



Downregulation of the expression of subgenomic chromosome A7 genes promotes plant height in resynthesized allopolyploid *Brassica napus*

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Abstract

Key message Homoeolog expression bias and the gene dosage effect induce downregulation of genes on chromosome A7, causing a significant increase in the plant height of resynthesized allopolyploid *Brassica napus*.

Abstract Gene expression levels in allopolyploid plants are not equivalent to the simple average of the expression levels in the parents and are associated with several non-additive expression phenomena, including homoeolog expression bias. However, hardly any information is available on the effect of homoeolog expression bias on traits. Here, we studied the effects of gene expression-related characteristics on agronomic traits using six isogenic resynthesized *Brassica napus* lines across the first ten generations. We found a group of genes located on chromosome A7 whose expression levels were significantly negatively correlated with plant height. They were expressed at significantly lower levels than their homoeologous genes, owing to allopolyploidy rather than inheritance from parents. Homoeolog expression bias resulted in resynthesized allopolyploids with a plant height similar to their female *Brassica oleracea* parent, but significantly higher than that of the male *Brassica rapa* parent. Notably, aneuploid lines carrying monosomic and trisomic chromosome A7 had the highest and lowest plant heights, respectively, due to changes in the expression bias of homoeologous genes because of alterations in the gene dosage. These findings suggest that the downregulation of the expression of homoeologous genes on a single chromosome can result in the partial improvement of traits to a significant extent in the nascent allopolyploid *B. napus*.

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Introduction

Polyoids are organisms that contain three or more sets of chromosomes. They are mainly grouped as autopolyploid and allopolyploid organisms, depending on whether the multiple chromosome sets are identical or divergent (Comai 2005; Otto 2007; Zhao et al. 2022). The successful cultivation of allopolyploids indicates that they have an evolutionary advantage owing to their increased diversity and plasticity (Chen 2007; Levin 1983; Ramsey and Schemske 1998). Allopolyploids, especially important crops such as wheat, cotton, and oilseed rape, have been studied extensively to understand their genetics, evolution, genomics, and gene functions (Li et al. 2022). As a young allotetraploid species, *B. napus* has shown great potential for phenotypic variation and phenological adaptation in the past 400–500 years (Bus et al. 2011; Lu et al. 2019). A recent study helped identify 628 associated loci-related causative candidate genes for 56 agronomically important traits, including plant architecture and yield-related traits,

which provides insights into the genomic basis for improving rapeseed varieties and a valuable genomic resource for genome-assisted rapeseed breeding (Hu et al. 2022).

When two diverged genomes merge into a single cell, duplicate copies of genes with similar or redundant functions might result in altered their gene expression patterns (Doyle et al. 2008; Li et al. 2014; Yoo et al. 2013). Allopolyploids may undergo genetic changes, epigenetic changes, dosage regulation, and orthologous/homoeologous protein interactions, leading to non-additive (deviated from the mid-parent value) gene expression. These changes could result in homoeolog expression bias, expression-level dominance, and transgressive expression, and the dosage balance could affect gene expression (Cao et al. 2023; Li et al. 2021, 2023; Song and Chen 2015). Homoeolog expression bias is defined as the unequal relative expression of homoeologs in progeny (Grover et al. 2012; Li et al. 2014). For example, approximately 36.5 and 78.1% of gene pairs showed expression bias with a preference towards the A-genome in resynthesized (Wu et al. 2018) and natural (Li et al. 2020) allopolyploid *B. napus*, respectively. However, the mechanism by which homoeolog expression bias affects complex regulatory hierarchies and biological pathways that lead to heterosis and adaptive traits in allopolyploids remains unknown (Leitch and Leitch 2008).

The development of various aneuploid plants has facilitated the study of the effects of genomic changes, chromosomal variations, and gene dosages on gene expression levels and phenotypes (Gaeta et al. 2007; Zhu et al. 2015). The genomically imbalanced nature of whole-chromosome aneuploidy often has severe phenotypic consequences in all studied organisms (Birchler 2014; Henry et al. 2010; Williams and Amon 2009). Although plants with many types of aneuploidies are viable and fertile, they exhibit pleiotropic developmental defects and impaired fitness levels. In aneuploid wheat, the expression level of most genes is positively correlated with the chromosome dosage. The loss of a copy of chromosome 4B resulted in the formation of a narrow third seedling leaf and lowered plant fertility. In contrast, an extra copy of this chromosome resulted in a shorter spike and fewer spikelets than those observed for the euploid spike (Zeng et al. 2020). In *B. napus*, the comparative analyses of global transcript profiles with the euploid donor showed the dominant trans-acting effects of one copy of chromosome C2 on the majority of differentially expressed genes in the aneuploid plant. Monosomic plants lacking a chromosome C2 exhibited a similar morphology in euploid plants, but they flowered approximately ten days earlier (Zhu et al. 2015). Although these studies have reported on aneuploidy-induced genomic expression bias and phenotypic variations, as compared with the euploid, the association between them is not fully understood.

Due to a lack of integrated genotypic, gene expression, and phenotypic data, it is challenging to explain the developmental variation observed in allopolyploids. Allotetraploid *B. napus*, an oilseed crop, is a critical model plant used to study scientific problems related to hybridization and polyploidization (Li et al. 2022). Unlike natural *B. napus*, resynthesized *B. napus* has specific parents that help to determine whether the variation is inherited or a byproduct of allopolyploidization. In this study, resynthesized *B. napus* was generated using double haploid parents, resulting in each independent line starting off as genetically identical, thereby eliminating the side effects of allelic variation based segregation between different lines. We analysed the first ten generations of inbred *B. napus* lines and observed the emergence of diverse phenotypes. Previous studies confirmed the dominance of the C subgenome in these resynthesized lines (Bird et al. 2020), but the effect of non-additive gene expression on phenotypes has not been analysed. Therefore, this study aims to study the effect of gene expression-related characteristics on traits and the distribution of genes controlling target traits in the nascent allopolyploid *B. napus*.

Materials and methods

Plant materials, phenotype measurement, and sequencing data

Resynthesized *Brassica napus* allopolyploid lines (CCAA; $2n = 38$) EL100, EL200, EL300, EL400, EL600, and EL1100 were developed by hybridizing the double haploid (DH) *Brassica oleracea* line TO1000 (egg donor; C genome) with the DH *Brassica rapa* line IMB218 (pollen donor; A-genome) as described previously (Gaeta et al. 2007). Then, allotetraploid *B. napus* (S_0 generation) was self-fertilized to produce seeds. Three fertile seeds were randomly chosen from self-fertilized S_0 lines and planted for the phenotypic analysis of the next generation. All the plants were grown in a greenhouse under a 16 h-light/8 h-dark photoperiod. Fully grown plants were randomly selected from each line to determine the flowering time (day), pollen activity (%) (Peterson et al. 2010), plant height at the mature stage (cm), and seed number (Henry et al. 2009) using standard methods. Leaves were collected and split in half for DNA and RNA isolation. A library was constructed for Illumina sequencing and sequenced with paired-end 150 bp reads on a HiSeq 4000 platform (Novogene, Beijing, China). Genome and transcriptome sequencing data were obtained from a previous study (Bird et al. 2020).

Weighted gene co-expression network analysis and functional enrichment

B. napus reference genome (Chalhoub et al. 2014) and annotation files were downloaded from Ensembl Plants (<http://plants.ensembl.org/index.html>). Adaptor sequences and low-quality reads were filtered using fastp with default settings (Chen et al. 2018). The remaining sequences, known as clean reads, and the FPKM (fragments per kilobase of exon model per million mapped fragments) expression matrix were obtained by performing RNA-seq analysis using HISAT2, StringTie, and Ballgown (Pertea et al. 2016).

Samples in which genes exhibited an FPKM mean value ≥ 2 were retained, and logarithm-transformed values ($\log_2(\text{FPKM} + 1)$) were used to generate co-expression networks using the weighted gene co-expression network analysis (WGCNA) package in R-3.6.3 (Langfelder and Horvath 2008). In short, if a group of genes has the same expression change (such as first up-regulated and then down-regulated) in samples from different backgrounds, these genes may be co-regulated, functionally related, or on the same pathway, and they will be divided into the same module via WGCNA. Samples were clustered using the hclust function with “average” parameter in R-3.6.3 to detect whether they were abnormal samples. Independent networks were constructed from the retained samples. An adjacency matrix was constructed using a soft threshold power of 16. Network interconnectiveness was measured by calculating the topological overlap using the TOMdist function with a signed TOMType. Average hierarchical clustering using the hclust function was performed to group the genes based on the topological overlap dissimilarity measure (1-TOM) of their connection strengths. Network modules were identified using a dynamic tree cut algorithm with a minimum cluster size of 30 and a merging threshold function of 0.25. To visualize the expression trend of the module, the module eigengene (ME, first principal component) was plotted using ggplot2 in R-3.6.3. The module membership (MM) for each gene was calculated based on the Pearson correlation between the expression level and the ME, to identify hub genes within the module. Genes within the module with the highest MM were highly connected within that module. To relate the trait measurements with the module, the ME was correlated with the trait data. To associate individual genes with traits, we calculated the gene significance as the absolute value of the correlation between gene expression and trait data. Networks were visualized using Cytoscape 3.7.1 (<https://cytoscape.org/>).

Subsequently, GO functional enrichment (<http://geneontology.org/>) analysis was performed using the clusterProfiler package in R-3.6.3 (Yu et al. 2012). The terms were considered to be significantly enriched if the false discovery rate was ≤ 0.05 .

Homoeolog expression bias

Homoeologous gene pairs were obtained from Ensembl Plants, and their expression values were extracted from the expression matrix. Then, statistical methods were used to analyse the homoeolog expression bias. Statistical analyses were performed with one-way ANOVA analyses, followed by the LSD test at the 0.05 level using R-3.6.3.

Copy number variations and karyotyping

Clean reads obtained after whole genome resequencing were aligned to the reference genome using BWA software (Li and Durbin 2009). Alignment files were converted to BAM files using SAMtools (Li et al. 2009), and used for copy number variation (CNV) analysis with Control-FREEC. All the settings were set to default, except for ExpectedGC, which was set at 0.3–0.5 (Boeva et al. 2012; Cao et al. 2023). All the figures were plotted using ggplot2 in R-3.6.3 (Zhao et al. 2023). Fluorescence in situ hybridization (FISH) was performed on pollen mother cells during the metaphase stage of mitosis to verify the results of sequencing analysis. Probes used for FISH, tissue preparation, hybridization, karyotyping, and imaging have been described previously (Xiong et al. 2021, 2011; Xiong and Pires 2011).

Results

Diverse phenotypes of resynthesized *B. napus*

The growth and development of 18 resynthesized allopolyploid *B. napus* individuals (six lines and three generations) were different, and a variety of phenotypes with different characteristics, including flower time, pollen activity, plant height, and seed number, were observed (Table S1). To study the causes of phenotypic diversity, the gene expression levels of 18 individuals were surveyed using RNA-seq analysis, but libraries could not be generated for two individuals. However, representative generations from all six lines were included in these analyses.

Sixteen samples were examined, and genes with a mean FPKM < 2 were filtered. A total of 16008 genes were thus selected for subsequent WGCNA analyses. Because the samples analysed in this study exhibited a parallel curve for FPKM distribution (Fig. 1a), we hypothesized that they should show perfect homogeneity. Interestingly, the sample clustering tree was divided into three clades, i.e. S_1 , S_5 , and S_{10} , according to generations but not lines, indicating that generations had a greater effect on expression than lines (Fig. 1b). The trait heatmap showed that traits of the S_1 generation were relatively stable, but the increase in the number

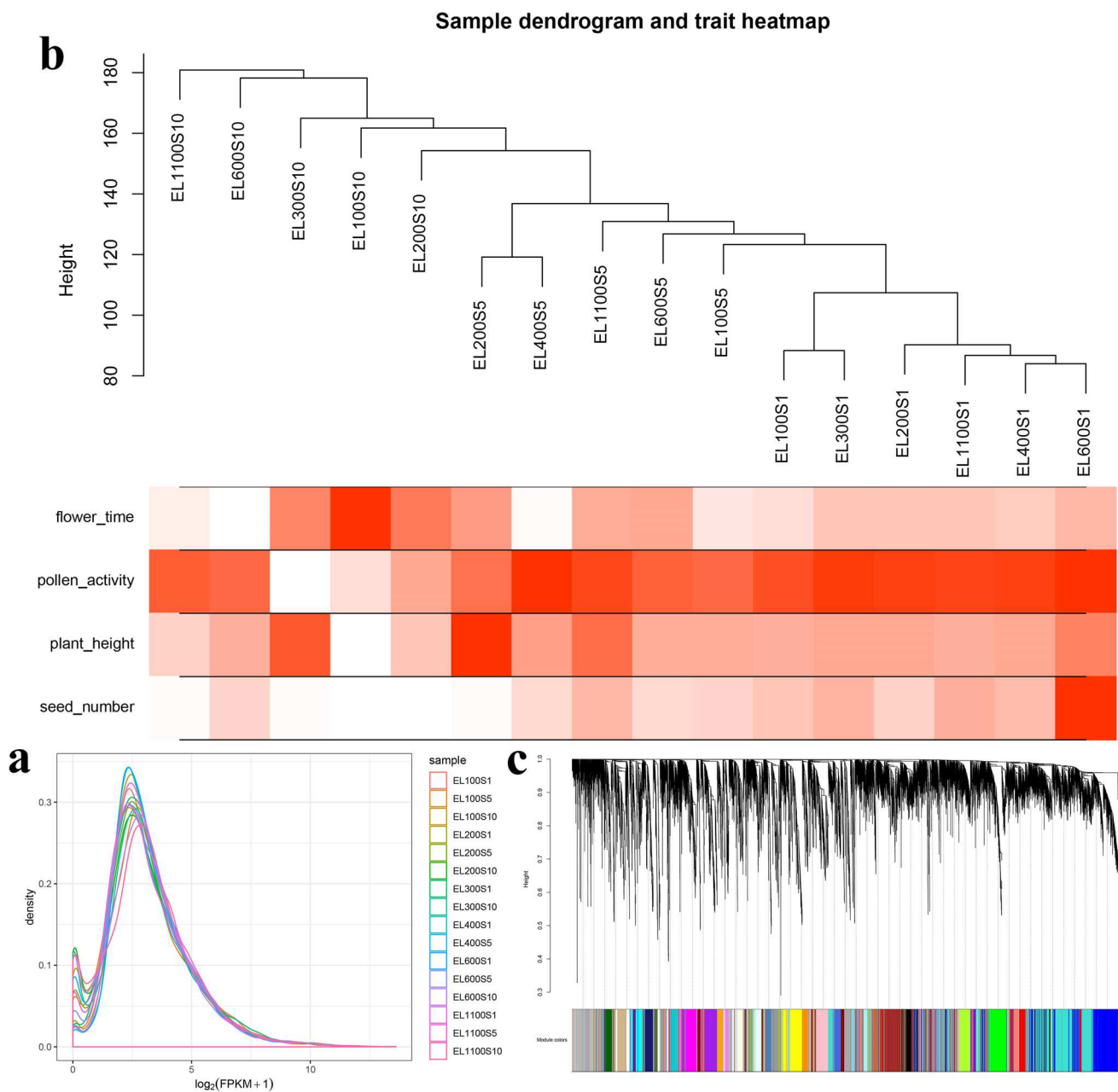


Fig. 1 *B. napus* samples clustering and traits heatmap. For the samples, six lines include EL100, EL200, EL300, EL400, EL600, and EL1100 and three generations include S_1 , S_5 , and S_{10} generations. **a** The original FPKM is logarithm-transformed ($\log_2(\text{FPKM} + 1)$) for

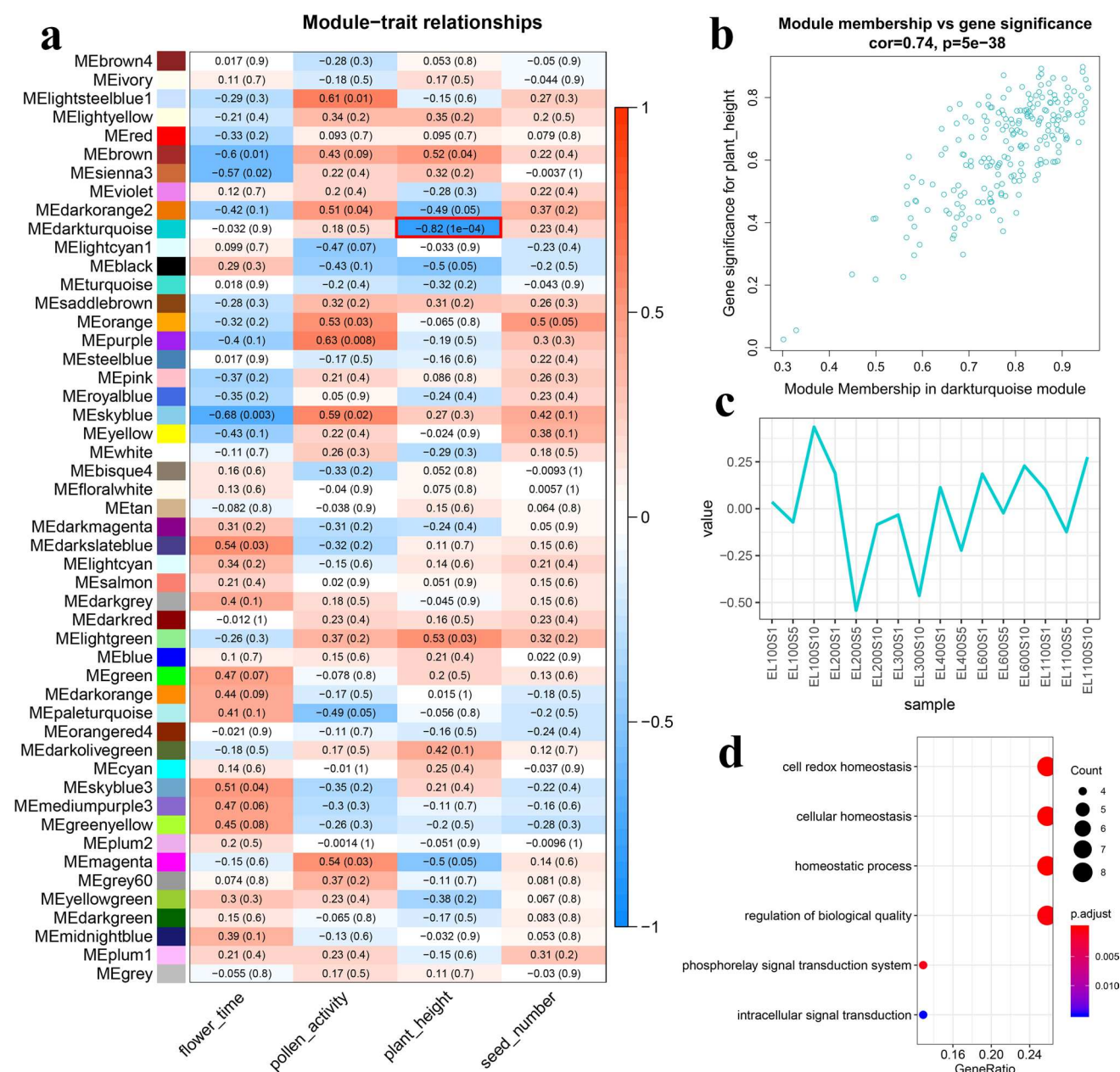
the density distribution curve. **b** In the heatmap, the trait values from high to low corresponded to the heatmap's colour from red to white. **c** Genes clustering to divide modules and different colours represent different modules (color figure online)

of generations led to diverse phenotypes, especially in the S_{10} generation (Fig. 1b; Table S1).

Expression of a set of genes on chromosome A7 is significantly negatively correlated with plant height

A co-expression network was built to identify 49 distinct functional modules (Fig. 1c). Module–trait relationships

showed that the darkturquoise module (including 212 genes; Supplemental Excel sheet) was significantly negatively correlated with plant height (Fig. 2a, highlighted by the red box). We then found that genes of the darkturquoise module were of high significance measures with plant height and the high module membership with the module eigengene (Fig. 2b). Besides, the line chart of the darkturquoise module eigengene (ME_{darkturquoise}) shows an overall trend of expression of genes in the darkturquoise module (Fig. 2c).



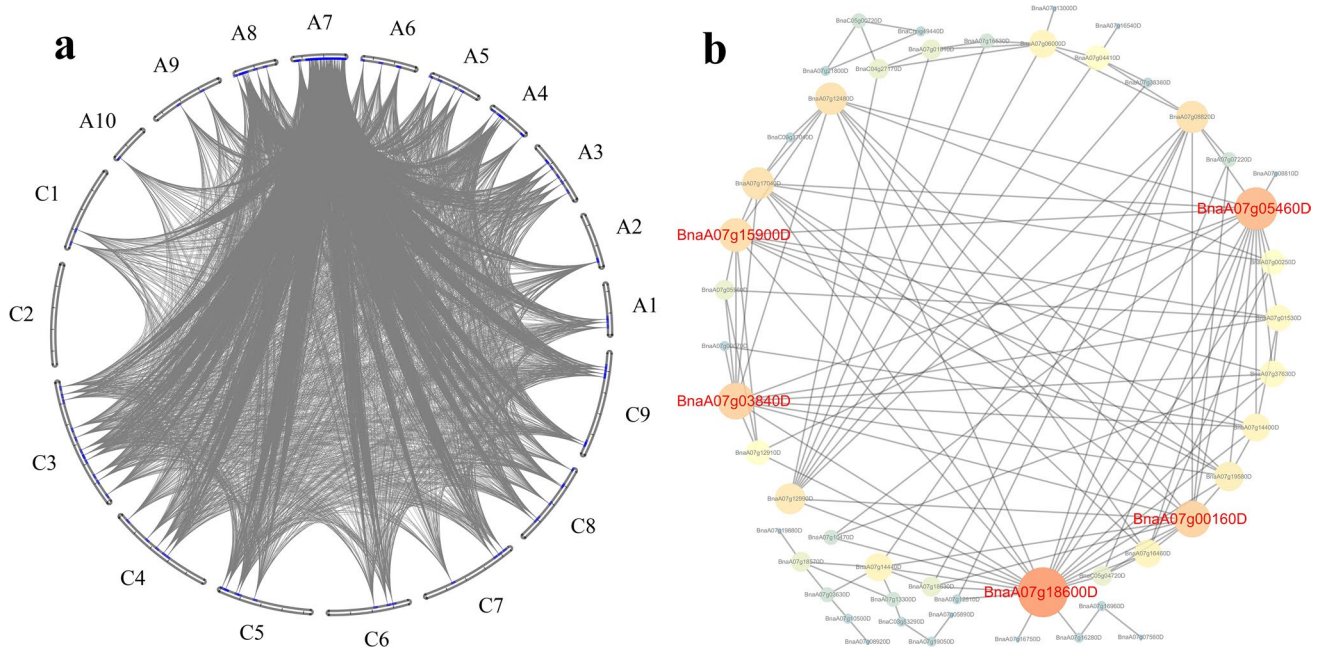


Fig. 3 Genes location and co-expression network of the darkturquoise module. **a** Two hundred and one genes are located on chromosomes. The grey lines show the co-expression of gene pairs. **b** In the co-

expression network, edge weight ≥ 0.10 , and the higher the connection degree is, the deeper the colour and the bigger the dot. The top five dots (hub genes) show their names in red (color figure online)

and 10 genes were located on chromosomes C3 and A8, respectively (Fig. 3a; Supplemental Excel sheet). Finally, this study helped to identify a total of 50 genes with an average gene connection degree of five in the co-expression network (only edge weight ≥ 0.10 were retained) of the darkturquoise module (Fig. 3b). We screened the hub genes based on the degree of connection. We found that all the hub genes were located on chromosome A7. For example, one hub gene *BnaA07g03840D* on this chromosome is homologous to the gene *AT2G15290 (PIC1)* of *Arabidopsis*, which previously was shown to regulate plant growth and development (Duy et al. 2011).

Effect of homoeolog expression bias on plant height

It was confirmed that almost all the key genes contributing to plant height were distributed in subgenomic chromosome A7 of the newly developed allopolyploid *B. napus* (Bn), but their homoeologous genes from C subgenome were not associated with plant height. Therefore, we hypothesized that this “lopsided” phenomenon was caused by homoeolog expression bias. Then, genes homoeologous to 116 genes on chromosome A7 with identity $> 80\%$ were retained (Supplemental Excel sheet). Finally, the expression matrix of 87 pairs of homoeologous genes was evaluated.

For the 87 pairs of homoeologous genes, the gene expression level of *B. rapa* (Br) was not significantly different from that of *B. oleracea* (Bo), A subgenome of *B. napus* (BnA),

and C subgenome of *B. napus* (BnC), but the gene expression level of BnA was significantly lower than that of BnC and Bo (Fig. 4a). These results indicate that these homoeologous genes in C subgenome were dominantly expressed and this homoeolog expression bias in *B. napus* was generated after hybridization rather than inherited from the parents. Notably, the average plant height of Bn was close to that of Bo, but significantly higher than that of Br (Fig. 4b). In addition, an independent analysis of 16 *B. napus* individuals showed that the gene expression level in BnA was generally lower than that in BnC (Fig. 4c). Particularly, in plants of EL200S5 and EL300S10, the expression level of these genes in BnA was significantly lower than that in homoeologous BnC genes, and interestingly these two plants had the first (EL200S5, 140 cm, Fig. 4e) and the second (EL300S10, 121 cm) highest plant height (Table S1). These results indicated that downregulation of the expression of subgenomic chromosome A7 genes promotes plant height in the allopolyploid *Brassica napus*.

However, one exception among 16 individuals was plant EL100S10, in which the expression level of these 87 genes in C subgenome was not dominantly expressed. On the contrary, EL100S10 showed that the expression of these genes of BnA was significantly higher than that of BnC (Fig. 4c), suggesting a transcriptome bias towards the A subgenome. This plant had a dwarf phenotype with delayed plant development and its height was much lower than that of parental *B. rapa* at the stage of material collections for

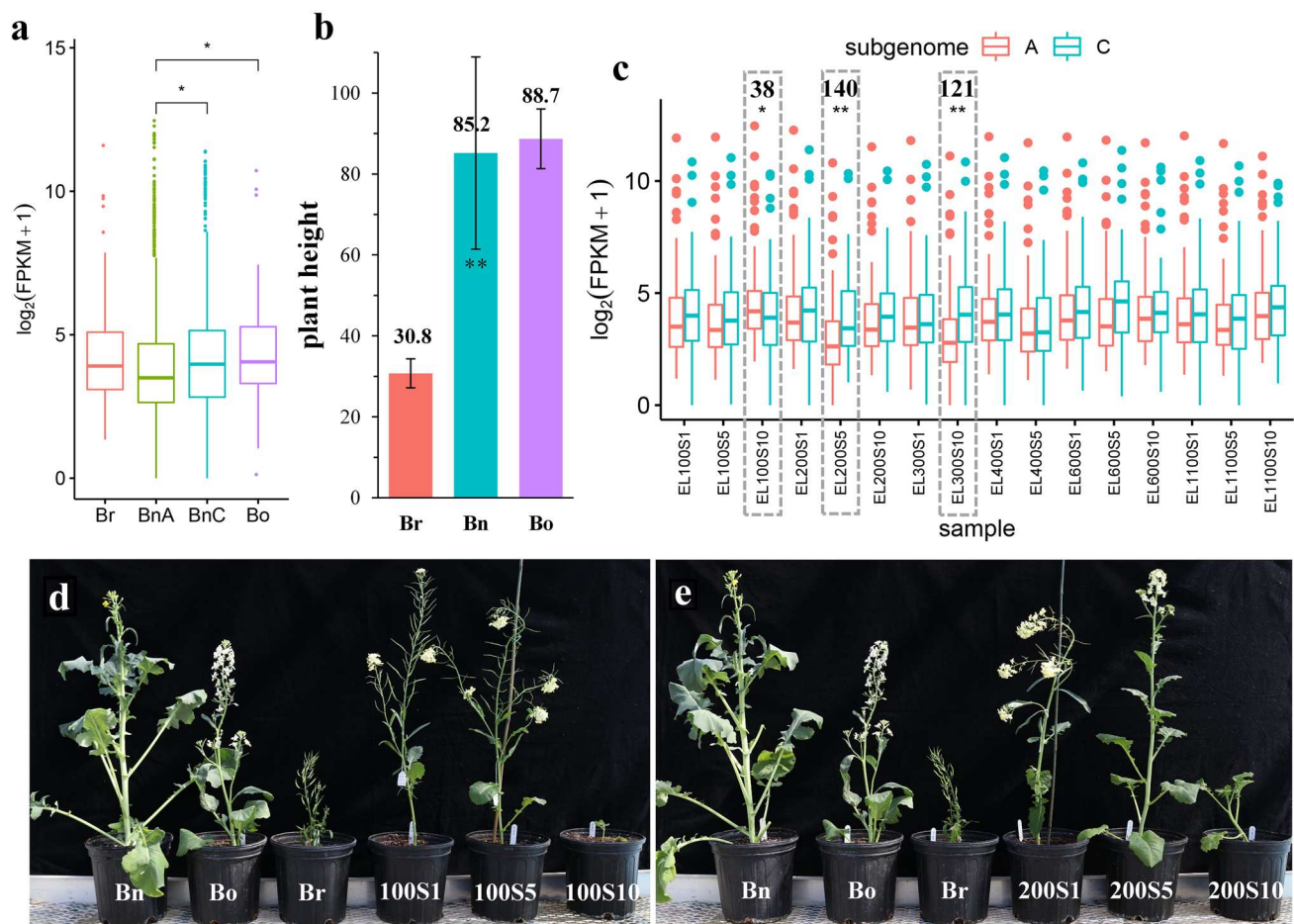


Fig. 4 Expression of genes on chromosome A7 within the darkturquoise module and their homoeologous genes, and the effect on plant height. **a** Comparison of the gene expression level of the 87 pairs of homoeologous genes in parental *B. rapa* IMB218 (Br), parental *B. oleracea* TO1000 (Bo), A subgenome of all resynthesized *B. napus* (BnA), and C subgenome of all resynthesized *B. napus* (BnC). **b** The plant heights (cm) of parents Br and Bo, and the average plant height of 16 resynthesized *B. napus* (Bn). Data as means $\pm$ SD ($n \geq 3$). **c** The gene expression level of the 87 pairs of homoeologous genes in

BnA and BnC of each resynthesized *B. napus* individual. The numbers in the boxplots represent plant height (cm). Statistical analyses were completed with a one-way ANOVA test. Asterisks represent statistically significant differences ($*P < 0.05$, $**P < 0.01$) between the indicated groups. **d** Phenotypes of the resynthesized line EL100 and their parents. From left to right are natural Bn, Bo, and EL100 at S₁, S₅, and S₁₀ generations. **e** Phenotypes of the resynthesized line EL200. From left to right are Bn, Bo, Br, and EL200 at S₁, S₅, and S₁₀ generations (color figure online)

RNA sequencing (Fig. 4d). At the mature stage, the height of this plant was the lowest among 16 individuals (38 cm; Table S1). This suggests that dominantly expressed in A subgenome of these genes may decrease plant height in resynthesized *B. napus*.

Gene dosage effect promotes plant height to the maximum extent

We have demonstrated that the homoeolog expression bias of a single chromosome substantially affected plant height. To further understand why different resynthesized individuals had different levels of bias gene expression on subgenomic chromosome A7, and why plant EL100S10 had a reverse subgenomic bias gene expression on the same chromosome,

we analysed the genome sequences including gene dosages of the three resynthesized plants (EL100S10, EL200S5, and EL300S10). Since extensive chromosomal variations including aneuploids had been reported in the self-fertilized progeny of resynthesized *B. napus* (Cao et al. 2023; Xiong et al. 2011), we speculated that the extreme phenotypic changes in plant height might be attributable to the gene dosage changes by chromosome number variations.

We extracted the dosages of 87 pairs of homoeologous genes and found that EL100S10 had two copies of the genes from C subgenome and three copies of genes from A subgenome. But EL200S5 and EL300S10 just had one copy of these genes from A subgenome and two copies from C subgenome (Fig. 5a). In combination with the expression profiles of these three samples (Fig. 4c, circled by the grey

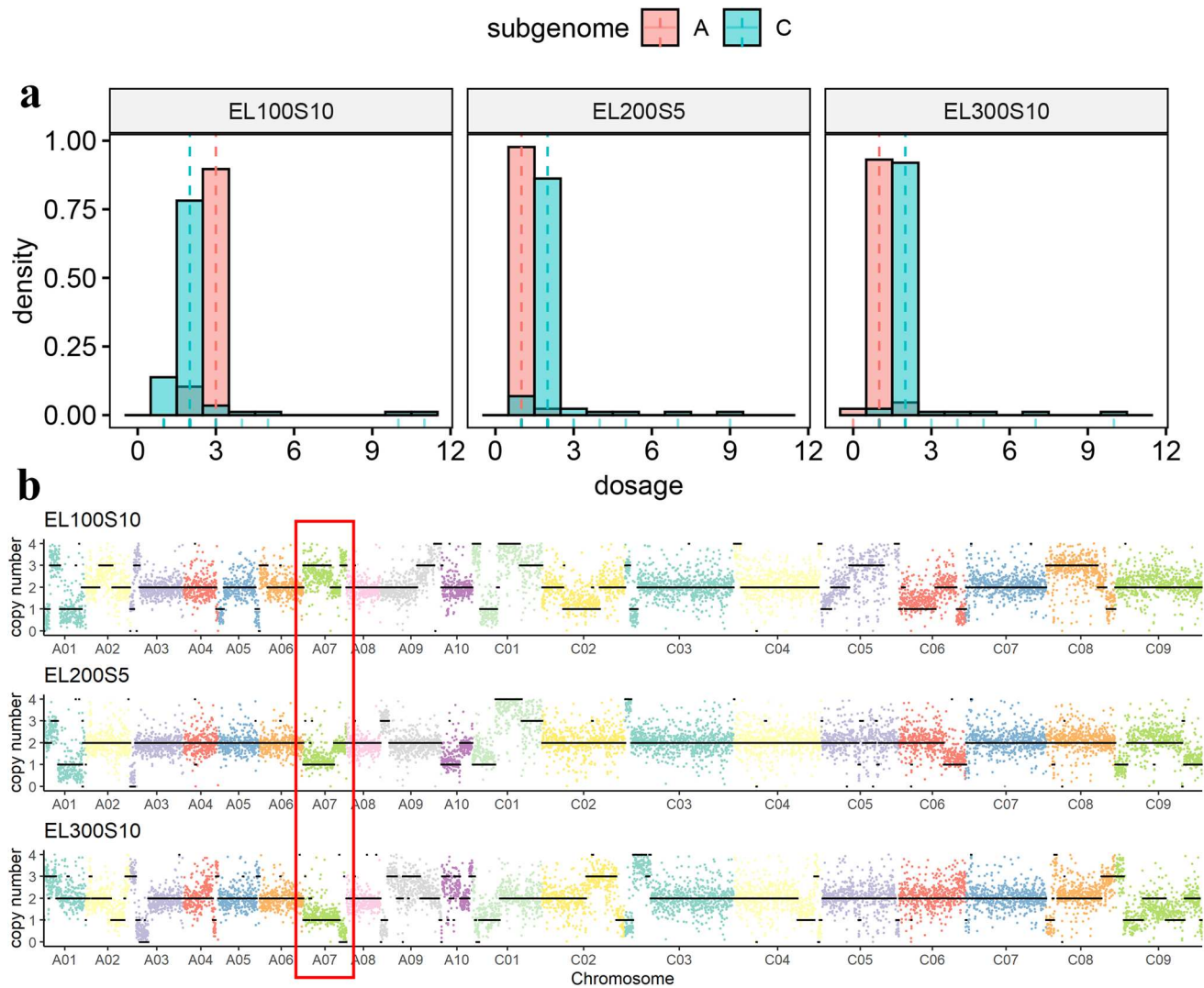


Fig. 5 Copy number variation and karyotyping in the three resynthesized *B. napus* individuals showing abnormal plant height. **a** Dosage variations of the 87 pairs of homoeologous genes from A and C subgenomes. The dotted line is the median. Density means proportion. **b** Molecular karyotyping and chromosome number of the three

resynthesized *B. napus* individuals. The scatter represents Ratio * 2 (expected ploidy), and the levels of the black line show the copy number of chromosomes. Chromosomes in the red box are A7 from A subgenome

dotted line), we found that the increase or decrease in gene copy number in the A subgenome resulted in a corresponding increase or decrease in the gene expression level. This result demonstrated that gene dosage impacted the gene expression from different subgenomes and the alterations in the gene dosages changed the direction of expression bias to BnA or BnC, which might lead to the plant height being the lowest or highest.

To verify whether the gene dosage alterations from different subgenomes were induced by chromosome number variations, we did the molecular and cytogenetic karyotyping of the three plants (Cao et al. 2023). The results of molecular karyotyping analyses revealed that EL100S10 gained one extra chromosome A7, while both EL200S5 and EL300S10

lost one chromosome A7 (Fig. 5b, highlighted by the red box). Cytogenetic karyotyping by multicolour FISH showed that all of the three plants were aneuploidies (Fig. S1). EL100S10 had three A7, one homoeologous C6, and two homoeologous C7 chromosomes, which made it have a 3/1 (or 3/2) ratio of homoeologous chromosomes from A and C subgenomes. EL200S5 and EL300S10 had one A7 and two corresponding homoeologous C chromosomes, indicating having a 1/2 ratio of homoeologous chromosomes from A and C subgenomes. These results demonstrated that homoeologous chromosome number variations of A7/C6C7 broke the balance of homoeologous gene copy number, which might further change the direction of expression bias to A or C subgenomes. Cytogenetic karyotypes were also performed

on other resynthesized *B. napus* with similar heights to *B. oleracea* and found that they had two normal A7 and two corresponding homoeologous C chromosomes (Fig. S2).

Discussion

Plant height is an important trait affecting plant reproductive success, as taller plants have been predicted to disperse their seed further (Thomson et al. 2011; Zu and Schiestl 2017). Plant height is often controlled by pollinator-mediated selection and is correlated with various other traits, such as flower number, flower size, and plant phenology (Zu and Schiestl 2017). The choice of plant height can contribute to plant trait evolution (Endler 1986). In rapeseed, plant height is one of the key factors of plant architecture that affect plant density and lodging, which are crucial for achieving a yield (Fan et al. 2021). Asymmetric epigenetic modifications contribute to homoeolog expression bias (Li et al. 2021). In a previous study, DNA methylation differences between subgenomes mirrored the observed gene expression bias towards the dominant C subgenome in all lines and generations based on the same experimental materials as the present study (Bird et al. 2020). Homoeolog expression biases have been shown to affect plant growth, development, and stress responses in several polyploid species (Powell et al. 2017), but their effect on plant height in *B. napus* has remained unknown.

In this study, the resynthesized *B. napus* tended to express homoeologous genes from Bo (BnC) at high levels and maintained the same expression level as the parent. In contrast, the expression of homoeologous genes from Br (BnA) was significantly down-regulated (Fig. 4a), leading to a significant increase in plant height (Fig. 4b). Interestingly, the genes related to plant height were almost all located on chromosome A7. Especially, the hub genes in the co-expression network were also located on this chromosome (Fig. 3). This result was consistent with many previous studies, in which the candidate genes for plant height were located on chromosome A7 by quantitative trait loci (QTLs) and genome-wide association study techniques (Li et al. 2016; Shen et al. 2018; Shi et al. 2009; Sun et al. 2016; Udall et al. 2006; Zheng et al. 2017). To verify that our results are independent of genome quality and transcriptome quantitative methods, we obtained the TPM (transcripts per million) expression matrix using Salmon software (Patro et al. 2017) and a newly improved assembled genome of *B. napus* ZS11 (Song et al. 2020). In summary, we obtained similar results from WGCNA using this and our aforementioned approach.

The hub genes impacted the plant height, including *BnaA07g03840D*, and its *Arabidopsis* homologous gene *AT2G15290* (*PIC1*)-overexpressing lines (PIC1ox), resulted in high phenotypic variation and were affected by oxidative stress and leaf chlorosis. PIC1ox plants were characterized

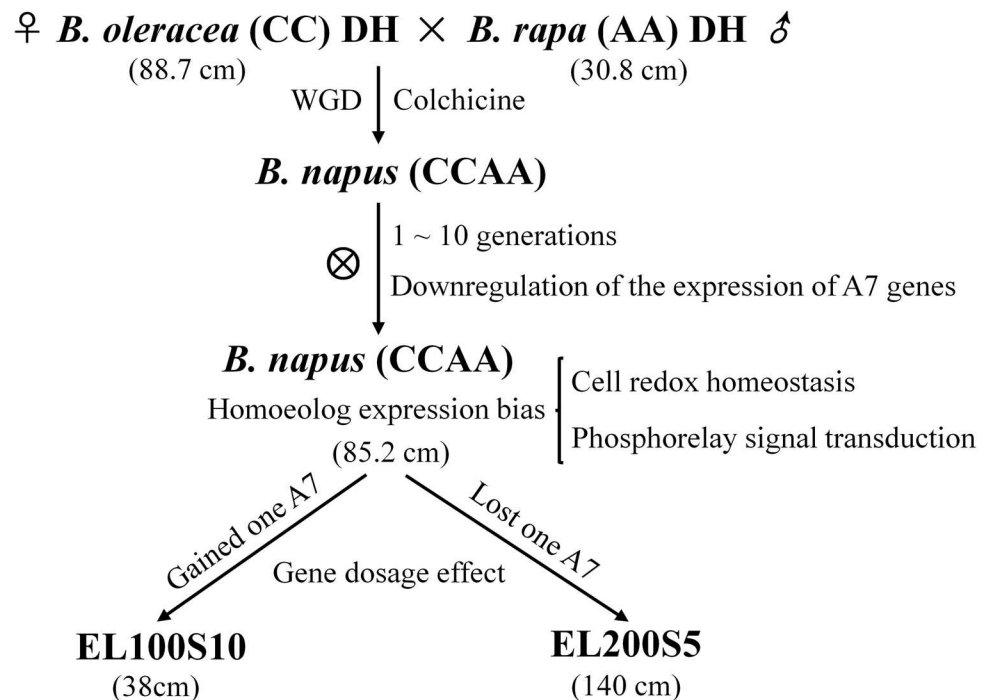
by reduced biomass and severely defective flower and seed development (Duy et al. 2011). The expression level of *BnaA07g03840D* in EL100S10 (FPKM = 22.8) was twice that of the average expression level in all samples (FPKM = 11.7). Characteristics such as severe dwarfness, delayed flowering time, and seedlessness in EL100S10 (Table S1) were consistent with those in PIC1ox, indicating a significant negative correlation between the gene expression level of BnA and plant height.

However, we do not believe that a single gene solely is responsible for differences in plant height variation observed in these resynthesized *B. napus*. First, the results of WGCNA showed that multiple genes were simultaneously up/down-regulated that are correlated with plant height (Fig. 2c), and previous studies have shown that plant height is controlled by multiple QTLs (Li et al. 2016). Second, the plant height of the hybrid Bn (CCAA) plant was the median of the height of the parents, assuming that Bo (CC) and Br (AA) carried a single gene for long and short stems, respectively, according to the parental phenotype. However, the results of karyotyping analysis showed that the highest (CCAA-A7) and shortest (CCAA + A7) plants lost and gained one chromosome A7, respectively. The plant height order was CCAA-A7 (140 cm) > CC (88.7 cm) > CCAA (85.2 cm) > CCAA + A7 (38 cm) > AA (30.8 cm) and did not conform to the theoretical single gene-associated plant order, i.e. CC > CCAA-A7 > CCAA > CCAA + A7 > AA. In conclusion, we believe that interactions between multiple genes cause variations in plant height.

Many homoeologous exchanges (HEs) in nascent allopolyploids (Hurgobin et al. 2018; Wu et al. 2020) increase the difficulty associated with karyotyping via whole genome resequencing, as HEs could result in a large number of CNVs. For example, CNV analysis of chromosome numbers showed that one copy of chromosome A1 and four copies of partial chromosome fragments of C1 could be found in EL100S10 and EL200S5 (Fig. 5b). In contrast, FISH results showed that there were four copies of C1 without A1 (Fig. S1). Genetic variation does cause expression bias and thus affects clustering of samples, and the variation accumulates with generations, showing significant generation dependence (Fig. 1b). But WGCNA is able to simultaneously analyse samples (no less than 15) with genetic variation to uncover which variation has the greatest impact on the target trait (height), even if the sample is aneuploid. Besides, the accumulation of variations in high-generation nascent allopolyploids seems conducive to breaking the genetic bottleneck (Wu et al. 2020), thus promoting extensive phenotypic diversity (Fig. 1b; Table S1).

In comparison, all aneuploid wheat strains of chromosomes 1 and 2, including those exhibiting nullisomy, monosomy, trisomy, and tetrasomy, had a significantly lower plant height (Zhang et al. 2017). Nevertheless, wheat 4A and 4D

Fig. 6 A model explaining the main observations described in this paper



monosomics are tall (Worland and Law 1985). The height of the diploid aneuploid ($2n = 2x + 2$) variety was significantly higher than that of the wild-type variety of the ‘Red Flash’ Caladium (Zhang et al. 2020). However, in this study, the plant height increased and decreased significantly in A7 monosomic and trisomic *B. napus*, respectively. Moreover, other chromosomal variations also affected phenotypes and might have no effect or hardly any effect on the plant height. This is consistent with the plant height of EL200S5 being substantially taller because of the loss of chromosome A7; however, its poor seed yield could be attributable to other chromosomal variations (Fig. 1b; Table S1). In conclusion, our results suggest that homoeolog expression bias in a single chromosome, especially dosage changes in the chromosome, considerably affected the plant height of newly developed allopolyploids (Fig. 6).

Due to the high stability of the natural *B. napus*, it is rare to gain or lose a whole chromosome. Nevertheless, homoeolog expression bias is commonly observed (Chalhoub et al. 2014; Li et al. 2020), and our findings confirmed that genomic expression bias causes phenotypic variations. Therefore, we hypothesized that the conclusion of this study is also applicable to natural *B. napus*. Our findings shed new light on the genetic regulation of homoeologs and have implications for understanding allopolyploid genome evolution and crop breeding. These variants, including the allotetraploids and aneuploids, exhibit great potential for the cultivar development, genetic study, and chromosome engineering of *B. napus*.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00122-023-04510-y>.

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Author contribution statement KZ and ZX designed the research. KZ, YB, YY, CL, LW, TL, LF, and YW performed data analysis. JD and JX completed the cytogenetic experiment. KZ and JX wrote the manuscript. PPE and ZX finalized the manuscript.

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Data availability Raw data from this project are available on the NCBI Sequence Read Archive Project PRJNA577908.

Declarations

Conflicts of interest The authors declare no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

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References

- Birchler JA (2014) Facts and artifacts in studies of gene expression in aneuploids and sex chromosomes. *Chromosoma* 123:459–469
- Bird KA, Niederhuth CE, Ou S, Gehan M, Pires JC, Xiong Z, VanBuren R, Edger PP (2020) Replaying the evolutionary tape to investigate subgenome dominance in allopolyploid *Brassica napus*. *New Phytol* 230(1):354–371
- Boeva V, Popova T, Bleakley K, Chiche P, Cappel J, Schleiermacher G, Janoueix-Lerosey I, Delattre O, Barillot E (2012) Control-FREEC: a tool for assessing copy number and allelic content using next-generation sequencing data. *Bioinformatics* 28:423–425
- Bus A, Korber N, Snowdon RJ, Stich B (2011) Patterns of molecular variation in a species-wide germplasm set of *Brassica napus*. *Theor Appl Genet* 123:1413–1423
- Cao Y, Zhao K, Xu J, Wu L, Hao F, Sun M, Dong J, Chao G, Zhang H, Gong X, Chen Y, Chen C, Qian W, Pires JC, Edger PP, Xiong Z (2023) Genome balance and dosage effect drive allopolyploid formation in *Brassica*. *Proc Natl Acad Sci U S A* 120:e2217672120
- Chalhoub B, Denoeud F, Liu SY, Parkin IAP, Tang HB, Wang XY, Chiquet J, Belcram H, Tong CB, Samans B, Correa M, Da Silva C, Just J, Falentin C, Koh CS, Le Clainche I, Bernard M, Bento P, Noel B, Labadie K, Alberti A, Charles M, Arnaud D, Guo H, Daviaud C, Alamery S, Jabbari K, Zhao MX, Edger PP, Chelaifa H, Tack D, Lassalle G, Mestiri I, Schnell N, Le Paslier MC, Fan GY, Renault V, Bayer PE, Golicz AA, Manoli S, Lee TH, Thi VHD, Chalabi S, Hu Q, Fan CC, Tollenaere R, Lu YH, Battail C, Shen JX, Sidebottom CHD, Wang XF, Canaguier A, Chauveau A, Berard A, Deniot G, Guan M, Liu ZS, Sun FM, Lim YP, Lyons E, Town CD, Bancroft I, Wang XW, Meng JL, Ma JX, Pires JC, King GJ, Brunel D, Delourme R, Renard M, Aury JM, Adams KL, Batley J, Snowdon RJ, Tost J, Edwards D, Zhou YM, Hua W, Sharpe AG, Paterson AH, Guan CY, Wincker P (2014) Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* 345:950–953
- Chen ZJ (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annu Rev Plant Biol* 58:377–406
- Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34:i884–i890
- Comai L (2005) The advantages and disadvantages of being polyploid. *Nat Rev Genet* 6:836–846
- Doyle JJ, Paterson AH, Soltis DE, Wendel JF (2008) Evolutionary genetics of genome merger and doubling in plants. *Annu Rev Genet* 42:443–461
- Duy D, Stube R, Wanner G, Philipp K (2011) The chloroplast per-mease PIC1 regulates plant growth and development by directing homeostasis and transport of iron. *Plant Physiol* 155:1709–1722
- Endler JA (1986) Natural selection in the wild. Princeton University Press
- Fan S, Zhang L, Tang M, Cai Y, Liu J, Liu H, Liu J, Terzaghi W, Wang H, Hua W, Zheng M (2021) CRISPR/Cas9-targeted mutagenesis of the BnaA03.BP gene confers semi-dwarf and compact architecture to rapeseed (*Brassica napus* L.). *Plant Biotechnol J* 19:2383–2385
- Gaeta RT, Pires JC, Iniguez-Luy F, Leon E, Osborn TC (2007) Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. *Plant Cell* 19:3403–3417
- Grover CE, Gallagher JP, Szadkowski EP, Yoo MJ, Fligel LE, Wendel JF (2012) Homoeolog expression bias and expression level dominance in allopolyploids. *New Phytol* 196:966–971
- Henry IM, Dilkes BP, Tyagi AP, Lin HY, Comai L (2009) Dosage and parent-of-origin effects shaping aneuploid swarms in *A. thaliana*. *Heredity (edinb)* 103:458–468
- Henry IM, Dilkes BP, Miller ES, Burkart-Waco D, Comai L (2010) Phenotypic consequences of aneuploidy in *Arabidopsis thaliana*. *Genetics* 186:1231–1245
- Hu J, Chen B, Zhao J, Zhang F, Xie T, Xu K, Gao G, Yan G, Li H, Li L, Ji G, An H, Li H, Huang Q, Zhang M, Wu J, Song W, Zhang X, Luo Y, Chris Pires J, Batley J, Tian S, Wu X (2022) Genomic selection and genetic architecture of agronomic traits during modern rapeseed breeding. *Nat Genet* 54:694–704
- Hurgobin B, Golicz AA, Bayer PE, Chan CKK, Tirnaz S, Dolatabadian A, Schiessl SV, Samans B, Montenegro JD, Parkin IAP, Pires JC, Chalhoub B, King GJ, Snowdon R, Batley J, Edwards D (2018) Homoeologous exchange is a major cause of gene presence/absence variation in the amphidiploid *Brassica napus*. *Plant Biotechnol J* 16:1265–1274
- Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. *BMC Bioinform* 9(1):1–13
- Leitch AR, Leitch IJ (2008) Perspective-genomic plasticity and the diversity of polyploid plants. *Science* 320:481–483
- Levin DA (1983) Polyploidy and novelty in flowering plants. *Am Nat* 122:1–25
- Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754–1760
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing S (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* 25:2078–2079
- Li A, Liu D, Wu J, Zhao X, Hao M, Geng S, Yan J, Jiang X, Zhang L, Wu J (2014) mRNA and small RNA transcriptomes reveal insights into dynamic homoeolog regulation of allopolyploid heterosis in nascent hexaploid wheat. *Plant Cell* 26:1878–1900
- Li F, Chen BY, Xu K, Gao GZ, Yan GX, Qiao JW, Li J, Li H, Li LX, Xiao X, Zhang TY, Nishio T, Wu XM (2016) A genome-wide association study of plant height and primary branch number in rapeseed (*Brassica napus*). *Plant Sci* 242:169–177
- Li M, Wang R, Wu X, Wang J (2020) Homoeolog expression bias and expression level dominance (ELD) in four tissues of natural allotetraploid *Brassica napus*. *BMC Genom* 21:330
- Li M, Sun W, Wang F, Wu X, Wang J (2021) Asymmetric epigenetic modification and homoeolog expression bias in the establishment and evolution of allopolyploid *Brassica napus*. *New Phytol* 232:898–913
- Li M, Hu M, Xiao Y, Wu X, Wang J (2022) The activation of gene expression and alternative splicing in the formation and evolution of allopolyploid *Brassica napus*. *Hortic Res* 9:uhab075
- Li Z, Li M, Wu X, Wang J (2023) The characteristics of mRNA m(6) A methylomes in allopolyploid *Brassica napus* and its diploid progenitors. *Hortic Res* 10:uhac230
- Lu K, Wei L, Li X, Wang Y, Wu J, Liu M, Zhang C, Chen Z, Xiao Z, Jian H, Cheng F, Zhang K, Du H, Cheng X, Qu C, Qian W, Liu L, Wang R, Zou Q, Ying J, Xu X, Mei J, Liang Y, Chai YR, Tang Z, Wan H, Ni Y, He Y, Lin N, Fan Y, Sun W, Li NN, Zhou G, Zheng H, Wang X, Paterson AH, Li J (2019) Whole-genome resequencing reveals *Brassica napus* origin and genetic loci involved in its improvement. *Nat Commun* 10:1154
- Otto SP (2007) The evolutionary consequences of polyploidy. *Cell* 131:452–462
- Patro R, Duggal G, Love MI, Irizarry RA, Kingsford C (2017) Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* 14:417
- Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL (2016) Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat Protoc* 11:1650–1667
- Peterson R, Slovin JP, Chen C (2010) A simplified method for differential staining of aborted and non-aborted pollen grains. *Int J Plant Biol* 1:e13

- Powell JJ, Fitzgerald TL, Stiller J, Berkman PJ, Gardiner DM, Man-ners JM, Henry RJ, Kazan K (2017) The defence-associated transcriptome of hexaploid wheat displays homoeolog expression and induction bias. *Plant Biotechnol J* 15:533–543
- Ramsey J, Schemske DW (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu Rev Ecol Syst* 29:467–501
- Shen YS, Xiang Y, Xu ES, Ge XH, Li ZY (2018) 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* 9:390
- Shi JQ, Li RY, Qiu D, Jiang CC, Long Y, Morgan C, Bancroft I, Zhao JY, Meng JL (2009) Unraveling the complex trait of crop yield with quantitative trait loci mapping in *Brassica napus*. *Genetics* 182:851–861
- Song Q, Chen ZJ (2015) Epigenetic and developmental regulation in plant polyploids. *Curr Opin Plant Biol* 24:101–109
- Song JM, Guan Z, Hu J, Guo C, Yang Z, Wang S, Liu D, Wang B, Lu S, Zhou R, Xie WZ, Cheng Y, Zhang Y, Liu K, Yang QY, Chen LL, Guo L (2020) Eight high-quality genomes reveal pan-genome architecture and ecotype differentiation of *Brassica napus*. *Nat Plants* 6:34–45
- Sun CM, Wang BQ, Yan L, Hu KN, Liu S, Zhou YM, Guan CY, Zhang ZQ, Li JN, Zhang JF, Chen S, Wen J, Ma CZ, Tu JX, Shen JX, Fu TD, Yi B (2016) Genome-wide association study provides insight into the genetic control of plant height in rapeseed (*Brassica napus* L.). *Front Plant Sci* 7:1102
- Thomson FJ, Moles AT, Auld TD, Kingsford RT (2011) Seed dispersal distance is more strongly correlated with plant height than with seed mass. *J Ecol* 99:1299–1307
- Udall JA, Quijada PA, Lambert B, Osborn TC (2006) Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (*Brassica napus* L.): 2. Identification of alleles from unadapted germplasm. *Theor Appl Genet* 113:597–609
- Williams BR, Amon A (2009) Aneuploidy: cancer's fatal flaw? *Cancer Res* 69:5289–5291
- Worland A, Law C (1985) Aneuploidy in semi dwarf wheat varieties. *Euphytica* 34:317–327
- Wu J, Lin L, Xu M, Chen P, Liu D, Sun Q, Ran L, Wang Y (2018) Homoeolog expression bias and expression level dominance in resynthesized allopolyploid *Brassica napus*. *BMC Genom* 19:586
- Wu Y, Lin F, Zhou Y, Wang J, Sun S, Wang B, Zhang Z, Li G, Lin X, Wang X (2020) Genomic mosaicism due to homoeologous exchange generates extensive phenotypic diversity in nascent allopolyploids. *Natl Sci Rev* 8(5):nwaa277
- Xiong ZY, Pires JC (2011) Karyotype and identification of all homoeologous chromosomes of allopolyploid *Brassica napus* and its diploid progenitors. *Genetics* 187:37–49
- Xiong ZY, Gaeta RT, Pires JC (2011) Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus*. *Proc Natl Acad Sci USA* 108:7908–7913
- Xiong Z, Gaeta RT, Edger PP, Cao Y, Zhao K, Zhang S, Pires JC (2021) Chromosome inheritance and meiotic stability in allopolyploid *Brassica napus*. *G3* 11:jkaa011
- Yoo MJ, Szadkowski E, Wendel JF (2013) Homoeolog expression bias and expression level dominance in allopolyploid cotton. *Heredity* (edinb) 110:171–180
- Yu GC, Wang LG, Han YY, He QY (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics-a J Integr Biol* 16:284–287
- Zeng D, Guan J, Luo J, Zhao L, Li Y, Chen W, Zhang L, Ning S, Yuan Z, Li A, Zheng Y, Mao L, Liu D, Hao M (2020) A transcriptomic view of the ability of nascent hexaploid wheat to tolerate aneuploidy. *BMC Plant Biol* 20:97
- Zhang A, Li N, Gong L, Gou X, Wang B, Deng X, Li C, Dong Q, Zhang H, Liu B (2017) Global analysis of gene expression in response to whole-chromosome aneuploidy in hexaploid wheat. *Plant Physiol* 175:828–847
- Zhang YS, Chen JJ, Cao YM, Duan JX, Cai XD (2020) Induction of tetraploids in 'Red Flash' caladium using colchicine and oryzalin: morphological, cytological, photosynthetic and chilling tolerance analysis. *Sci Hortic-Amst* 272:109524
- Zhao K, Jin N, Madadi M, Wang Y, Wu L, Xu Z, Wang J, Dong J, Tang SW, Wang Y, Peng L, Xiong Z (2022) Incomplete genome doubling enables to consistently enhance plant growth for maximum biomass production by altering multiple transcript co-expression networks in potato. *Theor Appl Genet* 135:461–472
- Zhao K, Bai Y, Zhang Q, Zhao Z, Cao Y, Yang L, Wang N, Xu J, Wang B, Wu L, Gong X, Lin T, Wang Y, Wang W, Cai X, Yin Y, Xiong Z. Karyotyping of aneuploid and polyploid plants from low coverage whole-genome resequencing. *BMC Plant Biol*. 2023;23(1):630. <https://doi.org/10.1186/s12870-023-04650-9>
- Zheng M, Peng C, Liu HF, Tang M, Yang HL, Li XK, Liu JL, Sun XC, Wang XF, Xu JF, Hua W, Wang HZ (2017) 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* 8:1246
- Zhu B, Shao Y, Pan Q, Ge X, Li Z (2015) Genome-wide gene expression perturbation induced by loss of C2 chromosome in allotetraploid *Brassica napus* L. *Front Plant Sci* 6:763
- Zu P, Schiestl FP (2017) The effects of becoming taller: direct and pleiotropic effects of artificial selection on plant height in *Brassica rapa*. *Plant J* 89:1009–1019

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