Fine mapping of the *BnaC04.BIL1* gene controlling plant height in *Brassica napus* L.

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**Abstract**

**Background:** Plant height is an important architecture trait which is a fundamental yield-determining trait in crops. Variety with dwarf or semi-dwarf phenotype is a major objective in the breeding because dwarfing architecture can help to increase harvest index, increase planting density, enhance lodging resistance, and thus be suitable for mechanization harvest. Although some germplasm or genes associated with dwarfing plant type have been carried out. The molecular mechanisms underlying dwarfism in oilseed rape (*Brassica napus* L.) are poorly understood, restricting the progress of breeding dwarf varieties in this species. Here, we report a new dwarf mutant *Bndwarf2* from our *B. napus* germplasm. We studied its inheritance and mapped the dwarf locus *BnDWARF2*.

**Results:** The inheritance analysis showed that the dwarfism phenotype was controlled by one semi-dominant gene, which was mapped in an interval of 787.88 kb on the C04 chromosome of *B. napus* by Illumina Brassica 60 K Bead Chip Array. To fine-map *BnDWARF2*, 318 simple sequence repeat (SSR) primers were designed to uniformly cover the mapping interval. Among them, 15 polymorphic primers that narrowed down the *BnDWARF2* locus to 34.62 kb were detected using a *F₂* family population with 889 individuals. Protein sequence analysis showed that only BnaC04.BIL1 (BnaC04g41660D) had two amino acid residues substitutions (Thr187Ser and Gln399His) between ZS11 and *Bndwarf2*, which encoding a GLYCOGEN SYNTHASE KINASE 3 (GSK3-like). The quantitative real-time PCR (qRT-PCR) analysis showed that the *BnaC04.BIL1* gene expressed in all tissues of oilseed rape. Subcellular localization experiment showed that BnaC04.BIL1 was localized in the nucleus in tobacco leaf cells. Genetic transformation experiments confirmed that the *BnaC04.BIL1* is responsible for the plant dwarf phenotype in the *Bndwarf2* mutants. Overexpression of *BnaC04.BIL1* reduced plant height, but also resulted in compact plant architecture.

**Conclusions:** A dominant dwarfing gene, *BnaC04.BIL1*, encodes an GSK3-like that negatively regulates plant height, was mapped and isolated. Our identification of a distinct gene locus may help to improve lodging resistance in oilseed rape.

**Keyword:** *Brassica napus*; semi-dominant; dwarf; single nucleotide polymorphism; gene mapping

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suitable for mechanization harvest [1]. To find available germplasm or genes associated with dwarfing plant type for *B. napus* breeding, some efforts have been carried out. For example, the dwarfness-associated genes in *B. napus*, including *DS-1* [2], *ndf-1* [3], *DS-3* [4], *DS-4* [5], *G7* [6], BnaDwf.C9 [7], have been positioned or identified. Additionally, the *Bndwf1* was fine-mapped on the A9 chromosome to a 152-kb interval [8]. However, the molecular mechanism(s) underlying the development of the dwarf phenotype in *B. napus* remain elusive. The lack of innovation on *B. napus* ideal type breeding is mainly due to absence of successfully applied cultivar in vast oilseed rape production region. Dwarfism is usually related to plant hormone biosynthesis and signal transduction, such as auxin [9, 10], gibberellin (GA) [1, 11], and brassinosteroid (BR) [12, 13]. Auxin affects plant height by regulating cell division, elongation, and differentiation [14]. GA mainly affects the elongation of stem and internode to regulate plant height [1]. Relationship between plant dwarf stature and genes in auxin and GA biosynthesis and signal transduction pathways has been well-documented [4, 15–19]. Defects in BR biosynthesis and signaling pathways can lead to dwarfing phenotypes. During BR biosynthesis, many synthases belong to the cytochrome P450 monooxygenase (CYP) gene. Defects of these synthases can lead to dwarfing phenotypes. For instance, *CPD* (constitutive photomorphogenesis and dwarfism) encodes a steroid 23α-hydroxylase enzyme, a member of CYP90A family, which acts in the conversion of cathasterone to teasterone in the BR biosynthetic pathway [20]. The loss-of-function mutations of *AtCPD* gene leads to the dwarfing phenotype, when over-expression of *CPD* gene can restore the plant height and plant type [20]. *BRD1* (*BR-deficient dwarf 1*) gene encodes the final catalytic enzyme (BR-C6 oxidase) in BR biosynthesis, mutation of which cause dwarfing phenotype. Rice *brd1* was the first report to describe the phenotypic characterization of a BR-deficient mutant in monocot plants, and showed the phenotype of leaf sheath small, leaves wrinkled, internodes short, fewer tillers. The exogenous application of BL can restore the plant type of the *brd1* mutants [21]. The *dwr4* (*CYP90B1*) in *Arabidopsis*, *dwr2* (*CYP90D*) and *dwrfl1* (*CYP724B1*) in rice encode P450 monooxygenase to involve in BR biosynthesis, loss-of-function mutations of which reduce the endogenous BR levels and consequently confer reduced plant height [13, 22, 23]. The *Arabidopsis DET2* (*de-etiolated 2*) is a key gene in BR biosynthesis and allow an assignment for this steroid’s role in plant development [24, 25]. The *Arabidopsis det2* mutant [24] and the maize *na1* (*nana plant1*) mutant [26] were the loss-of-function of *DET2* gene lead to the dwarfing phenotype, dark green leaves, and have reduced fertility.

BR signal transduction is a signaling cascade from the BR receptor to the expression of BR target genes, which plays an important role in various developmental and growth processes in plants [27]. Researches during the past several decades have accumulated extensive knowledge of BR signaling pathways in model plants [28–30], such as *Arabidopsis* and rice. It is well documented that BRs are perceived extracellularly by the BR-INSENSITIVE1/BR11-ASSOCIATED KINASE1 (BR11-BAK1) [31–33] complex. Afterwards, the binding between BRs and BR11-BAK1 complex could initiate signal transduction to BRASSINOLYSIS1/BRASSINOSTEROID INSENSITIVE1/BRASSINOSTEROID INSENSITIVE1/BRASSINOSTEROID INSENSITIVE2 (BIN2) [39, 40], as well somehow PROTEIN PHOSPHATASE 2A (PP2A) [41]. The transcriptional factor *BES1*/*BRZ1* affects plant growth and development in various aspects through the regulating expression of thousands of BR responsive genes. Among these genes, glycogen synthase kinase-3 (GSK3)-like kinase BIN2 is a key suppressor that regulates plant growth and development by determining the phosphorylation status of *BES1* and *BRZ1* [33, 34, 39, 40]. GSK3-like kinases are a highly conserved Ser/Thr kinases that are implicated in a wide range of cellular and developmental processes [42]. In *Arabidopsis*, the GSK3/SHAGGY-like family has 10 gene members that can be classified into four subgroups [43]. In this family, the *Arabidopsis* GSK3-like kinase (AT4G18710, BIN2/UCU1/DWF12/AtSK21) which belongs to the group II, has activity to negatively regulate the BR signal transduction by phosphorylating *BZR1*/*BES1* [39, 40, 44]. The gain-of-function *bin2* mutant was discovered to be insensitive to BRs in *Arabidopsis* and has the shaggy phenotypic characteristic of dwarfing architecture. It also confers curved leaves, and an impaired cell elongation [45]. The coding sequence of the *BIN2* gene, substitutes consecutive glutamate residues in the highly conserved TREE domain, which results in the negatively regulating growth by phosphorylating the *BES1* and *BZR1* proteins, that result in the degradation of *BZR1* to reduce its activity [40]. Based on sequence similarity of BIN2 with its two closest group II *Arabidopsis* homologs, BIN2-Like1 (BIL1) and BIN2-Like2 (BIL2), which belong to the AtSKs group [40]. It was further suggested that BIL1 and BIL2 may also be involved in BR signaling. Over-expression of *BIL1* or *BIL2* gene driven by their native
promoters in wild-type Arabidopsis plants exhibits the dwarf phenotype [46]. However, the evidence of BIL1 and BIL2 genes involved in BR signal transduction is still insufficient, and the mechanism of plant dwarf phenotype caused by overexpression of BIL1 and BIL2 genes remains to be elucidated. Therefore, it is urgent to further explore their participation and even related mechanism.

In this study, a pure dwarf mutant, Bndwarf2, was found in advanced selfing generation in a nearly pure line CB1501-1 in B. napus. To expedite this study, the dwarf gene BnaC04.BIL1 was isolated using map-based cloning. The BnaC04.BIL1 gene encoding a GSK3-like kinase, belongs to GSK II subfamily. Genetic transformation experiments confirmed that the BnaC04.BIL1 was responsible for the plant dwarf phenotype in the Bndwarf2 mutants. Our study clarifies the role of BnaC04.BIL1 in the regulation of plant height, which may help to improve lodging resistance in oilseed rape.

Results
Characterization of the Bndwarf2 Mutant
A pure dwarf mutant, Bndwarf2 was obtained in advanced selfing generation in a nearly pure line CB1501-1 in B. napus. The Bndwarf2 mutant showed obvious dwarf phenotype after 6 d dark germination compared to Zhongshuang 11 (ZS11, a conventional B. napus cultivar), which was used as a parent to map-based clone the gene responsible for the dwarfism (Fig. 1a). At seedling stage, the Bndwarf2 mutant plants had shorter hypocotyls and shorter petioles (Fig. 1b, c). The leaves of Bndwarf2 mutants showed darker green, thickened, and wrinkled leaves, and had significant higher Chl a, Chl b, and Chl contents than those of ZS11 (Table S1). At flowering stage, the Bndwarf2 mutant showed significant difference in plant height from ZS11 (Figure S1). While at maturity stage, the Bndwarf2 mutant showed dwarf stature (33.62 ± 1.12 cm) with no apical dominance,
that was significantly lower than that for ZS11 (193.54 ± 4.80 cm) (Fig. 1d). The siliques of Bndwarf2 mutants were significantly shorter compared to that of ZS11 (Fig. 1e). In addition, the Bndwarf2 mutants had lower 1000-seeds weight and compact plant architecture (Fig. 1f; Table S2). The F1 plants (105.30 ± 5.16 cm) generated by cross of ZS11 with Bndwarf2 were in-between that of ZS11 and Bndwarf2.

Inheritance of the dwarf trait
To investigate the genetic regulation mechanism for Bndwarf2, the F1 (ZS11 × Bndwarf2) and RF1 (Bndwarf2 × ZS11) plants were obtained by crossing Bndwarf2 with ZS11, all had the dwarf trait, indicating that dwarf trait was controlled by dominant genes. The phenotypic segregation ratio of dwarf plants to tall plants in the F2 population was in a Mendelian model of 1:2:1 (69 homozygous dwarf plants vs. 140 hybrid dwarf plants vs. 78 tall plants, $\chi^2 < \chi^2_{0.05}$) (Figure S2). Among 289 BC1 individuals, 139 as dwarf types and 150 as tall types, also approximately fitted an expected Mendelian inheritance ratio of 1:1 (dwarf plants vs. tall plants). In subsequent segregating F2:3 populations, the genetic regulation was confirmed (Table 1). These results indicated that the dwarf trait was controlled by a semi-dominant nuclear gene, which was named as BnDWARF2 in the subsequent study.

Map-based cloning
To map BnDWARF2, 94 plants (70 dwarf plants and 24 tall plants) from the F2 population were used for single nucleotide polymorphism (SNP) marker genotyping. Although the chip (Illumina, Inc) has 52,157 SNP markers, only 7457 polymorphic markers were used to construct the SNP genetic linkage map after removing the invalid or non-polymorphism markers. The BnDWARF2 locus was primarily mapped within the 787.88-kb on C04 chromosome between the SNP marker M33367 and M35244 (Fig. 2a). To fine map the BnDWARF2 locus, 318 primer pairs of simple sequence repeat (SSR) markers were designed to uniformly cover the preliminary mapping interval. A further 889 individuals from the F2:3 populations, finally narrowed down the BnDWARF2 locus to a 34.62-kb region between SSR markers S3 and S4 (Fig. 2b). No other markers to further narrow the mapping interval were found for this mapping population and its parents. A total of 5 putative genes (BnaC04g41640D, BnaC04g41650D, BnaC04g41660D, BnaC04g41670D, and BnaC04g41680D) were localized in the 34.62-kb region according to the gene annotation of the B. napus reference genome (Fig. 2c; Table 2). Gene cloning was performed for the mapping interval, and the results showed that only BnaC04g41660D (BnaC04.BIL1) gene had 10 SNPs differences between ZS11 and Bndwarf2. The BnaC04.BIL1 had two amino acid residues substitutions at aa-187 (Thr-to-Ser mutation, named Thr187Ser) and aa-399 (Gln-to-His mutation, named Gln399His) (Fig. 2e).

Table 1

| Population | Homozygous dwarf plants | Hybrid dwarf plants | Tall plants | Expectation | $\chi^2$ | P value |
|------------|-------------------------|---------------------|------------|-------------|--------|--------|
| F1         | 0                       | 30                  | 0          | 1:2:1       | 0.74   | 0.69   |
| RF1        | 0                       | 30                  | 0          | 1:2:1       | 0.35   | 0.56   |
| F2         | 69                      | 140                 | 78         | 1:2:1       | 1.71   | 0.43   |
| BC1        | 0                       | 139                 | 150        | 1:1         | 0.35   | 0.56   |
| F2:3       | 206                     | 451                 | 232        | 1:2:1       | 1.71   | 0.43   |
Fig. 2  Map-based cloning of BnDWARF2. a The BnDWARF2 locus was mapped primarily on C04 chromosome between the SNP markers M33367 and M35244. b The BnDWARF2 locus was fine-mapped in the 34.62 kb region between SSR markers S3 and S4. The numerals indicate the number of recombinants. c The genes in the mapping interval. d The gene structure and the mutation sites in BnaC04.BIL1. e The protein structure and the mutation sites of the BnaC04.BIL1 protein, and the STKc_GSK3 superfamily domain was predicted. Solid lines show the position of the amino acid transition. f The chromatogram of BnaC04.BIL1 at 1565–1585 bp in ZS11 and Bndwarf2 mutant, respectively. g The chromatogram of BnaC04.BIL1 at 2900–2920 bp in ZS11 and Bndwarf2 mutant, respectively. The black arrows denote the A1575T and G2910T substitutions, respectively.

Table 2  Information of 5 putative genes in the mapping interval

| Gene in B. napus | Homologue in A. thaliana | Gene function                                      |
|-----------------|-------------------------|---------------------------------------------------|
| BnaC04g41640D   | AT2G29770.1             | Galactose oxidase/kelch repeat superfamily protein |
| BnaC04g41650D   | unknown protein         |                                                   |
| BnaC04g41660D   | AT2G30980               | Encodes a GSK3-like protein kinase                |
| BnaC04g41670D   | AT2G30990.1             | Arginine N-methyltransferase, putative (DUF688)   |
| BnaC04g41680D   | unknown protein         |                                                   |
Expression patterns of \textit{BnaC04.BIL1} and the subcellular localization

To explore the possible function of \textit{BnaC04.BIL1} gene from \textit{Bndwarf2} mutant in different tissues, the transcription levels of \textit{BnaC04.BIL1} in leaves, roots, hypocotyls, stems, buds, flowers, siliques, and seeds were analyzed. The qRT-PCR analysis showed that the \textit{BnaC04.BIL1} gene was expressed in all tissues, which indicated that \textit{BnaC04.BIL1} expressed constitutively (Fig. 3a). The expression level of \textit{BnaC04.BIL1} was higher in leaves, hypocotyls, siliques, and seeds, while its level in buds and stems were lower.

Previous research showed that \textit{Arabidopsis} BIN2 \cite{52} was localized in the nucleus. To define the subcellular location of expression, pA7-GFP and BnaC04.BIL1-GFP constructs were then introduced into the tobacco leaf cells by the particle bombardment method. The merged image of BnaC04.BIL1-GFP and nuclear localization

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Expression pattern of \textit{BnaC04.BIL1} and subcellular localization of its encoding protein. \textbf{a} Expression pattern of \textit{BnaC04.BIL1} detected by qRT-PCR in bud, stem, flower, sique, seed, root, hypocotyl, and leaf from \textit{Bndwarf2}. The \textit{BnActin} gene was used as a reference gene and the expression level of bud was set to 1. The bud, stem, and flower samples are from flowering stage. The sique samples are from podding stage. The seed samples are from maturity stage. The root and hypocotyl samples are from 7-day-old seedlings grown on medium, and the leaf samples are from seedling stage. \textbf{b} Subcellular localization of \textit{BnaC04.BIL1} protein in tobacco leaf cells. Plasmids pA7-GFP and BnaC04.BIL1-GFP were introduced into tobacco leaf cells by particle bombardment, respectively. Bars = 20 μm}
\end{figure}
signal (NLS)-mCherry signals showed that BnaC04.BIL1 was localized to the nucleus (Fig. 3b). The result showed the BnaC04.BIL1 gene functions in the nucleus.

**Overexpression of BnaC04.BIL1 leads to plant dwarf**

To investigate BnaC04.BIL1 functioning in plant height, a construct was generated by inserting a 1233 bp BnaC04.

BIL1 ORF fragment from Bndwarf2 into the vector pBI-121 under the control of the CaMV35S promotor. The construct was introduced into ZS11 plants by Agrobacterium-mediated transformation. The plant height trait was compared between the ZS11 and OE-BnaC04.

BIL1 (OE-BIL1) transgenic plants by overexpressing the BnaC04.BIL1 gene. Notably, plant height in the OE-BIL1 transgenic plants was similar to the expected Bndwarf2 phenotype with obvious dwarf stature; meanwhile, the transgenic plants also displayed dramatically smaller seeds than the ZS11 plants (Fig. 4a-c; Table S3). At the seedling stage, the OE-BIL1 transgenic lines displayed darker green and wrinkled leaves compared to those of ZS11 (Fig. 4b). These results suggest that the BnaC04.

BIL1 gene not only controls the plant height, but also regulates the seed size. It follows that, the yield of per OE-BIL1 transgenic plants showed a significantly reduction compared to that of ZS11 (Table S3). The T2 progeny plants were examined from six independent T1 transgenic lines in growth chamber, which showed the expected Mendelian inheritance ratio of 3:1 in T2 progeny (dwarf vs. tall plants, \( \chi^2 < \chi^2_{0.05} \), \( \chi^2 = 3.84 \), \( P > 0.05 \); Table S4). The T2 progeny plants displayed perfect cosegregation between the transgene and the dwarf phenotype. Consistently, the expressions of BnaC04.BIL1 gene in homozygous T3 lines (OE-BnaC04.BIL1 transgenic genes) were significantly higher than those of ZS11 plants (Fig. 4d). These results confirmed that the BnaC04.

BIL1 is the causal mutation for the dwarfism and controls smaller seeds, which were also observed in Bndwarf2.

**Discussion**

Plant height is an important growth habit that is a fundamental yield determining trait in crops. In the 1960s and 1970s, the dwarf trait genes (Rht1 and sd1) were introduced into wheat and rice that were crucial to the first “Green Revolution” [1, 53]. The semi-dwarf architecture can help to increase harvest index, increase planting density, enhance lodging resistance, and thus be suitable for mechanization harvest [54]. However, there are few studies with respect to dwarf oilseed rape. Because of the lower mechanization level of oilseed rape production and few varieties suitable for mechanization harvest, oilseed rape production faces severe challenge.

Most of our knowledge about BIN2 functions came mostly from gain-of-function results. For example, genetic screening in Arabidopsis for BR-insensitive...
dwarf mutants resulted in the isolation of eight gain-of-function bin2 alleles [39, 40, 45]. Based on sequence similarity of BIN2 with its two closest group II Arabidopsis homologs, BIN2-Like1 (BIL1) and BIN2-Like2 (BIL2), which belong to the AtSKs group [46]. Overexpression of BIL1 or BIL2 gene driven by their native promoters in wild-type Arabidopsis plants exhibits the dwarf phenotype [46]. In our study, a gain-of-function mutation for BIL1 in oilseed rape has been discovered, and most importantly, it exhibits the BR-insensitive dwarf phenotype. For example, the Bndwarf2 mutant displayed the BR signaling phenotypes: shorter hypocotyls, shorter petioles, wrinkled leaves, and obvious dwarf compared with the ZS11 (Fig. 1; Figure S1; Table S2). These characteristics were similar to the phenotypes of BR-insensitive mutants such as bri1 [27], dwf12 [39], and ucl1 [45]. Through map-based cloning, the BnaC04.BIL was identified to be a BIN2-Like1 (BIL1), showing a Thr187Ser amino acid substitution residing in the conserved region (Fig. 2; Figure S3a). Genetic transformation experiments confirmed that the BnaC04.BIL1 was responsible for the plant dwarf phenotype in the Bndwarf2 mutants. Overexpression of BnaC04.BIL1 under the background of ZS11 reduced plant height compared with ZS11 (Fig. 4; Table S3). This result was consistent with previous reports, showing that overexpressing BIL1 gene confers the dwarf phenotype in Arabidopsis [46]. The genetic evidence clarifies the BnaC04.BIL1 can sharply change plant architecture in natural plant accessions in allotetraploid.

Further study has identified Bndwarf2, a dwarf and compact mutant in B. napus, and the dwarf trait is controlled by a semi-dominant nuclear gene (Table 1). The plant height of F1 derived from the cross of Bndwarf2 with the tall parent, decreased by about 50% compared to that of tall plant (Table S2). Particularly, the Bndwarf2 displayed an extreme reduction in height at maturity, which is different from the previously reported dwarf mutants in B. napus [4, 8, 55–57]. For example, the dwarf locus of bnc.dwf mutant was controlled by a recessive gene [56]. And, the dwarf trait of Bndwf1 mutant was controlled by a semi-dominant gene [8]. The F1 plants have compact properties such as shortened branch, shortened gap between siliques, shortened gap between branches and dwarfing plant height by BnDWARF2 gene (Fig. 1; Figure S1; Table S2). This finding implicates that the plant architecture of homozygous or heterozygous individuals derived Bndwarf2 mutant is compact (Fig. 1). This kind of compact architecture can be undoubtedly helpful to increase planting density, enhance lodging resistance and increase planting density, therefore the compact plant architecture is ideal for machinery production of oilseed rape.

The germplasm Bndwarf2 has compact plant type, and lacks strong growth vigor. However, the compact plant architecture can be used in hybrid cultivar development in which the compact type and hybrid vigor can be combined well. This is helpful to breeding of variety breeding with the objectives such as high-yield, good quality and suitable for machinery. On the other hand, the growth vigor in pure line or cultivar may be improved in some genetic background. Some reports have demonstrated that the genes in BIN2 regulation network can also interact with BIN2, leading to improvement of the growth inhibition caused by BIN2 gene overexpression caused by natural biological accession state or by transgenic [58–61]. We speculate that some gene may interact with BnaC04.BIL1 to attenuate its role in limit growth vigor as that the Arabidopsis homolog BIN2 crosstalk experiments have shown. Furthermore, expressions of some regulator genes may probably alter the expression level of BnaC04.BIL1 that is constitutively expressed in the various organs, and reduced expression level may improve the growth vigor. The subcellular localization analysis demonstrated that BnaC04.BIL1 exists in the nucleus (Fig. 3). Consistently, the Arabidopsis BIN2 functioned in nucleus to negatively regulate BR signaling [52]. In fact, previous results revealed that many genes regulated by BZR1 and/or BES1, and some proteins interacting with BZR1/BES1, were closely associated with the BR signaling [29, 62]. The BR signal transduction pathways was impairment to lead to the dwarfing phenotype.

**Methods**

**Plant materials and growth conditions**

A pure dwarf mutant, Bndwarf2 was found in advanced selfing generation in a nearly pure line CB1501-1 in B. napus from our germplasm bank of our lab in Nanjing Agricultural University. The populations for mapping the BnDWARF2 locus, were generated from the crosses between Bndwarf2 and the canola variety Zhongshuang 11 (ZS11). All oilseed rape materials were grown in growth chamber and the fields of the Jiangpu Agricultural Experimental Station at Nanjing Agricultural University.

Tobacco was grown in growth chamber. The illumination period was 14 h with temperature at 26 °C and 10 h with temperature at 20 °C. When tobacco leaves at 5-leaf stage were used for the subcellular localization.

**Map-based cloning**

SNP and SSR markers were used to map the dwarf gene. 70 dwarf plants, 24 tall plants and parents from F1 population were genotyped using a Brassica 60 K SNP Bead Chip Array (Illumina, Inc), which have a total of 52,157 SNP markers. The SNP genetic map was constructed by
The cDNA was used as the template for qRT-PCR analysis with 1000 replicates. First-strand cDNA synthesis was carried out using a Reverse Transcription System (Takara, Tokyo, Japan). Using this genomic sequence, SSR marker primers were designed by aid of SSR Hunter 1.3 [64], and Primer Premier 5.0 [65]. A total of 318 polymorphic SSR markers were obtained. These SSR markers helped to fine-map the *BnDWARF2* locus using a size-enlarged population comprised of *F*₂₃ plants.

To identify genes associated to the dwarf trait, sequence of the fine mapping interval was obtained from the *Brassica napus* Genome Browser for reference to next-step experiments. Then, all of the genes in the fine mapping interval were cloned from *Bndwarf2* and parent ZS11. And, the resulting sequences were aligned using ClustalX 1.83 and GeneDoc software. The specific primers of the genes are listed in Table S5.

**Sequence analysis**
The *B. napus* BIL1 genes were obtained by screening the *B. napus* Genome Browser (http://www.genoscope.cns.fr/brassicaneus/cgi-bin/gbrowse/colza/) with known *A. thaliana* BIL1 gene as a query. The Conserved Domain Database was used to search the protein functional in the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). Predicted *A. thaliana* BIL1 amino acid sequences were obtained from the TAIR website (http://www.arabidopsis.org/Blast). Moreover, the protein sequences of other species were obtained from the NCBI using the *A. thaliana* amino acid sequences as a query. The resulting protein sequences were aligned using ClustalX 1.83 [66]. Additionally, a phylogenetic tree was constructed using MEGA 7.0 [67] with maximum likelihood method, and the bootstrap values were estimated with 1000 replicates.

**RNA extraction and qRT-PCR**
Total RNA was extracted from various samples using TRIzol reagent (Sigma; http://www.sigmaaldrich.com/). First-strand cDNA synthesis was carried out using a Reverse Transcription System (Takara, Tokyo, Japan). The cDNA was used as the template for qRT-PCR analysis with specific primers (Table S5). The qRT-PCRs were carried out with SYBR Green Real-time PCR Master mix using a CFX96-2 PCR machine (BIO-RAD, USA). Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method with Actin as an internal control.

**Plant transformation**
The 1223-bp *BnaC04.BIL1* open reading frame was amplified from *Bndwarf2* using the primers *BnaC04*. *BILL-1/F* (Table S5) and cloned into the Xba I-BamHI I sites of the overexpression pBI121 vector with CaMV35S promoter to construct the 35S::*BnaC04.BIL1*-pBI121 plasmid. The 35S::*BnaC04.BIL1*-pBI121 plasmid was introduced into *Agrobacterium tumefaciens* strain EHA105 by a heat shock method. The positive *A. tumefaciens* were transformed into ZS11 with a modified floral dip method. Briefly, agrobacteria cultures carrying a target construct were collected by centrifugation and then resuspended in a solution containing 1/2 MS salts containing 3% Suc, 0.1% Silwet L-77, 2 mg/L 6-benzyladenine, and 8 mg/L acetylsyringone. The ZS11 plants at the flowering stage were used for the transformation. The head of a flowering plant was bent downward and dipped into a beaker containing the agrobacterial culture liquid for 3 min, and the treated plant head was loosely wrapped with a vegetable parchment paper. The plant for transformation was treated every week 1 to 2 times and then continued to grow until maturation. Seeds that experienced transgenic treatment were harvested. The transformant leaf were collected for PCR detection [68].

**Abbreviations**
*B. napus*: *Brassica napus*; GSK3: GLYCOCEN SYNTHASE KINASE 3; *BIN2*: BRASSINOSTEROID INSENSITIVE2; *BIL1*: BINARY-ASSOCIATED 1; qRT-PCR: quantitative real-time PCR; *ZS11*: Zhongshuang 11; SNP: Single nucleotide polymorphism; SSR: Simple sequence repeat; Thr187Ser: Thr-to-Ser mutation at 187; Gln399His: Gln-to-His mutation at 399; GA: gibberellin; BR: brassinosteroid; CYP: cytochrome P450 monooxygenase; CPD: CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM; BRI1: BR-DEFICIENT DWARF 1; DET2: DE-ETIOLATED 2; BRI1-BAK1: BR-INSENSITIVE1/BRASSINAZOLE-RESISTANT1/BRASSINAZOLERESISTANT1/BRASSINAZOLE-SUPPRESSOR1; CDG1: CONSTITUTIVE-DIFFERENTIAL GROWTH 1; BS1: BR SIGNALING KINASE1; BSU1: BR-SUPPRESSOR1; PP2A: PROTEIN PHOSPHATASE 2A; ORF: Open reading frame; OE-BIL1: OE-*BnaC04.BIL1*; NCBI: National Center for Biotechnology Information.

**Supplementary Information**
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**Additional file 1:** Figure S1. The plant height of ZS11, F₃, and *Bndwarf2* at flowering stage. Figure S2. The distribution of plant height in the F₃ population. Figure S3. Sequence analysis of *BnaC04.BIL1* protein. A multiple sequence alignment of amino acid sequences of *BnaC04.BIL1* protein. The conserved TREE and SYICSR motif were boxed in red. At, Cs, Cr, Cc, Cs, Dv, Ee, Bo, Br, Bn, Cc, Cc, Cc, Dv, Ht, Cc, Ap, Gm, Vv, Ac, and Mn denote Arabidopsis thaliana, Camelina sativa, Capsella rubella, Arabis alpina, Raphanus sativus, Eutrema salsugineum, Brassica oleracea, Brassica rapa, Brassica napus, Citrus sinensis, Quercus suber, Prunus persica, Hennaria umbratica, Carica papaya, Abrus precatorius, Gynecium max, Vitis vinifera, Actinidia chinenis, Morus notabilis, respectively. B Phylogenetic tree analysis of *BnaC04.BIL1* using Neighbor-joining method in MEGA 7.0 program. Bootstrap values from 1000 replicates were indicated at each node. The GSK3 group I is marked with green, and the GSK3 group II is marked with red, and the GSK3 group III is marked with yellow, and the GSK3 group IV is marked with blue. The *BnaC04* BIL1, *BnaC04.BIL1-Mut*, and *BnaC04.BIL1*-WT are labeled.

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JoinMap 4.1 mapping software [63], then the *BnDWARF2* locus was primarily mapped onto physical and genetic map. The mapping interval sequence was downloaded from the *Brassica napus* Genome Browser (http://www.genoscope.cns.fr/brassicaneus/cgi-bin/gbrowse/colza/).

Using this genomic sequence, SSR marker primers were designed by aid of SSR Hunter 1.3 [64], and Primer Premier 5.0 [65]. A total of 318 polymorphic SSR markers were obtained. These SSR markers helped to fine-map the *BnDWARF2* locus using a size-enlarged population comprised of *F*₂₃ plants.
Table S1. Leaf chlorophyll contents in the leaves of ZS11 and Bndwarf2 mutant.

Table S2. Agronomic traits comparisons in ZS11, F1, and Bndwarf2.

Table S3. Agronomic traits of OE-8Il transgenic lines.

Table S4. Genetic analysis of T, progeny derived from six independent T, transgenic plants.

Additional file 2: Table S5. Primers used in this study.

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Authors’ contributions
MY and RG designed the research. MY, SW, WL, WC, YW, XJ, PC, and PC performed the experiments. JH analyzed the SNP and sequencing data. RG advised on the experiments. MY wrote the manuscript. WS and RG revised the manuscript. All authors read and approved of the final manuscript.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are available in the Figshare repository (https://doi.org/10.6084/m9.figshare.14679021).

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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