Regional association and transcriptome analysis revealed candidate genes controlling plant height in Brassica napus

Rui Ren
Hunan Agricultural University
Wei Liu
Hunan Agricultural University
Min Yao
Hunan Agricultural University
Yuan Jia
Hunan Agricultural University
Luyao Huang
Hunan Agricultural University
Wenqian Li
Hunan Agricultural University
Mei Guan
Hunan Agricultural University
Zhongsong Liu
Hunan Agricultural University
Chunyun Guan
Hunan Agricultural University
Wei Hua
Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Ministry of Agriculture
Xinghua Xiong
Hunan Agricultural University
Lunwen Qian (qianlunwen@163.com)
Hunan Agricultural University

Research Article

Keywords: Brassica napus, GWAS, Plant height, Haplotype, Coexpression

Posted Date: April 4th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1460223/v1

Abstract

Background

Plant height is a key morphological trait of rapeseed, which not only plays an important role in determining plant architecture, but is also an important character related to yield. Presently, improvement of plant architecture is a major challenge in rapeseed breeding. This work was carried out to identify genetic loci related to plant height have been characterized in rapeseed.

Results

In this study, a genome-wide association study (GWAS) of plant height was performed using a *Brassica* 60K Illumina Infinium SNP array in 203 *Brassica napus* accessions. Eleven haplotypes carrying important candidate genes were detected significantly associated with plant height on chromosomes A02, A03, A05, A07, A08, C03, C06 and C09. Meanwhile, regional association analysis of 50 resequencing rapeseed inbred lines was used to further analyze these eleven haplotypes and revealed nucleotide variation in the *BnFBR12-A08* and *BnCCR1-C03* gene regions related to the phenotypic variation in plant height. Furthermo re, coexpression network analysis showed that *BnFBR12-A08* and *BnCCR1-C03* were directly connected with hormone genes and transcription factors, and formed a potential network regulation in plant height of rapeseed.

Conclusions

Combination of GWAS with haplotype analysis revealed a number of directly or indirectly gene related to plant height and provide insight into the genetic basis of plant height in rapeseed. Otherwise, our results will be favorable to develop haplotype functional markers to further improve plant height in rapeseed.

Background

Plant height, as one of the important agronomic traits of rapeseed, influences the establishment of an ideal plant type and high yield. The global production of cereal grains significantly increased in the 1960s and 1970s during the ‘Green Revolution’, which created semi-dwarf varieties with lodging resistance and high yields in rice and wheat through the modification of plant architecture [1, 2]. Breeding practices in many crops have demonstrated that the application of semi-dwarf varieties could effectively improve lodging resistance and reduce yield loss [3]. Moderate dwarfing of plants can strengthen stalks, improve the harvest index, and improve the utilization rate of fertilizer, pesticide and water [4]. Therefore, it is of great significance to identify some important genes of plant height and apply them to rapeseed breeding.

Plant height is mainly related to internode elongation, cell elongation, apical meristem growth and differentiation. In rice and *Arabidopsis* model plant, controlling the genetic mechanisms of plant height

has been more thoroughly investigated than in any other plant. In polyploid plants, some researchers identification genes are homologous to *Arabidopsis* genes controlling a similar metabolic pathway; however, to further elucidate the molecular mechanism of plant height are limited due to the complexity of the polyploid genome. In rapeseed, some researchers have identified many quantitative trait loci (QTLs) associated with plant height in bi-parental populations in the past few decades. For example, Wang et al. (2015) detected 27 QTLs related to plant height in a large doubled haploid (DH) population of 348 inbred lines [5]. Mei et al. (2009) identified seven QTLs association with plant height, accounting for 8.5–28.6% of phenotypic variation [6]. A total of 25 QTLs related to plant height were identified by a double haploid population composed of Y689 and Westar [7]. However, despite previously reported QTLs have been a good understanding of the plant height regulation pathway, two parents influence mapping accuracy and are limited in further revealing the molecular mechanisms of plant height. However, high-density SNPs and historical recombination events in large populations have provided the opportunity to methodically analyze the genetic architecture of plant height by GWAS. Li et al. (2016) performed a GWAS to detect eight SNPs significantly associated with plant height by 472 rapeseed accessions with a 60K single nucleotide polymorphism (SNP) array [8]. Zheng et al. (2017) performed a GWAS of plant height, and seven SNPs were significantly associated with plant height in 333 *Brassica napus* accessions [9]. Sixty-eight SNPs were identified as significantly associated with plant height by GWAS, and 48 SNPs overlapped the confidence interval of QTLs previously detected from bi-parental populations [10].

At present, some studies have suggested plant hormone genes related to plant height in rapeseed. For example, Liu et al. (2010) mapped the semi-dominant gene *ds-1* on the A6 chromosome, and changes in the amino acid sequence of the N-terminal conserved domain of the mutant protein in *ds-1* affect the GA signaling pathway, resulting in a reduction in plant height and internode shortening [11]. Zheng et al. (2020) used CRISPR/Cas9 editing technology to knock out the *BnMAX1* gene, which is related to the synthesis of strigolactone, and obtained dwarf and more branched plants [12]. *BnUC2* was identified to be related to semi-dwarf stature by fine mapping and cloning in *Brassica napus* [13]. Li et al. (2019) suggested a weak gain-of-function mutation in*iaa7.a03* that will reduce the length of internodes in rapeseed [14].

In this study, a GWAS of haplotype was performed to identify eleven haplotypes associated with plant height in 203 *Brassica napus* accessions with a 60K SNP array. Then, novel genes/loci affecting plant height in these eleven haplotype regions were further identified by using 50 resequenced accessions and transcriptomes. These results will facilitate the development of haplotype functional markers to further improve plant height in rapeseed.

Material And Methods

Plant material and phenotypic data

In this study, 203 semi-winter rapeseed accessions were collected from the breeding program of Southwest University in Chongqing, China (Table S1). All accessions with a randomized complete block

design were sown at Gross Gerau (49.941000°N, 8.501391°E) and Rauischholzhausen (50.760932°N, 8.880663°E) in 2013 (designated as E1) and 2014 (designated as E2), respectively. Meanwhile, we also planted these accessions at Chongqing (106.38°E, 29.84°N), China in 2013 (designated as E3) and 2014 (designated as E4). Each line was planted in a three-row plot, 0.05 m between plants within each row and 0.25 m between the rows, and 25 plants per row. Plant height was measured as the distance from the ground to the tip of the tallest panicle for each plot.

The R packages HMISC [15] and PSYCH [16] were used to calculate and analyze the plant height phenotypic distribution, mean value, standard deviation, correlation coefficient, minimum and maximum values under four environments. To calculate broad sense heritability (H^2) of plant height by the statistical software package SPSS Statistics (IBM Corp., Armonk, NY, USA), and the calculation formula is below:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + {\sigma_e^2}/{n}}$$

Where σ_g^2 and σ_e^2 is the genotypic and residual variance components, respectively, and estimates of the residual variance were divided by the number or environment n.

Genome-wide association analysis

For this study, 60k SNP loci were filtered by a heterozygosity rate > 0.25 and a minor allele frequency (MAF) < 0.05. Finally, 24,338 SNPs were used to carry out GWAS of plant height in 203 *Brassica napus* accessions. The population structure of 203 accessions was described in detail by Qian et al. (2014) [17]. TASSEL 5.0 software was used to calculate and analyze the relationships among 203 accessions [18]. To examine marker-trait associations using the mixed linear model (Q + K) as proposed by Yu et al. (2006) [19], the calculation formula is as follows:

$$\mathrm{y} = \mu + \mathrm{S}\alpha + \mathrm{Pv} + \mathrm{Zu} + \mathrm{e}$$

Where y, μ, α, v, u and e correspond to phenotypic, the overall mean, the fixed allelic effects, fixed population effects, vector of random genetic background effects and the vector of residuals. The matrix P is information of population structure, and S and Z are incidence matrices relating y to α and u, respectively. The R package qqman was used to display Q–Q and Manhattan plots in GWAS of plant height [20]. A significance threshold based on the false discovery rate (FDR) to avoid the loss of interesting loci under different environments [21]. The R package fdrtool was used to calculate FDR threshold, a threshold value of $-\log10^{(P)} = 3.5$ was used to estimate the significant association between SNP and the phenotype of plant height [22].

Regional association analysis

The resequencing of 50 rapeseed accessions was described in detail by Dong et al. (2018) [23] and Yao et al. (2021) [24]. To remove SNP loci with a heterozygous rate > 0.25 and an MAF < 0.05. Eventually, 533,046 high-quality SNPs were used to further analysis in eleven haplotype regions. The software package SPAGeDi [25] was employed to calculate the relative kinship. Principal component analysis (PCA, P) was calculated using the R package SNP Relate [26]. Based on the P and K matrices, the calculation was performed with the mixed linear model incorporated into TASSEL 5.0 software [18].

Haplotype analysis and gene content in the haplotype block regions

The R package 'LD heatmap' was used to identify haplotype blocks in the whole genome [27]. Haplotype alleles with frequency > 1% were used to test for significant phenotypic differences by a two-sample t-test (assuming unequal variance). Two databases, the *Brassica napus* Darmor-bzh reference genome v. 4.2 [28] (accessed from https://genomevolution.org/CoGe/) and the *Arabidopsis* genome (http://www.arabidopsis.org/) were used to further analyze and verify the most likely gene functions from significantly associated haplotype regions.

Transcriptome sequencing

Equivalent amounts of stem tips from 20 accessions at the stem elongation stage were collected for RNA extraction, and three biological replicates were performed. These samples were immediately flash frozen in liquid nitrogen and stored at − 80°C until RNA extraction. A total amount of 3 μg RNA per sample was used for library construction according to the manufacturer's instructions (Illumina Inc.). Illumina HiSeq 2500 platform was employed to sequence all accessions libraries and generated 125 bp paired-end raw reads. High-quality clean data was obtained by strictly filtering from raw reads. HISAT 2 (http://ccb.jhu.edu/software/hisat/index.shtml) was used to align the high-quality paired-end clean reads to the Darmor-bzh reference genome v.4.2 (accessed from https://genomevolution.org/CoGe/) using HISAT 2 [28]. The gene expression levels were calculated using HTSeq0.6.1 [29].

Coexpression analysis

The transcriptome data of stem tips from 20 semi-winter rapeseed varieties in China were collected to calculate the coexpression edges, and a soft threshold value of 0.9 was chosen in the weighted gene coexpression network analysis (WGCNA) R package [30]. Genes with pearson correlation coefficients (PCCs) ≥ 0.20 were used to visualize coexpression network by CYTOSCAPE3.6 [31]. GO enrichment was carried out with TBtools [32] and visualized using the R package ggplot2 [33].

Results

Phenotypic analysis of plant height in 203 rapeseed accessions

In this study, we observed extensive phenotypic variation for plant height in the 203 *B.napus* inbred lines. The frequency distribution of plant height for the four different environments is summarized in Fig. 1. In the E1, E2, E3 and E4 environments, the plant height ranged from 74 to 161 cm, 77 to 163 cm, 112 to

214.33 cm and 121 to 202.67 cm, with average values (± SD) of 119.66 ± 15.28 cm, 126.61 ± 16.91 cm, 171.80 ± 20.22 cm and 168.79 ± 16.10 cm, respectively, and variable coefficients of 12.77%, 13.36%, 11.76%, and 9.54%, respectively (Table 1). Plant height is significant positive correlations across all environments with correlation coefficients of 0.31 to 0.82 (Fig. 1). The values of broad-sense heritability (H^2) of plant height is 0.79 (Table 1), suggesting that plant height is stably inherited.

Table 1
Phenotypic characteristics for plant height in 203 Chinese semi-winter rapeseed accessions.

Environment	Min	Max	Mean ± SD	CV%	H^2
E1	74.00	161.00	119.66 ± 15.28	12.77	0.79
E2	77.00	163.00	126.61 ± 16.91	13.36	
E3	112.00	214.33	171.80 ± 20.22	11.76	
E4	121.00	202.67	168.79 ± 16.10	9.54	
SD, standard deviation; CV, coefficient of variation;					

We also analyzed the phenotypic variation in plant height in 50 resequencing accessions in four different environments. Plant height of four different environments was significantly correlated with each other with correlation coefficients of 0.24 to 0.77 (Figure S3). Plant height range was 92 to 152 cm, 80 to 163 cm, 146 to 203 cm and 121 to 195 cm, mean value was 118.38 ± 13.85 cm, 121.4 ± 16.21 cm, 176.91 ± 14.1 cm and 168.3 ± 15.5 cm, with variable coefficient 11.70%, 13.34%, 7.96% and 9.20%, respectively (Table S2).

Genome-wide haplotype analysis of plant height

A total of 56 SNPs significantly associated with plant height were identified by the K + Q model in a genome-wide scan (Fig. 2; Table S3). These significant SNPs associated with plant height were investigated at a high resolution by assaying haplotype blocks ($r^2 > 0.50$) in flanking chromosome segments. Finally, we checked 11 haplotypes which showed associated with plant height distribution on chromosomes A02, A03, A05, A07, A08 C03, C06 and C09, including 8 haplotype regions overlapping with previously reported QTLs (Figure S1; Figure S2; Table S3). To detect 19 candidate genes in these haplotype regions, which are involved in plant growth and development processes. Twelve of them are genes involved in hormone signaling (Figure S1; Figure S2; Table S3).

Regional association analysis in haplotype regions

Regional association analysis of 50 resequencing accessions detected three SNPs from the promoter region of FUMONISIN B1-RESISTANT12 (*BnFBR12-A08*, BnaA08g19830D) associated with plant height on the haplotype block (2,580,486–2,752,416 bp) of chromosome A08 (Fig. 3a). Four haplotype alleles were identified in the *BnFBR12-A08* gene region (Fig. 3a). Comparison in four haplotype alleles

corresponded to plant height phenotype, we identified *BnFBR12-A08*_Hap4 corresponding to accessions that were the shortest than other haplotype alleles (t-test: assuming unequal variance; Fig. 3b; Table S4).

A similar study was performed on the haplotype region (49,305,993–49,972,021 bp) of chromosome C03. Two SNPs in the promoter region of CINNAMOYL COA REDUCTASE 1 (*BnCCR1*; BnaC03g60490D) were associated with plant height by regional association analysis of 50 resequencing *B.napus* inbred lines (Fig. 3c). Three haplotype alleles were detected in *BnCCR1-C03* (Fig. 3c). Comparison in three haplotype alleles related to plant height, and identified that *BnCCR1-C03*_Hap1 corresponds to accessions have the shortest plant height compared to other haplotype alleles (t-test: assuming unequal variance; Fig. 3d; Table S4).

Coexpression network of candidate genes

To provide additional context for the proposed functions of *BnFBR12-A08* and *BnCCR1-C03*, we used gene expression data of 20 stem tips to construct a coexpression network in *B.napus*. This analysis yielded 12 gene modules, each represented by a different color in the output (Fig. 4a). The modules containing candidate genes that had been detected by WGCNA, *BnFBR12-A08* and *BnCCR1-C03* were in the yellow and blue modules, respectively (Figure S4). The yellow and blue module showed significantly negatively and positively correlated with plant height, and the correlation coefficient was − 0.35 and 0.53, respectively (Fig. 4b).

We processed gene ontology (GO) enrichment analysis for the blue module and yellow module, and a bubble chart marked out the top 10 GO terms for biological process, cellular component and molecular function. The blue module was significantly enriched in genes involved in the response to cytokinin (GO: 0009735), protein transport (GO: 0006810) and so on (Figure S5a; Table S6), and the yellow module was notably enriched in genes involved in the abscisic acid-activated signaling pathway (GO: 0009738), cellular response to auxin stimulus (GO: 0071365), signal transduction (GO: 0007165) and so on (Figure S5b; Table S6).

Based on the functional annotations, we classified the genes from *BnFBR12-A08* and *BnCCR1-C03* coexpressed network. In the *BnCCR1-C03* subnetwork, 52, 44, 8, 16, 4 and 47 genes corresponded to the abscisic pathway, auxin pathway, brassinosteroid pathway, cytokinin pathway, gibberellin pathway and transcription factors, respectively (Fig. 4c; Table S5). A total of 14 genes in the *BnFBR12-A08* subnetwork included 2, 3, 1 and 5 genes located in the abscisic acid pathway, auxin pathway, cytokinin pathway and transcription factors (Figure S6; Table S5).

Discussion

Rapeseed breeders are in the process of modifying plant height to obtain improved ideal plant types for increased yield and mechanized harvesting. In the model plant *Arabidopsis*, brassinosteroid, auxin, gibberellin, and strigolactone biosynthesis and signaling pathway genes are known to regulate plant height [34–36]. Strong selection can cause multilocus tight linkage of genomic regions associated with

phenotypic variation in complex traits. High-resolution genome analysis technologies provide an unprecedented level of insight into local linkage disequilibrium (LD) patterns of complex crop genomes [37, 38]. In our study, eleven haplotype blocks carrying nineteen genes were involved in plant growth and development processes that were significantly associated with plant height under four different environments. We detected haplotypes containing the candidate genes *SAUR30*, *TCP22*, and *GAI*, which were identified by previous QTL mapping [5]. The candidate gene *SAUR30* is small auxin-up RNAs (SAURs) that belong to the largest family of early auxin response genes. SAUR proteins as key effector outputs of hormonal and environmental signals regulation in plant growth and development [39]. TCP family genes are important transcription factors that regulate plant growth and development. The TCP genes regulate plant growth by responding to GA signals. *TCP22* has been shown to affect plant height in *Arabidopsis* [40]. The candidate gene *GAI* regulates plant growth by negative regulation of the gibberellin (GA) signal transduction pathway. The *gai* mutant shows a short plant in *Arabidopsis* [41]. At the same time, we also identified some new QTLS containing the candidate genes *BRI1* and *SKP2A* in the chromosome A05 and C09. Loss-of-function mutant of the brassinosteroid biosynthesis-associated gene *BRI1* presents a dwarf phenotype in *Arabidopsis* [42]. And *SKP2A* affects plant growth and development by positively regulating cell division [43].

Meanwhile, regional association analysis detected the structural variations of *BnFBR12-A08* and *BnCCR1-C03* related to the phenotypic variation of plant height in the haplotype region of chromosomes A08 and C03 in 50 resequencing rapeseed accessions. *FBR12* encodes a putative eIF-5A-2 protein regulation cell division and cell growth in a tissue- and development-specific manner [44]. *FBR12* affected the growth rate and organogenesis of *Arabidopsis thaliana* and reduced the initial rate of leaves and the number of adult organs, resulting in a dwarfing phenotype [44]. Ren et al. (2013) suggested that the eukaryotic translation initiation factor eIF5A-2 regulates cytokinin signaling in *Arabidopsis* [45]. *CCR1* encodes a cinnamoyl CoA reductase that is involved in lignin biosynthesis [46]. The *Arabidopsis* mutant of *ccr1* displayed a dwarf phenotype [47]. The *ccr1* dwarf phenotype is the consequence of the dramatically increased levels of FeA that keep the cellular oxidation state low, resulting in a prolonged phase of cell proliferation and dwarfism [48]. Bonawitz et al. (2013) suggested that lignin quantity or composition as primarily responsible for dwarfing in plants [49]. Previous studies have shown that auxin [50], cytokinin [51], gibberellin [52], abscisic acid [53] and brassinosteroid [54] affect the biosynthesis of lignin. Moreover, the coexpression network directly correlated *BnFBR12-A08* and *BnCCR1-C03*, which included many genes involved in auxin, cytokinin, gibberellin, abscisic acid and brassinosteroid biosynthesis and signaling pathway genes. These results suggest that these two genes may be associated with plant hormone genes to co-regulate the growth and development of oil rapeseed.

Haplotype blocks combining multiple SNPs show highly increased heterozygosity due to their inherent multiallelic nature and are thus much more informative than single biallelic SNPs [55]. Based on growing quantities of population sequence data to more precise identification of gene variants in haplotypes will provide a basis for more precise selection of environmentally resilient cultivars. For instance, nine haplotypes were detected in *ZmCCT*, and Hap5 showed excellent performance for both stalk rot resistance and flowering time and is thus expected to have potential value in future maize breeding

programs [56]. Three haplotypes were detected in *ARF18* from chromosome A09, and *ARF18*-Hap.C accessions showed the largest seed weight and the longest silique length in rapeseed [23]. Abbai et al. (2019) proposed developing next-generation tailor-made rice with superior haplotype combinations of target genes to meet future food and nutritional demands via haplotype-based breeding [57]. In this study, we detected that the favorable haplotype alleles *BnCCR1-C03*_Hap1 and *BnFBR12-A08*_Hap4 on chromosomes C03 and A08, respectively, contribute to the shortest plant heights. Our results will be beneficial for the development of haplotype functional markers to further improve plant height and provide important candidate gene information for the creation of dwarf accessions by using gene editing techniques in rapeseed.

Conclusion

Eleven haplotypes carrying nineteen genes were involved in plant growth and development processes and were significantly associated with plant height by GWAS, including 8 haplotype regions overlapping with previously reported QTLs. We revealed that the structural variation of *BnFBR12*-A08 and *BnCCR1*-C03 influenced the plant height in 50 resequencing accessions and transcriptome analysis of 20 accessions. *BnFBR12*-A08_Hap4 and *BnCCR1*-C03_Hap1 had the shortest plant height phenotypes compared with the other haplotypes. Our results will be beneficial for the development of haplotype functional markers to further improve plant height in rapeseed.

Abbreviations

GWAS: Genome-wide association study; QTL: Quantitative trait loci; SNP: Single nucleotide polymorphism; MAF: Minor allele frequency; FDR: False discovery rate; PCA: Principal component analysis; WGCNA: Weighted correlation network analysis; PCCs: Pearson correlation coefficients; H^2: Broad-sense heritability; GO: Gene ontology. *FBR12*: FUMONISIN B1-RESISTANT12; *CCR1*: CINNAMOYL COA REDUCTASE 1.

Declarations

Availability of data and materials

50 rapeseed accessions resequencing data in the current study is available from NCBI under BioProject accession PRJNA358784. The RNA-seq Illumina paired-end reads of the transcriptome in this study can be found in the NCBI SRA dataset under the following accession numbers: PRJNA779103.

Acknowledgments

We thank other lab members for their help in this article revision and drawing. We are grateful to the editors and reviewers for their constructive comments on the manuscript.

Funding

This study was funded by the National Nature Science Foundation of China (grant No. 32072100).

Authors' Contributions

Lunwen Qian and Xinghua Xiong conceived the research idea and plans. Rui Ren and Wei Liu prepared the manuscript. Rui Ren, Yuan Jia and Min Yao performed data mining and bioinformatics. Luyao Huang and Wenqian Li carried out reagents and the field experiments. Mei Guan, Zhongsong Liu, Chunyun Guan, Wei Hua, Xinghua Xiong and Lunwen Qian read and commented the manuscript. All authors read and approved the final manuscript.

Ethics approval and Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Hargrove TR, Cabanilla VL. The impact of semidwarf varieties on Asian rice-breeding programs. Bioscience. 1979;29:731–5.
2. Khush GS. Green revolution: preparing for the 21st century. Genome. 1999;42:646–55.
3. Hedden P. The genes of the Green Revolution. Trends Genet. 2003;19:5–9.
4. Shah L, Yahya M, Shah SMA, Nadeem M, Ali A, Ali A, et al. Improving lodging resistance: using wheat and rice as classical examples. Int J Mol Sci. 2019;20:4211.
5. Wang X, Wang H, Long Y, Liu L, Zhao Y, Tian J, et al. Dynamic and comparative QTL analysis for plant height in different developmental stages of *Brassica napus* L. Theor Appl Genet. 2015;128:1175–92.
6. Mei DS, Wang HZ, Hu Q, Li YD, Xu YS, Li YC. QTL analysis on plant height and flowering time in *Brassica napus*. Plant Breed. 2009;128:458–65.
7. Shen Y, Xiang Y, Xu E, Ge X, Li Z. Major co-localized QTL for plant height, branch initiation height, stem diameter, and flowering time in an alien introgression derived *Brassica napus* DH population. Front Plant Sci. 2018;9:390.
8. Li F, Chen B, Xu K, Gao G, Yan G, Qiao J, et al. A genome-wide association study of plant height and primary branch number in rapeseed (*Brassica napus*). Plant Sci. 2016;242:169–77.

9. Zheng M, Peng C, Liu H, Tang M, Yang H, Li X, et al. Genome-wide association study reveals candidate genes for control of plant height, branch initiation height and branch number in rapeseed (*Brassica napus* L.). Front Plant Sci. 2017;8:1246.
10. Sun C, Wang B, Yan L, Hu K, Liu S, Zhou Y, et al. Genome-wide association study provides insight into the genetic control of plant height in rapeseed (*Brassica napus* L.). Front Plant Sci. 2016;7:1102.
11. Liu C, Wang J, Huang T, Wang F, Yuan F, Cheng X, et al. A missense mutation in the VHYNP motif of a DELLA protein causes a semi-dwarf mutant phenotype in *Brassica napus*. Theor Appl Genet. 2010;121:249–58.
12. Zheng M, Zhang L, Tang M, Liu J, Liu H, Yang H, et al. Knockout of two *BnaMAX1* homologs by CRISPR/Cas9-targeted mutagenesis improves plant architecture and increases yield in rapeseed (*Brassica napus* L.). Plant Biotechnol J. 2020;18:644–54.
13. Huang C, Yang M, Shao D, Wang Y, Wan S, He J, et al. Fine mapping of the *BnUC2* locus related to leaf up-curling and plant semi-dwarfing in Brassica napus. BMC Genomics. 2020;21:1–12.
14. Li H, Li J, Song J, Zhao B, Guo C, Wang B, et al. An auxin signaling gene *BnaA3. IAA7* contributes to improved plant architecture and yield heterosis in rapeseed. New Phytol. 2019;222:837–51.
15. Harrell FE, Dupont C. Hmisc: harrell miscellaneous. R package version 4.1-1. R Found Stat Comput https//CRAN R-project org/package = Hmisc. 2018.
16. Revelle WR. psych: Procedures for Psychological, Psychometric, and Personality Research. Northwestern University, Evanston, Illinois. R package version 2.2.3, https://CRAN.R-project.org/package=psych. 2022.
17. Qian L, Qian W, Snowdon RJ. Sub-genomic selection patterns as a signature of breeding in the allopolyploid *Brassica napus* genome. BMC Genomics. 2014;15:1170.
18. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics. 2007;23:2633–5.
19. Yu J, Pressoir G, Briggs WH, Vroh Bi I, Yamasaki M, Doebley JF, et al. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet. 2006;38:203–8.
20. Turner SD. qqman: an R package for visualizing GWAS results using QQ and manhattan plots. Journal of Open Source Software. 2014;3:2731
21. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B. 1995;57:289–300.
22. Strimmer K. fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. Bioinformatics. 2008;24:1461–2.
23. Dong H, Tan C, Li Y, He Y, Wei S, Cui Y, et al. Genome-wide association study reveals both overlapping and independent genetic loci to control seed weight and silique length in *Brassica napus*. Front Plant Sci. 2018;9:921.
24. Yao M, Guan M, Yang Q, Huang L, Xiong X, Jan HU, et al. Regional association analysis coupled with transcriptome analyses reveal candidate genes affecting seed oil accumulation in *Brassica napus*.

Theor Appl Genet. 2021;134:1545–55.

25. Hardy OJ, Vekemans X. spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes. 2002;2:618–20.
26. Zheng X, Levine D, Shen J, Gogarten SM, Laurie C, Weir BS. A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics. 2012;28:3326–8.
27. Shin JH, Blay S, McNeney B, Graham J. LDheatmap: an R function for graphical display of pairwise linkage disequilibria between single nucleotide polymorphisms. J Stat Softw. 2006;16:1–10.
28. Chalhoub B, Denoeud F, Liu S, Parkin IAP, Tang H, Wang X, et al. Early allopolyploid evolution in the post-Neolithic Brassica napus oilseed genome. Science. 2014;345:950–3.
29. Anders S, Pyl PT, Huber W. HTSeq–a Python framework to work with high-throughput sequencing data. bioinformatics. 2015;31:166–9.
30. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9:1–13.
31. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics. 2011;27:431–2.
32. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, et al. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13:1194–202.
33. Villanueva RAM, Chen ZJ. Ggplot2: Elegant Graphics for Data Analysis, 2nd Edition. Meas-Interdiscip Res; 2019. p. 160–167.
34. Wang Y, Li J. Molecular basis of plant architecture. Annu Rev Plant Biol. 2008;59:253–79.
35. Y, Fan XY, Cao DM, Tang W, He K, Zhu JY, et al. Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. Dev Cell. 2010;19:765–77.
36. Clouse SD: Brassinosteroids. The Arabidopsis Book. Edited by: Somerville CR, Meyerowitz EM. Rockville MD: American Society of Plant Biologists; 2002.
37. Edwards D, Batley J, Snowdon RJ. Accessing complex crop genomes with next-generation sequencing. Theor Appl Genet. 2013;126:1–11.
38. Voss-Fels K, Snowdon RJ. Understanding and utilizing crop genome diversity via high-resolution genotyping. Plant Biotechnol J. 2016;14:1086–94.
39. Ren H, Gray WM. SAUR proteins as effectors of hormonal and environmental signals in plant growth. Mol Plant. 2015;8:1153–64.
40. Davière JM, Wild M, Regnault T, Baumberger N, Eisler H, Genschik P, et al. Class I TCP-DELLA interactions in inflorescence shoot apex determine plant height. Curr Biol. 2014;24:1923–8.
41. Dill A, Sun T. Synergistic derepression of gibberellin signaling by removing *RGA* and *GAI* function in *Arabidopsis thaliana*. Genetics. 2001;159:777–85.
42. Noguchi T, Fujioka S, Choe S, Takatsuto S, Yoshida S, Yuan H, et al. Brassinosteroid-insensitive dwarf mutants of *Arabidopsis* accumulate brassinosteroids. Plant Physiol. 1999;121:743–52.

43. Jurado S, Diaz-Trivino S, Abraham Z, Manzano C, Gutierrez C, del Pozo J c. SKP2A protein, an F-box that regulates cell division, is degraded via the ubiquitin pathway. Plant Signal Behav. 2008;3:810–2.
44. Feng H, Chen Q, Feng J, Zhang J, Yang X, Zuo J. Functional characterization of the Arabidopsis eukaryotic translation initiation factor 5A-2 that plays a crucial role in plant growth and development by regulating cell division, cell growth, and cell death. Plant Physiol. 2007;144:1531–45.
45. Ren B, Chen Q, Hong S, Zhao W, Feng J, Feng H, et al. The *Arabidopsis* eukaryotic translation initiation factor eIF5A-2 regulates root protoxylem development by modulating cytokinin signaling. Plant Cell. 2013;25:3841–57.
46. Lacombe E, Hawkins S, Van Doorsselaere J, Piquemal J, Goffner D, Poeydomenge O, et al. Cinnamoyl CoA reductase, the first committed enzyme of the lignin branch biosynthetic pathway: cloning, expression and phylogenetic relationships. Plant J. 1997;11:429–41.
47. Ruel K, Berrio-Sierra J, Derikvand MM, Pollet B, Thévenin J, Lapierre C, et al. Impact of *CCR1* silencing on the assembly of lignified secondary walls in *Arabidopsis thaliana*. New Phytol. 2009;184:99–113.
48. Xue J, Luo D, Xu D, Zeng M, Cui X, Li L, et al. *CCR1*, an enzyme required for lignin biosynthesis in *Arabidopsis*, mediates cell proliferation exit for leaf development. Plant J. 2015;83:375–87.
49. Bonawitz ND, Chapple C. Can genetic engineering of lignin deposition be accomplished without an unacceptable yield penalty? Curr Opin Biotechnol. 2013;24:336–43.
50. Khadr A, Wang GL, Wang YH, Zhang RR, Wang XR, Xu ZS, et al. Effects of auxin (indole-3-butyric acid) on growth characteristics, lignification, and expression profiles of genes involved in lignin biosynthesis in carrot taproot. PeerJ. 2020;8:e10492.
51. Guo J, Hu X, Duan R. Interactive effects of cytokinins, light, and sucrose on the phenotypes and the syntheses of anthocyanins and lignins in cytokinin overproducing transgenic *Arabidopsis*. J Plant Growth Regul. 2005;24:93–101.
52. Biemelt S, Tschiersch H, Sonnewald U. Impact of altered gibberellin metabolism on biomass accumulation, lignin biosynthesis, and photosynthesis in transgenic tobacco plants. Plant Physiol. 2004;135:254–65.
53. Liu C, Yu H, Rao X, Li L, Dixon RA. Abscisic acid regulates secondary cell-wall formation and lignin deposition in *Arabidopsis thaliana* through phosphorylation of NST1. Proc Natl Acad Sci. 2021;118.
54. Rao X, Dixon RA. Brassinosteroid mediated cell wall remodeling in grasses under abiotic stress. Front Plant Sci. 2017;8:806.
55. Stephens JC, Schneider JA, Tanguay DA, Choi J, Acharya T, Stanley SE, et al. Haplotype variation and linkage disequilibrium in 313 human genes. Science. 2001;293:489–93.
56. Li Y, Tong L, Deng L, Liu Q, Xing Y, Wang C, et al. Evaluation of *ZmCCT* haplotypes for genetic improvement of maize hybrids. Theor Appl Genet. 2017;130:2587–600.
57. Abbai R, Singh VK, Nachimuthu VV, Sinha P, Selvaraj R, Vipparla AK, et al. Haplotype analysis of key genes governing grain yield and quality traits across 3K RG panel reveals scope for the development of tailor-made rice with enhanced genetic gains. Plant Biotechnol J. 2019;17:1612–22.

Figures

Figure 1

Correlation coefficients and frequency distributions for plant height in 203 Chinese semi-winter rapeseed accessions. E1: field experiments in Gross Gerau, Germany in 2013. E2: field experiments in Rauischholzhausen, Germany in 2014. E3: field experiments in Chongqing, China in 2013. E4: field experiments in Chongqing, China in 2014. ***$p \leq 0.001$

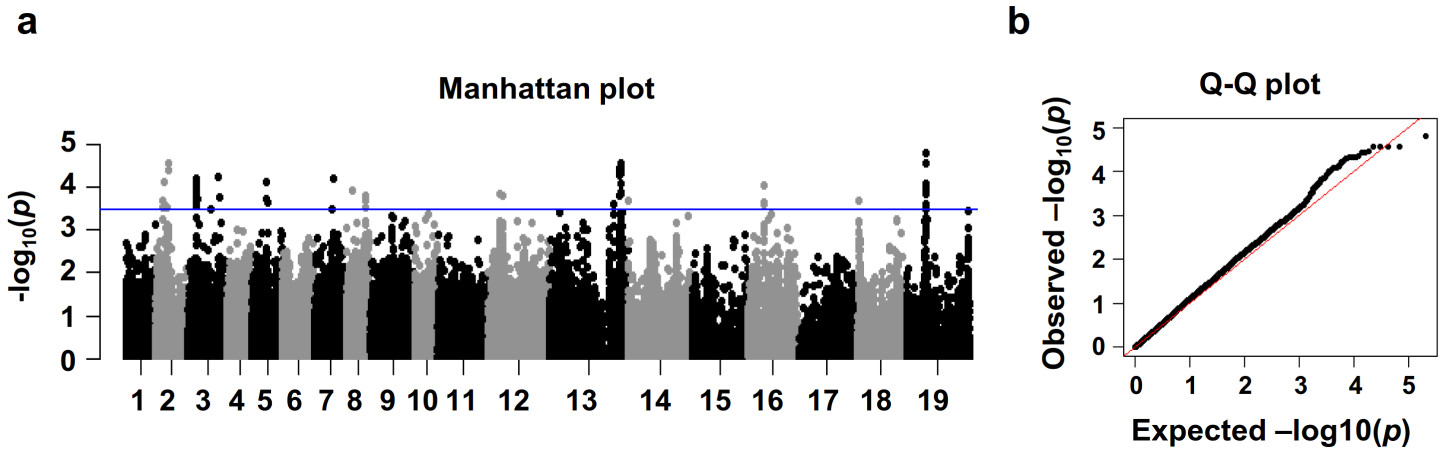

Figure 2

GWAS for plant height in 203 *B.napus* accessions. Genome-wide marker-trait associations for plant height was showed in Manhattan (a) and quantile-quantile (QQ) plots (b). A horizontal grey line represent the $-\log_{10}{}^{(p)}$ significance threshold of 3.5.

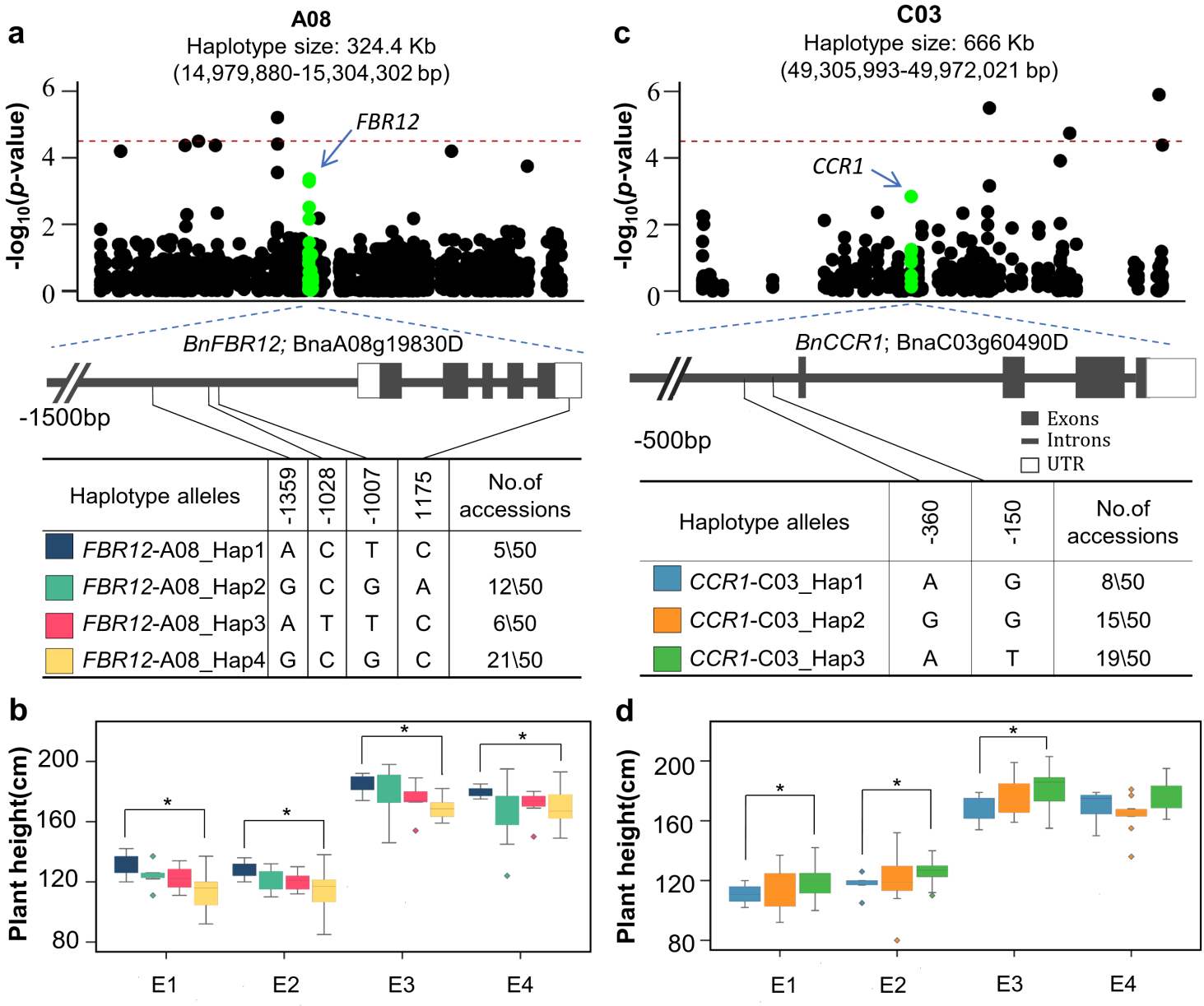

Figure 3

Regional association analysis on haplotype blocks using 50 re-sequenced rapeseed inbred lines. The green dots represent these SNPs located in *BnFBR12-A08* (a) and *BnCCR1-C03* (c) on chromosome A08 and C03, respectively. Four and three haplotype alleles showed frequency >1% were detected in the *BnFBR12-A08* (a) and *BnCCR1-C03* (c) haplotype region, respectively. (b) and (d) Boxplot showed phenotype comparative analysis among these haplotype alleles related to plant height phenotype in *BnFBR12-A08* and *BnCCR1-C03*, respectively. *$p \leq 0.05$

Figure 4

Co-expression network analysis of *BnFBR12-A08* and *BnCCR1-C03.* (a) Cluster dendrogram of WGCNA gene modules. (b) Module-trait relationships. (c) Co-expression network of *BnCCR1-C03* in *Brassica napus*. Red nodes represent *BnCCR1-C03* gene. These genes from the coexpression network are divided into the following categories: Abscisic acid pathway (Lime nodes), Auxin pathway (Cyan nodes), Brassinosteroid pathway (Lightcoral nodes), Cytokinin pathway (Pink nodes), Ethylene pathway (Magenta nodes), Gibberellin pathway (Lavender nodes) and Transcription factor (Dimgray nodes).

Supplementary Files

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

- SupplementaryMaterial1.pptx
- SupplementaryMaterial2.xlsx