Fine mapping of the BnUC2 locus related to leaf up-curling and plant semi-dwarfing in Brassica napus

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Abstract

Background Studies of leaf shape development and plant stature have made important contributions to the fields of plant breeding and developmental biology. The optimization of leaf morphology and plant height to improve lodging resistance and photosynthetic efficiency, increase planting density and yield, and facilitate mechanized harvesting is a desirable goal in Brassica napus.

Results Here, we investigated a B. napus germplasm resource exhibiting up-curled leaves and a semi-dwarf stature. In progeny populations derived from NJAU5737 and Zhongshuang 11 (ZS11), we found that the up-curled leaf trait was controlled by a dominant locus, BnUC2. We then fine mapped the BnUC2 locus onto an 83.19-kb interval on chromosome A05 using single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers. We further determined that BnUC2 was a major plant height QTL that explained approximately 70% of the phenotypic variation in two BC5F3 family populations derived from NJAU5737 and ZS11. This result implies that BnUC2 was also responsible for the observed semi-dwarf stature. The fine mapping interval of BnUC2 contained five genes, two of which, BnaA05g16700D (BnaA05.IAA2) and BnaA05g16720D, were revealed by comparative sequencing to be mutated in NJAU5737. This result suggests that the candidate gene mutation (BnaA05g16700D) in the conserved Degron motif GWPPV (P63S) was responsible for the BnUC2 locus. In addition, investigation of agronomic traits in a separation population indicated that plant height, main inflorescence length, and branching height were significantly reduced by BnUC2, whereas yield was not significantly altered. Our findings may provide an effective foundation for plant type breeding in B. napus.

Conclusions Using SNP and SSR markers, a dominant locus (BnUC2) related to up-curled leaves and semi-dwarf stature in B. napus has been fine mapped onto an 83.19-kb interval of chromosome A05 containing five genes. The BnaA05g16700D (BnaA05.IAA2) is inferred to be candidate gene responsible for the BnUC2 locus.

Background

Leaf morphology and plant stature are determining factors for dicot plant photosynthesis, dry material accumulation, lodging resistance, tolerance to high planting density, and amenability to mechanized harvesting. Slight up-curling of leaves and a semi-dwarf stature may benefit grain yield improvement [1, 2]. Research on leaf morphology and plant stature is important in the fields of plant developmental biology and crop genetic improvement. Leaf development from the shoot apical meristem comprises several stages, including leaf primordium formation, polarity establishment, and cell differentiation[3]. Leaf curling is due to abnormal leaf development caused by mutations of genes related to leaf development. Many genes may be involved in leaf development. Transcription factors, including KANADI [4–7], Class III HOMEODOMAIN LEUCINE-ZIPPER (HD-Zip III) [8–11], WUSCHEL RELATED HOMEOBOX (WOX) [12], and TB1-CYC-PCFs (TCPs) [13], participate in alteration of leaf polarity establishment, which leads to the up-curled leaf phenotype. Plant hormone biosynthesis and signal transduction is another major aspect that influences leaf shape. AUXIN/INDOLE–3-ACETIC ACID

(Aux/IAA) gene family mutations have been reported to cause leaf curling in plants [14–16]. The activity of auxin response factors (ARFs) affects the mutual antagonism between HD-ZIP IIIs and KAN that regulates leaf development. Mutation of Arabidopsis *ARF3* can cause leaf curling [17]. The *UCU1* gene encoding the SHAGGY/GSK3 protein involved in the signal transduction of auxin and BR can lead to down-curled leaves and short stature phenotypes [18]. In addition, some microRNAs that can specifically recognize the START domain of HD-ZIP III family genes, such as miRNA165 and miRNA166, may cause leaf curling by regulating HD-ZIP III family gene expression [9, 19–22]. miRNA160 regulates leaf curling by controlling the expressions of auxin-responsive genes ARF10 and ARF17 [23, 24]. miRNA164 affects the development of leaf margins by regulating the expression of the *CUC1* gene, which may lead to a curled leaf phenotype [25].

Plant height is mainly determined by plant hormone biosynthesis, signal transduction, and related pathways. Although not described here, a well-documented relationship exists between the regulation of plant dwarf stature and genes involved in gibberellin and brassinosteroid biosynthesis and signal transduction pathways [26–31]. Auxin biosynthesis, polar transport, and signal transduction exert a crucial role in plant growth and development, including plant height determination. *YUCCA* encodes a flavin monooxygenase, a catalytic enzyme in the auxin biosynthesis pathway, and mutation of *YUCCA* leads to short stature in plants [32]. Phosphorylated glycoproteins PGP1 and PGP19/MDR1 are vectors for auxin transport. Because of their weaker auxin polar transport capacities, the *mdr1–1* single mutant and *mdr1–1/pgp1–1* double mutant of Arabidopsis are dwarfs [33, 34]. The auxin signal transduction pathway is mainly composed of transport inhibitor resistant1/auxin signaling F-box proteins (TIR1/AFBs), Aux/IAA proteins, and ARFs [35, 36]. Aux/IAA proteins, which act as repressors of auxin-regulated transcriptional activation, possess four conserved domains (domains I, II, III, and IV), and domain II contains a strongly conserved amino acid motif, GWPPV. In the presence of auxin, TIR1/AFBs can recognize this motif and bind to the Aux/IAA proteins, leading to the degradation of Aux/IAA proteins. ARF is then released to activate the expressions of auxin-response genes [37]. The GWPPV motif, called the Degron domain, is thus the core component of auxin signal transduction [38, 39]. Mutations of any sequence in the GWPPV motif or its flanks can lead to defective plant growth and development, including the generation of shorter hypocotyls and stature, leaf curling, reduction in the number of lateral roots, and loss of apical dominance [40–42]. In Arabidopsis and *B. napus,* mutations of Aux/IAA genes decrease plant height and lead to leaf curling [16, 43, 44].

Because of the important role of dwarf/semi-dwarf plant resources in crop genetic improvement, especially in dicots, their excavation and utilization has attracted the attention of agronomists and biologists. In *B. napus,* the dwarf phenotypes of *bzh* [45] and *NDF–1* [46] mutants are controlled by a major gene with additive effects, while the dwarf trait of 99CDAM [47] is caused by three pairs of recessive genes. Although some mutants related to plant height in *B. napus* have been reported, few genes have been studied to clarify their mechanical roles in plant development. The semi-dwarf nature of gibberellin-insensitive mutants, including *NDF–1* [46], *ds–1* [48], *ds–3* [49] and *banC.dwf* [50], and *ds–1* and *ds–3* are caused by a mutation in the VHYNP motif of the DELLA protein. In *ds–4* [16] and *sca* [44] mutants insensitive to auxin, a mutation in the GWPPV motif of the Aux/IAA7 protein is responsible for

their semi-dwarf/dwarf phenotypes. The dwarf characteristics of *banC.dwf* [50], *Bndwf1* [51] and *Bndwf/dcl1* [52] are controlled by a pair of dominant genes, while those of *ds–1* [48], *ds–3* [49], *ds–4* [16] and *sca* [44] are regulated by a pair of semi-dominant ones. Many dwarfing genes have a negative effect on crop agronomic traits, which restricts the breeding development of dwarf or semi-dwarf varieties [53]. The investigation of dwarf mutants, the identification of new dwarfing genes, and the elucidation of dwarfing mechanisms are therefore crucial for genetic improvement of *B. napus.*

Curled-leaf traits, including up-curling, down-curling, and wrinkling, are often observed in *B. napus.* Leaf up-curling is a useful trait that allows an increase in the planting density of *B. napus.* Published research on curled-leaf traits is limited. Wang et al. [52] found that the wrinkled, down-curved leaf type of *Bndwf/dcll,* which is short-statured, is controlled by a dominant gene. Li et al. [44] reported a *B. napus* mutant, *sca,* with crinkled leaves, a semi-dwarf stature, narrow branch angles, and upright siliques, and determined that the underlying gene related to the mutated traits is a semi-dominant gene. Zhao et al. [16] discovered that a semi-dominant gene is responsible for an extremely dwarf mutant, *ds–4,* with down-curved leaves. Yang et al. [54] uncovered an up-curling leaf locus (*BnUC1)* associated with a dominant gene.

In the present study, we investigated the pure *B. napus* line NJAU5737 (named *Bnuc2),* a new semi-dwarf mutant with up-curled leaves developed in our laboratory, analyzed the inheritance of the up-curled leaf trait, and fine mapped the *BnUC2* locus. We also evaluated the effects of the *BnUC2* locus on agronomic traits. Our results may provide an effective foundation for plant type breeding in *B. napus.* Our findings may also serve as a foundation for the semi-dwarf variety breeding of *B. napus* and exploration of the dwarfing mechanism.

Results

Performance of the up-curled-leaf mutant

Compared with the parent Zhongshuang11 (ZS11), seedlings of the parent NJAU5737 had shorter hypocotyls and stature. In addition, leaves of NJAU5737 were up-curled and slightly crinkled, whereas ZS11 had normal, flat leaves (Fig. 1a, b). At the mature stage, NJAU5737 plants were approximately 120–130 cm high. F_1 seedlings of NJAU5737 × ZS11 had up-curled, crinkled leaves (Fig. 1a, b), with mature plant heights that were intermediate between those of the two parents (Fig. 1c).

Inheritance of the up-curled leaf trait

Plants in F_1 (ZS11 × NJAU5737) and RF_1 (NJAU5737 × ZS11) generations, obtained by crossing NJAU5737 (up-curled leaves) with ZS11 (normal, flat leaves), all possessed up-curled leaves, which implies that the leaf-curling trait is controlled by dominant genes. The segregation ratio of up-curled to normal leaves in all obtained backcross populations with a recessive locus, from BC_1 to BC_6 to ZS11, was in good agreement with the expected Mendelian segregation ratio of 1:1 according to Chi-square tests

(Table 1). Furthermore, segregation in subsequently selfed BC_5F_2 family populations and two BC_5F_3 populations was in accord with the expected Mendelian segregation ratio of 3:1 (up-curled vs. normal leaves) (Table 1). These results indicate that leaf up-curling is controlled by a dominant locus (*BnUC2)*..

Genetic mapping of the *BnUC2* locus

Eight plants with up-curled leaves from the consecutive backcross BC_5 family population were genotyped along with the two parents, NJAU5737 and ZS11, using a *Brassica* 60 K SNP bead chip array (Illumina, US). The SNP chip data analysis uncovered a BC_5 plant (named BnUC2–5) with three segments, on chromosomes A05, C02, and C07, that differed from those in the recurrent parent ZS11. The A05 differential segment contained 227 polymorphic SNP markers covering a 6.05-Mb interval between SNP markers M10447 and M11106. The C02 differential segment included 198 polymorphic SNP markers encompassing a 3.81-Mb interval between SNP markers M25019 and M36509, and the C07 differential segment harbored 211 polymorphic SNP markers covering an interval of 4.17 Mb between SNP markers M34819 and M44556. These three segments were clearly candidates for the region harboring the *BnUC2* locus. As other plants had larger genomic disparities than BnUC2–5, the latter plant was selfed and backcrossed to build populations for mapping of the up-curled leaf locus. As a result, 286 BC_5F_2 individuals and 246 BC_6 individuals were obtained. Using these plants, 60 SSR-marker primer pairs were designed on the basis of the genomic sequences of the three differential segments. In SSR experiments, six polymorphic co-dominant SSR markers (BnaC02–12, BnaC02–14, BnaC07–04, BnaC07–05, BnaA05–21, and BnaA05–25) were found on the three segments.

All plants in BC_6 and BC_5F_2 family populations were analyzed using the six polymorphic markers. Calculations of recombination frequencies in JoinMap 4.1 based on the resulting data suggested that the *BnUC2* locus was located on the A05 differential segment.

Next, 70 SSR primer pairs were designed to map the *BnUC2* locus on the A05 chromosome. Five of these markers (BnaA05–121, BnaA05–127, BnaA05–133, BnaA05–256, and BnaA05–23) were found to be polymorphic (Additional file 2: Table S2). To construct linkage maps containing the up-curled-leaf trait locus *BnUC2* (Fig. 2a), 246 BC_6 and 286 BC_5F_2 plants were genotyped with the five polymorphic markers. A linkage map including the *BnUC2* locus was then generated in JoinMap 4.1 and used to localize the *BnUC2* locus to a 3.84-Mb interval between SSR markers BnaA05–133 and BnaA05–256. The arrangement of the markers on the linkage map was in good agreement with the physical genome map of *B. napus,* thus indicating that this preliminary mapping was reliable.

To fine map the *BnUC2* locus, 1,661 BC_5F_3 plants were obtained by selfing BC_5F_2 non-recombinant plants heterozygous at the *BnUC2* locus according to SSR marker analysis. In addition, we designed 160 SSR primers within the preliminary mapping interval, 10 of which were found to be polymorphic (BnaA05–385, BnaA05–336, BnaA05–442, BnaA05–730, BnaA05–364, BnaA05–375, BnaA05–347, BnaA05–343, BnaA05–94, and BnaA05–90; Additional file 2: Table S2). Next, the 1,661 BC_5F_3 plants were scanned

with polymorphic markers, and linkage maps were obtained using JoinMap 4.1 (Fig. 2b). Finally, the *BnUC2* locus was mapped onto the interval between SSR markers BnaA05–730 and BnaA05–336, with marker BnaA05–442 found to be co-segregated with the *BnUC2* locus as shown by distinct SSR bands (Fig. 3). The frequency of recombination between BnaA05–730 and BnaA05–336 was 0.06%, and the mapping interval was 83.19 kb long. No other polymorphic marker was detected in the mapping interval. The arrangement of the markers was completely consistent with the physical genome map of *B. napus* (Fig. 2c). These results verified the quality of the mapping.

Effect of the BnUC2 locus on plant height

To evaluate the effect of the *BnUC2* locus on plant height, all plants in the two BC_5F_3 family populations were genotyped with the co-dominant SSR marker BnaA05–442 co-segregating with *BnUC2,* and their heights were measured.

Analysis of variance (ANOVA) uncovered extremely significant variation with respect to the three marker-genotypes of BnaA05–442 co-segregating with leaf shape phenotype in the two family populations (BC_5F_3 [1] and BC_5F_3 [2]; Table 2). This result clearly indicated that the *BnUC2* locus affects plant height.

The effect of *BnUC2* genotype on average plant height was assessed in the BC_5F_3 (1) and BC_5F_3 (2) populations (Table 3). Compared with the heights of homozygous plants having normal, flat leaves, the average height of homozygous plants with up-curled leaves was reduced by 28.86% and 28.93% in BC_5F_3 (1) and BC_5F_3 (2) populations, respectively; these differences were extremely significant. Plant heights were also significantly lower in heterozygous plants with up-curled leaves, with reductions of 14.15% and 12.50% in BC_5F_3 (1) and BC_5F_3 (2) populations, respectively. We thus concluded that the *BnUC2* locus has a negative effect on plant height.

On the basis of these results, we concluded that the *BnUC2* locus not only controls leaf type; it can also reduce plant height, leading to a semi-dwarf phenotype. By regarding the *BnUC2* locus as a quantitative trait locus (QTL) for plant height, we were able to calculate its role. According to analysis of data from the BC_5F_3 (1) and BC_5F_3 (2) populations, the effect of the QTL was mainly additive (Table 3).The *BnUC2* locus explained 72.72% and 70.41% of phenotypic variation in plant height in the BC_5F_3 (1) and BC_5F_3 (2) populations, respectively. Frequency distributions in the two family populations were also analyzed (Additional file 3: Fig S1). Plant heights were not normally distributed but instead followed a multimodal distribution, consistent with the effect of a major gene. We thus concluded that the *BnUC2* locus is a major QTL for plant height.

Candidate gene analysis

Using the *B. napus* Genome Browser (http://www.genoscope.cns.fr/brassicanapus/), five genes were identified and annotated in the fine mapping interval (Table 4). Two of these genes, *BnaA05g16680D* and *BnaA05g16700D,* are homologous to AT3G23050.1 and AT3G23030.1 found in the Arabidopsis

Information Resource database that encode Aux/IAA7 and Aux/IAA2 proteins, respectively. These two Arabidopsis Aux/IAA proteins, which have been reported to act as repressors of auxin-regulated transcriptional activation, have four conserved domains (domains I, II, III, and IV) [37]. At high auxin concentrations, TIR1 interacts with Aux/IAA proteins via domain II to activate the degradation of Aux/IAA proteins by 26S proteasome, with ARFs then released [55]. Mutations of Aux/IAA family genes can lead to defective plant growth and development, including the generation of shorter hypocotyls and stature, leaf curling, reduction in lateral root number, and loss of apical dominance [14, 16, 41, 42, 56, 57]. *BnaA05g16680D* and *BnaA05g16700D* were thus considered to be candidate genes responsible for leaf up-curling and plant semi-dwarfing.

BnaA05g16690D in the mapping interval encodes an unknown protein, while *BnaA05g16710D* is homologous to *AT3G23020.1,* which encodes a tetratricopeptide repeat (TPR)-like superfamily protein. *BnaA05g16690D* and *BnaA05g16710D* have no previously reported relationship to leaf type and plant height regulation.

BnaA05g16720D is homologous to *AT3G23000.1,* which encodes a CBL interaction protein kinase 7 (CIPK7). CIPK7 is involved in the regulation of plant adversity stress and participates in plant signal transduction during response to abiotic stress conditions [58, 59]. No association has been reported between *BnaA05g16720D* and the regulation of leaf type and plant height.

To further assess the above gene candidates for the *BnUC2* locus, we performed comparative sequencing of NJAU5737 and ZS11, the parents of the mapping populations. The DNA sequences of *BnaA05g16680D, BnaA05g16690D,* and *BnaA05g16710D* were identical between the two parents, but differences were found in *BnaA05g16700D* and *BnaA05g16720D.* Two single-nucleotide transition mutations, leading to two amino-acid substitutions at positions 30 and 63 (Fig. 4), were present in *BnaA05g16700D (*named *BnaA05.IAA2)* of NJAU5737. The substitution at amino-acid position 63 was located in the Degron motif (GWPPV) of domain II, which is strongly conserved in most plant IAA family members. *BnaA05g16700D* is thus the gene most likely corresponding to the *BnUC2* locus.

Agronomic traits

To evaluate the effect of the *BnUC2* locus on plant agronomic traits, 20 plants with up-curled leaves and 20 with flat leaves were randomly sampled from the BC_6 population derived from NJAU5737 and recurrent parent ZS11. Plant height, branching height, main inflorescence length, and silique length were significantly smaller in up-curled-leaf plants than in flat-leaf plants, with no alterations observed in other analyzed traits (Table 5). These results indicate that the *BnUC2* locus significantly reduces plant height, branching height, and main inflorescence length but has no significant influence on yield.

Discussion

A plant type with upright leaves and a short stature is undoubtedly beneficial for the improvement of crop yield and lodging resistance [2]. In *B. napus,* the mechanisms underlying plant height are complex, and many plant height loci/QTLs have been reported [16, 44, 48, 49, 51, 60–64]. No technical breakthroughs have yet been achieved in the breeding of dwarf stature canola variety, however, and further mining and utilization of new dwarf or semi-dwarf germplasm resources is thus required. In the present study, we characterized a novel germplasm resource with up-curled leaves and a semi-dwarf stature. We identified a locus, *BnUC2,* in this germplasm resource that exhibited dominance in the studied populations but had mainly additive effects on plant height and explained 72.72% and 70.41% of phenotypic variation in plant height in the two BC_5F_3 family populations. These results suggest that the up-curled leaf trait can serve as an indicator trait for semi-dwarf variety breeding; at the same time, it can be useful for increasing planting density, with the aim of elevating *B. napus* yield. In addition, the locus does not have any significant effect on yield traits. Thus, the newly characterized germplasm resource harboring the *BnUC2* locus may aid *Brassica* variety breeding.

Our primary mapping of *BnUC2* positioned this locus within a 3.84-Mb interval, which corresponds to a recombination rate of 0.8% based on the linkage map. This value is far larger than the *B. napus* genome-level average of 400 kb/cM, this is most likely because the *BnUC2* locus is located near the chromosome A05 centromere, a location that affects chromosomal recombination.

On the basis of our mapping, gene sequencing, and bioinformatics analysis results, *BnaA05g16700D* (*BnaA05.IAA2)* is the gene most likely responsible for leaf up-curling and plant semi-dwarfing. This gene encodes an AUX/IAA2 protein that acts as a repressor of auxin-regulated transcriptional activation. Two single amino-acid changes were identified in the AUX/IAA2 protein. The substitution at amino-acid position 63, located in the domain-II Degron motif (GWPPV) conserved in most plants, is key to the phenotypic mutation [37]. Auxin, one of the most important hormones in plants [65, 66], is involved in the regulation of multiple traits, including leaf type and plant height [32, 40, 41, 56]. Mutations of genes related to auxin synthesis and signal transduction may lead to leaf curling and dwarf/semi-dwarf phenotypes. *BnaA05g16700D* (*BnaA05.IAA2)* is thus the best candidate gene and has a pleiotropic effect on leaf type and plant height. Another candidate gene, *BnaA05g16720D,* with coding sequence mutations and mainly involved in stress response [58, 59], could not be excluded completely.

Materials And Methods

Plant materials and analysis of inheritance of the up-curled leaf trait

The *Bnuc2* mutant exhibiting leaf up-curling and a semi-dwarf stature was derived from a pure canola line, NJAU5737. NJAU5737 was crossed with canola variety ZS11 to produce the F_1 generation. The F_1 individuals were then backcrossed with ZS11 to produce progeny populations. The self and backcross populations were examined to determine the segregation ratio of plants with up-curled vs. flat leaves. Chi-square tests were performed to test the genetic regulation of the up-curled leaf trait. BC_5, BC_6, and BC_5F_2

family populations derived from NJAU5737 and ZS11 were used for preliminary mapping of the up-curled leaf trait locus *BnUC2,* and the BC_5F_3 family population was used for fine mapping the *BnUC2* locus.

All materials were grown in fields at the Jiangpu Experimental Station of Nanjing Agricultural University (Jiangsu Province, China). Plants were sown uniformly in 2.5-m long rows, with 0.4 m between rows and 15 individuals per row.

SNP analysis

Eight BC_5 plants with up-curled leaves and the two parents (ZS11 and NJAU5737) were selected for SNP genotyping using a *Brassica* 60 K SNP bead chip array (Illumina, US). The genotyping was performed to detect chromosome segments differing between the backcross progeny population and ZS11, thereby preliminarily identifying the chromosome on which the *BnUC2* locus was located. The SNP marker was named using “M” plus an index number specified by Genome Studio v2011.1 (Illumina, US). The SNP analysis was identical to that of a previous study [51].

SNPs on 19 chromosomes were compared between the eight up-curled-leaf plants and the recurrent parent ZS11 to determine differential chromosome segments. The BC_5 plant having the fewest genome-level differences with ZS11, BnUC2–5, possessed three differential segments located on chromosomes A05, C02, and C07.

Genetic mapping of the BnUC2 locus

BnUC2–5 was selfed and backcrossed with ZS11, and 286 BC_5F_2 and 246 BC_6 individuals were obtained for mapping the *BnUC2* locus.

SSR-marker primers were designed in Primer Premier 5.0 [67] using the genomic sequences of the three differential fragments downloaded from the *B. napus* Genome Browser. SSR markers that were polymorphic between BnUC2–5, ZS11, and NJAU5737 were used to detect polymorphisms in all plants in BC_6 and BC_5F_2 family populations. Recombination frequencies between SSR markers and the *BnUC2* locus were calculated using JoinMap 4.1 software to determine the chromosome harboring the *BnUC2* locus.

For fine mapping of the *BnUC2* locus, we used 1,661 BC_5F_3 plants obtained from non-recombinant BC_5F_2 plants. On the basis of preliminary mapping results, polymorphic SSR markers were gradually developed, and a genetic map was constructed in JoinMap 4.1 to narrow the interval containing the *BnUC2* locus.

Polymerase chain reaction (PCR) amplifications of molecular markers were performed as described previously [54]. Total DNA extraction and linkage map construction were carried out according to previous reports [51].

Identification of genes in the mapping interval and comparative sequencing

To identify genes in the mapping interval, the genomic sequence of the mapping interval carrying the *BnUC2* locus was downloaded from the *B. napus* Genome Browser. Genes detected in the mapping interval were annotated and then cloned. Gene-amplification primers based on the genomic sequence were designed using Primer Premier 5.0 (Additional file 1: Table S1). Full-length sequences of genes in the mapping interval were amplified using genomic DNA and cDNA from parents NJAU5737 and ZS11.

PCR amplifications were performed in 50-μL reaction volumes using PrimeSTAR Max DNA polymerase (Takara, Tokyo, Japan). The PCR conditions were as follows: 94 °C for 2 min, followed by 35 cycles of 98 °C for 10 s, annealing for 5 s at the annealing temperature of each gene-amplification primer, and 72 °C for 30s, with a final extension of 72 °C for 5 min.

The PCR products were purified using a AxyPrep DNA Gel Extraction kit and sequenced by GenScript Biotech (Nanjing, China). The resulting sequences were aligned using ClustalX and GeneDoc software.

Agronomic traits

We randomly selected 20 up-curled-leaf and 20 flat-leaf plants from the BC_6 population to investigate traits for evaluation of the effect of leaf type on plant agronomic efficiency. The agronomic trait data included plant height, branching height, main inflorescence length, number of first effective branch, stem diameter, number of siliques on the main inflorescence, total siliques per plant, silique length, seeds per siliques, 1000-seed weight, and yield per plant. The mean values of all agronomic traits between up-curled-leaf and flat-leaf plants were compared by *t*-tests.

Heights of all plants in two BC_5F_3 family populations were determined, and an ANOVA was applied to estimate the genetic variance, error variance, and phenotypic variation of this trait.

Declarations

Additional files

Additional file 1: Table S1. The designed primers of comparative sequencing used in this study.

Additional file 2: Table S2. The designed SSR markers used in this study.

Additional file 3: Fig S1. Distribution of Plant height in the BC5F3 (1) and BC5F3 (2) populations.

Abbreviations

B. napus: Brassica napus; BnUC2: Up-curling leaf locus in *B. napus* genome; *Bnuc2:* Up-curling leaf mutant; Chl: Chlorophyll; PCR: Polymerase chain reaction; TIR1/AFB: Transport inhibitor resistant1/auxin signaling F-box; ARF: Auxin response factor; SNP: Single nucleotide polymorphism; SSR: Simple sequence repeat; TAIR: The Arabidopsis Information Resource; ZS11: Zhongshuang 11; ANOVA: Analysis of variance; QTL: Quantitative Trait Locus; HD-ZIP III: Class III HOMEODOMAIN LEUCINE-ZIPPER; WOX: WUSCHEL RELATED HOMEOBOX; TCP: TB1-CYC-PCFs; AUXIN/INDOLE–3-ACETIC ACID: Aux/IAA.

Notes

Acknowledgments

Not applicable.

Authors' contributions

RG conceived and designed the study. CH performed most of the experiments and wrote the manuscript. RG advised on the experiments and modified the manuscript.MY, DS, YW, and SW took part in the DNA extraction and marker experiments. JH and ZM carried out sequence analysis. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All the data generated or analyzed during this study are included in this published article and its supplementary information files.

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Tables

Table 1 Genetic segregation analysis of *BnUC2* in populations derived from NJAU5737 and the recurrent parent ZS11 in *Brassica napus*

Population	Up-curling leaf	Flat leaf	Total	Expected ratio	value
F_1	120	0	120		
RF_1	115	0	115		
BC_1	60	55	115	1:1	0.22
BC_2	42	56	98	1:1	2.00
BC_3	45	57	102	1:1	1.41
BC_4	58	70	128	1:1	1.13
BC_5	40	48	88	1:1	0.73
BC_6	127	119	246	1:1	0.26
BC_5F_2	211	75	286	3:1	0.23
BC_5F_3 (1)	641	227	868	3:1	0.61
BC_5F_3 (2)	588	205	793	3:1	0.31

Table 2 Analysis of variance of maker genotypes on plant heights in the BC_5F_3 (1) and BC_5F_3 (2) populations derived from NJAU5737 and the recurrent parent ZS11

Population	Source	*DF*	*SS*	*MS*	*F*	$F_{0.01}$
BC_5F_3 (1)	Marker genotype	2	265238.81	132619.41	1152.85**	4.63
	Error	865	99506.44	115.04		
	Total	867	364745.25			
BC_5F_3 (2)	Marker genotype	2	231933.09	115966.54	939.77**	4.63
	Error	790	97485.43	123.40		
	Total	792	329418.51			

"**" indicates significant differences at 0.01 probability level.

Table 3 Average heights of three marker genotypes in the BC_5F_3 (1) and BC_5F_3 (2) populations derived from NJAU5737 and the recurrent parent ZS11

Population	Genotype	Sample size	Mean ± *SD* (cm)
BC_5F_3 (1)	Normal flat leaf	227	170.60±11.95A
	Heterozygous up-curling leaf	430	146.46±11.75B
	Homozygous up-curling leaf	211	121.36±6.03C
BC_5F_3 (2)	Normal flat leaf	205	169.02±12.45A
	Heterozygous up-curling leaf	405	147.89±11.85B
	Homozygous up-curling leaf	183	120.12±6.94C

Values in a column followed by different letters indicate significant differences by *LSD* ($P = 0.01$)

Table 4 Gene annotation on the mapping interval

Gene in *B. napus*	Chromosome position	Homologue in *A. thaliana*	Gene annotations
BnaA05g16680D	11341560-11342921	*AT3G23050.1*	Aux/IAA7 protein
BnaA05g16690D	11375956-11376874		unknown protein
BnaA05g16700D	11384467-11385470	*AT3G23030.1*	Aux/IAA2 protein
BnaA05g16710D	11398936-11401491	*AT3G23020.1*	Tetratricopeptide repeat (TPR)-like superfamily protein
BnaA05g16720D	11401616-11403042	*AT3G23000.1*	CBL-interacting protein kinase 7

Table 5 Agronomic traits of flat leaf and up-curling leaf plants in the BC_6 derived from NJAU5737 and the recurrent parent ZS11

Trait	Flat leaf plants	Up-curling leaf plants
Plant height (cm)	175.64±13.67	149. 64±13.99[a]
Branching height(cm)	61.75±9.57	54.53±9.55[b]
Main inflorescence length (cm)	71.29±8.33	54.64±8.82[a]
Number of first effective branch	6.9±1.45	6.2±1.14
Stem diameter (mm)	20.38±3.33	18.223±3.20
Number of siliques on the main inflorescence	77.51±11.14	70.25±8.60
Total siliques per plant	395.38±89.29	351.63±95.13
Silique length (cm)	10.11±0.97	9.43±1.09[b]
Seeds per siliques	25.38±3.74	26.46±3.09
1000-seed weight (g)	4.75± 0.14	4.54± 0.11
Yield per plant (g)	24.61±11.68	21.08±9.35

[a] and [b] Indicate significant differences at 0.01 and 0.05 probability level by *t*-test, respectively. Data are shown as mean±*SD*, *n*=20

Figures

Figure 1

Performance of NJAU5737, (NJAU5737 ×ZS11) F1 and ZS11. a Leaf of NJAU5737 (left), (NJAU5737 ×ZS11) F1 (middle) and ZS11 (right) at the seedling stage. b Seedling of NJAU5737 (left), (NJAU5737 ×ZS11) F1 (middle) and ZS11 (right). c NJAU5737 (left), (NJAU5737 ×ZS11) F1 (middle) and ZS11 (right) at mature stage.

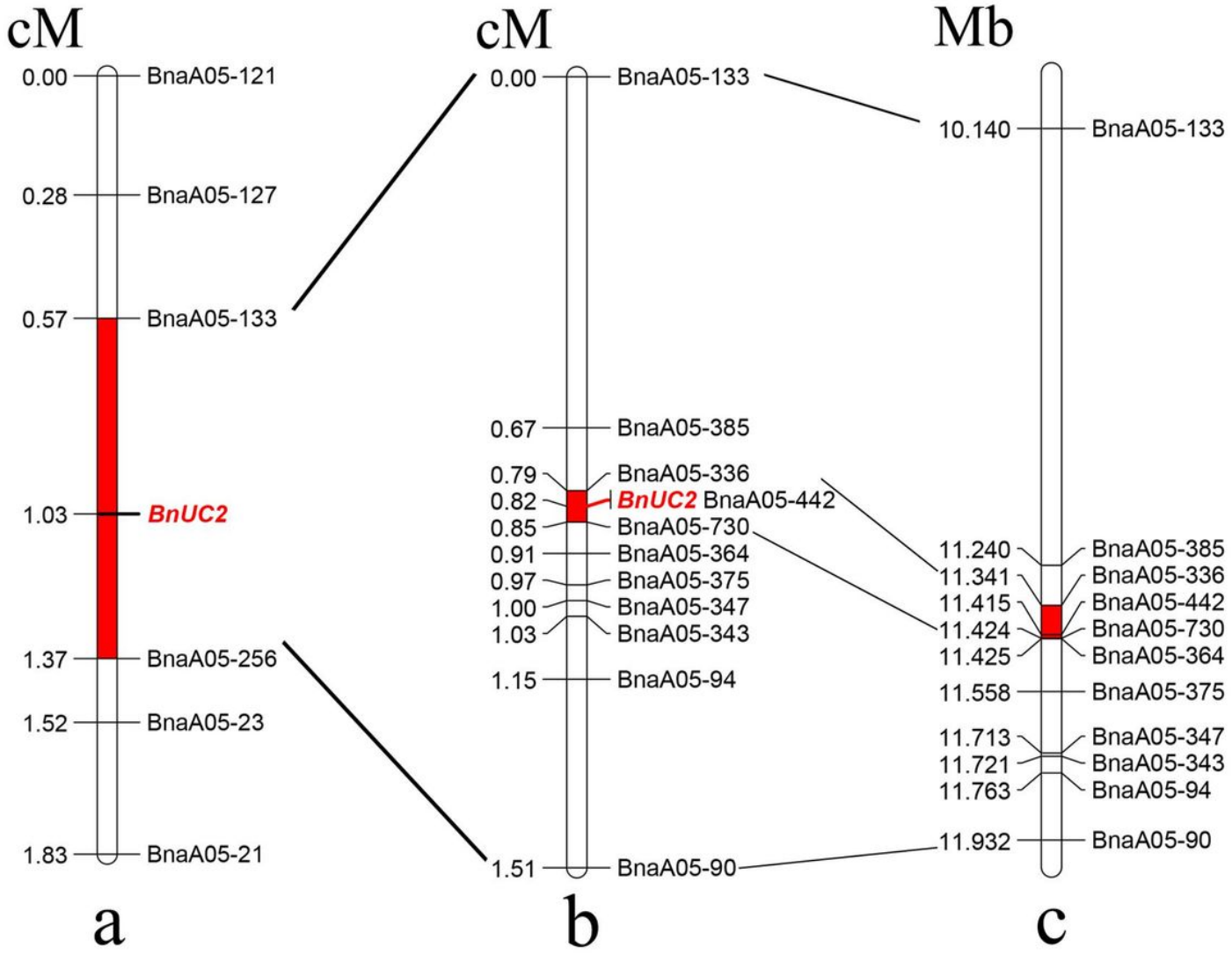

Figure 2

Genetic map and physical map of the BnUC2 locus. a. The primary genetic map of BnUC2 locus, the red indicates the mapping interval. B. Fine mapping of BnUC2 locus. c. The physical map of BnUC2 locus, the unit is Mb.

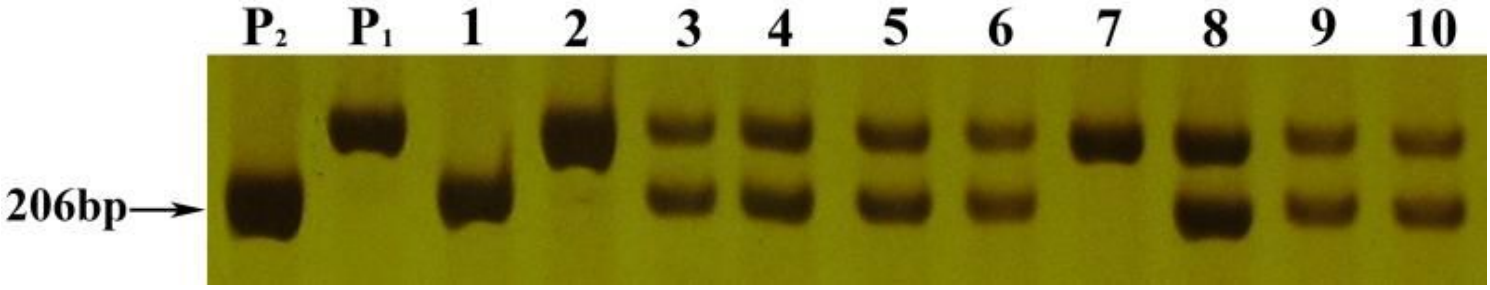

Figure 3

Partial marker experimental results for polymorphic marker BnaA05-442. Marker scan with progeny BC5F3 populations derived from the parents ZS11 and NJAU5737 was conducted. P1 and P2 indicates PCR products from the parents ZS11 and NJAU5737 plants, respectively. The number 3, 4, 5, 6, 8, 9 and 10 denote the PCR products from heterozygous plants with up-curling leaves, and 1 denote the PCR products from homozygous plants with flat leaves.

Figure 4

Amino acid sequence alignment of BnaA05g16700D (BnaA05.IAA2) in ZS11 and NJAU5737.

Supplementary Files

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