Deep sequencing of the transcriptome reveals distinct flavonoid metabolism features of black tartary buckwheat (*Fagopyrum tataricum* Garetn.)

Huipeng Yao, Chenglei Li, Haixia Zhao, Jianlan Zhao, Hui Chen, Tongliang Bu, Wang Anhu, Qi Wu

College of Life Science, Sichuan Agricultural University, Ya'an 625014, Sichuan, People’s Republic of China

**A B S T R A C T**

Black tartary buckwheat is recognized as ‘black pearl’ because of containing more rutin and other flavonoids as compared to yellow tartary buckwheat (traditional tartary buckwheat). Here, we show a genome-wide comparison of their transcriptomes by using an RNA-seq approach to elucidate the different molecular metabolism on the flowers from black tartary buckwheat (HEIFENG No1) and yellow tartary buckwheat (XIQIAO No2). Over 48.4 million paired-end reads were assembled into 57,800 unigenes, of which about 57.9% (33,472 unigenes) were annotated by BLAST searches in the NCBI non-redundant protein database. RPKM analysis showed that compared to YTB, the unigenes encoding phenylalanine ammonialyase (PAL), chalcone synthase (CHS) and chalcone isomerase (CHI) for early flavonoid synthesis and the unigene encoding quercetin 3-O-glucosyltransferase (UF3GT) for synthesizing rutin were at a higher level, but the unigene encoding Flavonol synthase (FLS) charging for kaempferol and quercetin synthesis at a lower level in BTB, which may be the reason for the higher content of rutin and the lower content of quercetin, the result obtained by HPLC, as confirmed by qRT-PCR analysis of these genes. The result will not only explain the molecular mechanism of flavonoid synthesis in balck tartary buckwheat, but also provide the basis for further genomics research on this species or its allies.

© 2016 Elsevier Ltd. All rights reserved.
1. Introduction

Buckwheat is a plant belonging to the genus Fagopyrum, which is a member of the eudicot family Polygonaceae comprising about 17 species (Ohnishi, 1991, 1998). One of the species, tartary buckwheat (Fagopyrum tataricum Garetn.), has gradually become a recognized model organism for research on rutin metabolism due to the abundance of rutin, which is 0.8–1.7% of the dry weight of the plant and 50–80% of total flavonoids content (Fabjan et al., 2003). Rutin, quercetin, isoquercetin and other flavonoids in tartary buckwheat have anti-diabetic activity (Zhang et al., 2011; Yao et al., 2003). Rutin, quercetin, myricetin and other polyphenols have anti-inflammatory activity (Karki et al., 2013), anti-inflammatory activity (Karki et al., 2013), anti-fatigue properties (Jin and Wei, 2011) and can be used to treat fatty liver disease (Tsai et al., 2012; Ushida et al., 2008; Li et al., 2003). The black pearl buckwheat (F. tataricum) is a phenomenon that is different from the traditional tartary buckwheat. It has dark black shell that is different from the traditional tartary buckwheat the colour which is yellowish white (yellow tartary buckwheat, YTB). The kaempferol content in BTB is also less than that in YTB, but the myricetin in BTB is the same as that in YTB.

2. Results and discussion

2.1. Flavonoids difference between BTB and YTB

To know more about flavonoid characters in BTB, we compared flavonoids content and total flavonoids content in BTB and YTB by using HPLC and ultrasonic methods. It is found that the valuable substance content such as total flavonoids and rutin in BTB are more than that in YTB (Table 1). For example, the average of the amount of total flavonoids from flowers in YTB and BTB was 49.07 μg/mg and 52.81 μg/mg (DW), which indicate that of BTB is 7.63% more than that in YTB (Fig. 11). Rutin content in BTB and YTB is about 35.93 μg/mg and 38.80 μg/mg, accounting for 73.2% and 73.5% in total flavonoids. The rutin content in BTB is 8.0% more than that in YTB. But as a precursor in rutin biosynthesis pathway, quercetin content in YTB and BTB is 3.06 μg/mg and 6.49 μg/mg, which indicate that quercetin content of BTB is 52.9% less than that of YTB. The kaempferol content in BTB is also less than that in YTB, but the myricetin in BTB is the same as that in YTB.

2.2. De novo assembly of sequences and construction of unigene library

In the present study, in order to ensure the inclusion of the maximum number of genes in the transcriptome, we performed the Illumina GAIIx platform run on each of two cDNA libraries constructed from flowers in Fagopyrum tataricum cultivars with contrasting seed F. tataricum cultivar XIQIAO No. 2 (YTB, yellow tartary buckwheat) and F. tataricum cultivar HEIFENG No. 1 (BTB, black tartary buckwheat) (Fig. 1). The total number of reads obtained was 21.6 million and 26.8 million with a mean raw read length of 101 bp in YTB and BTB, which corresponded to 4,361,917,824 bp and 5,417,027,031 bp, respectively (Table 2). After adapter trimming, B-trimming and low-quality end-trimming, the high quality reads were used for de novo assembly to generate 756,343 contigs and 912,830 contigs respectively. Due to the non-availability of a reference genome sequence, the assembly of the high quality reads was done using Trinity (v3.3.5), which resulted in 44,043 transcripts and 46,544 transcripts with an average read length of 908.14 bp and 896.60 bp in the two kinds of flowers, indicating an increased coverage and depth of sequencing by generating longer fragment lengths (Table 2). Finally, 82,128 unigenes were assembled in XIQIAO No. 2 unigene library and 87,062 unigenes were assembled in HEIFENG No. 1 unigene library and 57,800 unigenes were assembled in both types in F. tataricum. With an increase in the length, the number of all unigenes decreased (Fig. 2).

In 2011, Logacheva et al. performed flower transcriptome sequencing by 454 sequencing. Recently, Haifeng et al. performed root and shoot transcriptome sequencing by Illumina sequencing. In comparison with the results, we produced more assembled unigenes (57,800 vs 39,815 vs 25,401), although the average length of unigenes is not the longest in the data (954.83 vs 1184 vs 703) (Table 2). Therefore, the assembled unigene database in our study should provide a useful resource for future research on F. tataricum, especially on the flower.

Please cite this article in press as: Yao, H., et al., Deep sequencing of the transcriptome reveals distinct flavonoid metabolism features of black tartary buckwheat (Fagopyrum tataricum Garetn.), Progress in Biophysics and Molecular Biology (2016), http://dx.doi.org/10.1016/j.pbiomolbio.2016.11.003

References

Statistics of Table 2 unigenes to BLAST X analysis (e-value ≤ 2.3). Gene and pathway annotation and analysis from black tartary buckwheat (BTB); (B) A flower from black tartary buckwheat (BTB; (B) A flower from yellow tartary buckwheat (YTB).

Table 1
Flavonoid content (mg/500 mg fresh weight FW.%) in different species of F. tataricum.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Total flavonoid content</th>
<th>Rutin</th>
<th>Kaempferol</th>
<th>Quercetin</th>
<th>Myricetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTB</td>
<td>52.81 ± 1.56</td>
<td>38.80 ± 0.017</td>
<td>0.0556 ± 0.000</td>
<td>3.06 ± 0.000</td>
<td>0.43 ± 0.002</td>
</tr>
<tr>
<td>YTB</td>
<td>49.07 ± 0.91</td>
<td>35.93 ± 0.795</td>
<td>0.0934 ± 0.002</td>
<td>6.49 ± 0.003</td>
<td>0.40 ± 0.001</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SD (n ≥ 2).

2.3. Gene and pathway annotation and analysis

The sequence similarity search was done by subjecting the unigenes to BLAST X analysis (e-value ≤ 1e-5) against the NR, NT, TrEMBL, SwissProt, COG, GO and KEGG databases. From a total of 57,800 unigenes, 33,472 (57.91%) sequences were annotated by being aligned to the NR database (S1 Table); 26,250 (45.42%) hits had similarity with the NT database (S2 Table); 33,526 (58.00%) were matched to the TrEMBL database (S3 Table); 27,346 (47.31%) had similarity with the Swiss-Prot database (S4 Table); 12,099 (20.93%) were matched to the COG database (S5 Table); 28,762 (49.76%) unigenes showed a significant similarity to the sequences available in the GO database (S6 Table); 1,182 (2.04%) were matched to 158 pathways in the KEGG database (S7 Table, Table 3).

23,336 unigenes did not match any of the databases; this could be due to the presence of novel unigenes, the small size of the sequences or errors in sequencing (Table 4).

2.4. Gene ontology (GO)

Blast2GO was used to classify the transcripts into different plant gene ontology categories such as molecular functions, biological processed and cellular components. 57,800 unigenes were selected for GO annotation (Fig. 3).

Among the biological processes category, cellular process were the main group, representing the maximum number of unigenes (23,286, 40.29%), which were followed by metabolic processes (22,065, 38.17%), response to stimulus (16,592, 28.71%) and biological regulation (15,561, 26.92%), followed by developmental processes (12,776, 22.10%), cellular component organization or biogenesis (11,900, 20.59%), localization (10,329, 17.87%) and multicellular organismal processes (10,186, 17.62%).

The molecular function category, in decreasing order of abundance, included binding (18,172, 31.44%), catalytic activity (15,345, 26.55%), transporter activity (2,446, 4.26%), nucleic acid binding transcription factor activity (1,669, 2.89%), structural molecule activity (1,032, 1.79%), molecular transducer activity (921, 1.59%) and enzyme regulator activity (722, 1.25%).

The cellular responses category was represented by a large number of transcripts coding for proteins which belong to cell parts (25,173, 43.55%), cells (24,863, 43.02%), organelles (22,869, 39.57%), membranes (11,271, 19.5%), macromolecular complexes (5,587, 9.67%), membrane parts (5,178, 8.90%), cell junctions (3,541, 6.13%), extracellular regions (3,313, 5.73%) and membrane-enclosed lumens (1,849, 3.20%).

2.5. COG classification

Eukaryotic Orthologous Groups is another form of COG (Clusters of Orthologous Groups) which is unique to eukaryotes (Tatusov et al., 2003). It aids in identifying orthologous proteins and representing the phylectic classification of proteins coded in the whole genome of almost 21 organisms including bacteria, algae and eukaryotes. The unigenes obtained in our study were compared with the COG database and classified into 24 categories. The majority of unigenes belonged to general function prediction (3,338, 5.78%), which was followed by transcription (1,641, 2.84%), replication, recombination and repair (1,624, 2.81%) and signal transduction mechanisms (1,470, 2.54%) (Fig. 4). None of the unigenes belonged to extracellular structures.

Please cite this article in press as: Yao, H., et al., Deep sequencing of the transcriptome reveals distinct flavonoid metabolism features of black tartary buckwheat (Fagopyrum tataricum) accessions used. A flower from black tartary buckwheat (BTB); (B) A flower from yellow tartary buckwheat (YTB).

Fig. 1. Images of the flowers of the tartary buckwheat accessions used. (A) A flower from black tartary buckwheat (BTB); (B) A flower from yellow tartary buckwheat (YTB).
2.6. KEGG classification

The 8305 unigenes obtained in our study were compared with 158 KEGG pathways in the KEGG database. The majority of unigenes belonged to ribosome pathways (ko03010), including 539 unigenes, which was followed by plant hormone signal transduction (ko04075) (321), oxidative phosphorylation (ko00190) (303), protein processing in the endoplasmic reticulum (ko04141) (297) and glycolysis/gluconeogenesis (ko04075) (249) (Fig. 5).

2.7. Nucleotide content analysis and identification of SSRs

The GC content is an important criterion for establishing the phylogenetic and evolutionary relationships among various species, and its percentage varies among different organisms, ranging from 20% to 72%. The GC contents observed in BTB and YTB were around 45.97% and 46.20%, respectively (Table 5).

2.8. Transcripts differentially expressed between BTB and YTB

To investigate the expression level of unigenes in BTB and YTB,
the number of clean reads was compared between the libraries for each of 57,800 unigenes through Reads Per Kilobase of transcript per Million (RPKM) analysis (Grabherr et al., 2011). Of the 824 unigenes found to be differentially expressed by more than 2-fold between YTB and BTB, 191 unigenes were upregulated while 633 were downregulated in the BTB as compared to the YTB. Clustering algorithms and Treeview were used to analyze the expression profiles of these genes (Fig. 7). In the 683 annotated unigenes, 179 unigenes were upregulated while 504 were downregulated by more than 2-fold (S8 Table). Among these unigenes, 83 (12.2%) unigenes showed more than a 16-fold change in expression level and 374 (54.8%) unigenes showed a 2–4-fold change. The fold.
change distribution of unigenes differentially expressed between tartary buckwheats is shown in Fig. 8. Annotation of differentially expressed unigenes revealed that 683 unigenes belonged to 51 GO groups (Fig. 9) and 284 unigenes belonged to 25 functional categories (Fig. 10).

2.9. Analysis of the reason of total flavonoids and flavonol difference

In 57,800 unigenes, 252 unigenes were involved in flavonoid biosynthesis, 464 unigenes were involved in positive regulation of flavonoid biosynthesis and 73 unigenes were involved in flavonol biosynthesis (Table 5). In the unigenes, some key unigenes involved in flavonol metabolism were differentially expressed. The first key enzyme of flavonoid biosynthesis is phenylalanine ammonia-lyase (PAL, EC 4.3.1.24), which catalyses a reaction converting L-phenylalanine to ammonia and trans-cinnamic acid and is the first and committed step in the phenylpropanoid pathway. It is therefore involved in the biosynthesis of the polyphenol compounds such as flavonoids, phenylpropanoids, and lignin in plants (Fritz et al., 1976; Tanaka et al., 1989). Many experiments have shown that the expression of PAL is related to the expression of total flavonoids (Hu et al., 2009). The second key enzyme of flavonoid biosynthesis is chalcone synthase (CHS, EC 2.3.1.74), which can use 4-coumaroyl-CoA and malonyl-CoA to produce CoA, naringenin, chalcone, and CO2 and act as a central hub for the enzymes involved in the flavonoid pathway (Crosby et al., 2011). CHS controls the speed of total flavonoids production by interacting with chalcone isomerase (Jez et al., 2000). Chalcone isomerase (CHI, EC 5.5.1.6) is also the master of the conversion efficiency of pro-flavonoids (Muir et al., 2001), which catalyses a reaction converting chalcone to flavanone, which can generate flavonanes, flavones, flavonols, anthocyanidins, isoflavone, isoflavone and isoflavonol by the flavonoid branched metabolic pathways, respectively (Jez et al., 2000; Clain et al., 1997). According to S10 Table and Fig. 12, the expression of all unigenes of PAL, CHS and CHI in BTB is 22%, 14% and 16% more than that in YTB, which illustrates the reason that total flavonoids content from flowers in BTB is 7.6% more than that in YTB (Table 1, Fig. 11).

2.10. Real-time PCR analysis of the genes involved in the flavonoid biosynthesis pathway

To confirm the differences in expression level between the accessions found in RPKM analysis, five unigenes for flavonoid biosynthesis were chosen for qRT-PCR analysis (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0071110). These Fagopyrum_T2_Unigene_BMK.22919 (PAL), Fagopyrum_T1_Unigene_BMK.35714, CL15183Contig1 and CL16015Contig1 control the two metabolic pathways that have been confirmed (Sun, 2005). UFGT (UDP-glucose: Quercetin 3-O-glucosyltransferase) is responsible for the glycosylation of quercetin to produce rutin. The sum of 22 UFGT unigene RPKM values in BTB (683.26) was more than that in YTB (614.02), which explains the rutin content in BTB is more than that in YTB (Table 1, Fig. 11), although its precursor quercetin content in BTB is less than that in YTB (Table 1, Fig. 11).

3. Conclusion

In this study, we have described a genome-wide comparison between the tartary buckwheat transcriptomes derived from one source of YTB and two sources of BTB. We have identified various genes that were differentially expressed in both types, which are potentially associated with the morphological and functional features of BTB. We have also identified various genes with well-characterised roles in flavonoid metabolism, which may be responsible for the higher nutritional value of BTB compared to YTB. These results highlight the valuable characteristics of black tartary buckwheat.

4. Materials and methods

4.1. Plant materials

In 2013, two types of tartary buckwheat seeds (YTB, yellow tartary buckwheat (XIQIAO No2); BTB, black tartary buckwheat

Table 5

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Read Sum</th>
<th>Base Sum</th>
<th>GC (%)</th>
<th>N (%)</th>
<th>Q20%</th>
<th>Cycle Q20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTB</td>
<td>26819715</td>
<td>5417027031</td>
<td>45.97</td>
<td>0.00</td>
<td>97.72</td>
<td>100.00</td>
</tr>
<tr>
<td>YTB</td>
<td>21596099</td>
<td>4361917824</td>
<td>46.20</td>
<td>0.00</td>
<td>97.69</td>
<td>100.00</td>
</tr>
</tbody>
</table>
HEIFENG No1)) were grown under field conditions on a farm at Baoxing, Ya’an, Sichuan, China on April 13. The flowers from two buckwheats were collected during the flowering stage, on June 15 and some flowers were treated by Sample Protector for RNA/DNA (Takara, China) and stored at -80°C for mRNA extraction.

4.2. Determination of total flavonoids

Some flowers of two kinds of tartary buckwheats (500 mg each) were homogenised in 5 mL of 70% methanol containing 10% phosphoric acid (final, 0.1%) with a hand homogeniser. Flavonoids were extracted using an ultrasonic method. After cooling, the test tubes were centrifuged at 8000 x g for 10 min. The obtained supernatants were stored at -20°C. Extracts from F. tataricum were analyzed with a spectrophotometer according to a previous report with a little modification (Tomotake et al., 2007). The reaction process was: 1 mL diluted extracts containing flavonoids, 0.3 mL of 5% (W/W) NaNO2, and 4 mL of 70% methanol (V/V) were mixed for 6 min, and then 0.3 mL of 10% Al(NO3)3 (W/W) was added and mixed. Six minutes later, 4 mL of 1% NaOH (W/W) was added. The solution was diluted to 10 mL with 70% (V/V) methanol for measurement. After 15 min, the absorbance of the solution was measured at 500 nm with a UNICO WFJ2000 spectrophotometer (Unico, China). All samples were measured in triplicate.

4.3. Analysis of kaempferol, quercetin, rutin and myricetin content by HPLC

Two types of flowers were frozen in liquid nitrogen, ground to a fine powder using a mortar and pestle, and then extracted with 10 mL of 70% ethanol for 1 h at 60°C. After centrifugation, the supernatant was filtered through a 0.45-μm poly filter (Acrodisc Syringe Filters, Pall, Port Washington, NY) and analyzed by HPLC. The analysis was performed with a C18 column (250 mm × 4.6 mm, 5 μm; RStech, Daejon, Korea) at 30°C. The mobile phase was a gradient mixture of 0.2% acetic acid—water (A) and methanol (B). The solvent program was used as follows: 0 min solvent B 10%, 10 min solvent B 20%, 15 min solvent B 20%, 20 min solvent B 25%, 25 min solvent B 25%, 50 min solvent B 60%, 50.1 min solvent B 10%, and then constant solvent B 10% for 10 min (total, 60 min). The standard mixture of rutin, quercetin, kaempferol and myricetin were injected with a concentration range of 0.8–4.0 g/mL. The flow rate was maintained at 1.0 mL min⁻¹, the injection volume was 20 μL, and the detection wavelength was 280 nm. The concentration of flavones in the samples was calculated using the standard curve.

4.4. Total mRNA extraction

Total mRNA from the flowers of two kinds of tartary buckwheat...
was extracted using the RNAout kit (Tiandz, China) according to the manufacturer’s procedure respectively. The purity and concentration of the isolated RNA were determined using the AgilentG2939A and Agilent RNA 6000 Nano LabChip Kit (Agilent, CA, USA). Two types of samples were obtained, with 260/280 values of 2.2 and 2.19 and RNA integrity numbers (RIN) of 8.6 and 8.5, respectively. These two kinds of RNA samples were analyzed and pooled to prepare an equimolar concentration of total RNA and were used for

Fig. 8. The fold change distribution of differentially expressed genes between YTB and BTB.

Fig. 9. Functional categories of unigenes differentially expressed between YTB and BTB.
cDNA library construction respectively.

4.5. Transcriptome library construction and Illumina sequencing

The transcriptome library for Illumina sequencing was constructed according to the Illumina TruSeq RNA library protocol outlined in the TruSeq RNA Sample Preparation Guide respectively. To minimize the effect of transcriptome unevenness among individuals, equal quantities (5 μg) of total RNA isolated from YTB and BTB were pooled in 50 μl of RNase-free water and subjected to isolate poly(A) mRNA with poly-T oligo-attached magnetic beads (Invitrogen). Following purification, the mRNA was fragmented into small pieces using divalent cations at an elevated temperature. Then, the cleaved RNA fragments were reverse-transcribed to create the final cDNA library in accordance with the protocol for the mRNA-Seq sample preparation kit (Illumina, San Diego, USA). The average insert size for the paired-end libraries was 250 ± 50 bp. We then performed paired-end sequencing (101 bp) on an Illumina HiSeq™ 2000 apparatus (Illumina, USA) following the vendor’s recommended protocol.

4.6. Sequence assembly and data analysis

The raw data from the images acquired after sequencing were transformed by base calling into raw reads and stored in FASTQ format. FASTQ reads were subjected to quality check using SeqQC V2.1 (Genotypic Proprietary Tool). The adaptor sequences, homopolymers and low quality bases were trimmed/filtered from the
raw FASTQ data using the Custom Perl script to generate contigs database (Ravi and Mukash, 2012). Filtered contigs were de novo assembled using Trinity (Grabherr et al., 2011) (v3.3.5 with parameters: K-mer = 25, overlap = K-mer-1, group_pairs_distance = 500) to produce transcripts database. After de novo assembly, the transcripts from YTB, the transcripts from BTB and all transcripts from both types were clustered using BLAT (http://genome.ucsc.edu/cgi-bin/hgBlat) (parameters: tileSize = 8, -stepSize = 5) to produce three unigene libraries including the HEIFENG No. 1 unigene library, the XIQIAO No. 2 library and all unigene library having all unigenes by selecting the longest sequence in each cluster.

All unigenes were annotated by Getorf (Mortazavi et al., 2008) to look for open reading frame (ORFs) in the database and were annotated using BLAST software (Altschul et al., 1997) to align various databases such as NR, NT, TrEMBL, SwissProt, COG, GO and KEGG (Ashburner et al., 2000; Yangyang et al., 2006; Tatusov et al., 2000; Kanehisa et al., 2004) with the value of E less than 1e-5. The best hit for each unigene with the highest sequence similarity from the above databases was chosen and the annotations were retrieved. KOG was used to analyze, predict and classify unigenes with probable functions, whereas the Aracyc database was used to assign pathways (Tatusov et al., 2001). The Blast2GO program was used to obtain GO annotation according to molecular function, biological process and cellular component ontologies (Ana and Stefan, 2008; Fan et al., 2006).

4.7. Nucleotide content analysis and identification of SSR markers

The percentage compositions of the nucleotides A, T, G and C were calculated for each sequence and across the entire distribution of transcripts. Simple sequence repeats (SSRs) were detected using the MicroSAtellite tool. SSRs were detected by considering the 100 bp flanking sequences upstream and downstream of the SSRs. The parameters used for the development of SSRs are provided in Table 7.

4.8. Quantitative analysis and functional enrichment analysis of different gene expression

The RPKM value was used to calculate the unigene expression in our study (Grabherr et al., 2011). The unigenes of different gene expression (DGE) in two types of tartary buckwheats were selected by using the Ebseq software with FDR less than 0.01 and Log2 ratio greater than 1 (two-fold change) (Leng et al., 2013) and aligned to GO databases and COG databases with the value of E less than 1e-5.

4.9. Quantitative real-time PCR (qRT-PCR) analysis

Five unigenes involved in flavonoid synthesis were chosen for validation by qRT-PCR. Total RNA was isolated from YTB and BTB using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). For qRT-PCR, 1 μg of total RNA was reverse-transcribed using the SuperScript II First Strand Synthesis Kit (Invitrogen, Carlsbad, CA, USA) and an oligo(dt)20 primer. Transcription levels were analyzed by real-time PCR. Gene-specific primer sets were designed for real-time PCR, and the primers are listed in S11 Table. Gene-of-interest expression was normalized to that of the histone H3.
Fig. 14. qRT-PCR validation of RPKM analysis of the five unigenes involved in flavonoid biosynthesis of YTB and BTB.

Table 7
Parameters used for development of SSRs.

<table>
<thead>
<tr>
<th>S.no</th>
<th>SSR type</th>
<th>Unit size</th>
<th>Minimum number of repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mono nucleotide</td>
<td>Repeats 1</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Di nucleotide</td>
<td>Repeats 2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Tri nucleotide</td>
<td>Repeats 3</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Tetra nucleotide</td>
<td>Repeats 4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Penta nucleotide</td>
<td>Repeats 5</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Hexa nucleotide</td>
<td>Repeats 6</td>
<td>5</td>
</tr>
</tbody>
</table>

Maximal number of bases interrupting two SSRs in a compound microsatellite: 100.

housekeeping gene. Real-time PCR reactions were performed in triplicate on a MiniOption system (Bio-Rad Laboratories, Hercules, CA) with the QuantiTect SYBR Green PCR Kit (Qiagen). The PCR protocol was as follows: denaturation for 5 min at 95 °C, followed by 39 cycles of denaturation for 15 s at 95 °C, annealing for 15 s at 60 °C, and elongation for 20 s at 72 °C. PCR results were calculated as the mean of 3 replicated treatments. Significant differences between treatments were evaluated by standard deviation.

Data availability

The full data sets have been submitted to NCBI, Genebank under Bioproject, Accession: PRJNA271797.

Acknowledgements

This work was supported by the research grants from the Department of Education of Sichuan Province, China (13ZB0294) and Sichuan Agricultural University (00770114).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.pbiomolbio.2016.11.003.

References

Guo, X., Zhu, K., Zhang, H., Yao, H.P., 2007. Uri...

Please cite this article in press as: Yao, H., et al., Deep sequencing of the transcriptome reveals distinct flavonoid metabolism features of black tartary buckwheat (Fagopyrum tartaricum Gaertn.), Progress in Biophysics and Molecular Biology (2016), http://dx.doi.org/10.1016/j.pbiomolbio.2016.11.003