeds per silique and thousand-seed weight as wild type plants (Figure 6, B & C). OE₃₅ transgenic lines showed much higher seed yield per plant (0.12-0.22g) and biomass per plant (0.8 -1.2g), compared with the wild type plant (0.06g and 0.5g, respectively) (Figure 6, D and E), this might be attributed to the ectopic expression of *BnERF114.A1* greatly promoting the emergence and outgrowth of shoot branches (Figure 5, E), which increased number of siliques per plant (Figure 6, A).

Ectopic expression of *BnERF114.A1* influenced the efflux of IAA in the main inflorescence of Arabidopsis

Apical dominance of plants is related to the synthesis and distribution of auxin in plants; Auxin synthesized from the apex of the plant is polar transported down to the lateral branches, thus inhibiting the growth of the lateral buds. It is reported that, decapitation of Arabidopsis induced the expression of *AtERF114* in the five uppermost lateral branches beside the main inflorescences, consequently, the plants performed more lateral branches and cespitose phenotype (Mehrnia et al. 2013). In the present study, ectopic expression of *BnERF114.A1* inhibited apical dominance of Arabidopsis, so we suspected that either auxin synthesis and/or auxin polar transport was blocked. To test our hypothesis, we determined the expression levels of IAA biosynthesis genes *YUCCA1*, *YUCCA2*, *YUCCA4* and *YUCCA6*, which have been reported playing important roles in auxin-dependent apex dominance (Cheng et al. 2006). The results showed that expression of *YUCCA1*, *YUCCA2*, *YUCCA4* and *YUCCA6* was significantly decreased compared with wild type plants (Figure 7, A). We further determined the expression levels of IAA transport carriers (including *PINs*, *AUX1/LAXs* and *PGPs*) in the main inflorescences of transgenic lines and wild type Arabidopsis. The results showed that expression levels of *PIN1*, *PIN3*, *PIN5*, *PIN6*, *AUX1/LAXs*, *PGP2* and *PGP19* in transgenic lines significantly decreased in various degrees (Figure 7, B~D), however, the expression levels of *PIN4* and *PGP4* increased. These suggested that ectopic expression of *BnERF114.A1* inhibited both auxin synthesis and the flux of IAA in Arabidopsis. So, we assumed that IAA was accumulated in the main inflorescences of transgenic lines. To further confirm this, endogenous IAA contents of main inflorescences of wild type and transgenic lines OE₃₅-18-1 was measured by liquid chromatogram. As we expected, the IAA contents of main inflorescences from transgenic lines dramatically increased, compared to that of wild type (Figure 7, E). These results indicated that ectopic expression of *BnERF114.A1* influence endogenous IAA content of the main inflorescences and flux of IAA in Arabidopsis.

Discussion

Ideal plant architecture (ideotype) is an important objective in rapeseed breeding (Fu and Zhou 2013). Identification and characterization of more candidate genes for ideotype will contribute molecular designed breeding in this area. In this study, we characterized a new candidate ERF transcript factor gene *BnERF114.A1* that belongs to a subgroup X-a member of ERF supfamily. Our results indicated that *BnERF114.A1* located in nucleus and possessed transcription activity. *BnERF114.A1* expressed in proliferating tissues. Ectopic expression of *BnERF114.A1* in Arabidopsis reduced plant height, inhibited apical dominance, enhanced shoot branch numbers, and finally, increased seed yield per plant and biomass. However, it did not affect harvest index. *BnERF114.A1* regulated plant architecture by influencing the efflux of IAA and endogenous IAA content of the main inflorescences of Arabidopsis.

Previous study showed that *AtERF114* highly expressed in undifferentiated suspension culture cells and callus (Zimmermann et al. 2004). Menges et al. confirmed that *AtERF114* had an elevated expression level at S phase of cell cycle, suggesting *ERF114* related to cell proliferating and division (Menges et al, 2003). In Arabidopsis, *AtEBE* (*AtERF114*) prominently expressed in root tips, shoot apex, young leaves

and reproductive organs (Mehrnia et al. 2013). In the present study, consistently, *BnERF114.A1* generally expressed in active cell division tissues, such as leaf primordia, shoot apical meristem, leaf marginal meristem, tender cauline leaves and reproductive organs (Figure 4). In addition, previous studies showed that *ERF114* and its homologous genes like *ERF109/ERF115* could be induced by ROS signal which aroused by stress or wounding (Kong et al. 2018; Zhou et al. 2019; Canher et al. 2020). In the present study, strong GUS activity was also detected in senescent and wounded leaves (Figure 2, J and R), suggesting *BnERF114.A1* also responded to ROS signal.

AtEBE (AtERF114) stimulates axillary bud formation and outgrowth, while its RNAi lines showed inhibited bud outgrowth and less branches than WT plant (Mehrnia et al. 2013). In the present study, overexpression of *BnERF114.A1* in Arabidopsis remarkably inhibited apical dominance and promoted first-order lateral branches (Figure 5), indicating that *BnERF114.A1* probably had similar function to *AtEBE* in promoting emergence and outgrowth of axillary buds. According to previous research, *AtERF114* belongs to ERF transcription factor family X-a subgroup. This group is consist of eight members including ERF108~ERF115 (Nakano et al. 2006). Wound-induced JA could activate ERF109 that upregulate the expression of a tryptophan biosynthesis-related gene (*ASA1*) in auxin biosynthesis pathway, resulting de novo root regeneration (Zhang et al. 2019). In view of this, we determined the auxin level in *BnERF114.A1* transgenic lines. The result showed that overexpression of *BnERF114.A1* strikingly enhanced the accumulation of auxin in main inflorescens, suggesting that *BnERF114.A1* had similar effect on auxin accumulation like *AtERF109*.

Auxin plays a vital role in regulating plant shoot branching and maintaining apical dominance (Leyser 2003). Long distance transport of endogenous IAA is performed in a polar manner depending on auxin polar transport carriers. It has been demonstrated that auxin efflux carrier PIN1 expressed in the vascular tissues and root primordial, regulating adventitious roots development and floral buds formation (Okada et al. 1991; Benková et al. 2003). In rice, down-regulated expression of *OsPIN1* resulted in increased plant tiller number depending on auxin contents (Xu et al. 2005). Intercellular transport carrier PGP19 has the same location with PIN1 and can stabilize PIN1 on cytomembrance, coordinately regulating auxin long-distance transport (Gälweiler et al. 1998; Blakeslee et al. 2007; Titapiwatanakun et al. 2009). In our study, ectopic expression of *BnERF114.A1* caused decreased expression levels of *PIN1*, *PIN3*, *PIN5*, *PIN6*, *AUX1/LAXs*, *PGP2* and *PGP19* in Arabidopsis (Figure7, B~D), suggesting the efflux of IAA from apex to axillary bud being blocked. This was in line with the fact that IAA content was greatly increased in the main inflorescence of transgenic lines (Figure 7, E). The block of IAA efflux and accumulation of IAA in the main inflorescence resulted in the phenotype of reduced plant height and increased number of branches (Figure 5A).

YUCCAs play important roles in endogenous IAA biosynthesis. IAA synthesis-deficient double mutants *yuc1yuc4*, and *yuc2yuc6*, triple mutants *yuc1yuc2yuc6*, *yuc1yuc2yuc4* and *yuc1yuc4yuc6*, and quadruple mutant *yuc1yuc2yuc4yuc6* displayed noticeable reduced plant height and loss of apical dominance (Cheng et al. 2006). In the present study, ectopic expression of *BnERF114.A1* resulted in decreased expression level of *YUCCA1*, *YUCCA2*, *YUCCA4* and *YUCCA6* (Figure 7, A), but increased IAA content in

main inflorescence (Figure 7, E). We assumed that the increased IAA content might be the result of IAA accumulation when the efflux of IAA from apex to axillary bud being blocked. The decreased expression level of *YUCCAs* might be the result of feedback inhibition by IAA accumulation. This also explained that the blocked elongation of transgenic plant main stems occurred at approximately 10 days after bolting rather than the beginning of bolting stage.

In general, we isolated *BnERF114.A1*, an ortholog gene of *AtERF114* in *B. napus*, which is expressed in leaf primordia, shoot apical meristem, leaf marginal meristem and reproductive organs. Ectopic expression of *BnERF114.A1* reduced plant height, increased the number of shoot branching, and arrested apical dominance increased seed yield per plant in *Arabidopsis*. Ectopic expression of *BnERF114.A1* regulates plant architecture through blocking auxin efflux in apex from apex to basal position. The findings suggested *BnERF114.A1* was probably a valuable gene for rapeseed plant architecture molecular breeding.

Declarations

Conflict of interest The authors declare that they have no conflict of interest

Acknowledgements

This work was financially supported by Open Project Fund of Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, P.R.China (Grant no. KF2018006) and National Natural Science Foundation of China (Grant no. 31801393).

Author contribution statement

Wang X, Hu S and Zhao H conceived and designed the experiments. Lyu J and Guo Y conducted the experiments and analyzed the data. Du C, Yu H and Liu L conducted parts of experiments. Guo L developed recombinant vectors. Lyu J wrote the draft of the manuscript, and Hu S and Zhao H revised the the manuscript. All authors read and approved the final manuscript.

References

1. Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, Friml J (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115(5):591–602. [https://doi.org/10.1016/s0092-8674\(03\)00924-3](https://doi.org/10.1016/s0092-8674(03)00924-3)
2. Blakeslee JJ, Bandyopadhyay A, Lee OR, Mravec J, Titapiwatanakun B, Sauer M, Makam SN, Cheng Y, Bouchard R, Adamec J, Geisler M, Nagashima A, Sakai T, Martinoia E, Friml J, Peer WA, Murphy AS (2007) Interactions among PIN-FORMED and P-Glycoprotein auxin transporters in *Arabidopsis*. *Plant Cell* 19(1):131–147. <https://doi.org/10.1105/tpc.106.040782>
3. Canher B, Heyman J, Savina M, Devendran A, Eekhout T, Vercauteren I, Prinsen E, Matosevich R, Xu J, Mironova V, De Veylder L (2020) Rocks in the auxin stream: Wound-induced auxin accumulation and

- ERF115* expression synergistically drive stem cell regeneration. Proc Natl Acad Sci USA 117(28):16667–16677. <https://doi.org/10.1073/pnas.2006620117>
4. Cheng Y, Dai X, Zhao Y (2006) Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in *Arabidopsis*. Genes Dev 20(13):1790–1799. <https://doi.org/10.1101/gad.1415106>
 5. Cheng Y, Wang Q, Li Z, Cui J, Hu S, Zhao H, Chen M (2013) Cytological and comparative proteomic analyses on male sterility in *Brassica napus* L. induced by the chemical hybridization agent monosulphuron ester sodium. PloS One 8(11):e80191. <https://doi.org/10.1371/journal.pone.0080191>
 6. Christou P, McCabe DE, Swain WF (1988) Stable transformation of soybean callus by DNA-Coated gold particles. Plant Physiol 87(3):671–674. <https://doi.org/10.1104/pp.87.3.671>
 7. Domagalska MA, Leyser O (2011) Signal integration in the control of shoot branching. Nature reviews. Molecular Cell Biology 12(4):211–221. <https://doi.org/10.1038/nrm3088>
 8. Duan XJ (2015) Genome-wide association analysis of plant-type related traits in rapeseed (*Brassica napus* L.). Master, Southwest University, Chongqing, China
 9. Fu T (2008) On research and application of heterosis in rapeseed. Chinese Journal of Oil Crop Sciences 30:1–5
 10. Fu T, Zhou Y (2013) Progress and future development of hybrid rapeseed in China. Eng Sci(05):13–18. <https://doi.org/10.3969/j.issn.1672-4178.2013.05.002>
 11. Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. Science 282(5397):2226–2230. <https://doi.org/10.1126/science.282.5397.2226>
 12. Gietz RD, Schiestl RH (2007) Quick and easy yeast transformation using the LiAc/SS carrier DNA/PEG method. Nat Protoc 2(1):35–37. <https://doi.org/10.1038/nprot.2007.14>
 13. Heyman J, Canher B, Bisht A, Christiaens F, De Veylder L (2018) Emerging role of the plant ERF transcription factors in coordinating wound defense responses and repair. J Cell Sci 131(2):jcs208215. <https://doi.org/10.1242/jcs.208215>
 14. Jofuku KD, den Boer BG, Van Montagu M, Okamoto JK (1994) Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. Plant Cell 6(9):1211–1225. <https://doi.org/10.1105/tpc.6.9.1211>
 15. Kagaya Y, Hattori T (2009) *Arabidopsis* transcription factors, RAV1 and RAV2, are regulated by touch-related stimuli in a dose-dependent and biphasic manner. Genes Genet Syst 84(1):95–99. <https://doi.org/10.1266/ggs.84.95>
 16. Kagaya Y, Ohmiya K, Hattori T (1999) RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants. Nucleic Acids Res 27(2):470–478. <https://doi.org/10.1093/nar/27.2.470>
 17. Kebrom TH, Brutnell TP, Finlayson SA (2010) Suppression of sorghum axillary bud outgrowth by shade, phyB and defoliation signalling pathways. Plant Cell Environment 33(1):48–58.

<https://doi.org/10.1111/j.1365-3040.2009.02050.x>

18. Kong X, Tian H, Yu Q, Zhang F, Wang R, Gao S, Xu W, Liu J, Shani E, Fu C, Zhou G, Zhang L, Zhang X, Ding Z (2018) PHB3 maintains root stem cell niche identity through ROS-responsive AP2/ERF transcription factors in *Arabidopsis*. *Cell Reports* 22(5):1350–1363.
<https://doi.org/10.1016/j.celrep.2017.12.105>
19. Lakehal A, Dob A, Rahnesan Z, Novák O, Escamez S, Alallaq S, Strnad M, Tuominen H, Bellini C (2020) *ETHYLENE RESPONSE FACTOR 115* integrates jasmonate and cytokinin signaling machineries to repress adventitious rooting in *Arabidopsis*. *New Phytol* 228(5):1611–1626.
<https://doi.org/10.1111/nph.16794>
20. Leyser O (2003) Regulation of shoot branching by auxin. *Trends Plant Sci* 8(11):541–545.
<https://doi.org/10.1016/j.tplants.2003.09.008>
21. Li H, Li J, Song J, Zhao B, Guo C, Wang B, Zhang Q, Wang J, King GJ, Liu K (2019) An auxin signaling gene *BnaA3.IAA7* contributes to improved plant architecture and yield heterosis in rapeseed. *New Phytol* 222(2):837–851. <https://doi.org/10.1111/nph.15632>
22. Li Z, Cheng Y, Cui J, Zhang P, Zhao H, Hu S (2015) Comparative transcriptome analysis reveals carbohydrate and lipid metabolism blocks in *Brassica napus* L. male sterility induced by the chemical hybridization agent monosulfuron ester sodium. *BMC Genom* 16(1):206.
<https://doi.org/10.1186/s12864-015-1388-5>
23. Luo H, Tang C, Guan C, Wu M (2015) Research progress in rapeseed mechanized harvest. *Journal of Agricultural Mechanization Research* (1):1–8. <https://doi.org/10.13427/j.cnki.njyi.2015.01.001>
24. McSteen P, Leyser O (2005) Shoot branching. *Annu Rev Plant Biol* 56:353–374.
<https://doi.org/10.1146/annurev.arplant.56.032604.144122>
25. Mehrnia M, Balazadeh S, Zanol MI, Mueller-Roeber B (2013) EBE, an AP2/ERF transcription factor highly expressed in proliferating cells, affects shoot architecture in *Arabidopsis*. *Plant Physiol* 162(2):842–857. <https://doi.org/10.1104/pp.113.214049>
26. Menges M, Hennig L, Gruissem W, Murray JA (2003) Genome-wide gene expression in an *Arabidopsis* cell suspension. *Plant Mol Biol* 53(4):423–442.
<https://doi.org/10.1023/B:PLAN.0000019059.56489.ca>
27. Nakano T, Suzuki K, Fujimura T, Shinshi H (2006) Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice. *Plant Physiol* 140(2):411–432. <https://doi.org/10.1104/pp.105.073783>
28. Ohme-Takagi M, Shinshi H (1995) Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. *Plant Cell* 7(2):173–182. <https://doi.org/10.1105/tpc.7.2.173>
29. Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y (1991) Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. *Plant Cell* 3(7):677–684.
<https://doi.org/10.1105/tpc.3.7.677>
30. Pfaffl MW, Horgan GW, Leo D (2002) Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* 30(9):e36. <https://doi.org/10.1093/nar/30.9.e36>

31. Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400(6741):256–261.
<https://doi.org/10.1038/22307>
32. Plesch G, Ehrhardt T, Mueller-Roeber B (2001) Involvement of TAAAG elements suggests a role for Dof transcription factors in guard cell-specific gene expression. *Plant J* 28(4):455–464.
<https://doi.org/10.1046/j.1365-313x.2001.01166.x>
33. Porebski S, Bailey LG, Baum BR (1997) Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter* 15(1):8–15. <https://doi.org/10.1007/bf02772108>
34. Reinhardt D, Kuhlemeier C (2002) Plant architecture. *EMBO Rep* 3(9):846–851.
<https://doi.org/10.1093/embo-reports/kvf177>
35. Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M (2002) A mutant gibberellin-synthesis gene in rice. *Nature* 416(6882):701–702. <https://doi.org/10.1038/416701a>
36. Shimizu-Sato S, Mori H (2001) Control of outgrowth and dormancy in axillary buds. *Plant Physiol* 127(4):1405–1413. <https://doi.org/10.1104/pp.127.4.1405>
37. Shimizu-Sato S, Tanaka M, Mori H (2009) Auxin-cytokinin interactions in the control of shoot branching. *Plant Mol Biol* 69(4):429–435. <https://doi.org/10.1007/s11103-008-9416-3>
38. Titapiwatanakun B, Blakeslee JJ, Bandyopadhyay A, Yang H, Mravec J, Sauer M, Cheng Y, Adamec J, Nagashima A, Geisler M, Sakai T, Friml J, Peer WA, Murphy AS (2009) ABCB19/PGP19 stabilises PIN1 in membrane microdomains in Arabidopsis. *Plant J* 57(1):27–44.
<https://doi.org/10.1111/j.1365-313X.2008.03668.x>
39. Wang B, Smith SM, Li J (2018) Genetic regulation of shoot architecture. *Annu Rev Plant Biol* 69:437–468. <https://doi.org/10.1146/annurev-arplant-042817-040422>
40. Wang Y, Li J (2008) Molecular basis of plant architecture. *Annu Rev Plant Biol* 59:253–279.
<https://doi.org/10.1146/annurev.arplant.59.032607.092902>
41. Wang Y, Li J (2011) Branching in rice. *Curr Opin Plant Biol* 14(1):94–99.
<https://doi.org/10.1016/j.pbi.2010.11.002>
42. Xu M, Zhu L, Shou H, Wu P (2005) A *PIN1* family gene, *OsPIN1*, involved in auxin-dependent adventitious root emergence and tillering in rice. *Plant Cell Physiology* 46(10):1674–1681.
<https://doi.org/10.1093/pcp/pci183>
43. Ye BB, Shang GD, Pan Y, Xu ZG, Zhou CM, Mao YB, Bao N, Sun L, Xu T, Wang JW (2020) AP2/ERF transcription factors integrate age and wound signals for root regeneration. *Plant Cell* 32(1):226–241. <https://doi.org/10.1105/tpc.19.00378>
44. Zhang G, Zhao F, Chen L, Pan Y, Sun L, Bao N, Zhang T, Cui CX, Qiu Z, Zhang Y, Yang L, Xu L (2019) Jasmonate-mediated wound signalling promotes plant regeneration. *Nature Plants* 5(5):491–497.
<https://doi.org/10.1038/s41477-019-0408-x>

45. Zhang X, Henriques R, Lin SS, Niu QW, Chua NH (2006) *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* using the floral dip method. Nat Protoc 1(2):641–646.
<https://doi.org/10.1038/nprot.2006.97>
46. Zhou W, Lozano-Torres JL, Blilou I, Zhang X, Zhai Q, Smart G, Li C, Scheres B (2019) A jasmonate signaling network activates root stem cells and promotes regeneration. Cell 177(4):942–956.
<https://doi.org/10.1016/j.cell.2019.03.006>
47. Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W (2004) GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol 136(1):2621–2632.
<https://doi.org/10.1104/pp.104.046367>

Supplementary Tables

The supplementary tables are not available with this version.

Figures

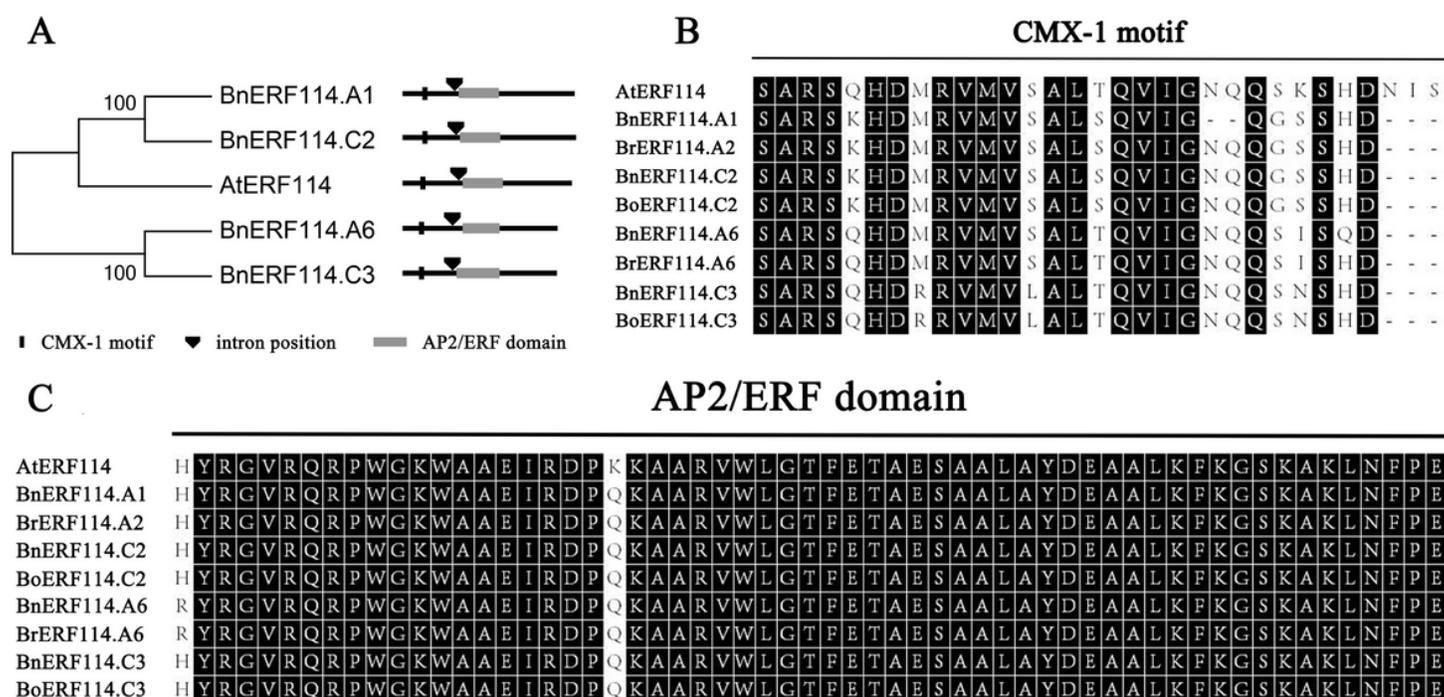


Figure 1

Molecular characteristics of BnERF114.A1. A, structure of ERF114 in *B. napus*; B, homology analysis of CMX-1 motif of ERF114 in *B. napus*, *B. rapa* and *B. oleracea*; C, homology analysis of AP2/ERF conserved domain of ERF114 in *B. napus*, *B. rapa* and *B. oleracea*.

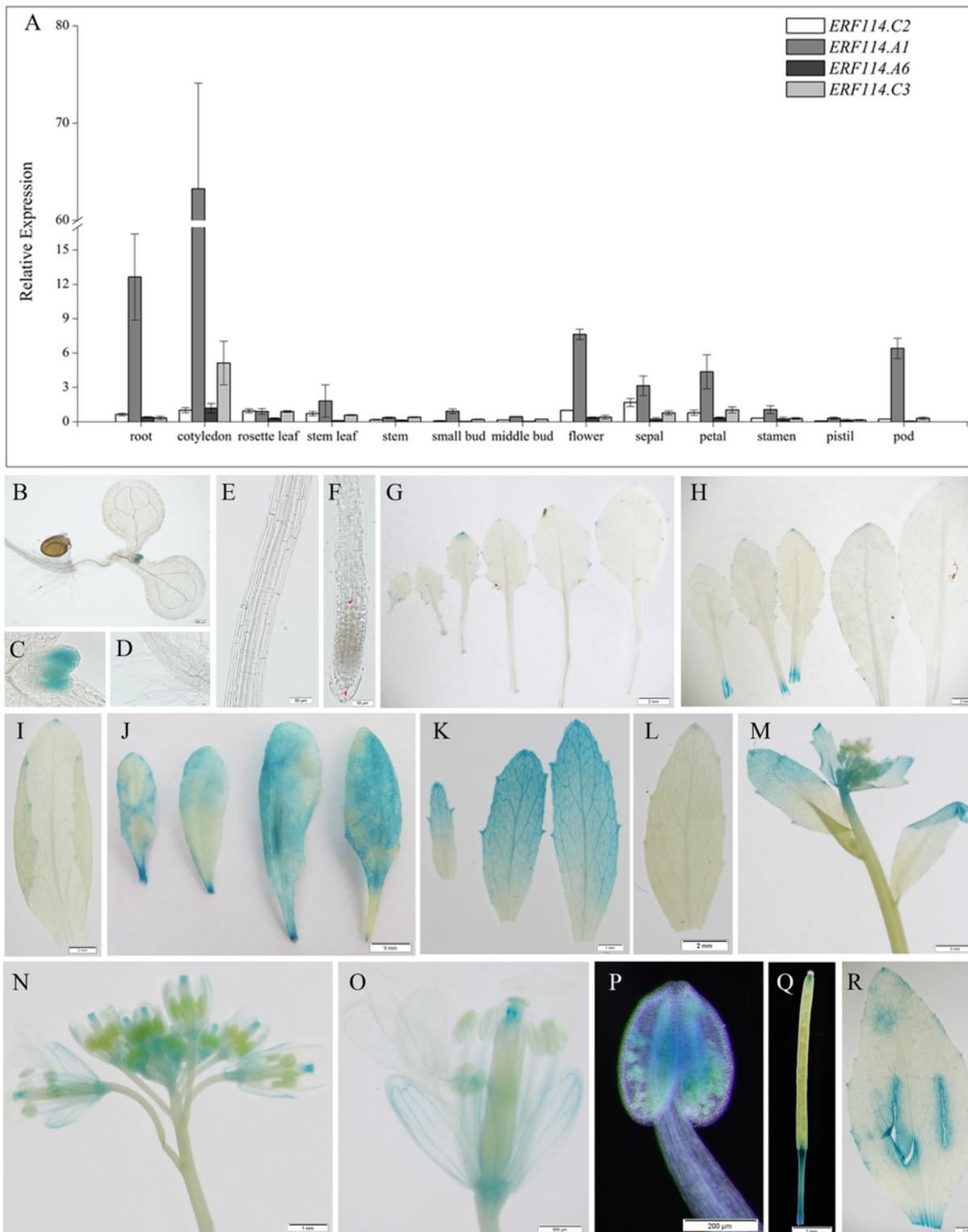


Figure 2

BnERF114s expression pattern. A, relative expression level of BnERF114s in different rapeseed tissues. Gene expression levels were normalized to the reference gene BnUBC21 (Gene ID: 106348550). Relative expression levels of BnERF114s were compared with that of BnERF114.C2 in flower. Each bar represented the average \pm SD from three independent biological samples. Different lowercase letter means significant difference at $P = 0.05$ level. B to R, BnERF114.A1 expression pattern identified by histochemical staining

of GUS using transgenic Arabidopsis OE114-46-3. B, cotyledon (Bar = 100 μ m); C, leaf primordium (Bar = 20 μ m); D, root hair (Bar = 50 μ m); E, elongation zone of root (Bar = 50 μ m); F, root tip (Bar = 50 μ m); G to J, the youngest rosette leaf to the oldest one, respectively (G~I, bars = 2 mm; J, bar = 9 mm); K, new cauline leaves (Bar = 1 mm); L, mature cauline leaf (Bar = 2 mm); M, main inflorescence (Bar = 2 mm); N, branched inflorescence (Bar = 1 mm); O, flower (Bar = 500 μ m); P, anther (Bar = 200 μ m); Q, pot (Bar = 2 mm); R, the scratched mature rosette leaf (Bar = 2 mm).

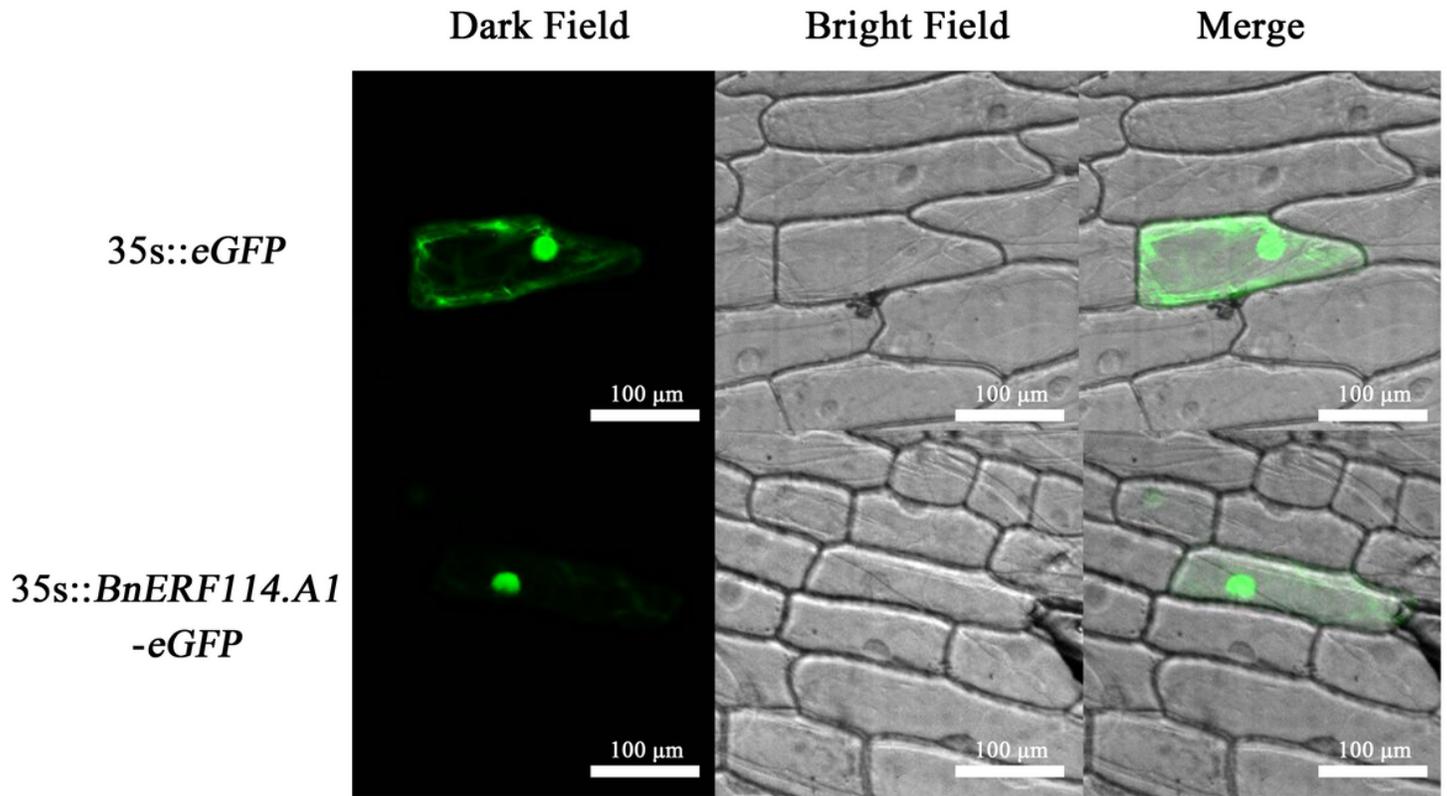


Figure 3

The transient expression of 35S:: BnERF114.A1-eGFP in onion epidermal cell. The wavelength of exciting light for eGFP was 488 nm. Bars = 100 μ m.

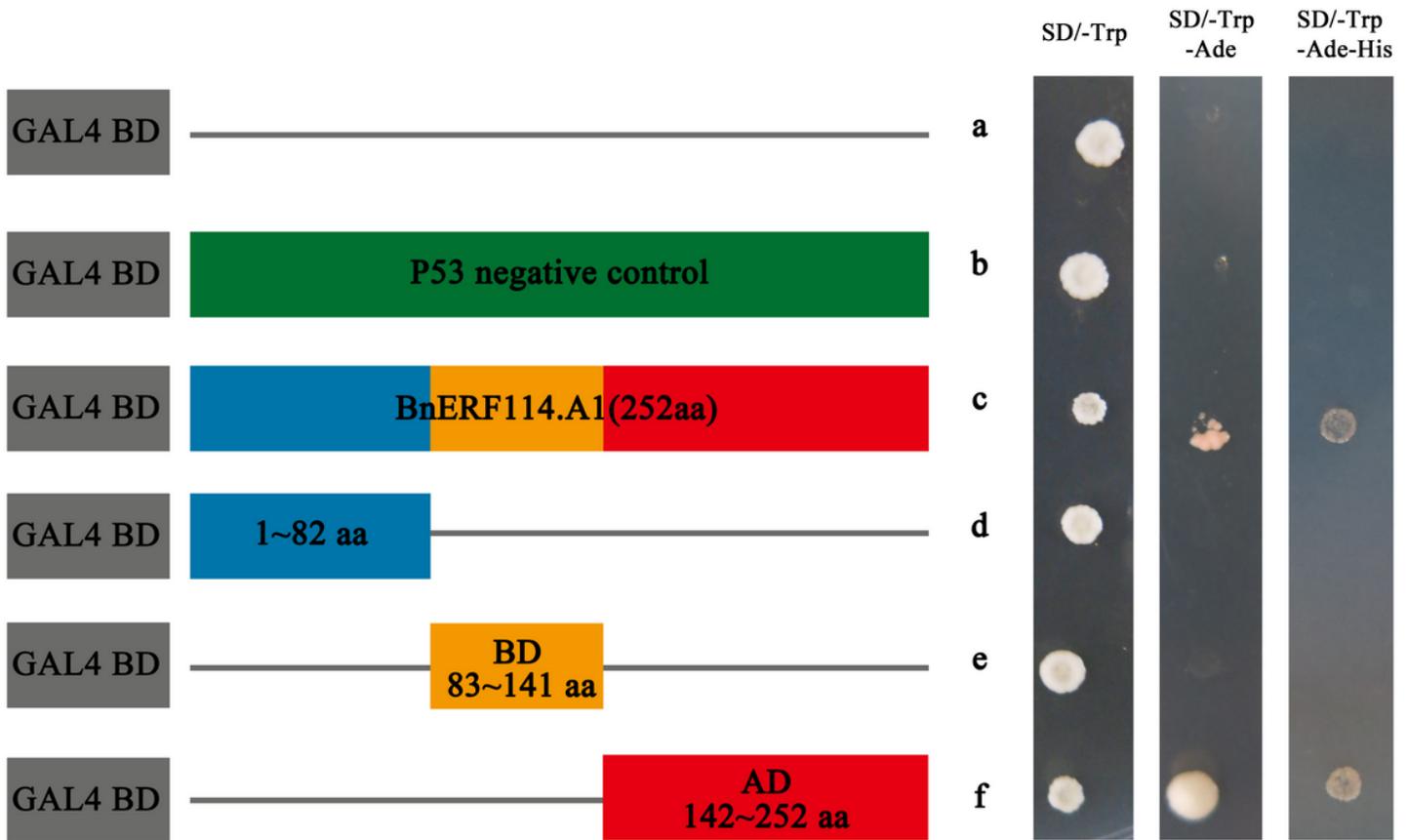


Figure 4

Transcriptional activity and active region analysis of BnERF114.A1. a ~ f indicate pGBKT7 (non-carrier vector), pGBKT7-P53 (P53 was a modified gene only containing DNA-binding domain, negative control), pGBKT7-BnERF114.A1, pGBKT7-BnERF114.A1(246-), pGBKT7-BnERF114.A1(-177-) and pGBKT7-BnERF114.A1(-333), respectively.

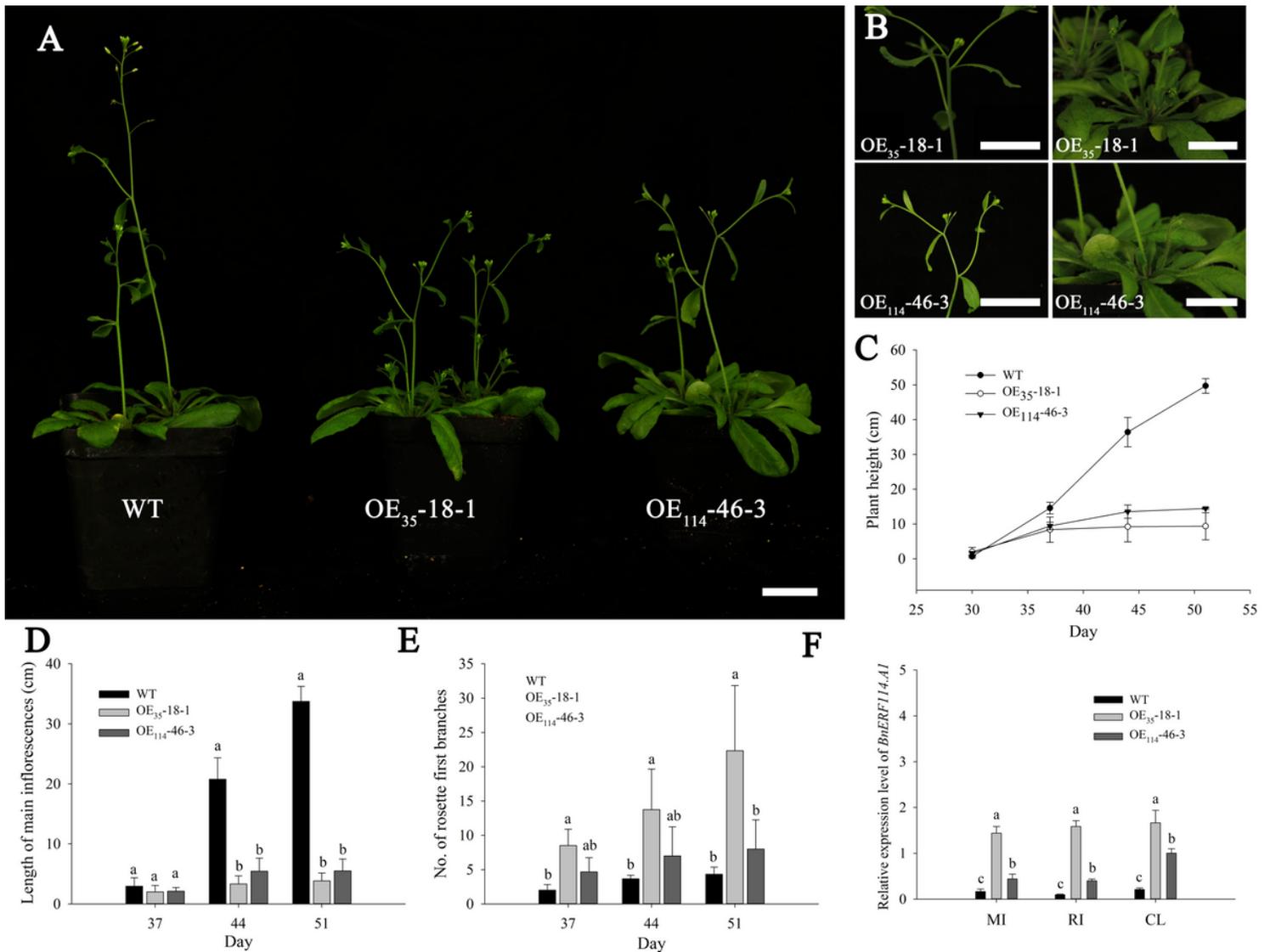


Figure 5

Effects of BnERF114.A1 ectopic expression on plant phenotype. WT, wild type Col-0 Arabidopsis; OE35-18-1, transgenic line expressing BnERF114.A1-eGFP-GUS under 35S promoting; OE114-46-3, transgenic line expressing BnERF114.A1-GUS under 114pro promoting. A, phenotype of transgenic plants OE35-18-1 and OE114-46-3 at 40-day stage; B, phenotype of main inflorescence and rosette first branches of OE35-18-1 and OE114-46-3 plant at 40-day stage; C, plant height of transgenic lines OE35-18-1 and OE114-46-3 at 30, 37, 44, 51-day stage (cm), the value of each data point represents the average \pm SD of 10 independent individuals; D, length of main inflorescence of transgenic lines OE35-18-1 and OE114-46-3 at 37, 44, 51-day stage (cm), the value of each bar represents the average \pm SD of 10 independent individuals; E, number of rosette first branches of transgenic lines OE35-18-1 and OE114-46-3 at 37, 44, 51-day stage (no.), the value of each bar represents the average \pm SD of 10 independent individuals. F, BnERF114.A1 expression level in main inflorescence (MI), rosette-first-branch inflorescence (RI) and young cauline leaf (CL) of OE35-18-1 and OE114-46-3 plant at 40-day stage. Gene expression levels are normalized to the reference gene AtUBC21 (At5g25760). The value of each bar represented the average \pm SD from three independent biological samples, and each independent biological sample contained 5

individuals. Relative expression level of BnERF114.A1 was compared with that in young cauline leaf of OE114-46-3. Different lowercase letter within one group means significant difference at P = 0.05 level in D, E, F. Bars = 2 cm in A and B.

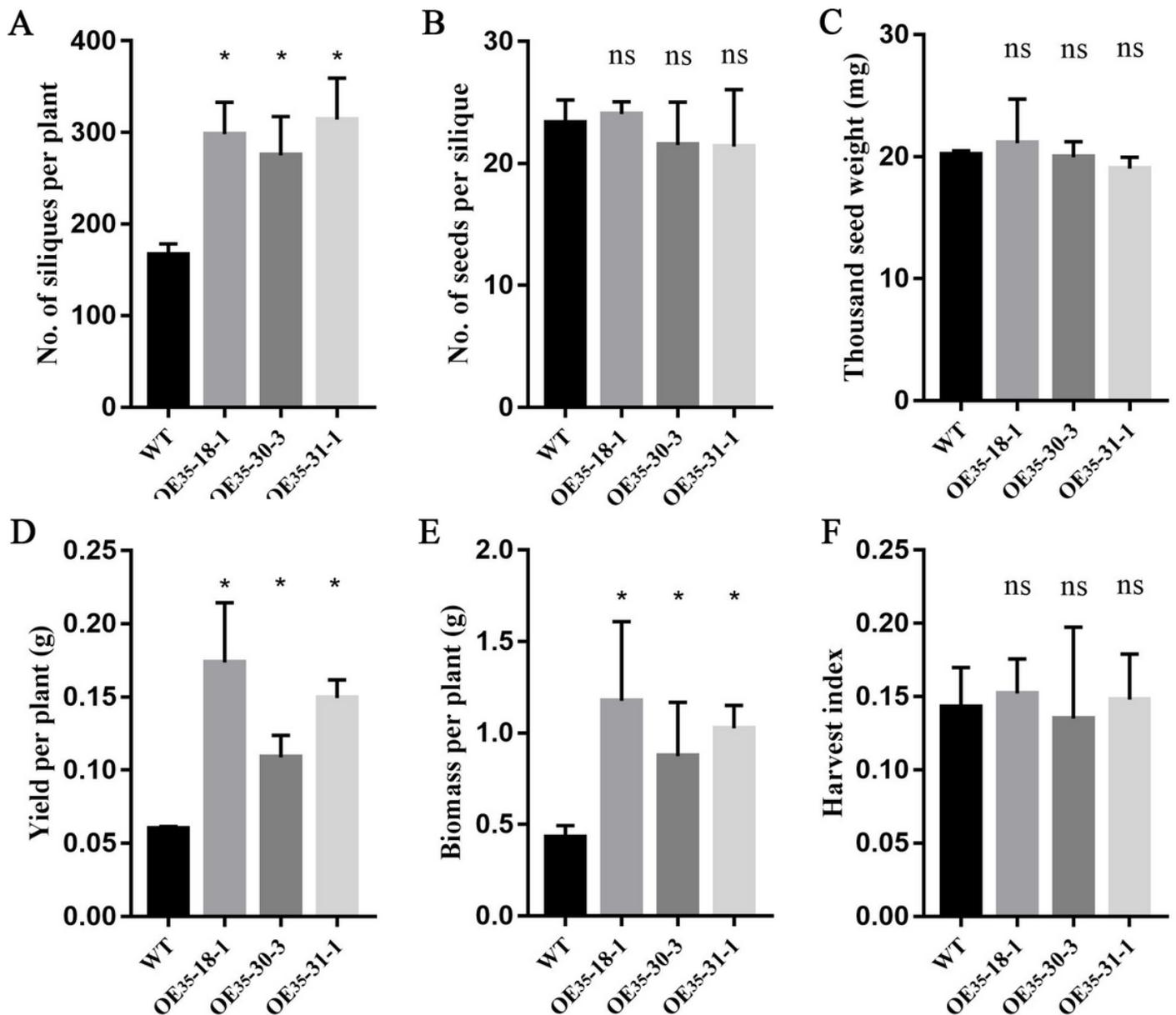


Figure 6

Yield related traits of BnERF114.A1 transgenic plants. A, number of siliques per plant; B, number of seeds per silique; C, thousand seed weight (mg); D, yield per plant (g); E, biomass per plant (g); F, harvest index. Data came from 20 individual plants containing 10 WT plants and 10 OE35 plants. Asterisk means significant difference at P = 0.05 level.

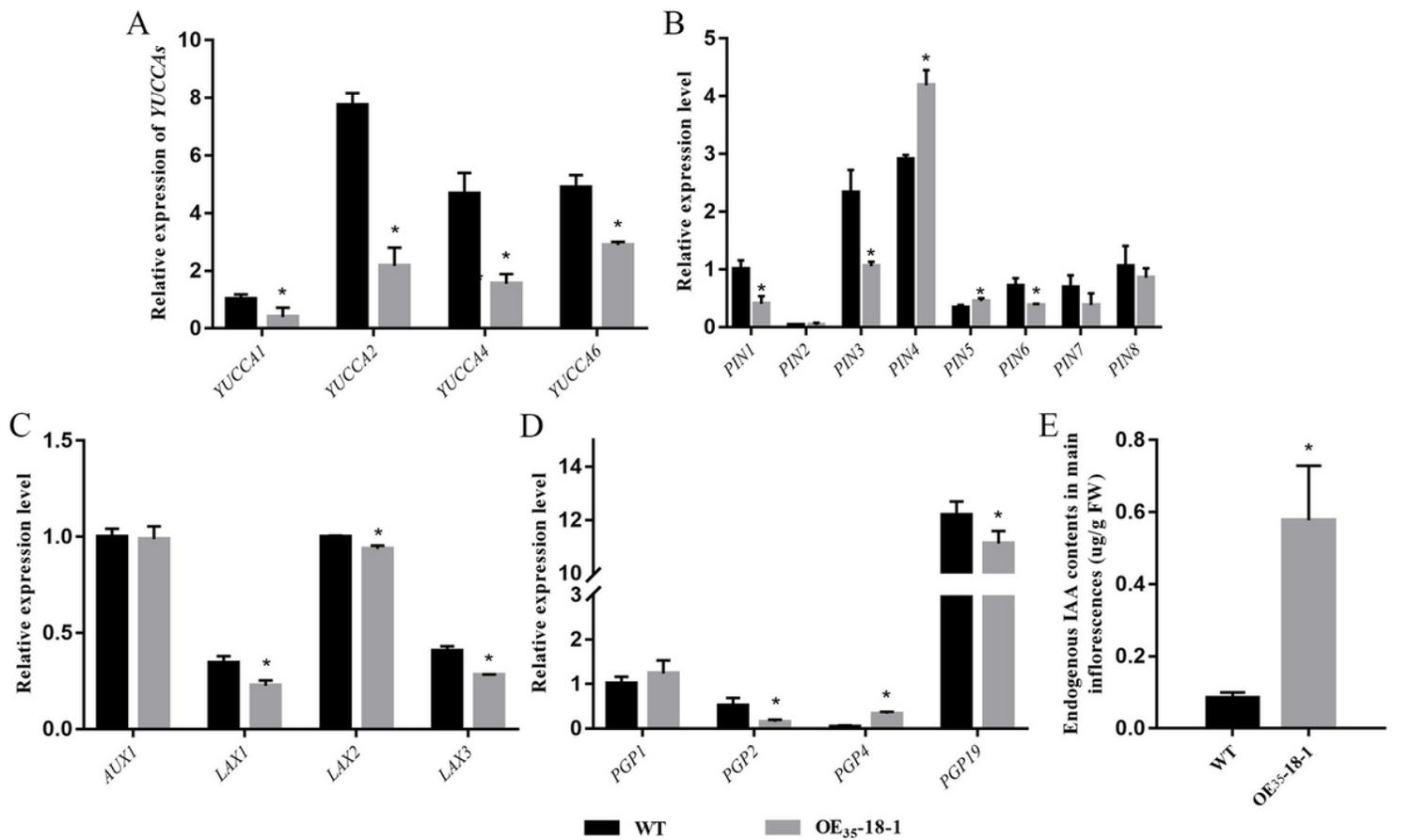


Figure 7

BnERF114.A1 affected endogenous IAA distribution. A, relative expression level of four YUCCA genes (AtYUCCA1, AtYUCCA2, AtYUCCA4, AtYUCCA6: At4g32540, At4g13260, At5g11320, At5g25620), and relative expression level of each gene was compared with AtYUCCA1 in WT; B, relative expression level of eight PIN family genes (AtPIN1 ~ AtPIN8: At1g73590, At5g57090, At1g70940, At2g01420, At5g16530, At1g77110, At1g23080, At5g15100), and relative expression level of each gene was compared with AtPIN1 in WT; C, relative expression level of four AUX/LAX family genes (AtAUX1, AtLAX1 ~ AtLAX3: At2g38120, At5g01240, At2g21050, At1g77690), and relative expression level of each gene was compared with AtLAX2 in WT; D, relative expression level of four PGP family genes (AtPGP1, AtPGP2, AtPGP4, AtPGP19: At2g36910, At4g25960, At2g47000, At3g28860), and relative expression level of each gene was compared with AtPGP1 in WT. Gene expression levels were normalized to the reference gene AtUBC21 (At5g25760). E, contents of endogenous IAA (µg/g fresh weight) in main inflorescences of Arabidopsis plants at 40-day stage. The value of each bar represented the average ± SD from three independent biological samples, and each independent biological sample contained 5 individuals. Asterisk means significant difference at P = 0.05 level.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Fig.S1.pdf](#)