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Analysis of small RNA changes in different *Brassica napus* synthetic allopolyploids

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Allopolyploidy is an evolutionary and mechanistically intriguing process involving the reconciliation of two or more sets of diverged genomes and regulatory interactions, resulting in new phenotypes. In this study, we explored the small RNA changes of eight F2 synthetic *B. napus* using small RNA sequencing. We found that a part of miRNAs and siRNAs were non-additively expressed in the synthesized *B. napus* allotetraploid. Differentially expressed miRNAs and siRNAs differed among eight F2 individuals, and the differential expression of miR159 and miR172 was consistent with that of flowering time trait. The GO enrichment analysis of differential expression miRNA target genes found that most of them were concentrated in ATP-related pathways, which might be a potential regulatory process contributing to heterosis. In addition, the number of siRNAs present in the offspring was significantly higher than that of the parent, and the number of high parents was significantly higher than the number of low parents. The results have shown that the differential expression of miRNA lays the foundation for solving the trait separation phenomenon, and the significant increase of siRNA alleviates the shock of the newly synthesized allopolyploidy. It provides a new perspective of small RNA changes and trait separation in the early stages of allopolyploid polyploid formation.

1 Analysis of small RNA changes in different *Brassica napus* synthetic 2 allopolyploids

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10 11 Abstract

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26 **Key words:** small RNA; synthetic *Brassica napus*; trait separation

Introduction

Polyploidy, or whole-genome duplication (WGD), is prevalent in nature and is particularly common in angiosperms, increasing biodiversity and providing new genetic material for evolution (Wendel 2000). Synthetic polyploidy is often associated with novel and presumably advantageous ecological attributes such as range expansion (Hijmans et al. 2007), novel secondary chemistry and morphology (Leitch and Leitch 2008), and increased pathogen resistance (Nuismer and Thompson 2001). Previous studies have investigated synthetic allopolyploids and show that various genetic (Song et al. 1995; Xiong et al. 2011) and epigenetic (Adams et al. 2003; Cui et al. 2013; Ge et al. 2013) changes, as well as alterations in gene expression levels (Wang et al. 2006; Chelaifa et al. 2010; Yoo et al. 2013) occur at the initial stage of allopolyploidization. At the genetic level, loss of parental and/or appearance of novel sequences at the initial stage of allopolyploidization are common events. Non-homologous chromosome exchanges occur in synthetic *B. napus*, resulting in the addition and/or deletion of sequences (Gaeta et al. 2007). At the epigenetic level, changes in small RNA and DNA methylation patterns occur at the initial stage of allopolyploidization. Shen et al. reported higher siRNA and DNA methylation levels in F1 hybrids (Shen et al. 2017). The role of heredity and epigenetics leads to changes in gene expression, which in turn leads to novel phenotypes (Chen 2007).

Non-coding small RNAs are widely found in eukaryotes, which are endogenous with a length of about 20-24 nt. Many studies have shown that small RNAs play an important role in gene expression regulation through transcriptional level gene silencing, or post-transcriptional level gene silencing (Baumberger and Baulcombe 2005). Their first report was the phenomenon of RNA interference in nematodes (Lee et al. 1993), and later the phenomenon of gene silencing or inhibition was discovered (Napoli et al. 1990; Carvalho et al. 1992; Hannon 2002). Shortly after these studies, the researchers confirmed that post-transcriptional gene silencing in plants is associated with small RNA activity (Hamilton and Baulcombe 1999). These small RNAs regulate various biological processes by interfering with the translation of mRNA. In plants, small RNAs can be divided into two major categories depending on their synthesis and function: miRNA and siRNA. miRNAs and siRNAs are considered to be highly conserved and are important gene expression regulators in plants (Jones-Rhoades et al. 2006; Axtell and Bowman 2008). This small RNA has been used as a means of molecular biology to control gene expression at the transcriptional and post-transcriptional levels.

Oilseed rape (*B. napus*, AACC, $2n = 38$), which is generally thought to be naturally crossed and doubled between *B. rapa* (AA, $2n = 20$) and *B. oleracea* (CC, $2n = 18$), was formed 7500 years ago and is a good model for exploring allopolyploids (U 1935; Chalhoub et al. 2014). In addition, oilseed rape is now one of the most important oilseed crops in the world and is inseparable from people's lives. However, due to the short history of domestication between 300 and 400 years ago, the genetic basis of oilseed rape was narrower than that of the parental species (Go'mez-Campo C 1999), which further led to the restriction of oilseed rape breeding and utilization of heterosis. Therefore, it is necessary to explore the molecular mechanism of the distant hybridization between *B. rapa* and *B. oleracea* in order to obtain stable synthesis *B. napus* and to expand the germplasm resources of *B. napus*.

Previous studies have explored sRNA changes and regulatory patterns in different generations of resynthesized *B. napus* (Fu et al. 2016b). However, these patterns in allopolyploids with different traits are not been fully examined. The present study analyzed small RNA changes of eight F2 synthetic *B. napus*. We found that a part of miRNAs and siRNAs were non-additively expressed in the synthesized *B. napus* allotetraploid. Differentially expressed miRNAs and siRNAs differed among eight F2 individuals. The differential expression of miR159 and

miR172 was consistent with that of flowering time trait, and the number of siRNAs present in the offspring was significantly higher than that of the parent. It provides a new perspective of small RNA changes and trait separation in the early stages of allopolyploid polyploid formation.

Materials and methods

Plant materials

For this study, we used 10 accessions, including the female parent Cai-Xin, male parent Chinese kale, and eight F2 synthetic allopolyploids (Fig. 1). First, by embryo rescuing, F1 haploid (AC) hybridization between Cai-Xin (P1) and Chinese kale (P2) was performed. Then, F1 allopolyploids (AACC) were obtained by colchicine doubling (Wei et al. 2017). Seeds were collected by F1 (AACC) budding self-pollination. The eight F2 plants and the parents were planted in the greenhouse of the Chinese Academy of Agricultural Sciences Institute of Vegetables and Flowers (Beijing, China). We investigated the field traits during the flowering period: flower time, flower size.

sRNA library construction and sequencing

Young leaves next to bud (5 cm in length) were collected, frozen in liquid nitrogen, and stored at -80°C until extraction. RNA was extracted from three biological replicates using TRIzol reagent (Invitrogen, Life Technologies) following standard protocols. The quality and quantity of the extracted RNA were assessed using the agarose gel electrophoresis, NanoPhotometer® spectrophotometer, Qubit and Agilent 2100. Then a linker was added to both ends of the small RNA and reverse transcription to synthesize cDNA. Subsequently, after PCR amplification, the target DNA fragment was separated by PAGE gel electrophoresis, and the cDNA library was recovered by gelatinization. The final PCR products were sequenced using Hiseq 2500 at Nuohe company (Beijing, China).

Identification of miRNAs and siRNA clusters

After trimming adaptor sequences at the 5' and 3' ends of the sequenced reads, the cellular structural RNAs (e.g., rRNAs, snoRNAs, snRNAs) were removed using in-house Perl scripts. Clean reads of 18 to 30 nt were aligned to the *B. rapa* (Wang et al. 2011) and *B. oleracea* (Liu et al. 2014) genome by the bowtie2 software (Langmead and Salzberg 2012) with parameter setting for a perfect match. The sequences of P1 were aligned to the *B. rapa* (Wang et al. 2011), the sequences of P2 were aligned to *B. oleracea* (Liu et al. 2014), and the sequences of F2 were aligned to the merge genome of *B. rapa* and *B. oleracea* (Wang et al. 2011; Liu et al. 2014). A miRNA was considered as conserved if its mature sequence had two or fewer nucleotide mismatches compared with the miRNAs in miRBase (<http://www.mirbase.org>, release 21) (Meyers et al. 2008).

After removing sRNAs aligned to *MIRNAs*, the remaining sRNA reads were then used to identify siRNA clusters. Only reads mapped to unique loci were counted for subsequent analyses. A siRNA cluster or locus was defined as a genomic region matched by at least three sRNA reads. If one cluster resided within 200 nt of another, they were merged and regarded as a single cluster.

Differential expression analysis of miRNA and siRNA

sRNAs were counted as miRNA reads when they were fully or partly (≥ 1 nt) overlapping with the mature miRNA sequence. The expression levels of miRNAs were normalized to reads per million (RPM) that was

calculated using the formula $RPM = \text{number of miRNA reads} / \text{total number of clean reads} \times 10^6$.

The expression level of a siRNA cluster was estimated by uniquely mapped reads. Only reads with a full-length perfect match were accepted as hits. And the expression levels were normalized to reads per million (RPM) for further analysis. The expression differences of miRNAs and siRNA clusters were determined by DEGseq (Wang et al. 2010).

miRNA target prediction and GO enrichment analysis of target genes

The targets of miRNAs in *B. rapa* and *B. oleracea* were predicted using the psRNATarget (Dai et al. 2018). Default parameters were used to filter candidates.

GO enrichment analysis was implemented by the Goseq R package (<http://www.bioconductor.org/packages/release/bioc/html/goseq.html>) in which gene length bias was corrected. AgriGO (a Web-based tool and database for gene ontology analysis; <http://bioinfo.cau.edu.cn/agriGO/>) (Du et al. 2010), was also used in this study. GO terms with a corrected $FDR \leq 0.05$ were considered to be significantly enriched.

Results

sRNAs of resynthesized *B. napus* and its parents

The reads obtained by parental sequencing were 16.1 million and 14.0 million, respectively. The reads of eight F2 plants were 15.3 million, 15.2 million, 15.8 million, 13.2 million, 16.0 million, 18.3 million, 14.5 million, 11.0 million (Fig. 2a). After removing low quality reads, we got 10.6 – 15.7 million clean reads (96.79% - 98.97% of total reads). Among them, 21-24nt reads account for the largest proportion, which was 15% - 16% in the ten samples (Fig. 2b). The reads size distribution is not consistent with that of previous reports (Fu et al. 2016b). It may be due to different materials used in the experiment.

On average, average 93.69% of the total reads in the eight F2 samples, 96% and 70% of two parents samples, could be perfectly mapped to the *B. rapa* genome and *B. oleracea* genome with no mismatch (Fig. 2c). The alignment ratio of the eight F2 samples was relatively high, which was inconsistent with previous research results (Shen et al. 2017). May be because the method of comparison was different. The high ratio indicates that our method was reasonable and the utilization of reads was relatively high. In addition, the perfectly mapped reads consisted of various types of sRNAs, including miRNA, rRNAs, tRNAs, snoRNAs, snRNAs, and unannotated sRNAs (Fig. 2d). A large fraction of perfectly mapped unique reads (approximately 88.58 %) was not annotated and probably includes new siRNA candidates (Fig. 2d).

Identification and comparison of miRNAs in eight synthetic *B. napus* and its parents

We identify known miRNAs by aligning clean reads with miRBase databases 22.1. 72050 – 173239 known miRNAs of eight F2 samples, 22208 and 1410 know miRNAs of two parent samples were detected. The number of miRNAs found in F2 was much higher than that of the parent, indicating that hybridization and polyploidization caused changes in the number of miRNAs (Additional table 1).

To further explore the changes in miRNAs caused by hybridization and doubling, we compared the expression quantity between eight F2 plants and mid-parent value (MPV). Under the assumption of additive expression, average expression in each of the eight F2 samples was quite different with the MPV.

A total of 33 conserved bra-miRNAs in the eight F2 samples were found to be non-additively expressed ($P \leq 0.05$,

FDR $\leq$ 0.05). Of the 33 miRNAs, 11, 1, 9, 8, 5, 4, 11 and 6 were non-additively activated in eight F2 allotetraploids, respectively, while 7, 8, 5, 7, 7, 8, 6 and 5 were non-additively repressed in eight F2 allotetraploids, respectively (Fig. 3a). A total of 13 conserved bol-miRNAs in the eight F2 samples were found to be non-additively expressed ($P\leq$ 0.05, FDR $\leq$ 0.05). Of the 13 miRNAs, 5, 5, 2, 3, 3, 4, 4 and 4 were non-additively activated in eight F2 allotetraploids, respectively, while 5, 4, 7, 5, 5, 6, 8 and 5 were non-additively repressed in eight F2 allotetraploids, respectively (Fig. 3b). The target genes of these non-additively expressed miRNAs were predicted from the gene models in the *B. rapa* and *B. oleracea* genome annotation via psRNATarget analysis (Wu et al., 2012). A total of 300 target transcripts were predicted for 33 conserved bra-miRNAs. A total of 269 target transcripts were predicted for 13 conserved bol-miRNAs.

Compared with all annotated *B. rapa* genes, these target genes were significantly ($P < 0.01$) enriched for 16 biological process, 4 cell cellular component and 19 molecular function GO terms, including the ATP biosynthetic process, phospholipid transport and auxin efflux transmembrane transporter activity (Fig. 3c). Compared with all annotated *B. oleracea* genes, these target genes were significantly ($P < 0.01$) enriched for 6 biological process, 1 cell cellular component and 8 molecular function GO terms, including phospholipid transport, ATP biosynthetic process and ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism (Fig. 3d).

These results suggest that the protein amino acid phosphorylation pathways and miRNA-mediated regulation of genes of the pathways might be a potential regulatory process contributing to heterosis.

Identification and comparison of siRNAs in eight synthetic *B. napus* and its parents

To characterize the effects of siRNAs on genome stability and gene expression, we surveyed siRNA density and expression level in different allotetraploids. In eight F2 samples, about 18744–38962 siRNA clusters were identified. 19 and 101 siRNA clusters were identified in two parents. To further investigate the expression level of the siRNA clusters between F2 and the two parents, we found that high_parent in the eight F2 samples were 38963, 26014, 27654, 20226, 22592, 37995, 25629, 18746, much higher than low_parent 81, 81, 80, 80, 80, 80, 79, 81 (Fig. 4c). Most of the siRNAs in the progeny showed high_parent expression. The results indicate that hybridization and polyploidization caused a surge in siRNA. siRNA has the effect of mitigating polyploidy shock, which was consistent with the previous conclusions (Fu et al. 2016b).

In addition, the number of siRNAs in eight F2 plants was 249275 – 516295, which was much higher than the number of parents (1260) (Fig. 4a). AACC1 had the highest number of siRNAs, AACC6 followed by. The results showed that there was a difference in number of siRNA clusters in eight F2 plants.

Based on comparison of expression levels, we found that 387 - 679 of the siRNA clusters were differentially expressed between F2 and its two parents ($P\leq$ 0.05, pairwise Student's t-test). Interestingly, to AA genome, the number of siRNA clusters in F2 plants with an expression level higher than the MPV (220, 194, 172, 221, 225, 307, 149, 174 in eight F2 samples respectively) was significantly higher than the number of siRNA clusters with an expression level lower than the MPV (8, 7, 7, 3, 3, 7, 7, 7 in eight F2 samples respectively). To CC genome, the number of siRNA clusters in F2 plants with an expression level higher than the MPV (208, 130, 151, 177, 162, 242, 126, 113 in eight F2 samples respectively) was significantly higher than the number of siRNA clusters with an expression level lower than the MPV (76, 75, 77, 51, 50, 77, 77, 76 in eight F2 samples respectively) (Fig. 4b). In both *B. rapa* and *B. oleracea* genomes, the number of up-regulated siRNAs expression in F2 plants is much higher than the number of down-regulated expression. The results showed that there are more up-

regulated expression siRNAs to AA genome, while there are more down-regulated expression siRNAs to CC genome. Therefore, two genomes (AA\CC genomes) showed significant differences in response to WGD. Consistent with this observation, the majority of siRNA clusters had a $\log_2(F1/MPV)$ value of 7 (Fig. 4d), significantly deviating from the null expectation [$\log_2(F1/MPV) = 0$]. This result indicates that the distribution trend of eight F2 plants was consistent, AACC1 changed the most, and AACC8 changed the least. In short, there was a difference in siRNA differential expression of eight F2 plants.

Flowering trait analysis

Among the differentially expressed miRNAs, we found that bra-miR159 was down-regulated in AACC2 and up-regulated in other individuals. We used psRNATarget to predict the 14 target genes of bra-miR159 online, which were MYB101 and MYB65 transcription factors. These two transcription factors promote the expression of the LHY gene, which in turn promotes flowering (Liu and Chen 2009; Wu et al. 2009; Anwasha and Thomas 2010) (Fig. 5a). In addition, bol-miR172 was up-regulated in AACC1 and down-regulated in other individuals. It was predicted that bol-miR172a has 6 target genes, which were AP2-like and AP2 transcription factors. These two transcription factors inhibit the expression of FT (Wu et al. 2009; Wollmann et al. 2010; Zhu and Helliwell 2011), which in turn delays flowering (Fig. 5b). The results of field traits showed that AACC1 flowered at the earliest and AACC2 flowered earlier. The results show that small RNA could affect the trait by regulating the expression of the target gene.

Discussion

Changes in miRNA in the eight synthetic *B. napus* allopolyploids

Small RNA plays an important role in polyploid inheritance and gene expression by altering chromatin structure and regulating gene expression. Therefore, studying the expression level of small RNA is helpful to study the regulation mechanism of polyploid gene expression (Yao et al. 2007; Ng et al. 2012; Xie and Zhang 2015). Studies have analyzed the expression and distribution of miRNAs and siRNAs in allotetraploids of *A. thaliana* and found that small RNAs act as a buffer to buffer the genomic shocks of Arabidopsis polyploids (Ha et al. 2009). Xie et al. studied *G. hirsutum* (genomic AADD) and found that cotton has an increased miRNA relative to its two diploid ancestors (Xie and Zhang 2015). In addition, in the Brassica, Fu et al. found that the number and expression levels of miRNAs in the newly synthesized *B. napus* from different generations increased compared with the parents (Fu et al. 2016a). These conclusions are consistent with our findings, indicating that the process of hybridization and doubling of the multiploidization results in a general increase in miRNAs. In addition, we found that the number of miRNAs in the newly synthesized *B. napus* was much higher than that of the parents. Loss of miRNAs and new phenomena also exist (Additional Table 1). Our results are inconsistent with the results of Fu's research, possibly due to material differences: the parent we use was the follower Chinese cabbage, and the parent used by Fu was *B. campestris*. It is speculated that due to genetic differences among different subspecies, the number of differentially expressed miRNAs in newly synthesized *B. napus* is inconsistent.

Besides, there are few reports on the changes in sRNAs between different traits in allopolyploid. We found that the number of miRNAs differed between different individuals. Some miRNAs are expressed much higher in one F2 plant than other F2 plants. Some miRNAs are expressed in one F2 plant and are not expressed in another F2

plant. This result indicates that the initial genome of allopolyploid formation was unstable, and there are differences in miRNA expression in self-crossing progeny.

All of these results suggest that miRNAs play important roles in the regulation of interspecific hybridisation and polyploidization processes, and hence in subsequent evolution of polyploidy crops.

Insights into non-additive miRNA regulation in the synthetic *B. napus* allopolyploids

Non-additive gene expression occurs during polyploidization, which results in the complexity of gene expression and phenotype. The miRNA sequence regulates its target sites. Therefore, the miRNA gene is non-additively expressed or is itself mutagenized to form a base mutation, resulting in the generation of a new target site or the loss of the old target site, which is easy to generate new phenotype. The comparative analysis of microRNAs and siRNAs between *Arabidopsis A. thaliana* and its relatives *A. arenosa* and their parents showed that the expression patterns were highly variable between tetraploids and diploids, and most miRNAs were non-additive (Ha et al. 2009). Li et al. simulated the early generations of common wheat by using new synthetic wheat derived from crossing between *Triticum turgidum* (AABB) and *Agilops tauschii* (DD) and chromosome doubling. It was found that a high proportion of miRNAs were non-additively expression, which resulted in differential expression of important target genes. Dynamic regulation of some homologous genes mediated by small RNA may be responsible for the heterosis of new hexaploid wheat (Li et al. 2014). In our study, high-throughput sequencing was used to compare miRNA expression between eight F2 plants and their parents. Our results show that 48.9% of miRNAs are not-additively expressed in the synthetic *B. napus*. Fu et al. found that approximately 86.6% of miRNAs were non-expressively expressed in different generations of synthetic *B. napus* (Fu et al. 2016a). The reason for this difference may be the different materials used in the experiment. Fu's experimental materials were different plants of different generations, and our materials were different plants of the same generation. The experimental results show that the differences between generations may be greater than the differences between the same generation.

In addition, small RNA affects the performance of field traits by regulating the expression of target genes. Guan et al. found miR828 and miR858 regulate homoeologous MYB2 gene in polyploidy cotton, which in turn affects the fabric trait (Guan et al. 2014). In allopolyploid *Arabidopsis*, target genes of miR163 encode a family of small molecule methyltransferases involved in secondary metabolite biosynthetic pathways (Ng et al. 2011). In our experiment, non-additively expression differs among eight plants. The differential expression of bra-miR159 and bol-miR172 in eight F2 plants was consistent with the field traits, which further explained the differences in flowering traits among eight individuals. The results further indicate that epigenetic variation is widespread in the early stage of allopolyploid formation and could explain the phenomenon of trait separation.

The non-additively expression of miRNAs in synthetic heteropolyploids may increase polyploid fitness. In *Arabidopsis* allotetraploids, non-additively expressed genes are involved in multiple biological processes, which may provide an evolutionary mechanism for heterologous polyploid selection and adaptability (Wang et al. 2006). In our experiments, the relevant target genes of non-additively expressed miRNAs were significantly enriched in ATP-related pathways. These findings suggest that non-additive mRNA/miRNA may play an important role in the growth and non-additive phenotypes of polyploids.

Changes in siRNA in the eight synthetic *B. napus* allopolyploids

siRNA is generally produced by endogenous transposons and repetitive sequences in plants. Generally, siRNA induces epigenetic modification by RNA-directed DNA methylation. Similar to miRNAs, siRNA is non-additive

in newly synthesized allopolyploids, which may promote the variation and adaptability of polyploids (Shen et al. 2017). In this study, we studied the relationship of the siRNA clusters expression levels between eight F2 plants and parents. The number of up-regulated expression of siRNA cluster between eight F2 plants and the parents was found to be greater than the number of down-regulated expression. Ha et al. found that the newly synthesized Arabidopsis allopolyploid siRNA clusters were reduced (Ha et al. 2009). This indicates that the expression patterns of siRNA among different species are inconsistent, and the expression of siRNA of other allopolyploids needs further investigation. Moreover, it may be due to different analysis methods. Predecessors generally use allopolyploid genomes for data comparison, such as rapeseed, Arabidopsis, and cotton. Our experimental method is to compare with the *B. rapa* and *B. olearea* genomes respectively. In the early stage of allopolyploid formation, genomic information of synthetic allopolyploid was closer to the parent than to the allopolyploid after evolution, so our method could identify more differences. In addition, the number of non-additively expressed siRNAs in eight F2 plants was inconsistent, AACC1 had the largest variation and AACC8 had the smallest variation. The experimental results further indicated that the siRNA increased significantly in the early stage of allopolyploid formation to alleviate the genome shock, and there was a difference between the different allopolyploids, which may be related to the trait separation.

Author Contribution statement

R.S. and Y.W designed the experimental design and wrote the article. FL, SJZ, SZ, and HZ contributed to the contributed to the interpretation of the results and coordinated the study. Y.W. performed the experiment. All the authors read and approved the final manuscript.

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Compliance with ethical standards

Competing interests

The authors declare that they have no conflict of interest.

Data Accessibility Statement

The small RNA data we sequenced would be uploaded to genebank database after the article is published.

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- 422

Figure 1

Fig. 1. Plant materials

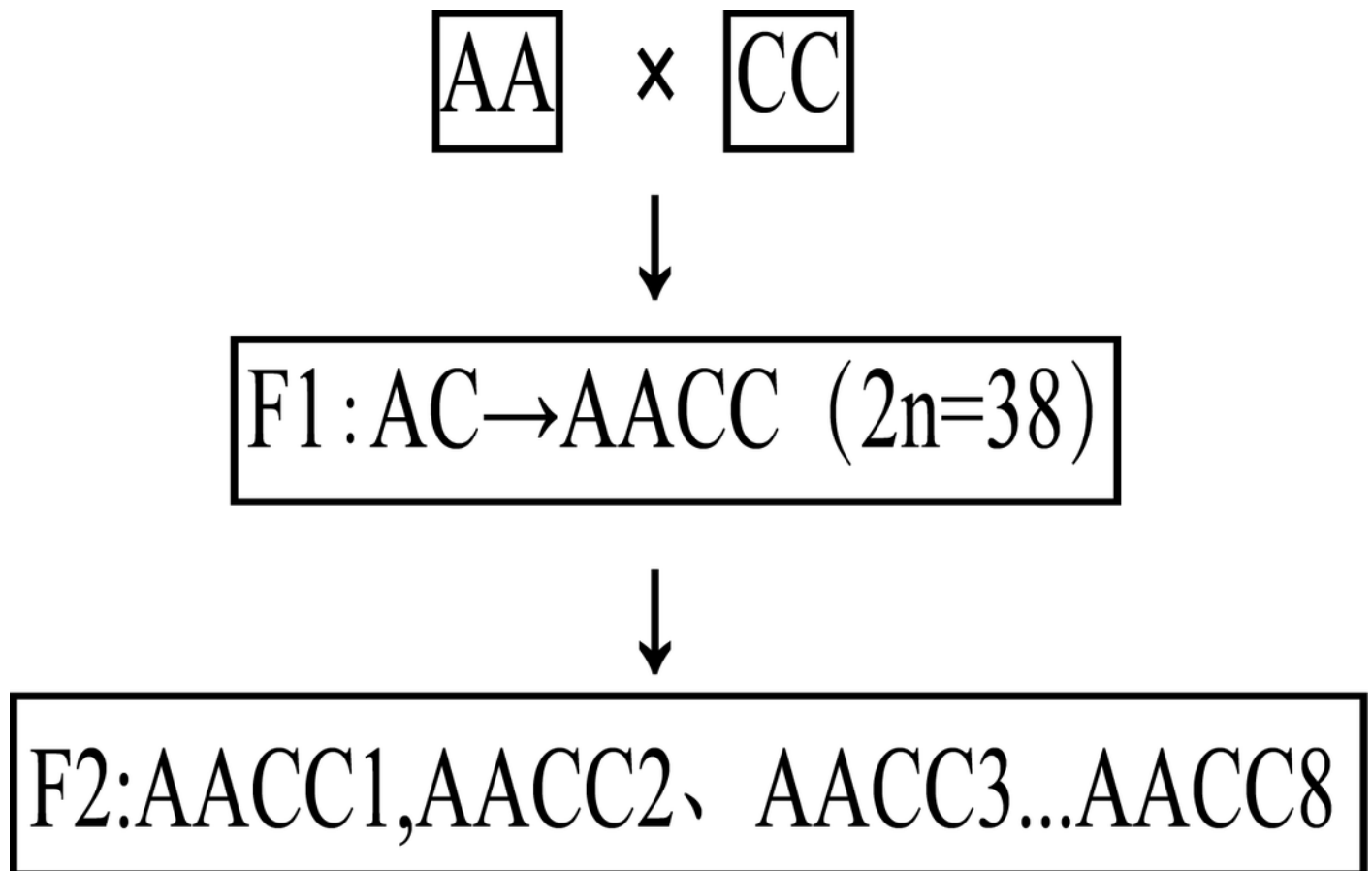


Figure 2

Fig. 2. Small RNA sequencing quality analysis and length distribution

a: Number of clean reads for small RNA sequencing in each sample; b: Distribution of small RNA in each sample; c: Ratio of all sRNAs in each sample to the genome (*B. rapa* and *B. oleracea*); d: Annotated information of small RNA in each sample and its proportion to total small RNA.

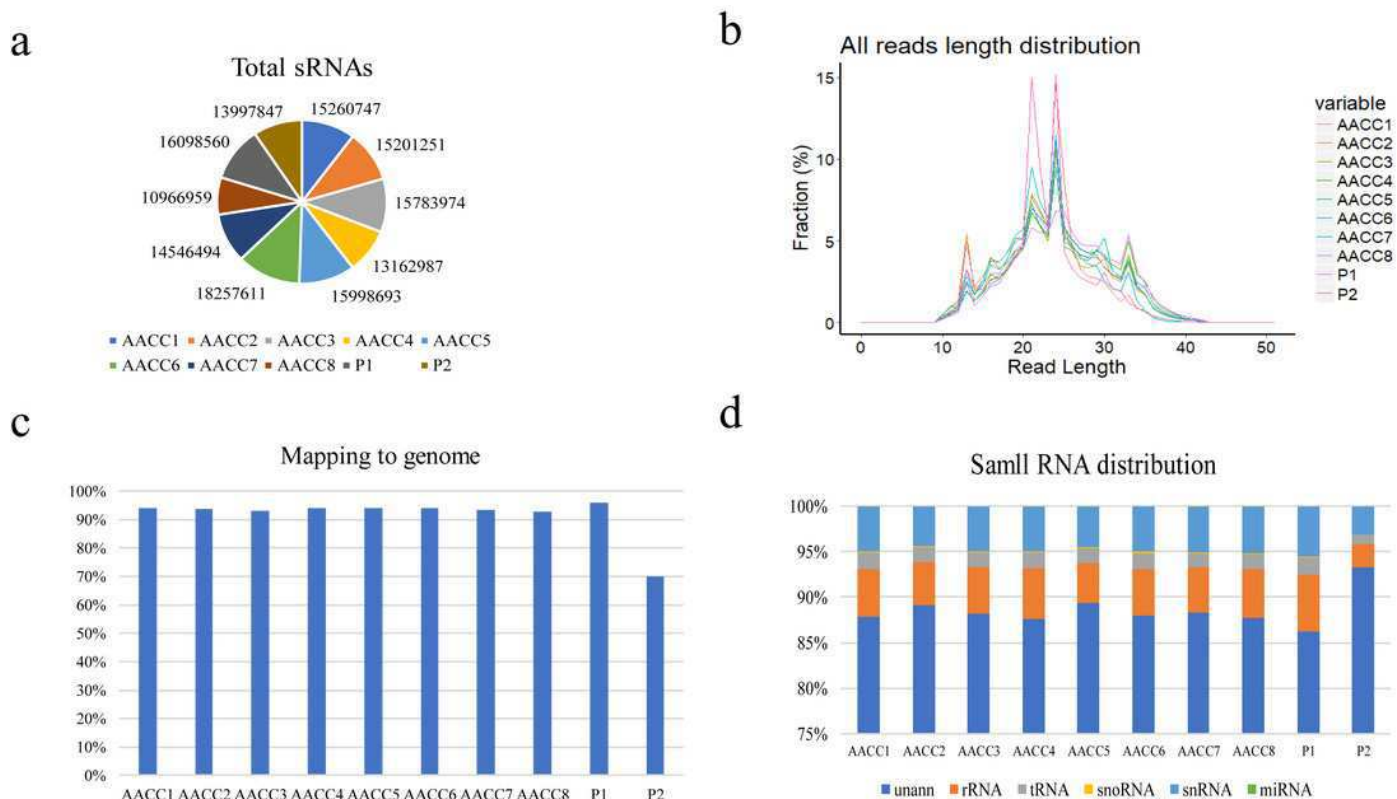


Figure 3

Fig. 3. Changes of miRNAs and their targets in the F2 hybrids.

a: Expression changes of the differentially expressed conserved miRNAs in the F2 hybrids compared with the MPV when aligning *B. rapa* genome; b: Expression changes of the differentially expressed conserved miRNAs in the F2 hybrid compared with the MPV when aligning *B. oleracea* genome; c: Gene Ontology terms of the differentially expressed miRNAs targets in F2 hybrids when aligning *B. rapa* genome; d: Gene Ontology terms of the differentially expressed miRNAs targets in F2 hybrids when aligning *B. rapa* genome.

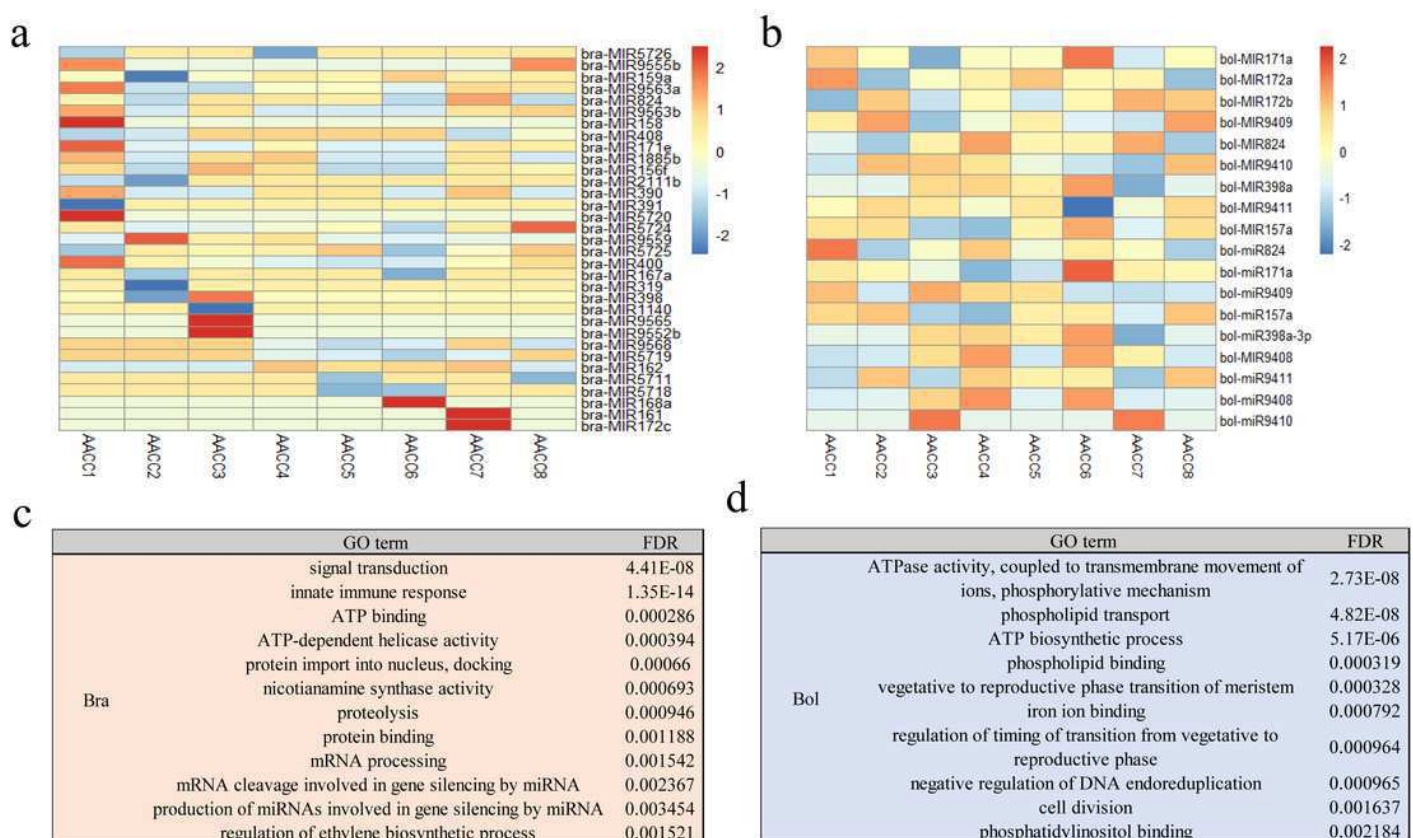


Figure 4

Fig. 4. Expression patterns of the siRNA clusters in F2 hybrids

a: Number of the siRNA clusters in F2 hybrids and MPV; b: Number of siRNA clusters that were up- or down-regulated in F2 hybrids compared with MPV; c: Number of siRNA clusters that were high- or low-parent in F2 hybrids; d: Distribution of the number of the siRNA clusters based on their expression level changes between F2 hybrids and MPV.

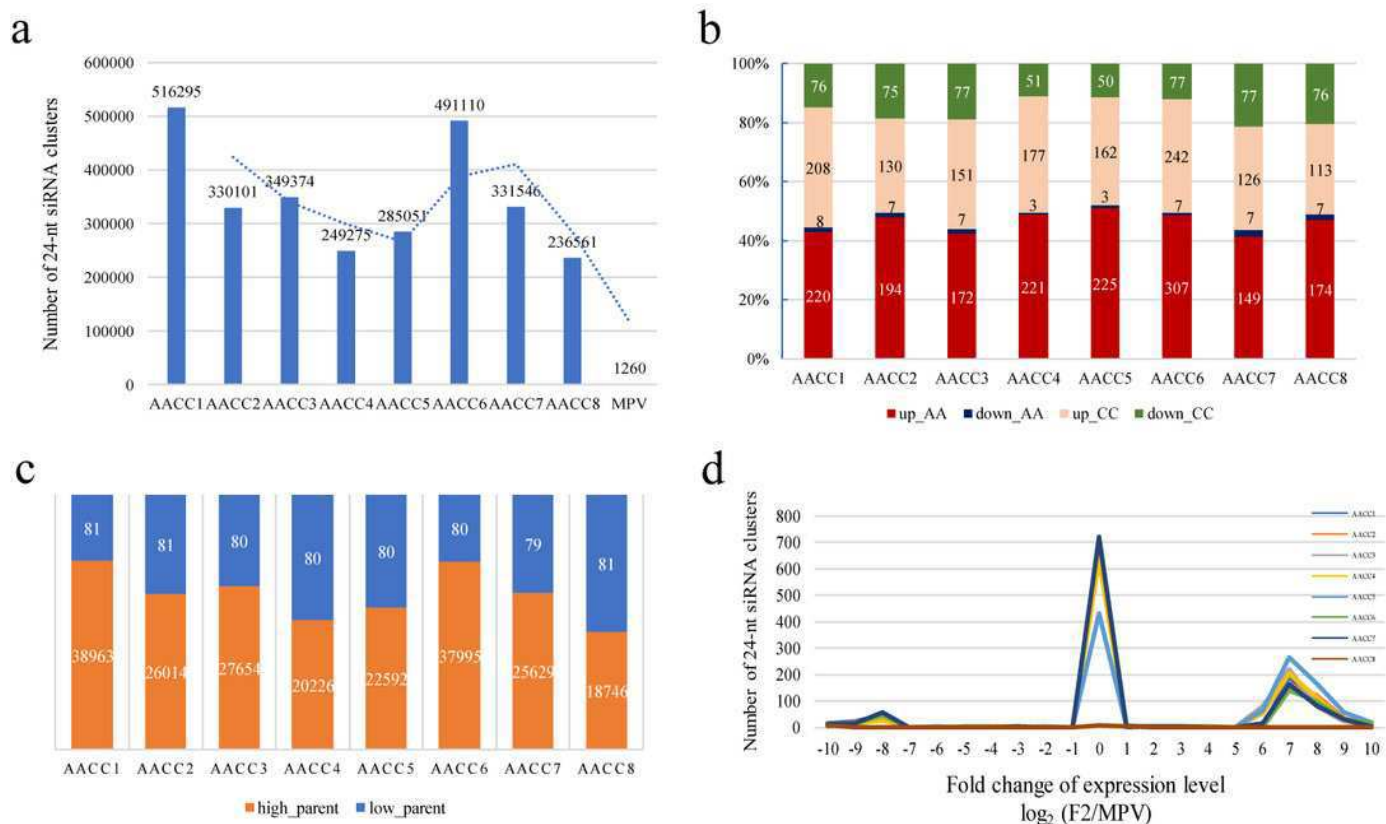


Figure 5

Fig. 5. miR159 and miR172 difference analysis of in F2 hybrids

a: miR159 difference analysis in the F2 hybrids; b: miR172 difference analysis in the F2 hybrids.

