Characterization and Fine Mapping of a New Dwarf Mutant in *Brassica Napus*

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Research article

Keywords: Brassica napus, dwarf, grain yield, BSA, fine mapping

Posted Date: September 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-55292/v1

Version of Record: A version of this preprint was published at BMC Plant Biology on February 26th, 2021. See the published version at https://doi.org/10.1186/s12870-021-02885-y.

Abstract

Background: Plant height is an important plant architecture character closely related to yield performance of many crops. Reasonable reduction of plant height of crops is beneficial for enhancing lodging resistance and improving yield.

Results: In the present study, we described a *Brassica napus* dwarf mutant *bnd2* induced by ethyl methanesulfonate (EMS) mutagenesis. Compared to wild type, *bnd2* showed shorter stature, shorter hypocotyl, as well as shorter petiole leaves. We crossed the *bnd2* mutant with its wild type and found that the ratio of the mutant to the wild type in the F_2 population was close to 1:3, indicating that *bnd2* is a recessive mutation of a single locus. Following bulked segregant analysis (BSA) by resequencing, *BND2* was located into the 13.77 Mb-18.08 Mb interval of chromosome A08, with a length of 4.31 Mb. After fine mapping with SNP and InDel markers, the gene was narrowed to a 140-Kb interval ranging from 15.62 Mb to 15.76 Mb. According to reference genome annotation, there are 27 genes in the interval, and one of them *BnaA08g20960D* has a SNP type variation in the intron between the mutant and its parent, which may be the candidate gene conferring to *BND2*. The hybrid line derived from a cross between the mutant *bnd2* and a commercial cultivar L329 has similar plant height but higher grain yield than the commercial cultivar, suggesting that the allele *bnd2* is benefit for hybrid breeding of lodging resistance and high yield in rapeseed.

Conclusion: In this study, we found a fresh resource and a new locus for dwarf in rapeseed, which may be benefit for functional analysis of genetic mechanism of plant architecture and grain yield in rapeseed.

Background

Brassica napus (*B. napus*, oilseed rape) is an important oil crop in the world. It plays a vital role in ensuring the supply of edible oil, improving food structure, promoting the development of aquaculture and light textile industry [1]. *B.napus* belongs to Brassica oil crops of Cruciferae [2]. It is a heterotetraploid crop formed by natural distant hybridization of two basic diploid species of *Brassica rapa* and *Brassica oleracea*. The whole genome contains about 100,000 protein coding genes [3]. The plant is not only high in the conventional rape varieties, but also increased by more than 20 cm on average in the hybrid varieties due to widespread heterosis in *B. napus* [4]. However, what a serious problem companies with higher plant height is prone to lodging. Higher plant height has become a major factor restricting the mechanized harvesting and yield increase of rape [5]. It was reported that a 15%-30%, even more than 60% reduction in yield was caused by lodging as a result of higher plant height in *Brassica napus* [6]. Dwarfism of *Brassica napus* are crucial for increasing both lodging resistance and yield production [7]. However, dwarf phenotypes sometimes are associated with poor agronomic traits, resulting in a poor yield. Therefore, it's of great value for rapeseed breeding to cultivate varieties which can control plant height and can be used for cross breeding with no change in plant height but higher yield performance.

To date, a lot of dwarf mutants were identified, but the alleles useful for breeding are rare in rapeseed. For example, a dwarf mutant *NDF-1* in *B. napus* was about 70 cm tall, and all agronomic characters except for the weight of the seed are much lower than its original parents. The decrease in the number of siliques per plant and seeds per silique leads to a decline in yield [8]. Mutant *bndf-1* with a height of 75 cm had more effective branches, but the plant height was too short, resulting in fewer siliques and lower yield [9]. Semi-dwarf mutant *ds-1* was only 69.3 cm high, and showed a lower yield per plant due to the decrease of the number of siliques per plant [10]. Semi-dwarf mutant *dw-1*, approximately 95 cm high, showed a more number of siliques per plant, but decreased in yield per plant due to the significantly decreased seeds per plant [11]. The mutation line '*GRC1157*' was only ~ 90 cm at maturity and showed obvious reductions in main inflorescence length, silique numbers per main inflorescence and seeds per silique [12]. Semi-dwarf mutant *ds-3* with a height of about 70 cm displayed fewer total nodes, shorter internodes and main inflorescences, and the position of the first main branch was lower than that of the wild type [13]. There are also some mutants that reduce plant height but have no effect on yield. The dwarf mutant *DW 871* had an average plant height of 139.1 cm. Compared with the homologous high stem strain, it had more first effective branches, but there was no significant difference in the number of effective siliques, the number of seeds per plant, thousand-seed weight, and yield per plant [14]. The EMS-mutagenized *sca* mutant with a plant height of ~ 80 cm was resulted from a single semi-dominant gene, which encodes an Aux/IAA protein (BnaA3.IAA7). The mutant had more siliques per plant, with a similar thousand-seed weight, but each silique had fewer seeds resulting in a similar yield per plant compared to wild type [15]. In addition, mutants *Bndwf1* [16], *ds-4* [17], *G7* [18] showed a height of 80–110 cm, 23.4 cm, 30 cm respectively, but there were no more description of yield-related traits. In this study, we described a dwarf mutant *bnd2* (*B. napus dwarf 2*) induced by EMS mutagenesis [19]. The *bnd2* mutant showed a reduction in plant height, and grain yield compared to wild type. However, the hybrid line F_1 produced by crossing the mutant *bnd2* with a commercial variety L329 showed no increase in plant height but increase in grain yield compared to the variety L329, suggesting that *bnd2* was a new locus for dwarf and useful for hybrid breeding of lodging resistance and high yield in *B. napus*.

Bulked segregant analysis (BSA) is a rapid method to detect molecular markers associated with target traits in mapping population [20]. The combination of BSA and Next generation sequencing (BSA-seq) accelerates the cloning of genes for important traits [21]. BSA-seq has been successfully used to map important agronomic traits in many crops such as rice [22, 23], potato [24], and soybean [25]. In this study, the locus *bnd2* for dwarf was primary mapped using BSA-sEq. Finally, *bnd2* was fine mapped into a 140-Kb interval, where a gene with unknown function was identified as its candidate. Our findings may lay a foundation for cloning of the gene conferring for *bnd2*, and provide a new locus for lodging-resistant hybrid breeding in *B. napus*.

Results

Phenotypic characteristics of the dwarf mutant bnd2

A *B. napus* mutant, *bnd2*, was isolated and screened from the EMS-mutagenized seeds of the cultivar "2B" (wild type, WT) [19]. At the seedling stage, *bnd2* showed reduced hypocotyl length and shorter petiole leaves compared to WT, respectively (Fig. 1a-e). At the flowering stage, the *bnd2* mutant exhibited an extremely dwarf and compact stature, and the flowering period of *bnd2* was slightly longer than that of WT (Fig. 1f and g). At maturity stage, the plant height of *bnd2* was 100.65 ± 8.09 cm (n = 10), which is only 59.8% of that of WT (168.2 ± 7.61 cm, n = 10) (Fig. 1h and i, Additional file 1: Table S1). In addition, the first branch height, internode length, internode number and main inflorescence length of *bnd2* were 41%, 76.7%, 69%, and 85.2% of that of WT, respectively. These results suggested that the dwarf traits were associated with lower position of first branch, shorter internode length, less internode number and reduced main inflorescence length (Fig. 1h-j, Additional file 1: Table S1). Accordingly, *bnd2* produced fewer yield per plant (YPP) (48.4% of WT) due to shorter silique length (83.1% of WT), fewer seeds per silique (SPS) (92.1% of WT) and less thousand-seed weight (TSW) (90% of WT) compared to WT, although similar siliques per plant (SPP) were observed both in *bnd2* and WT (Fig. 1k-n, Additional file 1: Table S1, Additional file 2: Figure S1).

Cell elongation and expansion in stem is decreased in bnd2

To look into the underlying cellular basis of the dwarf phenotype in *bnd2*, we performed paraffin section observation on the cross section and longitudinal section of the stem of *bnd2* and WT at the early bolting stage. As shown in Fig. 2, the parenchyma cells of *bnd2* were closely arranged with irregular shapes and different sizes compared to WT (Fig. 2a and b). The cell area and length were significantly reduced in both cross and longitudinal sections in *bnd2* plant (Fig. 2c-e). Indeed, cell area in both cross and longitudinal sections were decreased by more than 48.2% and 50.5%, and cell length were decreased by more than 31.6% and 16.6%, respectively. These results suggest that the reduction of parenchyma cell area and length in stem were likely to be the main causes for the dwarfism of the mutant *bnd2*.

Inheritance of the dwarf phenotype in the mutant bnd2

To analyze the inheritance of the dwarf mutant *bnd2*, *bnd2* was used to make crosses with its original parent WT and another commercial cultivar L329. The resulting heterozygous BC_1F_1 plants (*bnd2*/WT) displayed intermediate plant height between that of WT and the mid-parent value, suggesting that the allele *BND2* is semi-dominant to the allele *bnd2* (Fig. 3a-c). In addition, according to the plant height of BC_1F_2 generation crossed by 2B and *bnd2*, the 236 BC_1F_2 individuals could be classified into two groups: one has the dwarf phenotype of *bnd2* (dwarf plants, n = 49) and another has high plant height similar or close to WT (tall plants, n = 187) group. The BC_1F_2 generation was in line with an expected Mendelian inheritance ratio of 1:3 (dwarf plants: tall plants, $\chi^2 = 2.04 < \chi^2_{0.05,1} = 3.84$) (Fig. 3d). Another F_2 population was conducted from the cross between *bnd2* and another commercial cultivar L329 which possessed a normal plant height of ~ 159 cm. There were 75 plants with dwarf phenotype and 188 plants with plant height similar or close to that of L329 in F_2 population, also showing a Mendelian segregation ratio of 3:1 (tall plants: dwarf plants, $\chi^2 = 1.46 < \chi^2_{0.05,1} = 3.84$) (Additional file 3: Figure S2). Taken

together, these results suggested that the dwarfism phenotype of *bnd2* was controlled by a single recessive gene.

Genetic mapping of the dwarf mutant bnd2 by BSA-seq

To map the gene conferring for *bnd2*, the $F_{2:3}$ population derived from cross between *bnd2* and L329 was used to perform bulked segregant analysis (BSA) resequencing. In the $F_{2:3}$ population (n = 157), 25 extremely dwarf and 23 extremely tall homozygous lines were selected to make two bulks, such as a short bulk, and a high bulk. Through sequencing in two bulks and their parents, 105,361,953, 89,416,611, 99,097,181 and 109,214,266 clean reads were harvested for the L329 parent, the mutant *bnd2* parent, the high bulk and the short bulk, respectively (Additional file 4: Table S2). The sequencing data showed that the percentage of bases with a quality score of more than 30 in two pools and two parents (Q30) reached more than 92.99%, and Q20 reached more than 97.78% (Additional file 4: Table S2). In addition, the average GC content was 37.35% and the average genome coverage was 74.57% with an average coverage depth of 21.66 X (Additional file 4: Table S2). Therefore, we consider that the quality of the sequencing data is consistent with expectations and can be used for further analysis. According to aligning with the 'Darmor-*bzh*' reference genome [26], 1,157,351 polymorphisms (containing 948,896 single nucleotide polymorphisms (SNPs) and 208,455 insertions/deletions (InDels)) were identified between the two pools. The G' value and SNP-index were calculated from the short bulk and the high bulk, the △(SNP-index) was drawn based on the physical positions of the reference genome (Fig. 4a and b). And only one significant △(SNP-index) peak was identified and located into the 4.31 Mb region from 13.77 Mb to 18.08 Mb on chromosome A08 (Fig. 4c), suggesting that it was the candidate locus harboring the *BND2* gene.

Fine mapping and candidate gene analysis

To fine mapping the *BND2* locus, six insertion/deletion (InDel) markers (ID1421, ID1470, ID1482, ID1530, ID1656, ID1667) were developed from the 4.31-Mb region harboring *bnd2* based on the BSA-seq result. Then, in the $F_{2:3}$ population (*bnd2*/L329) with 543 lines, the six markers were used to genotype 107 recessive lines with dwarf statue, as well as two controls, such as 25 wild type lines with tall stature and 25 heterozygous lines with segregation in plant height (Fig. 5). According to fine genotypes of these lines, *bnd2* was furtherly fine mapped into the 1.26-Mb interval flanked by two InDel markers ID1530 and ID1656 (Fig. 6a). In order to further narrow the candidate interval, six pairs of new polymorphic markers were developed in the region of *bnd2*, such as the single nucleotide polymorphism (SNP) markers SNP1540, SNP1552, SNP1553, SNP1557 and SNP1562 and the InDel marker ID1576 (Fig. 6b). Subsequently, *BND2* was narrowed down to an interval from 15.62 Mb to 15.76 Mb, and the physical distance was 140.0 Kb (Fig. 6b). After fine mapping and the annotation information of reference genome 'Darmor-*bzh*', there are 27 genes in the 140 kb candidate interval, 14 of which were not cloned or had unknown functions (Fig. 6c). By analyzing the annotation results of all mutations in the candidate interval, one SNP occurred in the candidate gene, *BnaA08g20960D* (Fig. 6d), which encodes an Inositol-

pentakisphosphate 2-kinase family protein, where a single nucleotide substitution from C to T occurs in the fifth intron region. Therefore, we take this gene as a key candidate gene.

The potential application of bnd2 in hybrid rapeseed breeding

Due to the low yield of *bnd2*, it cannot be used in inbreed rapeseed breeding. In order to test its potential application in hybrid breeding, we crossed the *bnd2* mutant (*bnd2/bnd2)* with a commercial cultivar L329 (*BND2/BND2*) to get their hybrid line F_1 (*BND2/bnd2*). The plant height of the F_1 hybrid was similar to L329 (Fig. 7a and b, Additional file 5: Table S3). While the yield per plant (YPP) of F_1 was significantly higher than both of *bnd2* and L329, showing an increase of 32.7% than L329 due to more seeds per silique (SPS), and three times as much as *bnd2* (Fig. 7c, Additional file 5: Table S3). This result suggested that the introduce of *bnd2* in the hybrid line can produce a hybrid of no increase on plant height but higher grain yield due to the semi-dominant effect of *BND2* to *bnd2* and the heterosis between two lines.

Discussion

Plant height is an important plant architecture character closely related to yield performance of many crops, while too high plant height tends to increase the risk of lodging. Although many dwarf genes in rapeseed have been identified and reported, only a few varieties could be used as practical breeding resources [8–18]. Compared with rice and wheat [27], dwarf mutants in rapeseed are rare. In the present study, we described a new dwarf mutant *bnd2* isolated from an EMS-mutagenized seed in *B. napus* [19]. The mutant *bnd2* displayed a height of about 100 cm at maturity, and the decrease in plant height was due to a lower position of first branch, shorter internodes and reduced main inflorescence length. The reduction of first branch height and main inflorescence length are conducive to lodging resistance. *bnd2* had a poor biological yield performance due to the limitation of plant height, so it has limited benefit for inbred breeding of high-yield cultivars. While it was reported that a *Brassica rapa* dwarf mutant *Brrga1-d*, which showed significant reduction in seed yield, had no significant influence on the seed yield for hybrid lines containing dwarf allele in *B. napus* [28]. And *sca* mutant, which had relatively short height, showed midway height between corresponding parents and significantly higher yield per plant (YPP) after making crosses with three rapeseed cultivars, 4312, ZS11 and ZY821 [15]. In this study, as shown in Fig. 3, the heterozygous BC_1F_1 plants (*bnd2*/WT) derived from backcross *bnd2* with its wild type parent, displayed intermediate plant height between that of WT and the mid-parent value, suggesting that the allele *BND2* is semi-dominant to the allele *bnd2*. While the F_1 plants (*bnd2*/L329) derived from cross *bnd2* with the commercial cultivar L329 showed no significant difference on plant height, but had significant increase on the grain yield compared to the variety L329, suggesting that by combining with the semi-dominant effect of *bnd2* and heterosis between two lines, the allele *bnd2* may be a potential gene resource for lodging-resistance and high-yield breeding in hybrid rapeseed.

In the fine mapping interval of *bnd2*, a candidate gene *BnIPK1* was annotated to encode an Inositol 1,3,4,5,6-Pentapentaphosphate 2 kinase, which catalyzes the terminal step in the biosynthetic pathway of phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakisphosphate [InsP6]) [29]. Over the last two decades, with the

discovery of *IPK1* in budding yeast [30], *IPK1* homologous genes were subsequently isolated from *Schizosaccharomyces pombe* [31], human [32], *Drosophila* [33], maize [29, 34] and *Arabidopsis thaliana* [35]. As a product of *IPK1*, phytic acid not only acts as a storage form in seeds, but also involved in hormones and signal transduction processes [36]. *AtIPK1* has been reported to be essential for sustaining plant growth. *atipk1-1* displayed reduced size and leaf epinasty [34], and the other two mutants *atipk1-2* and *atipk1-3* showed more serious growth retardation in *Arabidopsis* [37]. Similarly, Lee et al. found that *atipk1-1* mutant were significantly smaller than the wild type (Columbia-0) [36]. In addition, the seed yield of *atipk1* mutant was only 52% of that of wild type due to many pods of the mutant contained abortive seeds. In this study, *bnd2* was mapped into a 140-Kb interval harboring the homologous gene of *AtIPK1*, *BnaA08g20960D*, where a single nucleotide substitution from C to T occurs in the fifth intron region between *bnd2* and its wild type parent 2B. Then it is considered as the candidate gene for the dwarf phenotype of *bnd2*, however, the molecular basis needs to be further examined.

Conclusion

In this study, we described a new dwarf mutant *bnd2* isolated from EMS mutagenesis. The mutation of *BND2* decreased plant height and grain yield in the background of inbred line, but maintained the plant height and increased grain yield in the background of hybrid line. Through BSA-seq and fine mapping, *bnd2* was mapped to a 140.0-Kb region on chromosome A08 in *B. napus*. In summary, we identified a dwarf mutant *bnd2* which may be useful for hybrid breeding with lodging resistance and high yield, and the fine mapping results will be benefit for functional analysis of genetic mechanism of plant architecture and grain yield in rapeseed.

Methods

Plant materials and growth

B. napus 2B was used as a wild type in this study. 2B is a maintainer line of bolima cytoplasmic male sterile line. The *B. napus* dwarf mutant *bnd2* was isolated and screened from 2B seeds induced by 0.8% EMS solution in our previous study [19]. Another commercial cultivar L329 (Xiangyou 15) described previously [38] was used to construct the $F_{2:3}$ population and the F_1 hybrid line for *BND2*'s genetic analysis and evaluation of its potential value in hybrid breeding. Plants of all generations including their parents were grown in the filed in Ningxiang, Hunan province.

Agronomic traits analysis

Plants of all generations including their parents were grown in an irrigated field. Each plot in the field is about 2 m wide, 2 m long, with a row spacing of 33 cm. Ten plants were planted in each row. The agronomic traits were measured and counted at maturity stage. Ten plants from plot were randomly selected for agronomic traits analysis. The plant height (PH), internode length (IL), internode number (IN), first branch height (FBH), main inflorescence length (MIL), number of effective primary branches (NPB),

number of siliques on raceme (NSR), siliques per plant (SPP), length of siliques (LS), seeds per silique (SPS), thousand-seed weight (TSW) and yield per plant (YPP), were measured and counted as previously described [39, 40]. Significant differences were determined by Student's *t*-test using SPSS version 25 (SPSS Inc, Chicago). The segregation ratio was calculated by Chi square test.

Microscopy analysis

The second internode stem segment from the top to the bottom of *bnd2* and WT plants at the early stage of bolting were fixed in FAA (formalin-acetic acid-alcohol) solution for 16–20 h, and then subjected to dehydration and transparency. The tissues were then immersed and embedded in paraffin wax, and sectioned to 6–10 μm (Leica rm2265). After staining with 0.05% toluidine blue, the samples were examined and photographed by a reverse fluorescence phase contrast microscope.

Genetic mapping and BSA-seq

To map the *BND2* locus, the mapping population containing 157 lines was conducted by *bnd2* and L329 in the $F_{2:3}$ generation obtained from the self-pollinated F_2 population. Young leaves were collected from 157 $F_{2:3}$ lines for genomic DNA extraction using the method of SDS extraction as described by Dellaporta *et al* [41]. The DNA concentration and purity were detected by Nanodrop one (Thermo Fisher, China). 25 extremely dwarf and 23 extremely tall homozygous lines were selected to make a short bulk and a high bulk, and the two parents *bnd2* and L329 were used for BSA resequencing.

The paired end (PE) library was constructed according to the manufacture's instructions (NEBNext®Ultra™ FS DNA Library Prep Kit for Illumina®). The genomic DNA was randomly broken into 300–500 bp fragments to construct PE library. PE150 was sequenced on Illumina NovaSeq platform. The reference genome used for mutation detection is 'Darmor-*bzh*' v4.1 [26]. Burrows-Wheeler Alignment tool (BWA, version 0.7.15) was used to compare the PE reads with the reference genome sequence to get the comparison result in SAM format which was then converted to the BAM format using SAMtools (version 1.3.1). Picard tool (version 1.91) was used to sort the reads in the BAM file Sort to remove polymerase chain reaction (PCR) duplication, and variant calling including single nucleotide polymorphism (SNP) and insertion/deletion (InDel) was performed by the HaplotypeCaller of Genome analysis toolkit (GATK, version 3.7). The candidate region was determined based on Δ(SNP-index) and G' value [42] calculated by OTLseqr (version 0.7.5.2) [43], and ANNOVAR (version 2016FeB1) was used to annotate variants and predict the effect of variants on gene function (Genoseq Technology Co. Ltd., Wuhan, Hubei, China).

Development of molecular markers and their genotyping

According to the BSA-seq results and the positions of SNP and InDel on chromosomes contained in the target gene candidate region, and based on the 'Darmor-*bzh*' sequence of the *B. napus* reference genome, DNA sequences of SNP/InDel were extracted by extending 250 bp forward (5 'end) and back (3' end) respectively, and Primer Premier (version 5.0) was used to design SNP/InDel markers. For all markers, two parents L329 and *bnd2* were used for polymorphism screening, and markers with polymorphism were used for PCR amplification and genotype identification of $F_{2:3}$ population. For InDel markers, 3% agarose

gel electrophoresis was used to separate PCR products. While for SNP markers, PCR products were first identified by 1% agarose gel electrophoresis and if bands between the *bnd2* and L329 were clear, then sent the PCR products to sequencing (TsingKe Biological Technology Co. Ltd., Changsha, Hunan, China). PCR sequencing results were analyzed with Sequencher (version 5.0). The band type consistent with *bnd2* (P_1) was recorded as A, the band type consistent with L329 (P_2) was recorded as B, and both band types were recorded as H, and the deletion was not recorded. The corresponding mapping markers sequences are listed in Additional file 6: Table S4.

Abbreviations

BND2: Brassica napus dwarf 2; EMS: ethyl methanesulfonate; BSA: bulked segregant analysis; BSA-seq: BSA and Next generation sequencing; SNP(s): single nucleotide polymorphism(s); InDel(s): insertion(s) and deletion(s); YPP: yield per plant; SPS: seeds per silique; SPP: siliques per plant; PH: plant height; IL: internode length; FBH: first branch height; MIL: main inflorescence length; NPB: number of effective primary branches; NSR: number of siliques on raceme; LS: length of siliques; TSW: thousand-seed weight; PCR: polymerase chain reaction; IPK1: Inositol 1,3,4,5,6-Pentapentaphosphate 2 kinase 1.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its additional files). Any material generated during the current study is available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Basic Research Program of Shenzhen Municipal Science and Technology Innovation Committee (No. JCYJ20170818112212721), the Basic Research Program of Changsha Municipal Science and Technology (No. kq1901028), and the Natural Science Foundation of Hunan province (No. 2018JJ3036). All the funders only provided funds but did not take participation in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contribution

XL, FX, DM and XZ designed and carried out the research. XL and FX performed the experiments. WZ, JY, XL, MZ, PY provided technical assistance to XL and FX. XL, CC, XL, DM and XZ analyzed the data. XL wrote the manuscript. CC, XL, DM and XZ revised the manuscript. All authors read and approved.

Acknowledgements

We thank Dr. Zhongsong Liu for providing L329 (xiangyou 15) seeds, Prof. Wusheng Peng for 2B seeds.

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Figures

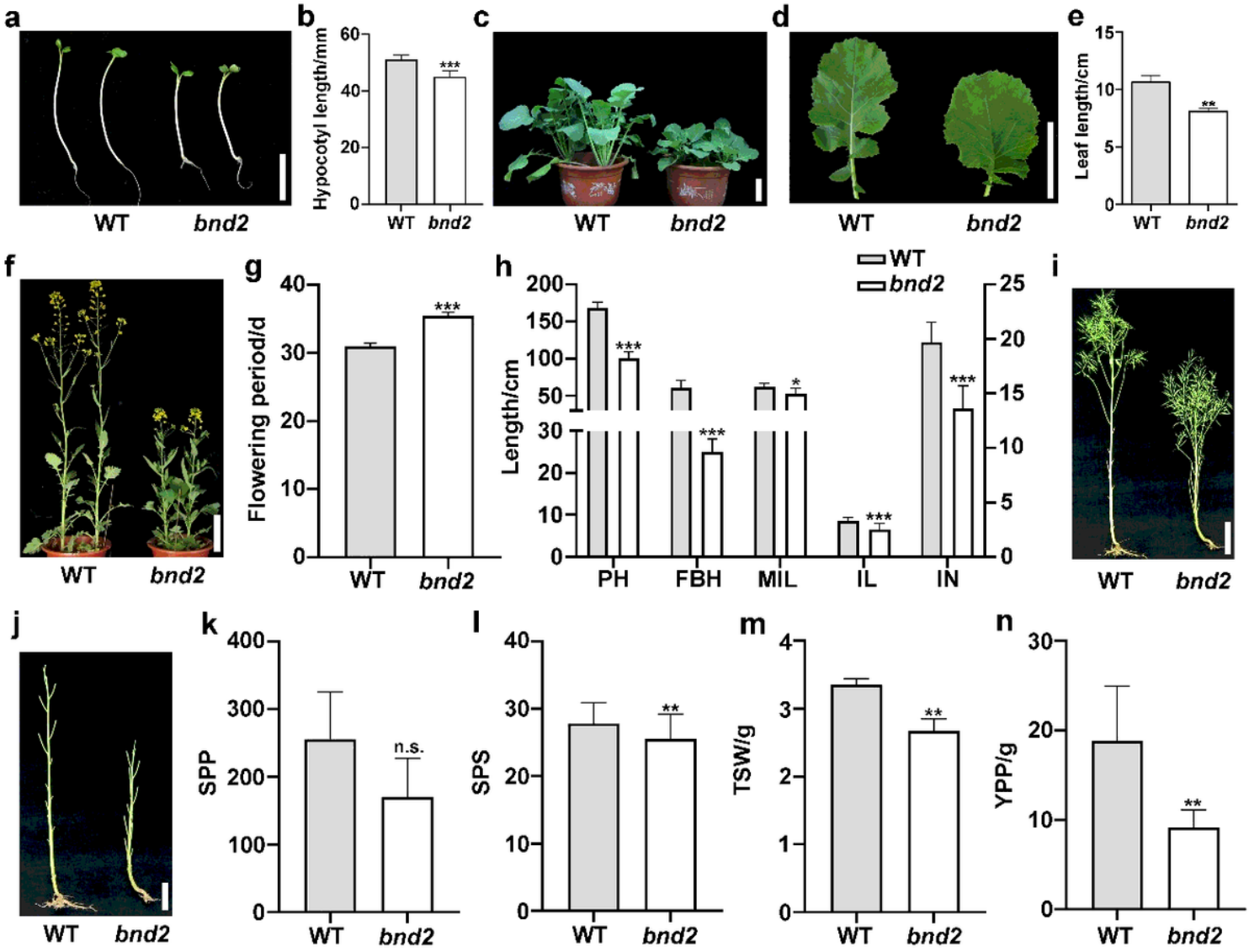

Figure 1

Phenotype characteristics of the mutant bnd2. a-b The hypocotyls length of one-week-old wild type (WT) and bnd2 seedlings. c-e Plants (c) and leaves (d-e) of WT and bnd2 at 5-week-old seedling stage. f-g Plants at peak flowering stage (f) and flowering period (g) of WT and bnd2. h Effects of the bnd2 mutant on plant height (PH), first branch height (FBH), main inflorescence length (MIL), internode length (IL) and internode number (IN). i-j Comparison of whole plant phenotype (i), internodes (j) between WT and bnd2. k-n Comparison of yield-related traits between WT and bnd2 including siliques per plant (SPP), seeds per silique (SPS), thousand-seed weight (TSW) and yield per plant (YPP). All values in (b, e, g, h, k-n) are mean ± standard deviation (SD) (n=10). Bars=20 cm in (a, f, i, j) and 5 cm in (c-d). Significance of difference was determined by Student's t-test (n.s., not significant; *, P<0.05; **, P<0.01; ***, P<0.001).

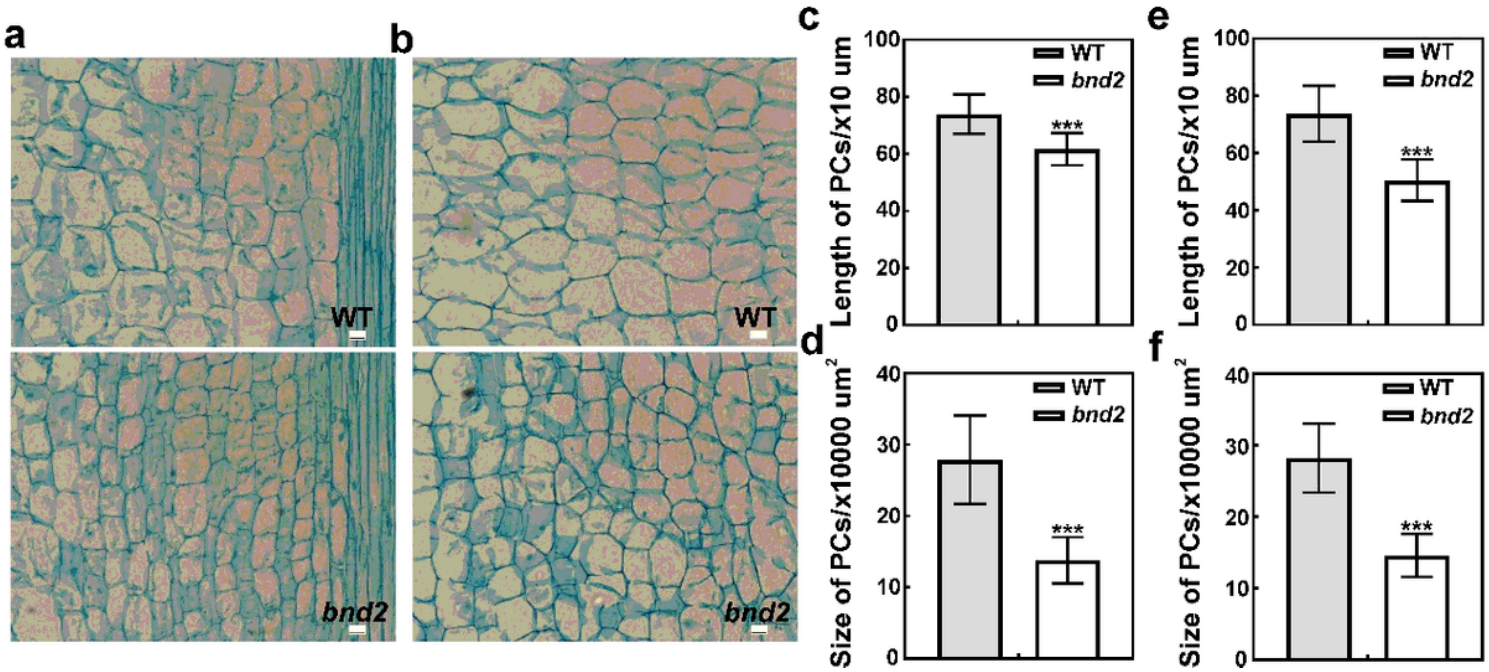

Figure 2

Cell elongation and expansion in stem of the mutant bnd2. a-b The longitudinal sections (a) and the cross sections (b) of parenchyma cells in the 2nd to 3rd internodes of WT and bnd2. c-d Statistical analysis of the length (c) and size (d) of the parenchyma cells shown in (a). e-f Statistical analysis of the length (e) and size (f) of the parenchyma cells shown in (b). All values in c-f are shown as mean ± SD (n=58, 110 of WT and bnd2 cells in c and d; n=68, 107 of WT and bnd2 cells in e and f). Bars=10 μm. The significance of the difference was determined by Student's t-test (***, P<0.001).

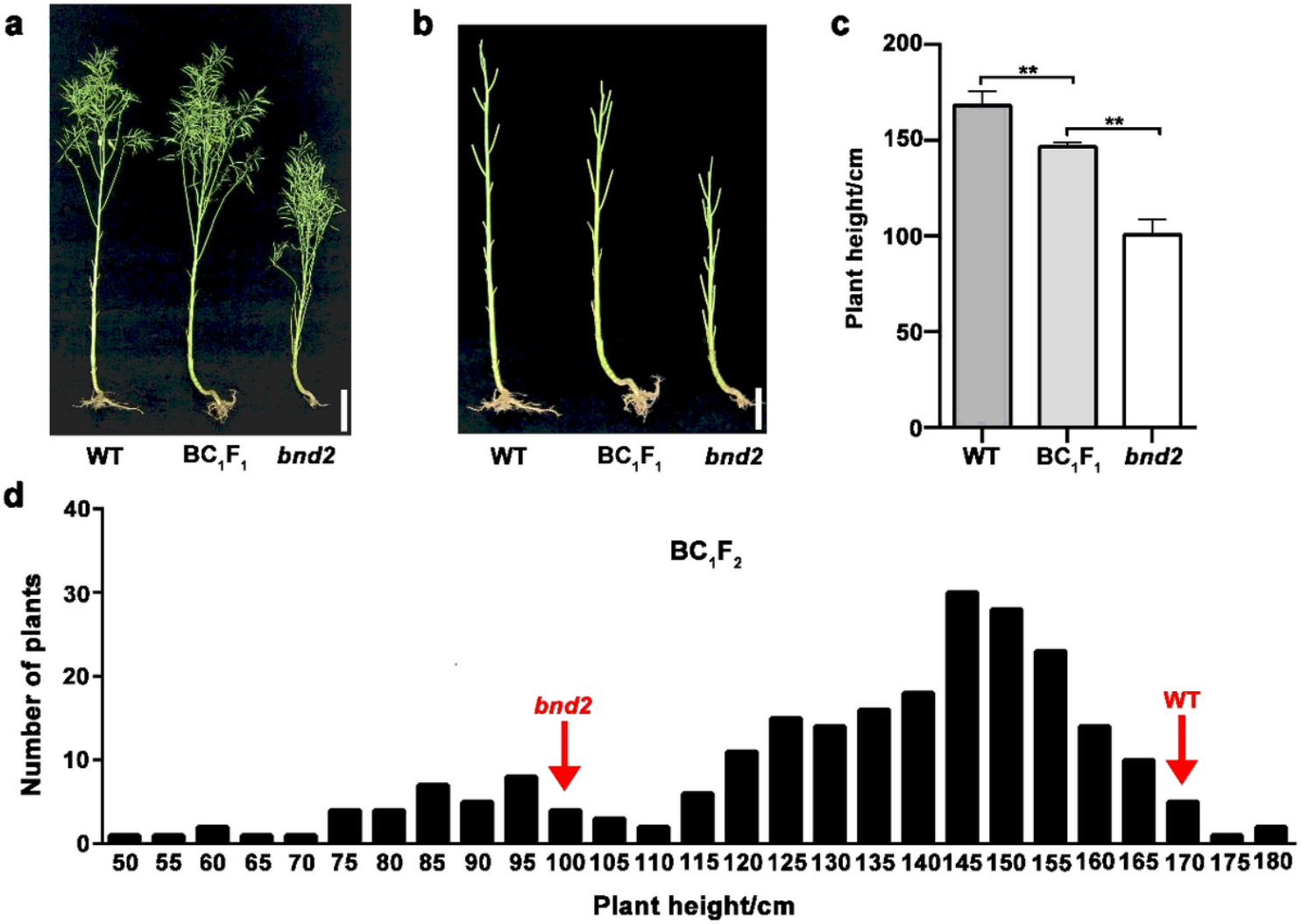

Figure 3

Phenotype and trait inheritance of bnd2 in the backcross population. a-b Performance of WT (left), bnd2 (right) and their F1 hybrid (middle) at maturity. c Plant height comparison among WT, bnd2 and their F1 at the maturity stage. Values are shown as mean ± SD (n=25). Bars=20 cm. The significance of difference was determined by Student's t-test (**, P<0.01). d Frequency distribution of plant height in the BC1F2 population containing 236 individuals derived from the cross of WT and bnd2.

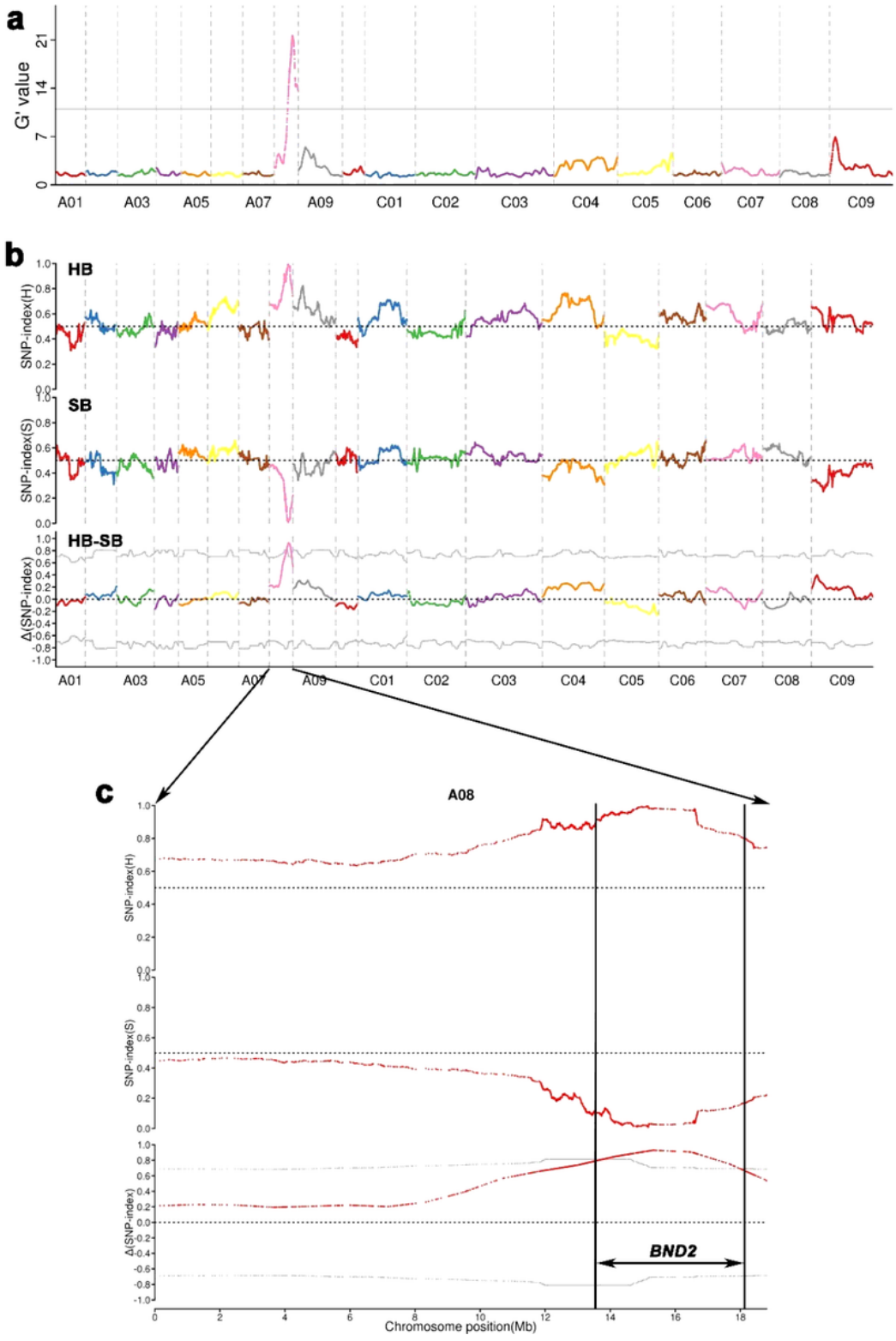

Figure 4

Primary mapping of BND2 by bulked segregant analysis using resequencing. The physical position (unit: MB) of each chromosome in Brassica napus is represented as the x-axis. a The G'-value was represented as the y-axis. b The SNP index of 3-Mb interval with 10-kb sliding window each time was represented by the y axis. The △(SNP-index) was calculated by subtracting the SNP index of the short bulk (SB) from

that of the high bulk (HB). The dotted line was the threshold of △(SNP-index) set as the mean of △(SNP-index) ± 3*SD. c The BND2-containing genomic interval was identified by using the threshold line.

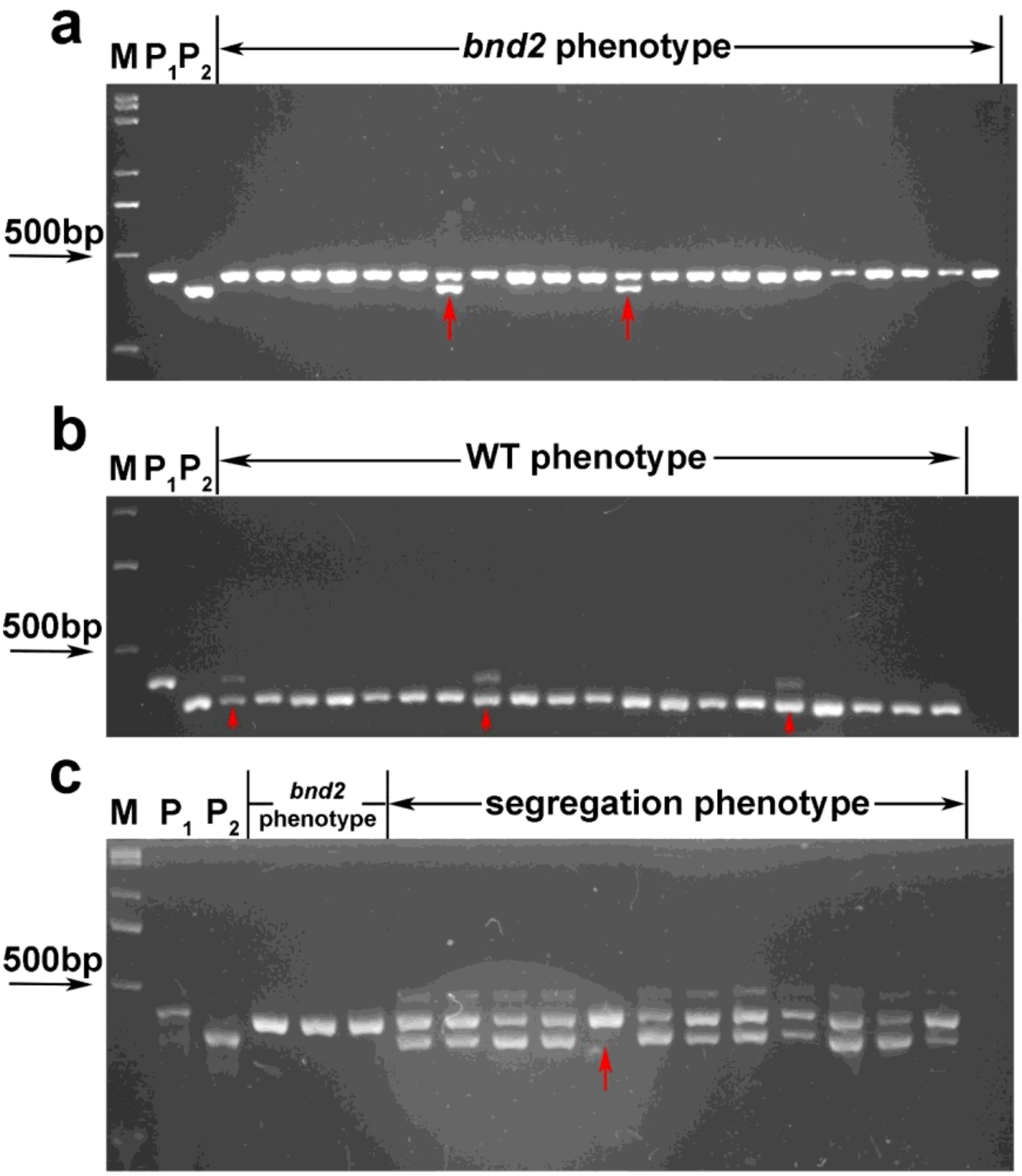

Figure 5

The genotypes of some F2:3 lines derived from cross between bnd2 and L329 at the marker ID1656. a The genotypes of the lines with the bnd2 phenotype. b The genotypes of the lines with WT phenotype. c The genotype of the lines with phenotype segregation. M means DNA Marker. P1 means the mutant

parent bnd2. P2 means the WT parent L329. The red arrow indicates the recombinants between BND2 and the marker ID1656.

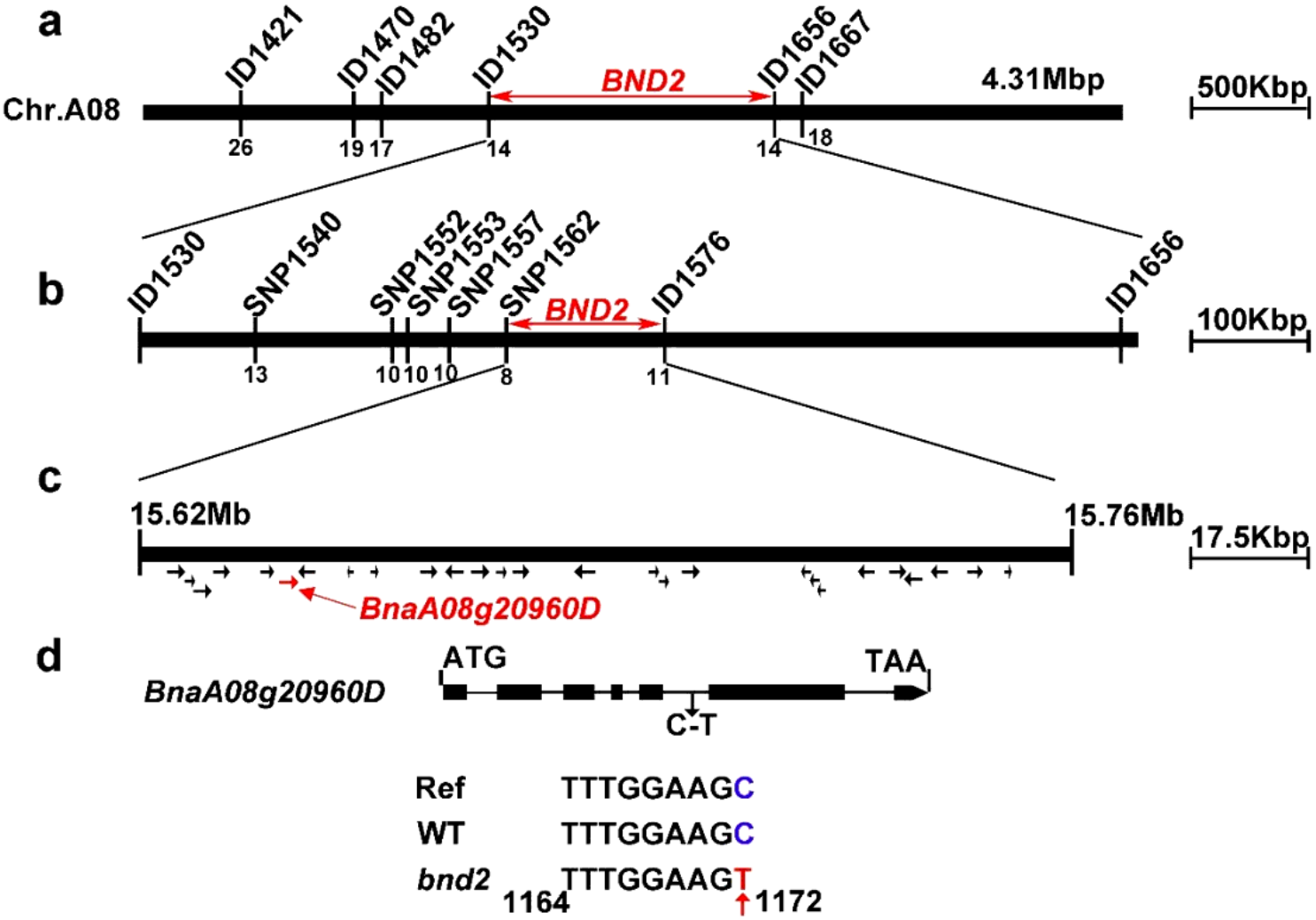

Figure 6

Fine mapping of BND2. a The BND2 locus was primary mapped into the interval flanked by the markers ID1530 and ID1656. Numbers below each marker is the number of recombinants. b The BND2 locus was finally mapped to a 140-Kb flanked by SNP1562 and ID1576. c Relative physical position of the BND2 locus. Numbers above chromosome A08 indicate physical distance (unit:Mb). The region contains 27 annotated genes according to the 'Darmor-bzh' reference genome. The candidate gene, BnaA08g20960D is marked in red. d Structure of the BnaA08g20960D gene, a single nucleotide substitution (C-T) between bnd2 and its wild type parent 2B was identified in the fifth intron. Exons and introns were represented as black boxes or black lines, respectively.

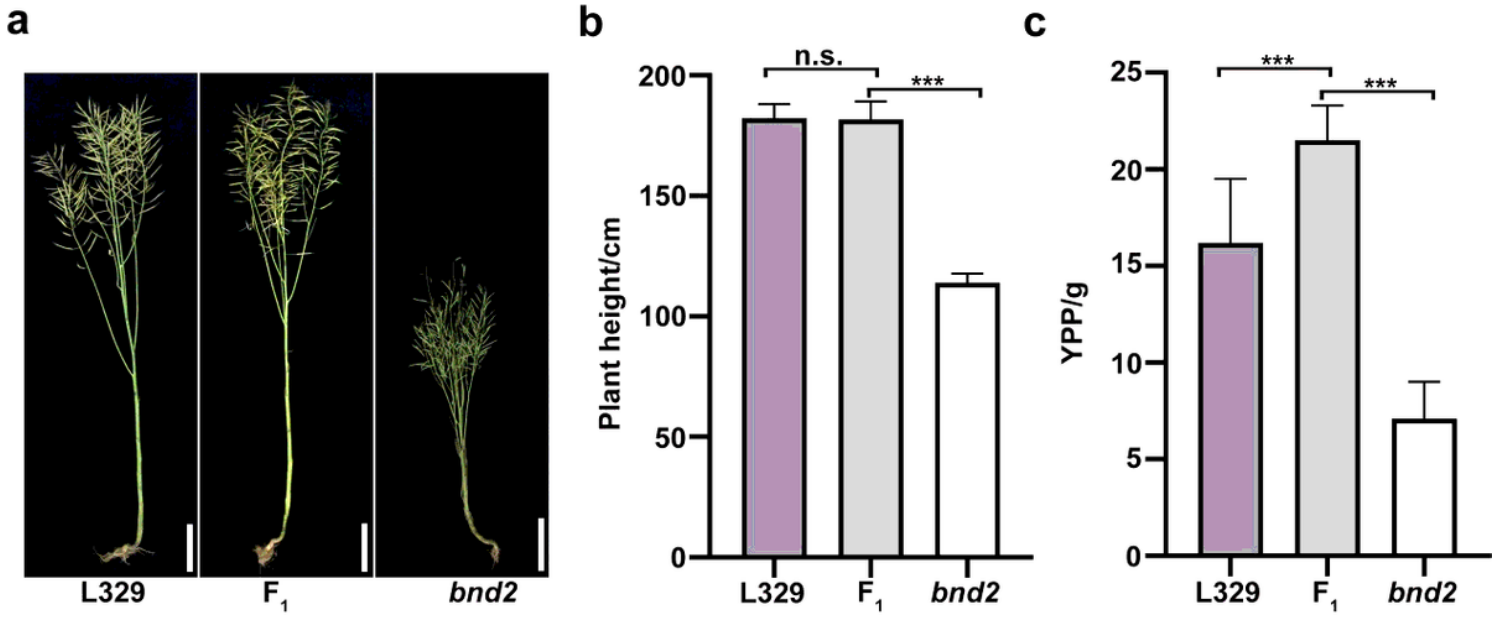

Figure 7

Performance on plant height and grain yield of bnd2 in the hybrid line. a Phenotypes of L329 (left), bnd2 (right) and their hybrid (F1, middle) at the maturation stage. b-c Plant height (b) and yield per plant (c) of L329, bnd2 and their F1 hybrid at maturity. Values are shown as mean ± SD (n=10). Bars=20 cm. The significance of difference was determined by Student's t-test (n.s. not significant; ***, P<0.001).

Supplementary Files

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