Introduction

Branching is a major factor influencing plant architecture (Jiao et al. 2010, Jin et al. 2008, Takeda et al. 2003, Wang and Li 2006, Xiang et al. 2010). Lateral branches develop from the axillary meristem usually undergo two distinct steps. The first is the initiation of a new axillary meristem at the axil of a leaf and subsequent generation of a few lateral leaves to form an axillary bud. The second is the outgrowth of axillary buds to form shoot or lateral branches (Xing et al. 2010). Several mutants defective in axillary meristem initiation and/or outgrowth have been molecularly analyzed to understand the regulation of branching in various species. In Arabidopsis, the integrated control network that regulates branching includes phytohormone (e.g., auxin, cytokinin, and strigolactones) biosynthesis, transport and signaling genes (Ehrenreich et al. 2007, Janssen et al. 2014, Li et al. 2016, Plackett et al. 2012), floral meristem identity genes (Li et al. 2016, Liljegren et al. 1999, Liu et al. 2013), flowering time genes (Hiraoka et al. 2013, Li et al. 2016), and node-patterning genes (Ehrenreich et al. 2007, Li et al. 2016, Teo et al. 2014). Since the MOC1 gene was first isolated as a key regulator in controlling rice branching (Li et al. 2003), genes involved in tillering or branching via the protein degradation pathway, phytohormone signaling pathways and post-transcriptional regulation (Liang et al. 2014), such as TAD1 (Xu et al. 2012), TE (Li et al. 2009), LAX1 (Komatsu et al. 2003), OsTBL1 (Takeda et al. 2003), OsMADS57 (Guo et al. 2013), FC1 (Doebley et al. 1997),
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D10 (Arite et al. 2007), D27 (Lin et al. 2009), D17 (Zou et al. 2006), D3 (Ishikawa et al. 2005), D14 (Arite et al. 2009), DLT (Tong et al. 2009), OsmirR393 (Xia et al. 2012) and miR444a (Guo et al. 2013), have been identified and functionally characterized. In maize, more branches are produced in *tbi* (Doebly et al. 1997) deficient mutants and *bif2* mutants fail to initiate branch meristems (McSteen and Hake 2001). In tomatoes, the initiation of axillary meristems is prevented in *Hake* 2001. In *B. napus*, lateral branching is strongly affected by planting density and fertilizer level (Xiang et al. 2010). High plant density can decrease light quantity and change light quality, thus leading to reduced branching (Xiang et al. 2010). Low phosphorous may induce the biosynthesis of strigolactone resulting in fewer tillers (Bouwmeester et al. 2007, Xiang et al. 2010), while high nutrient levels may inhibit strigolactone synthesis resulting in more tillers (Umehara et al. 2008, Xiang et al. 2010).

In addition to genetic factors, there are also reports of environmental regulation of branching. For instance, branching is strongly affected by planting density and fertilizer level (Xiang et al. 2010). High plant density can decrease light quantity and change light quality, thus leading to reduced branching (Xiang et al. 2010). Low phosphorous may induce the biosynthesis of strigolactone resulting in fewer tillers (Bouwmeester et al. 2007, Xiang et al. 2010), while high nutrient levels may inhibit strigolactone synthesis resulting in more tillers (Umehara et al. 2008, Xiang et al. 2010).

From an agronomic viewpoint, seed yield of *B. napus* L. is usually a factor of branch number and distribution, especially the primary branches and some early secondary branches. These traits indirectly influence rapeseed cultivar yield by affecting major yield-component traits, such as number of siliques per plant (Li et al. 2016). Thus, the ability to increase branching through genetic manipulation would be desirable for enhancing seed yield in *B. napus* L.

Protein S-acyltransferases (PATs) contain DHHC-CRD domains and are transmembrane proteins. The main function of PATs is to mediate the S-acylation of target proteins (Yuan et al. 2013). S-acylation is an important secondary modification that regulates membrane association, trafficking, and target protein function. However, little is known about the characteristics of PATs in plants (Yuan et al. 2013). Furthermore, PAT regulation of branching and seed yields remains unknown, especially in *B. napus* L. (Xiang et al. 2010, Zhou et al. 2017). OsPAT15 is an alternatively spliced model of *Os02g0819100* (Accession No. NM_001055066.1) in rice and the two transcript variants encompass DHHC domains and are DHHC-type zinc finger proteins (Zhou et al. 2017). BLAST searches within NCBI revealed several potential orthologues of OsPAT15 in Arabidopsis thaliana, Sorghum bicolor, Zea mays, Triticum aestivum, Aegilops tauschii, and Hordeum vulgare. Moreover, there are 24 PATs in *B. napus* L. (Yuan et al. 2013).

Previous investigations indicated that some DHHC-type proteins with S-acyl transferase activity can regulate cell phenotype or plant architecture, such as *Akr1* in yeast (Roth et al. 2002) and *AtTIP1* in Arabidopsis (Hemsley et al. 2005). *AtTIP1* was also named *AtPAT24* (Batistic 2012). Moreover, our previous studies indicated that OsPAT15 regulates plant architecture by altering the tiller in rice (Zhou et al. 2017). Whether OsPAT15 has DHHC-type S-acyl transferase activity and plays a similar effective role in the dicot *B. napus* L. is not known, and if so, how it regulates the branching and seed yield in these crop plants remains to be determined.

In this study, we aimed to verify the S-acyl transferase activities of OsPAT15, and performed heterogeneous expression of a novel DHHC-type zinc finger protein and analyzed its effects on plant branching and seed yield in *B. napus* L.

Materials and Methods

Plant material

The Zhonghua 11 rice cultivar used in this study was collected by our laboratory. The *B. napus* L. N2522 cultivar is a winter oilseed rape and was provided by the Academy of Seed Industry of Hunan Yahua. The leaves of 20-day-old seedlings of *Arabidopsis* Col-4 were used to isolate RNA and clone *AtTIP1* (*At5g20350*, Accession No. NM_122042).

Amino acid sequence alignment analysis

Amino acid sequence alignment of *AtTIP1*, OsPAT15, and Os02g0819100 was performed using GENEDOC software.

Complementation of *Akr1A* defects with OsPAT15 cDNA

The analysis of S-acyl transferase activity for OsPAT15 and Os02g0819100 was carried out as described (Hemsley et al. 2005) with minor modifications. Briefly, *AKR1* (BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0) and *akr1A* (BY4741; MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; YDR264c::kanMX4) haploid yeast (*Saccharomyces cerevisiae*) were obtained from the EUROScarf collection (http://www.uni-frankfurt.de/fb15/mikro/euroscarf/). The cDNAs of OsPAT15, Os02g0819100, and *AtTIP1* were cloned into the pYES2 vector (Invitrogen, Carlsbad, CA, USA) under the GAL1 promoter. The OsPAT15 C76A mutant (*MOSPAT15*) and Os02g0819100 C125A mutant (*MOS02g0819100*) were created by site-directed mutagenesis using crosslinking PCR before being recombined into pYES2. *AKR1* controls were introduced into pYES2 as a positive wild type (WT). Transformants were generated using lithium acetate/polyethylene glycol-mediated transformation (Gietz et al. 2002) and plated onto proper selective nutrient medium (Sigma-Aldrich, St. Louis, MO, USA).
USA). Single colonies from each transformation were streaked onto selective glucose medium (Sigma-Aldrich) twice before single colonies of equal size were picked and pointed onto complete selective galactose medium and grown at 25°C or 30°C for 48 h to visualize colonies. Three replicates were performed per genotype using independent transformants, and each was repeated in triplicate for each temperature. For the observation of cell morphology, cultures of each genotype were grown in selective liquid medium at 25°C or 30°C until mid-log phase. Cell morphology was observed using a Nikon ECLIPSE TE2000-U inverted light microscope.

**Construction of the expression cassette and plant transformation mediated by Agrobacterium tumefaciens**

The OsPAT15 gene from rice cDNA was amplified using the reverse transcription polymerase chain reaction (RT-PCR) method and specific 5′ (5′-CATGCCATGGATGGCGCGAGAGGGAAG-3′) and 3′ (5′-GGAGATCTATTGCTTCCTTTTGTGATCCTGA-3′) primers (restriction sites for NcoI and BglII are shown in boldface and the sequence corresponding to OsPAT15 is in italics). The amplified product was sub-cloned into the pUCM-T easy vector (shenggong Co., Ltd., Shanghai, China) and the sequence was confirmed by DNA sequence analysis. The OsPAT15 coding region was excised from the pUCM-T easy vector by NcoI and BglII digestion and inserted into the NcoI and BglII sites of the binary vector pCAMBIA1301 (Fig. 2A). The binary vector with foreign OsPAT15 was introduced into Agrobacterium tumefaciens strain GV3101 by electroporation using a Gene Pulser II system (Bio-Rad, Hercules, CA, USA) and the stability of positive constructs selected using 100 mg L⁻¹ kanamycin were confirmed by PCR and transformed into B. napus L. plants by Agrobacterium tumefaciens-mediated floral dip transformation (Zhang et al. 2006). Briefly, the inflorescences of B. napus L. plants grown in a field were dipped into the Agrobacterium tumefaciens inoculum containing OsPAT15 constructs (OD₆₀₀ = 0.6) and a surfactant (0.1% Silwet L-77, V/V) for 30 s twice every 3 days and the seeds were harvested from inoculated plants to screen for primary transformants. T₁ seeds were screened in a field using hygromycin (200 mg L⁻¹) and those with a 3:1 segregation ratio were selected to establish homozygous T₂ lines and for subsequent seed production. The segregation ratio was determined based on the number of green to bleached seedlings. Thirty-five independent transgenic lines containing the 35S::OsPAT15-GUS construct and their daughter lines were selected for further study. Twenty-four independent Os0290819100-GUS transgenic plants and 29 independent pCAMBIA1301-GUS transgenic lines were also obtained by Agrobacterium tumefaciens-mediated floral dip transformation method, respectively.

**Histochemical assay of β-glucuronidase (GUS) activity in T₂ transgenic plants**

Histochemical GUS assay was performed at 37°C for 3 h by vacuum infiltration of seedlings in 0.05 mol/L phosphate buffer (pH 7.0) containing 0.5 mg/mL X-gluc, followed by washing using 90, 80, and 70% ethanol solutions for 1 h. 20-day-old transformed seedlings (T₂) with the target gene and corresponding WT seedlings were stained.

**Molecular characterization of transgenic B. napus L. plants**

DNA from 20-day-old T₂ seedlings was extracted from WT and hygromycin-resistant transgenic lines using the hexadecltrimethylammonium bromide (CTAB) method (Murray et al. 1980). To detect the hygromycin resistance hph gene, PCR was performed using primers (hph-F: 5′-CCACGGCCTCCAGAAGAAAGATGT-3′, hph-R: 5′-TTGAGGTGGTTAGCGAGGCTGAC-3′) and a general PCR program: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. For the OsPAT15-GUS fused gene fragment, PCR was performed using primers (OsPAT15-GUS-F: 5′-TCATAACATGAAACAAATAGTGAC-3′, OsPAT15-GUS-R: 5′-CGCATCTTCCAGTAAAGGTAATG-3′) and a general PCR program: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. All PCR products were analyzed using 1.0% agarose gel electrophoresis.

Total RNA was purified from 100 mg of 20-day-old B. napus L. seedlings using Trizol reagent (Invitrogen) and treated with RNase-free DNase I (Promega Biotech Co., Madison, WI, USA). The DNase-digested RNA samples were used for reverse transcription by Superscript III reverse transcriptase (Invitrogen). Samples were stored at −80°C to serve as cDNA stocks for PCR analysis. Gene expression analysis of OsPAT15 transcript levels was performed using semiquantitative RT-PCR in a 20 μL solution containing 1 μL cDNA stock using rTaq DNA polymerase (TaKaRa Co., Dalian, China) and a programmable thermal cycler (Biometra T-gradient 96050-801, Germany). The PCR amplification program consisted of an initial denaturation step of 5 min at 94°C, followed by 22 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 55°C, and 30 sec extension at 72°C. The sense primer for OsPAT15 was 5′-CGTGGGCTATGCAAACCTACAA-3′ and the antisense primer was 5′-TGCGGATACTTCGTCCACTCT-3′. BnACTIN7, as an internal control, was amplified using Genbank Accession Number AF111812 with a sense primer (5′-TGGTTGGGATGGGTAAAAGA-3′) and an antisense primer (5′-CGAGGAGATGGCCTGAGGAC-3′).

To investigate whether the expression levels of S-acyl transferase genes in B. napus L. were altered in overexpressed oil rape plants, BnPAT15 (LOC106372531, NCBI Reference Sequence: XM_013812760.1) and BnPAT16 (LOC106372532, NCBI Reference Sequence: XM_013880605.1), two homologous genes of OsPAT15 in
The branch and seed yield increased by heterologous expression of OsPAT15 in Brassica napus L.

Results

Analysis of OsPAT15 S-acyl transferase activity in vivo

Previously, we cloned the novel zinc finger gene OsPAT15 from rice Oryza sativa L., which is an alternatively spliced model of Os02g0819100 (Accession No. NM_001055066.1) (Zhou et al. 2017). The amino acid sequence deduced from the open reading frame of OsPAT15 (OsDHHC1) (BankIt1948221, Accession No. KX783131) revealed that the cDNA encodes a protein with a length of 223 amino acids. BLAST analysis, combined with multiple sequence alignment analysis, showed that OsPAT15 is highly homologous to AtTIP1 from Arabidopsis (Hemsley et al. 2005) and also has three motifs: DPG, DHHC-CRD, and TTXE (Fig. 1A). To verify whether OsPAT15 has S-acyl transferase activity similar to AtTIP1, we performed a point mutation experiment. Specifically, we changed DHHC to DHHA in the DHHC-CRD motif in OsPAT15 (C76A, named MOsPAT15) and Os02g0819100 (C125A, called MOsOs02g0819100) by cross-bridging PCR. Then, OsPAT15 and the two DHHA mutants were transformed into yeast akr1Δ mutants, which are deficient in S-acyl transferase and

Phylogenetic analysis

Phylogenetic analysis was performed following the method of Yuan et al. (2013).

Immunoblot analysis

Total protein was isolated from 60-day-old B. napus L. seedlings according to the protocol described by Zhou et al. (2012). The enhanced chemiluminescence immunoblot analysis procedure followed the method of Zhou et al. (2017). The blot was probed with appropriate primary antibodies (anti-GUS antibody) and secondary antibody conjugated with horseradish peroxidase (Beyotime Institute of Biotechnology, China). Equal loading was verified using histone H1. All other chemicals were acquired from Sigma-Aldrich.

Investigation of agronomic traits

To investigate branching and seed yield, WT and T2 transgenic seedlings were sown in an irrigated field in early October 2013, 2014 and 2015. The cultivation of all transgenic Brassica in the irrigated field is legal in this study. The field was divided into six parts (WT, OeOsPAT15-GUS-2, OeOsPAT15-GUS-6, OeOsPAT15-GUS-9, Os02g0819100-GUS and pCAMBIA1301-GUS). Each part consisted of 3 plots. Each plot was 8.25 m² (length × width, 2.5 m × 3.3 m). Seedlings were sown with 0.25 m between seedlings and 0.33 m between rows (the universal B. napus L. cultivation standard in southern China). WT and transformed B. napus L. plants were cultivated under the same agronomic regime. At the floral transition stage, 10 individuals WT and transgenic plants were randomly selected and pulled to measure fresh weight and dry weight. At harvest time, another 10 individual plants were randomly selected from each plot, and primary branch number and valuable branch number were recorded (primary branch number consists of branches producing fertile pods; valuable branch number consists of the early secondary branches and later produced branches that extend and produce fertile pods or carry seeds). At the same time, the fresh and dry weights of each plant were also analyzed. Silique number per plant was recorded for statistical analysis. All seeds from each plant were harvested by hand, dried, threshed, and weighed. Thousand seed weight was also recorded. Each investigation of agronomic traits was repeated three times.

Fig. 1. Determining the S-acyl transferase activity of OsPAT15 in yeast akr1Δ mutants. A: Alignment analysis of the amino acid sequences of AtTIP1, OeOsPAT15, and Os02g0819100. B: The morphology of yeast cells with different genotypes in liquid culture at 25°C and 30°C. A description of the genotypes and references are also given.
present aberrant morphologies and temperature sensitivity (normal growth occurring at 25°C and aberrant growth occurring at 30°C) (Hemsley et al. 2005). As shown in Fig. 1B, at 25°C, all genotypes showed normal growth and morphology, but akr1Δ yeast showed slower growth; at 30°C, OsPAT15 + akr1Δ and Os02g0819100 + akr1Δ yeast both showed normal cell morphologies as did AKR1 and AtTIP1 + akr1Δ, while the DHHA mutants (OsPAT15 + akr1Δ and Os02g0819100 + akr1Δ) failed to complement the aberrant morphology of akr1Δ. Similar results were obtained across three replicates. These results indicate that yeast akr1Δ loss-of-function mutants can be rescued by the expression of OsPAT15 and that OsPAT15 is an S-acyl transferase.

Molecular characterization of OsPAT15 transgenic plants

To obtain OsPAT15 transgenic plants, an expression cassette containing the GUS gene, target gene (OsPAT15), and hygromycin resistant selectable marker gene under the control of the CaMV35S promoter was constructed and introduced into B. napus L. plants by Agrobacterium tumefaciens-mediated transformation (Fig. 2A). Thirty-five hygromycin-resistant independent lines were acquired after the first screening with hygromycin (Fig. 2B). Three T2 hygromycin-resistant independent lines with a segregation ratio of 3:1 (35S::OsPAT15-GUS-2, 35S::OsPAT15-GUS-6, and 35S::OsPAT15-GUS-9), which showed increased primary branches, were selected for further studies. Moreover, GUS staining signals (Fig. 2C), hph genes (Fig. 2D), and OsPAT15-GUS fused gene fragments (Fig. 2E) were observed in these three hygromycin-resistant lines but not in WT plants, indicating that the expression cassette was successfully introduced into B. napus L. genomic DNA. To further confirm that OsPAT15 was truly expressed in these hygromycin-resistant independent lines, the transcription levels of OsPAT15 were determined by semiquantitative RT-PCR using gene-specific primers. The housekeeping gene, BnACTIN7, was used as a control. We found that high transcript levels of OsPAT15 were present in transgenic seedlings but not in WT seedlings (Fig. 2F). Moreover, an immunoblot assay also showed that the OsPAT15-GUS fusion protein accumulated in transgenic B. napus L. plants but not in WT (Fig. 2G). These results suggest that OsPAT15 was successfully overexpressed in B. napus L. and that transgenic plants were obtained.

Branching and plant weight of OsPAT15 transgenic plants

To determine the phenotypic changes associated with increased exogenous OsPAT15 in transgenic plants, branching and plant weight were analyzed. Compared to WT, OsPAT15 overexpressed transgenic plants had a higher number of primary branches, and their first and last branches were at significantly lower positions on the main stems (Fig. 3A). Statistical results showed that observed primary branch numbers were increased by 1.58–1.76-fold in OsPAT15 overexpressed B. napus L. lines compared to WT (Fig. 3B). Importantly, valuable branch numbers were also increased by 1.56–1.67-fold in OsPAT15 overexpressed lines compared to WT (Fig. 3C). At the floral transition stage, the fresh and dry weights of OsPAT15 overexpressed transgenic plants were increased by 1.42–1.64-fold and 2.04–2.38-fold, respectively (Fig. 3D–3E). Similarly, at the harvest stage, the fresh weight was increased 1.84–2.12-fold and the dry weight was increased 2.26–2.43-fold in OsPAT15 overexpressed lines compared to WT (Fig. 3F–3G). Moreover, the primary branches, valuable branches, fresh weight, and dry weight were not markedly altered in B. napus L. plants.
The branch and seed yield increased by heterologous expression of OsPAT15 in Brassica napus L.

Transformed with the pCAMBIA-1301-GUS empty vector and Os02g0819100-GUS, respectively. Interestingly, transgenic B. napus L. plants showed longer retention of leaves than WT, although there was no obvious difference in developmental timing (Fig. 3A). Whether this was due to OsPAT15 remains to be determined.

Silique number and seed yield of OsPAT15 transgenic plants

In addition to branching and plant weight, we determined whether the increased OsPAT15 expression was accompanied by an increase in seed yield. As shown in Fig. 4A, the silique numbers in transgenic lines were increased by 1.86–1.89-fold compared with WT, but there were no significant differences in the lengths of siliques (Fig. 4B) and seed number of each silique (Fig. 4C) between the transgenic lines and WT. Furthermore, the seeds number of each line was increased by the seed yield per plant and per planted plot of the transgenic lines were increased by 2.31–2.43-fold (Fig. 4D) and 2.39–2.51-fold (Fig. 4E), respectively, even though the thousand seeds weight of transgenic lines did not significantly differ from WT (Fig. 4F). These results

Fig. 3. Branching and plant weight in WT and OsPAT15 overexpressed transgenic plants. Data are means ± standard deviation (SD; n = 30) in B–G; (*) represents significant difference of transgenic plant/WT as determined by Student’s t-test at P < 0.05. Blue dashed line indicated 1-fold, green dashed line indicated 2-fold. A: Phenotype of B. napus L. plants. B: Comparison of primary branch number in WT and transgenic plants. C: Comparison of valuable branch number in WT and transgenic plants. D: Investigation of the fresh weights of WT and transgenic plants at the floral transition stage of 2013, 2014 and 2015. E: Investigation of the dry weights of WT and transgenic plants at the floral transition stage of 2013, 2014 and 2015. F: Investigation of the fresh weights of WT and transgenic plants at the harvest stage of 2013, 2014 and 2015. G: Investigation of the dry weights of WT and transgenic plants at the harvest stage of 2013, 2014 and 2015.
Many DHHC-type zinc finger protein genes have S-acyl transferase activity (Batisti 2012, Hemsley et al. 2005, Mitchell et al. 2006, Yuan et al. 2013), such as Akr1 in yeast (Roth et al. 2002) and AtTIP1 in Arabidopsis (Hemsley et al. 2005). The yeast S-acyl transferase Akr1 localizes to the Golgi apparatus (Roth et al. 2002). AtTIP1 (AtPAT24) mainly localizes in the Golgi (Batistic 2012, Li et al. 2016, Li and Qi 2017, Qi et al. 2013). We hypothesized that OsPAT15 can replace the function of Akr1 in yeast similar to AtTIP1. We transformed OsPAT15 into akr1Δ mutant yeast. The result indicated that OsPAT15 complements the function of Akr1. At the same time, the detailed mechanism by which OsPAT15 regulates branch generation in rape remains to be determined. We are using an approach that combines genetics and biochemistry to investigate how these might influence branching.

**OsPAT15 as an S-Acyl transferase**

Many DHHC-type zinc finger protein genes have S-acyl transferase activity (Batisti 2012, Hemsley et al. 2005, Mitchell et al. 2006, Yuan et al. 2013), such as Akr1 in yeast (Roth et al. 2002) and AtTIP1 in Arabidopsis (Hemsley et al. 2005). The yeast S-acyl transferase Akr1 localizes to the Golgi apparatus (Roth et al. 2002). AtTIP1 (AtPAT24) mainly localizes in the Golgi (Batistic 2012, Li et al. 2016, Li and Qi 2017, Qi et al. 2013). We hypothesized that OsPAT15 can replace the function of Akr1 in yeast similar to AtTIP1. We transformed OsPAT15 into akr1Δ mutant yeast. The result indicated that OsPAT15 complements the function of Akr1. At the same time, the detailed mechanism by which OsPAT15 regulates branch generation in rape remains to be determined. We are using an approach that combines genetics and biochemistry to investigate how these might influence branching.

In this study, we determined that the rice OsPAT15 gene encodes an S-acyl transferase and can functionally substitute for the S-acyl transferase Akr1 in yeast akr1Δ mutants. When OsPAT15 from rice was heterologously expressed in B. napus L., the OsPAT15 transgenic plants showed increased branching and siliques. These results demonstrate that OsPAT15 may regulate branching and seed yield by acting as an S-acyl transferase.

**Discussion**

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**Expression levels of BnPAT15 and BnPAT16 are not altered in OsPAT15 transgenic plants**

Phylogenetic analysis indicated that BnPAT15 and BnPAT16 are closely related to OsPAT15 (Fig. 5A). The semi-quantitative RT-PCR results show that the expression levels of BnPAT15 and BnPAT16 were not significantly altered in OsPAT15 transgenic plants (Fig. 5B). This suggests that OsPAT15 did not affect the expression levels of BnPAT15 and BnPAT16 in transgenic plants.

**Overexpression of OsPAT15 improved seed yield by positively regulating branching in B. napus L.**

B. napus L. (AACC, 2n = 38) is an allopolyploid species resulting from hybridization and polyploidization between the two diploid species B. rapa (2n = 20, AA) and B. oleracea (2n = 18, CC) (Hansen et al. 2003, Zhou et al. 2012), and has a complex genome structure with high homology between its A- and C-subgenomes in which both
The branch and seed yield increased by heterologous expression of OsPAT15 in Brassica napus L.

**Fig. 5.** Phylogenetic analysis of the predicted protein S-acyltransferases (BnPATs) of *B. napus* L. A: Phylogenetic tree of 24 predicted S-acyltransferase proteins of *B. napus* L. and OsPAT15, the data in brackets are the Accession No. of each BnPAT. B: Expression levels of BnPAT15 and BnPAT16 in OsPAT15 overexpressed *B. napus* L.

homologous and non-homologous exchanges were extensively observed (Cai et al. 2014, Chalhoub et al. 2014, Liu et al. 2016). This situation imposes a huge challenge to identifying and functionally characterizing genes from *B. napus* L. However, it was shown that modulating the expression of an exogenous gene in *B. napus* L. by genetic engineering allows for the analysis of gene function by manipulating and controlling development and other physiological processes in a way that is both effective and feasible. For example, heterologous expression of the anthocyanin pathway gene transcription factors DELILA and ROSEAL from the snapdragon *Antirrhinum majus* can enhance *B. napus* L. antioxidant activity (Nie et al. 2013); expression of the global regulator IrrE from *Deinococcus radiodurans* can improve the salt tolerance of *B. napus* L. (Wang et al. 2016); and overexpression of diacylglycerol acyltransferase 1 (SsDGAT1) from *Sapium sebiferum* (L.) Roxb increased seed oil content and altered oil quality in *B. napus* L. (Peng et al. 2016). Currently, association analysis of genes involved in the genetic control of branching and seed yield in *B. napus* L. is still rare, except for a study on the overexpression of a gibberellin 2-oxidase gene from *Arabidopsis thaliana*, which was shown to increase branching (Zhou et al. 2012). Here, we overexpressed the rice DHHC-type zinc finger gene OsPAT15 in *B. napus* L. and characterized the transgenic lines.

OsPAT15 transgenic plants generally have more branches, more siliques, higher biomass weight, and higher seed yield relative to non-transgenic plants. The increase in seed yield in transgenic plants may be the direct result of increased branching and siliques. It is worth mentioning that the number of branches and siliques produced in transgenic plants is positively related to the expression level of OsPAT15. Transgenic lines with higher OsPAT15 expression levels produced about 0.9-fold more branches and 0.8-fold more siliques than WT under the same field conditions. Similar results were found in previous studies. For example, the overexpression of *AtGA2ox1* in low-input turfgrass (*Paspalum notatum* Flugge) resulted in a significantly increased number of tillers per area, thus enhancing the quantity of transgenic bahia grass under weekly moving (Agharkar et al. 2007). Ervin and Koski (1998) indicated that more forceful branching can be attained by superexpression of the plant growth regulator trinexapac-ethyl (Primo). In perennial rye grass, overexpression of *ATH1* resulted in the outgrowth of normally quiescent lateral meristems into extra leaves (Valk et al. 2004). Our results revealed that OsPAT15 leads to increased branch production, and likely functions in pathways related to the outgrowth of lateral meristems to regulate the generation of branches and seed output in *B. napus* L. Previously study indicated that overexpression of *AtDHHC1* can increase the number of shoot branch in *Arabidopsis* (Xiang et al. 2010); and then overexpression of OsDHHC1 (OsPAT15) can increase the number of tiller number and seeds yield in rice, but OsPAT15-RNAi rice plant had not markedly decreased in tillers and seeds yield (Zhou et al. 2017). At the same time, we had also transformed Os02g0819100 into *B. napus* L., but the branches and seeds yield of these transgenic plants were not markedly increased (Figs. 3B, 3C, Fig. 4E).

**The transcription levels of phylogenetically closely related S-Acyl transferases in *B. napus* L. were not altered in transgenic plants**

In *B. napus* L. genome sequence, the *PAT* gene family has 24 members and some PAT-like genes. Except BnPAT3, BnPAT8, BnPAT18, BnPAT19, BnPAT20, BnPAT21, BnPAT22, all of other BnPATs have DHHC-CRD domain (Supplemental Fig. 1). The semiquantitative RT-PCR results showed that the expression levels of BnPAT15 and BnPAT16, which are phylogenetically close to OsPAT15, were not significantly altered in OsPAT15 transgenic plants. This indicates that *OsPAT15* did not affect the transcription
levels of \( \text{BnPAT15} \) and \( \text{BnPAT16} \) in transgenic plants. Whether \( \text{BnPAT15} \) and \( \text{BnPAT16} \) would vary in protein level is a subject of further study.

\( \text{OsPAT15} \) transgenic \( B. \text{napus} \) can balance the plant numbers and products in a unit field. The modern high-density agriculture is planting more numbers of plant can obtain more products in a unit field. The advantage of \( \text{OsPAT15} \) transgenic \( B. \text{napus} \) is that it seems to be available to obtain more products with planting the same or less numbers of plant in a unit field, which can decrease cost of buying \( B. \text{napus} \) seeds or seedlings.

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