Single-Molecule Real-Time Transcript Sequencing of Turnips Unveiling the Complexity of the Turnip Transcriptome

Hongmei Zhuang, Qiang Wang,1 Hongwei Han, Huifang Liu, and Hao Wang1
Institute of Horticultural Crops, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, Xinjiang, China
ORCID ID: 0000-0003-2257-9335 (H.W.)

ABSTRACT To generate the full-length transcriptome of Xinjiang green and purple turnips, Brassica rapa var. Rapa, using single-molecule real-time (SMRT) sequencing. The samples of two varieties of Brassica rapa var. Rapa at five developmental stages were collected and combined to perform SMRT sequencing. Meanwhile, next generation sequencing was performed to correct SMRT sequencing data. A series of analyses were performed to investigate the transcript structure. Finally, the obtained transcripts were mapped to the genome of Brassica rapa ssp. pekinensis Chiifu to identify potential novel transcripts. For green turnip (F01), a total of 19.54 Gb clean data were obtained from 8 cells. The number of reads of insert (ROI) and full-length non-chimeric (FLNC) reads were 510,137 and 267,666. In addition, 82,640 consensus isoforms were obtained in the isoform sequences clustering, of which 69,480 were high-quality, and 13,160 low-quality sequences were corrected using Illumina RNA seq data. For purple turnip (F02), there were 20.41 Gb clean data, 552,829 ROIs, and 274,915 FLNC sequences. A total of 93,775 consensus isoforms were obtained, of which 78,798 were high-quality, and the 14,977 low-quality sequences were corrected. Following the removal of redundant sequences, there were 46,516 and 49,429 non-redundant transcripts for F01 and F02, respectively; 7,774 and 9,385 alternative splicing events were predicted for F01 and F02; 63,890 simple sequence repeats, 59,460 complete coding sequences, and 535 long-non coding RNAs were predicted. Moreover, 5,194 and 5,369 novel transcripts were identified by mapping to Brassica rapa ssp. pekinensis Chiifu. The obtained transcriptome data may improve turnip genome annotation and facilitate further study of the Brassica rapa var. Rapa genome and transcriptome.

Brassica crop renowned for their wide genetic and diverse phenotypes. B. rapa (AA, 2n = 20), B. oleracea (CC, 2n = 18), and B. napus (AACC, 2n = 38) are the three most well known Brassica crops (Wang et al. 2011; Lin et al. 2014). Several subspecies in B. rapa have been cultivated for particular phenotypic characteristics, including turnips, Chinese cabbage, broccoli, cauliflower, and the oilseed field mustard (Qi et al. 2017). Turnips (Brassica rapa ssp. rapa) represent an important morphological type of B. rapa species, cultivated in Europe since 2,500-2,000 B.C. and spread to other parts of the world afterward (Gray 1883; Zhang et al. 2014; Qi et al. 2017). Turnips are cultivated as fodder crop or vegetables, and its leaves and tubers are consumed depending on the region. The shoots and leaves are consumed in southern European countries, while fleshy root in northern and eastern Europe and China (Zhang et al. 2014). Turnips (Brassica rapa ssp. rapa), known to residents of Xinjiang as Qiamagu, are also known as rutabaga, round root, and dish plant, among other names. Xinjiang turnips are a cruciferous brassica biennial herbaceous plant belonging to the same species of inland turnips while differing greatly in shape and flavor (Ma et al. 2016). Turnips are a characteristic indigenous vegetable commonly eaten by people of all ethnic groups in Xinjiang. Residents eat the fat fleshy roots, which have a high nutrient content and are rich in protein.
and mineral elements (Shi et al. 2011). In many areas of southern Xinjiang, turnip is regarded as an indispensable food and is eaten every day. Turnips are considered by local ethnic minorities to be as important as ginseng is to the Han people, who regard ginseng as the “holy fruit of longevity” (Kortensniemi et al. 2015). In addition to being nutrient-rich, turnips have been shown to have medicinal value, and all the properties of Xinjiang turnips, such as its taste, functions, usage, and dosage, are recorded in detail in the Chinese Materia Medica Volume 4 (Fernandes et al. 2007; Li 2018). For the above reasons, the turnip is widely planted in Xinjiang, and the production area has increased from year to year. Structural and functional genomics are the basis for understanding plant biology (Saito and Matsuda 2010). Wang et al. previously reported the annotation and analysis of the draft genome sequence of *Brassica rapa* ssp. *pekinesis* at Chifu (https://www.ncbi.nlm.nih.gov/genome/?term=Brassica+rapa) in 2011 (Wang et al. 2011). Turnip is one of the subspecies of *Brassica rapa*. However, to date, the genome and transcriptome of *Brassica rapa var. Rapa* have not been investigated.

The transcriptome can reflect changes in gene expression during various physiological and biochemical processes (Yang et al. 2014). Hence, different transcriptome sequencing techniques have been developed and applied with various merits and demerits, of which short-read sequencing is considered as a potent method for profiling the transcriptome (Yang et al. 2018). Nevertheless, these techniques are mostly unsuitable for the analysis of specific biological processes due to the limitations of short read lengths, including assembly, detection of gene isoforms, and complex genomic regions (Rhoads and Au 2015). These limitations can be overcome by using single-molecule real-time (SMRT) sequencing, which was developed by Pacific BioSciences (PacBio) (Roberts et al. 2013). This approach uses real-time sequencing without the requirement of pausing between read steps, and is categorized as third-generation sequencing (Schadt et al. 2010).

SMRT can process read lengths of more than 20 kb for full-length transcripts or longer length fragments (Rhoads and Au 2015). Full-length transcripts can greatly elevate the veracity in annotating the genome and in characterizing the transcriptome, and can be used for the analysis of exon-intron structure and alternative splicing, which is helpful to completely understand the transcriptional behavior of genomic loci (Dong et al. 2015). Alternative splicing (AS) is one of the common ways to diversify the functional characteristics of the transcriptome and proteome in eukaryotic organisms (Reddy et al. 2013; Kalsotra and Cooper 2011). AS has been implicated in the regulation of plant development because it occurs in approximately 40–60% of intron-containing transcripts in different tissues and developmental stages in *Arabidopsis thaliana* (Marquez et al. 2012), *Zea mays* (Thatcher et al. 2014), and *Oryza sativa* (Dong et al. 2018).

Most previous research on the Xinjiang turnip has focused on its medical physiology, such as its content of linoleic acid, flavonoids, saponins, vitamins (B, C, and PP), calcium, iron, and other effective elements, nutrients, and amino acids. However, there has been little further investigation into *Brassica rapa var. Rapa*. Therefore, SMRT sequencing was used in this study to generate the full-length transcriptome of two *Brassica rapa* ssp. *Rapa* turnsips varieties (Xinjiang purple turnip and green turnip, named based on their root skin colors), followed by analysis of simple sequence repeat (SSR) and AS, and prediction of coding sequence and long non-coding RNA (lncRNA). This study was expected to improve the annotation of the turnip genome (*Brassica rapa var. Rapa*) and to provide a valuable resource for basic research and the molecular breeding of turnips.

![Figure 1](image-url) The phenotypes of the fleshy root of green and purple turnips.

### MATERIALS AND METHODS

**Workflow presentation**

The analysis process of this study is shown in Figure S1. Briefly, the third generation transcriptome sequencing data were used to assemble a complete transcript for structural optimization, while the second-generation transcriptome data were used for correction and quantitative analysis of the third generation transcriptome data (Figure S1).

**Plant materials and RNA sample preparation**

Green and purple *Brassica rapa var. Rapa* turnips were planted and grown under the same conditions in the experimental greenhouse at the Comprehensive Laboratory of the Xinjiang Academy of Agricultural Sciences (Xinjiang, China). Skin samples (phloem and periderm) were collected from the fleshy root of turnips using a knife blade at five stages during the vegetative period of green (P01) and purple turnips (P02), including the seedling stage, the succulent root enlargement stage, and the early, middle, and mature periods of succulent root expansion. The phenotypes of the fleshy root of green and purple turnips are shown in Figure 1. Three biological replicates were used, and a total of 15 samples were collected from each strain. Total RNA isolation was conducted using the RNasy Plus Mini Kit (Qiagen, Valencia, CA, USA), and their purity and concentration were measured using an OD260/280 reading on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA). The integrity assessment was conducted utilizing the RNA Nano 6000 Assay Kit on an Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The total RNA samples of five developmental stages from each strain were mixed for the following analysis.

**PacBio Iso-Seq library construction and sequencing**

The isofrom-sequencing (Iso-seq) library was constructed based on the protocol described by Pacific Biosciences (P/N00-377-100-05 and P/N100-377-100-04). The full-length mRNA cDNA was synthesized using the SMARTerTM PCR cDNA synthesis kit. Then, the BluePippin was used to screen full-length cDNA fragments and build cDNA libraries of different sizes. Next, the full-length cDNA was amplified and extended by PCR, followed by terminal repair. The cDNA was connected to the SMRT dumbbell-type connector, and exonuclease digestion was performed. BluePippin was used for secondary screening to obtain the sequencing library. The accurate quantification of libraries was performed using Qubit 2.0, followed by the detection of library size using Agilent 2100. The libraries were then sequenced on PacBio RSII according to the target data volume.
The PacBio RSII database binned lengths of 0-1 kb, 1-2 kb, 2-3 kb, 3-6 kb, and >6 kb. 2 turnsips and 5 vegetative growth stages were analyzed, and 18 cells were used in total.

**Illumina cDNA library construction and sequencing**

Purification of mRNA from 3 μg total RNA of each sample was completed utilizing poly-T oligo-attached magnetic beads, followed by fragmentation using fragmentation buffer. Then, the purified mRNA was used to synthesize cDNA using random hexamers (first strand), Nase H, and DNA polymerase I (second strand). After purification, end repair, adenylation, and adapter ligation, the cDNA fragments were enriched by PCR amplification, and the cDNA libraries were constructed and sequenced on an Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA).

**Pacific biosciences long-read processing**

Raw reads were processed into error-corrected reads of insert (ROIs) according to the conditions full passes ≥ 0 and sequence accuracy > 0.75. By detecting whether each ROI sequence contains a 5’ primer, 3’ primer, and poly-A tail, the sequences can be divided into full-length sequences (including all three elements) and non-full-length sequences. Based on the poly-A tail signal and the 5’ and 3’ cDNA primers in ROIs, the full-length non-chimeric (FLNC) transcripts were identified. The ICE algorithm in SMRT Analysis (v2.3.0) software was used to obtain a consistent sequence (consensus) isoform, and the full-length consensus sequences were polished using Quiver. Full-length transcripts with post-correction accuracy above 99% (high-quality isoforms) were generated for further analysis. The redundant sequences were removed from the high-quality and corrected low-quality transcript sequences of each sample using CD-HIT software (Li and Godzik 2006).

**AS detection**

The three-generation transcripts were mapped onto *Brassica rapa* genome (http://brassicadb.org/brad/datasets/pub/BrassicaceaeGenome/Brassica_rapa/V3.0/) using gmap with parameters setting as: similarity = 0.9 and Coverage = 0.85. Then AS events were analyzed using Astalavista.

**SSR detection**

MISA (MIcroSAtellite identification tool, http://pgrc.ipk-gatersleben.de/misa/) is a software used to identify SSRs, and it can identify 7 types of SSR by analyzing transcript sequences. SSR analysis was performed on transcripts more than 500 bp long using MISA.

SSR detection was analyzed using MISA based on the following criteria:

- Definition (unit_size,min_repeats): 1-10 2-6 3-5 4-5 5-5 6-5
- Interruptions (max_difference_between_2_SSRs): 100

For the definition of SSR, mono-nucleotide repeats 10 times or more; di-nucleotide repeats 6 times or more; tri-nucleotide, tetra-nucleotide, penta-nucleotide and hexa-nucleotide repeats 5 times or more. The distance between two SSRs is less than 100 bp and is defined as SSR compliant.

**Prediction of long non-coding RNAs (IncRNAs)**

The IncRNAs were predicted by analyzing the coding potential of transcripts using four widely used computational approaches, including the coding potential calculator (CPC) (Kong et al. 2007), coding-non-coding index (CNCI) (Sun et al. 2013), coding potential assessment tool (CPAT) (Wang et al. 2013), and Pfam protein structure domain analysis. Furthermore, the target transcripts of the obtained IncRNAs were predicted using the LncTar tool (Haas et al. 2013).
Prediction of coding sequence (CDS)

TransDecoder is a software used to predict CDSs, and can identify potential CDSs from transcript sequences based on the alignment of open reading frame length and the log-likelihood score of amino acid sequences to protein domain sequences in the Pfam database. The prediction of CDSs and corresponding amino acid sequences was performed using TransDecoder (version 3.0.0) (Xanthopoulou et al. 2016).

Functional annotation of transcripts

Functional annotation was performed for the non-redundant transcript sequences by mapping to NR, Swissprot, Gene Ontology (GO), Clusters of Orthologous Groups of proteins (COG), euKaryotic Ortholog Groups (KOG), Protein Family (Pfam), and KEGG databases using BLAST (version 2.2.26).

Transcript mapping to field mustard (Brassica rapa)

The published representative genome for field mustard (Brassica rapa) (assembly Brapa_1.0) was downloaded from NCBI as the reference transcriptome to identify potential novel transcripts. Next, the obtained transcripts were mapped to the reference transcriptome using BLASR, a long sequence alignment software commonly used in third-generation sequencing. The parameter was set as -bestn 1, and other parameters were set as default. The mapping rate (the percentage of mapped bases to the total number of bases in the sequence) and matching rate (the percentage of exactly matched bases to the total number of mapped bases) were calculated, and the known transcripts were determined to have mapping and matching rates over 70%, otherwise the transcripts were defined as novel transcripts.

Data Availability Statement

The raw data were available at NCBI Sequence Read Archive (SRA) repository with Accession Number SRP218278. Supplemental material available at figshare: https://doi.org/10.25387/g3.11790984.

RESULTS

SMRT sequencing statistics

PacBio RSII was used in SMRT sequencing data output mode. For Xinjiang green turnip (F01), after data preprocessing from 1,202,336 polymerase reads (Table 1), a total of 19.54 Gb clean data were obtained in 8 cells. A total of 510,137 ROIs were screened based on the data filtering criteria described in the methods section (Table 2). Additionally, 267,666 FLNC sequences were screened. The percentage of full-length transcripts was more than 46.35%, and the percentage

| Table 3 Full-length sequence statistics |
|-----------------------------------------|
| Samples | cDNA Size | Reads of Insert | Number of five prime reads | Number of three prime reads | Number of poly-A short reads | Number of full-length reads | Number of full-length non-chimeric reads | Average full-length non-chimeric read length | Full-Length Percentage (FL%) | Artificial Concatemers (%) |
| F01 All | 510,137 | 350,417 | 346,956 | 55,722 | 2,172 | 49.89% | 0.32% |
| F01 3-6K | 141,357 | 95,442 | 94,654 | 14,477 | 3,299 | 56.54% | 0.17% |
| F01 2-3K | 180,575 | 111,538 | 110,080 | 14,477 | 3,299 | 48.08% | 0.18% |
| F01 1-2K | 230,897 | 147,167 | 142,222 | 14,477 | 3,299 | 47.23% | 0.55% |
| F02 All | 94,602 | 78,798 | 78,286 | 14,477 | 3,299 | 83.29% | 5.04% |
| F02 above6kb | 1,329 | 10,003 | 2,200 | 94,602 | 14,977 | 300 bp; Number of non-full-length reads: the number of non-full-length ROIs; Number of FLNC reads: the number of full-length non-chimeric ROIs; Average FLNC read length: average length of full-length non-chimeric sequences; Full-length percentage (FL%): the percentage of ROIs that were full-length sequences; Artificial concatemers (%): the percentage of ROIs that were full-length chimeric sequences.

| Table 4 Results of Iterative Clustering for Error Correction (ICE) clustering analysis |
|-----------------------------------------|
| Samples | Size | Number of consensus isoforms | Average consensus isoforms read length | Number of polished high-quality isoforms | Number of polished low-quality isoforms | Percent of polished high-quality isoforms (%) |
| F01 0to1kb | 9,164 | 879 | 8,568 | 596 | 93.50% |
| F01 1to2kb | 29,434 | 1,392 | 25,900 | 3,534 | 87.99% |
| F01 2to3kb | 34,914 | 2,424 | 29,270 | 5,644 | 83.83% |
| F01 3to6kb | 8,117 | 3,548 | 5,724 | 2,393 | 70.52% |
| F01 above6kb | 1,011 | 9,556 | 18 | 993 | 1.78% |
| F02 All | 82,640 | 2,082 | 69,480 | 13,160 | 84.08% |
| F02 0to1kb | 10,600 | 894 | 10,003 | 597 | 94.37% |
| F02 1to2kb | 33,144 | 1,393 | 29,904 | 3,240 | 90.22% |
| F02 2to3kb | 28,963 | 2,397 | 24,579 | 4,302 | 84.86% |
| F02 3to6kb | 33,144 | 3,454 | 14,245 | 5,594 | 69.28% |
| F02 above6kb | 1,329 | 9,048 | 67 | 1,244 | 5.04% |
| F02 All | 94,602 | 2,200 | 78,798 | 14,977 | 83.29% |
of artificial concatemers sequences was 0.42%, suggesting a moderate SMRTbell concentration (Table 3).

Similarly, for Xinjiang purple turnip (F02), there were 1,202,336 polymerase reads (Table 1), and a total of 20.41 Gb clean data were obtained in 8 cells. In addition, 552,829 ROIs (Table 2) and FLNC sequences were obtained. The percentage of full-length transcripts was more than 47.23%, and the percentage of artificial concatemers sequences was 0.32%, suggesting a moderate SMRTbell concentration (Table 3).

Clustering analysis and correction of isoform sequences
In this study, using the SMRT analysis software RS_IsoSeq module, clustering analysis was carried out on the total length of the sequence, and the lengths of the transcriptome data for two different species of turnips were compared. From the Xinjiang green turnip (F01), 82,640 consensus isoforms were obtained, of which high-quality isoforms (69,480 high-quality isoforms) accounted for 84.08%. In addition, 13,160 low-quality isoforms were obtained, which were corrected using Illumina RNA-seq to improve the sequence accuracy (Table 4).

For Xinjiang purple turnip (F02), 93,775 consensus sequences were obtained, of which 78,798 were high-quality isoforms, accounting for 83.29%. A total of 14,977 low-quality isoforms were obtained, which were corrected using Illumina RNA-seq to improve the sequence accuracy (Table 4).

AS detection
Using Brassica rapa genome (http://brassicadb.org/brad/datasets/pub/BrassicaceaeGenome/Brassica_rapa/V3.0/) as a reference genome, AS events were identified. A total of 7,774 AS events were identified in Xinjiang green turnips (F01) (Table S1), including 39 mutually exclusive exon, 5,066 intron retention, 747 alternative 5' splice site, and 1,512 alternative 3' splice site (Figure 2A). For Xinjiang purple turnips (F02), a total of 9,385 AS events were identified (Table S2), including 52 mutually exclusive exon, 6,140 intron retention, 437 exon skipping, 882 alternative 5' splice site and 1,874 alternative 3' splice site (Figure 2B).

SSR detection
In all, 83,767 transcript sequences (total 175,104,191 bp) were used to evaluate the SSR, and a total of 63,890 SSRs were identified. In addition, 40,271 SSR-containing sequences, 14,540 sequences containing more than one SSR, and 7238 SSRs that exist as complexes were detected. Moreover, it was found that mononucleotide repeats were the most common type of SSR, followed by dinucleotide repeats and trinucleotide repeats (Figure 3).

Prediction of CDS
According to the method described above, a total of 81,371 open reading frames were identified, including 59,460 complete open reading frames. The protein sequence length of complete open reading frames is shown in Figure 4.

Functional annotation of transcripts
Functional annotations were performed for the 83,820 non-redundant transcripts. BLAST software (version 2.2.26) was used to compare the obtained transcript sequences with the NR, SwissProt, GO, COG, KOG, Pfam, and KEGG databases to obtain annotation information for the transcripts. In total, 35,506 transcripts were annotated in the COG database, 77,189 in the GO database, 35,918 in the KEGG database, 53,357 in the KOG database, 70,213
in the Pfam database, 65,292 in the SwissProt database, 80,379 in the eggNOG database, and 82,983 in the NR database. Moreover, 83,065 transcripts were annotated in at least one of the eight databases (Table 5). In NR annotation, the species with the sequence most homologous to each Brassica rapa var. Rapa transcript were predicted. The greatest number of sequences, approximately 60.48%, aligned to Brassica rapa, followed by Brassica napus (35.06%), Eutrema salsugineum (0.80%), Arabidopsis thaliana (0.67%), and Camelina sativa (0.51%) (Figure 5). In GO annotation, the transcripts were significantly enriched in 20 biological processes terms, 17 molecular function terms, and 16 cellular component terms (Figure 6). The results showed that most of the transcripts were mainly functioned in biological processes including cellular process, metabolic process, single-organism process, response to stimulus, biological regulation. Catalytic activity, binding and transporter activity were the major molecular functions of the transcripts. The cellular component functions of those transcripts were mainly cell part, cell and organelle. The COG database is based on bacteria, algae, and the system evolution of eukaryote relationship building. In COG annotation, the transcripts were most enriched in function R (general function prediction only, 20.37%), followed by function K (transcription, 11.31%) and function L (replication, recombination and repair, 11.18%) (Figure 7).

Prediction of lncRNAs
lncRNAs are not protein-coding, and as a result, screening transcripts for coding potential can identify potential lncRNAs. Based on the four methods described above, a total of 535 lncRNA transcripts were predicted (Figure 8). In addition, the target transcripts of the lncRNAs were further predicted using the LncTar tool, and 92 lncRNA were found to target at least one transcript (Table S3).

Transcript mapping to field mustard (Brassica rapa)
In SMRT sequencing, a total of 46,516 transcripts and 49,429 transcripts were obtained for Xinjiang green turnips (F01) and Xinjiang purple turnips (F02), respectively. Based on the method described above, these transcripts were mapped to field mustard (Brassica rapa) to identify potential novel transcripts. For Xinjiang green turnips (F01), 41,322 known transcripts were detected, 810 transcripts of which completely mapped (100% mapping and matching rate) to field mustard (Brassica rapa), and 5,194 novel transcripts were obtained. Similarly, for Xinjiang purple turnips (F02), 44,060 known transcripts were detected, 1,199 transcripts of which completely mapped (100% mapping and matching rate) to field mustard (Brassica rapa), and 5,369 novel transcripts were obtained (Table 6).

DISCUSSION
In recent years, transcriptome research has greatly accelerated due to the high accuracy of short reads generated by next generation sequencing. Nevertheless, short reads shows lower contiguity in sequence assembly, making analysis of complex genomic regions and specific biological processes difficult (Hackl et al. 2014; Koren et al. 2012). SMRT sequencing, which exhibits more advantages in transcriptome research than short-read sequencing due to the generation of full-length transcripts, has been comprehensively investigated (Sharon et al. 2013). Despite the relatively high error rate of third-generation sequencing, this disadvantage can be corrected by accurate short reads (Dong et al. 2015). In our current study, 267,666 FLNC reads were obtained from Xinjiang green turnip (F01). In addition, 82,640 consensus transcript sequences were obtained in sequence clustering analysis, of which 69,480 were high-quality, and

Table 5 Transcripts annotation statistics

| Anno_Database          | Annotated number | Length (300-1000) | Length (≥1000) |
|------------------------|------------------|------------------|----------------|
| COG_Annotation         | 35506            | 3433             | 32073          |
| GO_Annotation          | 77189            | 8147             | 69042          |
| KEGG_Annotation        | 35918            | 4064             | 31854          |
| KOG_Annotation         | 53357            | 5431             | 47926          |
| Pfam_Annotation        | 70213            | 6820             | 63393          |
| Swissprot_Annotation   | 62592            | 6126             | 56466          |
| eggNOG_Annotation      | 80379            | 8604             | 71775          |
| NR_Annotation          | 82983            | 9020             | 73963          |
| All_Annotated          | 83065            | 9035             | 74030          |
the 13,160 low-quality sequences were corrected using Illumina RNA-seq data. Similarly, a total of 274,915 FLNC sequences and 93,775 consensus transcript sequences (14,977 corrected sequences) were obtained from Xinjiang purple turnip (F02).

Following the removal of redundant sequences, there were 46,516 and 49,429 non-redundant transcripts for F01 and F02, respectively. A series of annotation analyses were performed on those transcripts. In NR annotation, the species with the sequence most homologous to each *Brassica rapa* var. *Rapa* transcript was predicted. 60.48% of sequences aligned to *Brassica rapa*, followed by *Brassica napus* (35.06%). Both *Brassica rapa* and *Brassica napus* belong to the *Brassica* species. *Brassica rapa* (AA, 2n = 20), *Brassica oleracea* (CC, 2n = 18), and their allopolyploid derivative, *Brassica napus* (AACC, 2n = 38) are the three most well known Brassica crops (Qi et al. 2017; Wang et al. 2011). Each of these Brassica species includes several cultivated subspecies that were domesticated for different use

**Figure 5** Homologous species distribution of *Brassica rapa* var. *Rapa* transcripts annotated in the NR database.

**Figure 6** Gene ontology (GO) functional annotation of *Brassica rapa* var. *Rapa* transcripts. Green represents biological process, blue represents molecular function, and red represents cellular component. The x-axis shows the GO categories, the y-axis (right) represents the number of transcripts, and the y-axis (left) represents the percentage of transcripts.
with diverse morphological characteristics. It has been considered in a long time as classic textbook example of artificial selection during plant domestication and breeding (Wang et al. 2011; Cheng et al. 2016; Qi et al. 2017). Wang et al. annotated and analyzed of the draft genome sequence of *Brassica rapa* ssp. *pekinesis* Chiifu, and modeled 41,174 protein coding genes in the *B. rapa* genome (Wang et al. 2011). They suggested that the remarkable morphological plasticity of Brassica species was probably benefited from the variation in the number of gene families members occurred in genome. Notably, the obtained transcripts for F01 and F02 were mapped to the *Brassica rapa* genome, and 5,194 and 5,369 novel transcripts were identified. These might be explained the morphological difference of *Brassica rapa* ssp. *Rapa* from other *Brassica rapa* crops.

AS is one of the common ways to diversify the functional characteristics of the transcriptome and the proteome in eukaryotic organisms (Reddy et al. 2013; Kalsotra and Cooper 2011). AS is implicated in the regulation of plant development, as it occurs in approximately 40–60% of intron-containing transcripts in different tissues and developmental stages in *Arabidopsis thaliana* (Marquez et al. 2012), *Zea mays* (Thatcher et al. 2014), and *Oryza sativa* (Dong et al. 2018). In Xinjiang green turnip (F01) and purple turnip (F02), a total of 620 and 872 AS events were predicted, respectively. Non-coding RNAs (ncRNA) refer to RNAs that lack the ability to encode proteins, and were initially regarded as inessential transcriptional "noise." Research advances have demonstrated the crucial regulatory roles of ncRNAs in various biological processes (Do et al. 2019). Plant IncRNAs had been reported to participate in photomorphogenesis, auxin transport, flowering, etc. (Yang et al. 2020; Shafiq et al. 2016; Liu et al. 2015). Based on the four commonly used methods described above, a total of 535 IncRNA transcripts were predicted in our study. In addition, the target transcripts of 92 IncRNAs were further predicted. Nevertheless, further study is needed to understand the functions and biological processes they are involved in.

**CONCLUSION**

In conclusion, the full-length transcriptome of *Brassica rapa* var. *Rapa* was first investigated using SMRT sequencing, which may facilitate further studies into the genetic data of *Brassica rapa* var. *Rapa* and may help to clarify the annotation of the turnip genome as well as serve as a reference for other brassica species. The results of

![Figure 7](image-url)  
*Figure 7*: Clusters of orthologous groups of proteins (COG) annotation of *Brassica rapa* var. *Rapa* transcripts. The x-axis shows the COG categories, and the y-axis represents the number of transcripts.

![Figure 8](image-url)  
*Figure 8*: Venn diagram of the number of lncRNAs predicted by Coding Potential Calculator (CPC), Coding-Non-Coding Index (CNCI), Coding Potential Assessment Tool (CPAT), and Pfam Protein Structure Domain Analysis.
Table 6 Statistics of transcripts mapping to field mustard (Brassica rapa)

| matching rate | 100% | 90–100% | 80–90% | 70–80% | total |
|---------------|------|---------|--------|--------|-------|
| 100%          | F01  | 810     | 12335  | 814    | 381 | 14340 |
|               | F02  | 1199    | 13400  | 917    | 355 | 15871 |
| 90–100%       | F01  | 924     | 21452  | 1354   | 492 | 24222 |
|               | F02  | 1364    | 21986  | 1451   | 517 | 25318 |
| 80–90%        | F01  | 128     | 1538   | 224    | 80  | 1970  |
|               | F02  | 158     | 1574   | 227    | 80  | 2039  |
| 70–80%        | F01  | 54      | 542    | 128    | 66  | 790   |
|               | F02  | 82      | 567    | 112    | 71  | 832   |

this study are of great significance to further study the dynamic changes in transcription and the differential expression of transcripts during the growth and development of turnips.

ACKNOWLEDGMENTS
This research was funded by the National natural Science Foundation of China (Study on the Physiological and Molecular Regulation Mechanism of Glucosinolate Synthesis in Brassica rapa var. rapa (No. 31960600)), the Project of Renovation Capacity Building for the Young Sci-Tech Talents Sponsored by Xinjiang Academy of Agricultural Sciences (Study on the physiology of glucosinolates synthesis and the regulation mechanism of gene expression in Turnip (Brassica rapa var. Rapa)) (No. xjnkq-2019003) and Open Project of Key Laboratory of Crop Breeding in Southern Zhejiang (Physiological study on synthesis of glucosinolates from Turnip (Brassica rapa var. Rapa)) (No. 2020SZCB03).

LITERATURE CITED
Cheng, F., R. Sun, X. Hou, and H. Zheng, 2016 Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in Brassica rapa and Brassica oleracea. Nat. Genet. 48: 1218–1224. https://doi.org/10.1038/ng.3634
Do, T., Z. Qu, and I. Searle, 2019 Purification and functional analysis of plant long noncoding RNAs (lncRNA), pp. 131–147 in Plant Long Non-Coding RNAs, Springer, New York. https://doi.org/10.1007/978-1-4939-9045-0_7
Dong, C., F. He, O. Berkowitz, J. Liu, P. Cao et al., 2018 Alternative splicing plays a critical role in maintaining mineral nutrient homeostasis in rice (Oryza sativa). Plant Cell 30: 2267–2285. https://doi.org/10.1105/tpc.18.00051
Dong, L., H. Liu, J. Zhang, S. Yang, G. Kong et al., 2015 Single-molecule real-time transcript sequencing facilitates common wheat genome annotation and grain transcriptome research. BMC Genomics 16: 1039. https://doi.org/10.1186/s12864-015-2257-y
Fernandes, F., P. Valentão, C. Sousa, J. A. Pereira, R. M. Seabra et al., 2007 Chemical and antioxidative assessment of dietary turnip (Brassica rapa var. rapa L.). Food Chem. 105: 1003–1010. https://doi.org/10.1016/j.foodchem.2007.04.063
Gray, A., 1883 THE ORIGIN OF CULTIVATED PLANTS. Science ns-1: 12–14. https://doi.org/10.1126/science.ns-1.11.12
Haas, B. J., A. Papanicolaou, M. Yassour, M. Grabherr, P. D. Blood et al., 2013 De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat. Protoc. 8: 1494–1512. https://doi.org/10.1038/nprot.2013.084
Hackl, T., R. Hedrich, J. Schultz, and F. Förster, 2014 proovread: large-scale high-accuracy PacBio correction through iterative short read consensus. Bioinformatics 30: 3004–3011. https://doi.org/10.1093/bioinformatics/btu392
Kalsotra, A., and T. A. Cooper, 2011 Functional consequences of developmentally regulated alternative splicing. Nat. Rev. Genet. 12: 715–729. https://doi.org/10.1038/nrg3052
Kong, L., Y. Zhang, Z.-Q. Ye, X.-Q. Liu, S.-Q. Zhao et al., 2007 CPC: assess the protein-coding potential of transcripts using sequence features and support vector machine. Nucleic Acids Res. 35: W345–W349. https://doi.org/10.1093/nar/gkm391
Koren, S., M. C. Schatz, B. P. Walenz, J. Martin, J. T. Howard et al., 2012 Hybrid error correction and de novo assembly of single-molecule sequencing reads. Nat. Biotechnol. 30: 693–700. https://doi.org/10.1038/nbt.2280
Kortesniemi, M., A. L. Vuorinen, J. Sinkkonen, B. Yang, and H. Kallio, 2015 NMR metabolomics of ripened and developing oilseed rape (Brassica napus) and turnip rape (Brassica rapa). Food Chem. 172: 63–70. https://doi.org/10.1016/j.foodchem.2014.09.040
Li, W., and A. Godzik, 2006 Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22: 1658–1659. https://doi.org/10.1093/bioinformatics/btl158
Li, Z., 2018 Study on inversion temperature in low pressure superheated steam drying of green turnip slice. Nongye Gongcheng Xuebao (Beijing) 34: 279–286.
Lin, K., N. Zhang, E. I. Severing, H. Nijveen, F. Cheng et al., 2014 Beyond genomic variation–comparison and functional annotation of three Brassica rapa genomes: a turnip, a rapid cycling and a Chinese cabbage. BMC Genomics 15: 250. https://doi.org/10.1186/1471-2164-15-250
Liu, X., L. Hao, D. Li, L. Zhu, and S. Hu, 2015 Long non-coding RNAs and their biological roles in plants. Genetics Proteomics Bioinformatics 13: 137–147. https://doi.org/10.1016/j.igpb.2015.02.003
Ma, G.-C., Y.-R. Wang, and Z.-Y. Xuan, 2016 Analysis and comparison of nutritional compositions in Xinjiang turnip (Brassica rapa L.). Science & Technology of Food Industry. 37: 360–364. https://doi.org/10.13386/j.isn002-0306.2016.04.064
Marquez, Y., J. W. S. Brown, C. Simpson, A. Barta, and M. Kalyna, 2012 Transcriptome survey reveals increased complexity of the alternative splicing landscape in Arabidopsis. Genome Res. 22: 1184–1195. https://doi.org/10.1101/gr.134106.111
Qi, X., H. An, A. P. Ragsdale, T. E. Hall, R. N. Gutenkunst et al., 2017 Genomic inferences of domestication events are corroborated by written records in Brassica rapa. Mol. Ecol. 26: 3373–3388. https://doi.org/10.1111/mec.14151
Reddy, A. S. N., Y. Marquez, M. Kalyna, and A. Barta, 2013 Complexity of the Alternative Splicing Landscape in Plants. Plant Cell 25: 3657–3683. https://doi.org/10.1105/tpc.113.117523
Rhoads, A., and K. F. Au, 2015 PacBio Sequencing and Its Applications. Genetics Proteomics Bioinformatics 13: 278–289. https://doi.org/10.1016/j.igpb.2015.08.002
Roberts, R. J., M. O. Carneiro, and M. C. Schatz, 2013 The advantages of SMRT sequencing. Genome Biol. 14: 305. https://doi.org/10.1186/gb-2013-14-6-405
Saito, K., and F. Matsuda, 2010 Metabolomics for Functional Genomics, Systems Biology, and Biotechnology. Annu. Rev. Plant Biol. 61: 463–489. https://doi.org/10.1146/annurev.arplant.043008.092035
Schadt, E. E., S. Turner, and A. Kasarskis, 2010 A window into third-generation sequencing. Hum. Mol. Genet. 19: R240–R249. https://doi.org/10.1093/hmg/ddq416
Shafq, S., J. Li, and Q. Sun, 2016 Functions of plants long non-coding RNAs. Biochim. Biophys. Acta 1859: 155–162. https://doi.org/10.1016/j.bbagen.2015.06.009
Sharon, D., H. Tilgner, F. Grubert, and M. Snyder, 2013  A single-molecule long-read survey of the human transcriptome. Nat. Biotechnol. 31: 1009–1014. https://doi.org/10.1038/nbt.2705

Shi, Y. J., J. Gao, and J. J. Zhao, 2011  Effects of Salt Stress on Seeds Germination of Turnip (Brassica rapa L.). Xinjiang Nongye Kexue 48: 487–492.

Sun, L., H. Luo, D. Bu, G. Zhao, K. Yu et al., 2013  Utilizing sequence intrinsic composition to classify protein-coding and long non-coding transcripts. Nucleic Acids Res. 41: e166. https://doi.org/10.1093/nar/gkt646

Thatcher, S. R., W. Zhou, A. Leonard, B.-B. Wang, M. Beatty et al., 2014  Genome-Wide Analysis of Alternative Splicing in Zea mays: Landscape and Genetic Regulation. Plant Cell 26: 3472–3487. https://doi.org/10.1105/tpc.114.130773

Wang, L., H. J. Park, S. Dasari, S. Wang, J.-P. Kocher et al., 2013  CPAT: Coding-Potential Assessment Tool using an alignment-free logistic regression model. Nucleic Acids Res. 41: e74. https://doi.org/10.1093/nar/gkt006

Wang, X., H. Wang, J. Wang, R. Sun, J. Wu et al., 2011  The genome of the mesopolyploid crop species Brassica rapa. Nat. Genet. 43: 1035–1039. https://doi.org/10.1038/ng.919

Xanthopoulou, A., F. Psomopoulos, I. Ganopoulos, M. Manioudaki, A. Tsaftaris et al., 2016  De novo transcriptome assembly of two contrasting pumpkin cultivars. Genom. Data 7: 200–201. https://doi.org/10.1016/j.gdata.2016.01.006

Yang, F., L. H. Huang, and A. B. Zhang, 2014  High-throughput transcriptome sequencing technology and its applications in Lepidoptera. Acta Entomol. Sinica 57: 991–1000.

Yang, L., Y. Jin, W. Huang, Q. Sun, F. Liu et al., 2018  Full-length transcriptome sequences of ephemeral plant Arabidopsis pumila provides insight into gene expression dynamics during continuous salt stress. BMC Genomics 19: 717. https://doi.org/10.1186/s12864-018-5106-y

Yang, Z., Z. Yang, C. Yang, Z. Wang, D. Chen et al., 2020  Identification and genetic analysis of alternative splicing of long non-coding RNAs in tomato initial flowering stage. Genomics 112: 897–907. https://doi.org/10.1016/j.ygeno.2019.06.005

Zhang, N., J. Zhao, F. Lens, J. de Visser, T. Menamo et al., 2014  Morphology, carbohydrate composition and vernalization response in a genetically diverse collection of Asian and European turnips (Brassica rapa subsp. rapa). PLoS One 9: e114241. https://doi.org/10.1371/journal.pone.0114241

Communicating editor: I. Parkin