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403 **Transcriptome analysis reveals candidate genes for dietary fiber**  
404 **metabolism in *Rosa roxburghii* fruit grown under different light**  
405 **intensities**

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## 432 **Abstract**

433 The fruit of the perennial rosebush *Rosa roxburghii* were valued for their high levels of ascorbic acid  
434 (AsA), superoxide dismutase activity, and cancer preventing effects. The high cellulose and low pectin  
435 content of *Rosa roxburghii* fruit results in an undesirable fibrous texture and hence needs to be addressed.  
436 However, little is known about the molecular mechanisms underlying dietary fiber metabolism in this  
437 fruit. Here, we report that the contents of cellulose, pectin, and lignin were increased by shading  
438 treatments at the maturation stage of fruit development. Under 50% shading, the soluble pectin content  
439 increased by 16.39%, which may improve the fruit palatability. However, deeper shading of 100% caused  
440 the lignin content to increase by 28.86%, which conversely may lower fruit quality. Based on  
441 transcriptome analysis, we identified candidate genes involved in dietary fiber metabolism, including  
442 *cellulose synthase (CesA) 1, 2, 3, and 5*, *â-1,4-xylosyltransferase (IRX)*, *arabinosyltransferase (ARAD) 1*  
443 *and 2*, *galacturonosyltransferase (GAUT)*, *cellulolytic enzyme (Cx)*, and *pectin methylesterase (PME)*, in  
444 which *CesA1*, *CesA2*, *CesA3*, *IRX*, *ARAD2*, and *GAUT3* significantly responded to shading and positively  
445 correlated with the content of their corresponding component. Furthermore, *cinnamyl alcohol*  
446 *dehydrogenase (CAD)* was significantly regulated by shading treatment and positively correlated with  
447 increasing lignin concentration. These results may facilitate a better understanding of the molecular  
448 mechanisms of dietary fiber metabolism in *R. roxburghii* fruit under low light conditions and provide a  
449 framework for future crop improvement.

450 **Keywords:** Dietary fiber · Gene expression · Molecular mechanisms · *Rosa roxburghii* fruit · Shading  
451 treatment

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## 454 **1 Introduction**

455 *Rosa roxburghii* Tratt is a perennial rosebush native to China that is becoming more commonly cultivated,  
456 especially in Guizhou Province, due to the nutritional and health-promoting properties of its fruit. While  
457 direct consumption of the fruit remains low, due in part to its fibrous texture, an increasing number of  
458 health care products, cosmetics, and functional foods containing *R. roxburghii* fruit extracts are available  
459 (Xu et al. 2019). To date, the cultivation area of this species in China stands at over 130,000 hectares. The  
460 mature fruit of *R. roxburghii* contains large quantities of total dietary fiber (23.80%), cellulose (8.70%),  
461 hemicellulose (5.10%), and lignin (1.40%), but relatively low levels of total pectin (3.40%), of which  
462 insoluble pectin is 2.8% and soluble pectin only 0.6% (Liu et al. 2015a). The high ratio between the

463 content of dietary fiber and pectin is one of the principal causes of undesirable organoleptic properties in  
464 fruit of *R. roxburghii*, and hence an important target for future breeding programs.

465 Cellulose, hemicellulose, lignin, and pectin are the four major components of *R. roxburghii* fruit dietary  
466 fiber. The quantity of these cell wall polymers in the developing and ripening fruit depends on their  
467 synthesis, remodeling, and degradation (Galanakis 2011). Our previous research suggested that the  
468 lignin-related activities of peroxidase (POD), cinnamoyl-CoA reductase (CCR), cinnamoyl-CoA  
469 reductase (4CL), and shikimate O-hydroxycinnamoyltransferase (HCT) play a crucial role in lignin  
470 biosynthesis (Lu et al. 2020).

471 Cellulose is a linear polysaccharide of (1 → 4)-linked β -D-glucosyl residues, whose synthesis is  
472 catalyzed by the *CesA* genes, which encode the probable catalytic subunits of the plant cellulose synthase  
473 enzyme complex, visible in the plasma membrane as rosettes (Schneider et al. 2016). At least 10 *CesA*  
474 isoforms exist in *Arabidopsis thaliana*, which exert distinct role/s in the cellulose synthesis process  
475 (Takata & Taniguchi 2015). In apple (*Malus domestica*), seven *CesA* genes were found to be  
476 downregulated during the transition from tight cluster flowers to anthesis (Guerriero et al. 2014). It has  
477 been suggested that *hydrolase-cellulase* (*Cx*) may also be implicated in the metabolism of (hemi-)  
478 cellulose in citrus (Dong et al. 2009).

479 Hemicelluloses are a diverse group of heterogeneous polysaccharides whose function is to cross-link  
480 cellulose microfibrils and encompass the heteromannans, xyloglucan, heteroxylans, and mixed-linkage  
481 glucan (Pauly et al. 2013). Polysaccharide synthesis is mediated mostly by glycosyltransferases (GT). The  
482 synthesis of the backbone of xyloglucan is catalyzed by members of the GT2-CSL superfamily. Group  
483 CSL-C catalyzes the xyloglucan backbone, CSL-A catalyzes the mannan and glucomannan backbone, and  
484 CSL-F catalyzes the mixed-linkage glucan backbone. The xylan backbone is thought to be synthesized by  
485 GT43 proteins with beta-1,4-xylosyltransferase activity (Lee et al. 2012). On the other hand, the synthesis  
486 of heteromannans and mixed-linkage glucan is catalyzed by *cellulose synthase-like* (*CSL*), which shares  
487 several common features with *CesA* genes. Evidence exists that all *CSL* gene products are also integral  
488 membrane proteins and contain the D, DxD, D, and QxxRW motifs (Saxena et al. 1995). Additionally,  
489 xyloglucan endo-trans-glycosylase/hydrolase (Miedes & Lorences 2009), β-xylosidase (Figueroa et al.  
490 2010; Takizawa et al. 2014), and α-L-arabinofuranosidase (Figueroa et al. 2010) are enzymes associated  
491 with hemicellulose degradation.

492 Pectic polysaccharides, a group of complex polysaccharides, are commonly referred to as pectin, which  
493 comprise mainly homogalacturonan and rhamnogalacturonan I and II (Smith 2013; Salima et al. 2018).  
494 Homogalacturonan is the most common form among them, which consists of residues of

495 (1-4)- $\alpha$ -D-galacturonic acid arranged linearly and catalyzed by *galacturonosyltransferase* (*GAUT*)  
496 (Sterling et al. 2006). Enzymes involved in pectin degradation, such as polygalacturonase (PG) and pectin  
497 methylesterase (PME), have been extracted from fruits. The activity of PG and PME was found to  
498 gradually increase during the development of orange (*Citrus*  $\times$  *sinensis*) fruit (Zeng et al. 2006). However,  
499 the PG activity decreased gradually during the development of strawberry (*Fragaria*  $\times$  *ananassa*) fruit  
500 (Figueroa et al. 2010). In tomato (*Solanum lycopersicum*), *PME* genes make up a small gene family  
501 including at least four genes (Mutsumi et al. 2015).

502 Light is an important environmental factor affecting fruit growth and development (Zhang et al. 2019).  
503 Studies have shown that light affects the synthesis and accumulation rate of dietary fiber components  
504 mainly by two aspects. One is to promote the synthesis of sugars, phenylalanine, and other organic  
505 compounds by affecting photosynthesis and the other is to directly promote the synthesis of dietary fiber  
506 components by regulating related enzyme activities in the biosynthetic pathway. The accumulation of  
507 dietary fiber components in fruit cell walls was closely related to low light (Zhang et al. 2019).  
508 Furthermore, low light intensity reduced the cellulose content in cotton (*Gossypium hirsutum*) (Chen et al.  
509 2014) and decreased the lignin content in tea (*Camellia sinensis*) (Wang et al. 2012). Under low light  
510 conditions, photorespiration is increased, and therefore, the ethylene/sugar ratio also increases, and the  
511 abscission rate of reproductive structures is higher (Millenaar et al. 2010). This can, for instance, result in  
512 reduced yield and fiber quality in cotton (Echer et al. 2019). In melon (*Cucumis melo*), low light intensity  
513 decreased the pectin content (Toshiyuki *et al.*, 2006) and reduced the hemicellulose content (Suparjo et al.  
514 1990). Shading treatment also inhibits the activity of phenylalanine ammonium-lyase (PAL), cinnamyl  
515 alcohol dehydrogenase (CAD), 4CL, and POD, which had significant effects on lignin accumulation (Liu  
516 et al. 2019). A comparison of rice (*Oryza sativa*) plants with low light-resistant and low light-susceptible  
517 genotypes showed that shade-tolerant plants had a higher lignin content (Wang *et al.*, 2015). The  
518 enzymatic activities of PAL, CAD, 4CL, and POD were also higher in shade-resistant plants compared to  
519 shade-susceptible plants (Hussain et al. 2020). In shade-grown japonica rice, the expression of genes  
520 involved in secondary cell wall synthesis, namely *PAL*, *caffeic acid O-methyltransferase* (*COMT*),  
521 *caffeoyl-CoA O-methyltransferase* (*CCoAOMT*), *CCR*, and *CAD2*, and primary cell wall synthesis genes  
522 *CesA1*, *CesA3*, and *CesA8* was significantly downregulated (Wu et al. 2017).

523 The high cellulose and low pectin content of *R. roxburghii* fruit causes an undesirable fibrous texture.  
524 Although the genes involved in dietary fiber metabolism have been extensively studied in many plant  
525 species, dietary fiber accumulation processes and their underlying molecular mechanisms remain largely  
526 unexplored in *R. roxburghii*. In this study, we treated *R. roxburghii* fruit with two different shading  
527 intensities during fruit development. We then screened the genes involved in the synthesis and

528 accumulation of dietary fiber components from fruit transcriptome sequences and analyzed the correlation  
529 between gene expression and the content of the corresponding dietary fiber component. This study may  
530 facilitate a better understanding of the molecular mechanisms of dietary fiber metabolism in *R. roxburghii*  
531 fruit under low light conditions and provide a framework for future crop improvement.

## 532 **2 Materials and methods**

### 533 **2.1 Plant material**

534 Samples were collected from 8-year-old plants of *Rosa roxburghii* ‘Guinong 5’ (Fan et al. 2011), which  
535 were grown in the fruit germplasm repository of Guizhou University, Guizhou, China, in 2019  
536 (26°42.408'N, 106°67.353'E). To study the effect of shading on the accumulation of dietary fiber,  
537 developing fruits were covered at 15 days after anthesis (DAA) with a translucent white bag (light  
538 transmittance measured at 50%) or an opaque yellow bag (light transmittance measured at 0%), or left  
539 uncovered as a control, thereby giving shade levels of 0, 50, and 100%. Then, 80, 60, and 40 fruit were  
540 collected at three different developmental stages at 30, 60, and 90 DAA, which represented the young  
541 fruit stage, fruit development stage, and mature stage, respectively (Fig. 1). After collection, samples were  
542 immediately frozen in liquid nitrogen and stored at -80°C for further use.

### 543 **2.2 Determination of cellulose content**

544 Cellulose content was determined through anthrone colorimetry as per Chen et al. (2010), with minor  
545 modifications. Air-dried fruit tissue (0.2 g) was digested in 60 mL of 60% H<sub>2</sub>SO<sub>4</sub> for 30 min in a  
546 cold-water bath at 4°C. The digested cellulose solution was transferred to a volumetric flask which was  
547 then filled to 100 mL with 60% H<sub>2</sub>SO<sub>4</sub>. The mixture was shaken well and filtered through a Brinell funnel.  
548 Next, 1.5 mL of filtrate was added to a 100 mL volumetric flask, diluted with distilled water in a  
549 cold-water bath, shaken well, and then 2 mL was taken into a tube with a plug. Then, 0.5 ml of 2%  
550 anthrone reagent was added, and 5 mL of H<sub>2</sub>SO<sub>4</sub> was added along the wall of the tube, shaken well, and  
551 allowed to stand for 12 min. Absorbance was measured at 620 nm.

### 552 **2.3 Determination of hemicellulose and lignin content**

553 Kiln-dried tissue (0.2 g) was weighed into a 100 mL beaker and 10 mL of 60% Ca (NO<sub>3</sub>)<sub>2</sub> was added and  
554 the mixture was heated for 10 min before filtration. The residue was washed with distilled water 3 times  
555 before oven drying at 70°C. The residue was transferred to a 250 mL conical flask to which 10 mL of 2 M  
556 HCl was added, and the flask was submerged in a water bath at 100°C for 50 min. After cooling and

557 filtering, the residue was washed with distilled water three times and the volume of the filtrate was  
558 measured. Then, 0.5 mL of filtrate was added to 1.5 mL of DNS solution, and again incubated in a water  
559 bath at 100 °C for 10 min. After cooling, the volume was adjusted to 25 mL and the absorbance was  
560 measured at 540 nm according to Jin et al. (2017). The lignin content was measured using a lignin content  
561 determination kit (Solarbio, Beijing, China), according to the manufacturer's instructions.

#### 562 **2.4 Determination of pectin content**

563 Carbazole colorimetry was used as per Einhorn-Stoll et al. (2018), with minor modifications. Fresh fruit  
564 tissue (2 g) was weighed, ground in a mortar, and washed into a conical flask with 50 mL of 95% ethanol,  
565 then extracted with 95% ethanol at 70 °C 3 times. Filtered, discarded the filtrate and washed the residue  
566 with 95% ethanol until there was no soluble sugar in the filtrate. The residue was washed into a conical  
567 flask with 40 mL of ddH<sub>2</sub>O and incubated in a 50 °C water bath for 1 h before filtering. The residue and  
568 filter paper were washed with a small amount of distilled water. The filtrate was transferred into a 50 mL  
569 volumetric flask and used for the determination of soluble pectin (WSP). The residue was washed into a  
570 triangular flask with 80 mL of 0.5 M H<sub>2</sub>SO<sub>4</sub>, heated in a water bath (100 °C) for 1 h, cooled and filtered,  
571 then transferred to a 100 mL volumetric flask, and used for the determination of insoluble pectin.

#### 572 **2.5 Differentially expressed genes and functional enrichment**

573 Transcriptome data of *R. roxburghii* fruit were analyzed as per our previous study (Lu et al. 2020).  
574 Differential expression analysis of two conditions/groups was performed using the DESeq R package  
575 (1.10.1). DESeq was used to determine the differential expression in digital gene expression data using a  
576 model based on the negative binomial distribution. The resulting *P*-values were adjusted using the  
577 Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted  
578 *P*-value < 0.05 found by DESeq were assigned as differentially expressed. To annotate the unigene  
579 sequences of *R. roxburghii*, a BLASTx search ( $E < 10^{-5}$ ) was used to search against Kyoto Encyclopedia  
580 of Genes and Genomes (KEGG) databases based on sequence similarity.

#### 581 **2.6 Real-Time Quantitative PCR Analysis**

582 Candidate differentially expressed genes (DEGs) involved in dietary fiber metabolism were selected for  
583 validation by real time quantitative PCR (qRT-PCR). Total RNA was extracted through a TaKaRa  
584 MiniBEST Plant RNA Extraction Kit (TaKaRa, Inc., Dalian, China). RNA quality was evaluated by  
585 agarose gel electrophoresis and the NanoDrop system (Implen, Los Angeles, CA, USA). cDNA was  
586 synthesized with the PrimeScrip RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, Inc.,

587 Dalian, China). The primer sequences used for qRT-PCR are listed in Table S1. qRT-PCR was performed  
588 on an ABI ViiA 7 DX system (Applied Biosystems) using SYBR Premix Ex Taq II (TaKaRa) with the  
589 ubiquitin gene as an endogenous control. Data analysis was performed using the  $2^{-\Delta\Delta CT}$  method. Values  
590 for mean expression and standard deviation (SD) were calculated from the results of three independent  
591 experiments.

## 592 **2.7 Statistical analysis**

593 All experiments were conducted in at least triplicate, and data were expressed in means  $\pm$  standard  
594 deviations. Statistical analysis was performed using SPSS 20.0 software (SPSS 20.0, IBM, Armonk, NY,  
595 USA), and the differences among mean values were tested by one-way ANOVA (SPSS 20.0, IBM,  
596 Armonk, NY, USA), taking a level of  $p < 0.05$  as significant to Duncan's multiple range test. The original  
597 8.0 software was used to draw the graph. TBtools software was used to draw the heat map of gene  
598 expression (Chen et al. 2020).

## 599 **3 Results**

### 600 **3.1 Effect of shading on fruit coloring of *R. roxburghii***

601 The effect of shading treatment on the color change of *R. roxburghii* fruit during fruit development is  
602 shown in Fig. 1. The young fruit (30 DAA) had a green coloration in the control sample, which was  
603 slightly paler in the 50% shade treatment, turning to a pale yellow-white color in the 100% shade  
604 treatment. The developing fruit (60 DAA) displayed a yellow-green color in the control group, which was  
605 much paler in the 50% shade treatment and had more pink-red hues in the 100% shade group. The mature  
606 fruit (90 DAA) had a pale-yellow color in the control group, which darkened to a more vivid yellow in the  
607 50% shade group, and further to a rich yellow with hints of orange in the 100% shade group. Shade  
608 treatments, especially 100% shade, caused a significant reduction in fruit weight.

### 609 **3.2 Content of dietary fiber components in *R. roxburghii* fruit and its response to shading**

610 The changes in the content of dietary fiber components of the *R. roxburghii* fruit during development  
611 under different shade treatments are presented in Fig. 2. During fruit development, cellulose content  
612 showed a linear downward trend in the control group, but a more uniform concentration during  
613 development in the shaded samples. In the young fruit stage (30 DAA), shading treatment significantly  
614 inhibited the accumulation of cellulose in the fruit, while in the later stage of fruit development, it  
615 significantly promoted the accumulation of cellulose, and the effect became more obvious with the  
616 increase of shading; the content of cellulose increased by 12.26% and 18.25% under 50% and 100%



617 shading, respectively (Fig. 2a). The accumulation of hemicellulose in *R. roxburghii* fruit showed a  
618 downward trend in the whole development process, but shading had no obvious effect on the  
619 accumulation of hemicellulose (Fig. 2b). The lignin content increased slowly first and then decreased  
620 rapidly in the control group, but shading effected lignin accumulation. Before 60 DAA, shading inhibited  
621 the accumulation of lignin, but at 90 DAA, under the 50% and 100% shading treatments, shade promoted  
622 the accumulation of lignin by 13.07% and 28.86%, respectively (Fig. 2c). Pectin content first decreased at  
623 60 DAA and then slightly increased or slightly decreased further at 90 DAA depending on the treatment.  
624 At the mature stage, both the 50% and 100% shading treatments promoted the accumulation of soluble  
625 pectin by 16.39% and 18.78%, respectively. However, only 100% shading promoted the accumulation of  
626 total pectin and insoluble pectin by 27% and 28.6%, respectively (Fig. 2d-f).

### 627 **3.3 Clustering and Kyoto Encyclopedia of Genes and Genomes pathway enrichment of the** 628 **differentially expressed genes**

629 To study the transcriptional regulation of the genes involved in fruit development and maturation, 17,470  
630 differentially expressed unigenes were classified into 18 types of clusters based on the modulation of  
631 expression patterns (Fig. 3). The gene expression patterns of cluster 1 (548 DEGs), cluster 2 (1,208  
632 DEGs), cluster 3 (231 DEGs), cluster 12 (732 DEGs), cluster 15 (1,747 DEGs), and cluster 17 (207 DEGs)  
633 exhibited similar changes during fruit development. The gene expression levels showed a rapid  
634 increase/decrease from 30 to 60 DAA, but insignificant changes were observed from 60 to 90 DAA.  
635 These genes may exert important functions in the young fruit. The gene expression patterns of cluster 6  
636 (88 DEGs), cluster 8 (179 DEGs), and cluster 9 (299 DEGs) were similar. The gene expression levels  
637 showed a rapid increase/decrease from 30 to 60 DAA, followed by a rapid decrease/increase from 60 to  
638 90 DAA. The genes in this group may function in the middle stages of fruit development. The gene  
639 expression patterns of cluster 4 (3,824 DEGs), cluster 5 (1,108 DEGs), cluster 7 (1,521 DEGs), cluster 14  
640 (331 DEGs), cluster 16 (87 DEGs), and cluster 18 (377 DEGs) exhibited similar changes during fruit  
641 development. The gene expression levels remained stable from 30 DAA to 60 DAA, and then a rapid  
642 decrease/increase from 60 to 90 DAA was observed. Hence, the genes in this group may exert their  
643 functions during the maturation stage of fruit development. Additionally, similar gene expression patterns  
644 were found in cluster 10 (457 DEGs) and cluster 11 (1021 DEGs), which showed a trend of continuous  
645 increase/decrease from 30 to 90 DAA. The genes in this group may function during all stages of fruit  
646 development.

### 647 **3.4 Genes mediating dietary fiber metabolism**

648 A total of 139 DEGs were found to be associated with dietary fiber metabolism. After removing the  
649 partially assembled transcripts and obvious discrepancies, 33 unigenes encoding enzymes were identified,  
650 including *CesA* (12), *CSL* (1), *IRX* (4), *ARAD* (2), *GAUT* (8), *Cx* (1), *PG* (2), and *PME* (2) as illustrated in  
651 Fig. 4. Of these 33 unigenes, 18 were significantly positively correlated with the content of total dietary  
652 fiber, cellulose, hemicellulose, and total pectin. They belonged to five different gene expression clusters  
653 (Table S2): *PME* (2-3k.c45874/2/1923) belonged to cluster 4; *CesA2* (3-6k.c17146/1/4436 and  
654 2-3k.c13506/4/2290), *CesA3* (3-6k.c2764/2/3959), *CesA5* (1-2k.c16341/1/1287), *IRX*  
655 (1-2k.c22164/1/1427), and *GAUT*(1-2k.c54278/1/1356) to cluster 5; *CesA1* (3-6k.c21066/11/3801) to  
656 cluster 7; *Cx* (2-3k.c56230/7/2136) to cluster 11; and *CesA3* (2-3k.c53599/4/2184 and  
657 3-6k.c6339/1/3496), *ARAD* (1-2k.c25067/1/1220 and 2-3k.c54088/9/2163), and *GAUT*  
658 (1-2k.c21559/1/1082, 2-3k.c26947/2/2489, 2-3k.c2467/1/2261, 2-3k.c50854/1/2501, and  
659 2-3k.c28445/1/4160) to cluster 13. As shown in Fig. S1, there was a strong correlation between RNA-seq  
660 data and qPCR data for most of the genes. These results confirmed the accuracy of our transcriptome  
661 profiling. In addition, lignin biosynthesis-related genes in *R. roxburghii* fruit were identified, including  
662 *4CL*, *HCT*, *coumaroylquinic acid (coumaroylshikimate) 3'-monooxygenase (C3'H)*, *CcoAMT*, *CCR*, *CAD*,  
663 *COMT*, and *POD* (Lu et al. 2020).

### 664 **3.5 Effect of shading on dietary fiber metabolism-related gene expression**

665 The expression of genes encoding intermediates in dietary fiber biosynthetic pathways across fruit  
666 development are shown in Fig. 5. During the development of fruit of *R. roxburghii*, the expression levels of  
667 *CesA1*, *CesA2*, and *CesA5* showed a downward trend, while those of *Cx* and *CesA3* showed a downward trend  
668 first and then an upward trend. This indicated that *CesA1*, *CesA2*, *CesA5*, and *Cx* are strongly correlated with  
669 the rate of cellulose accumulation. Shading significantly affected the expression of cellulose-related genes  
670 during fruit development. Shading of 50% inhibited the accumulation of cellulose and up-regulated the  
671 expression of *CesA1*, *Cx*, and *CesA3* before 60 DAA. However, at the mature stage (90 DAA), cellulose  
672 accumulation was promoted, and the expression of *CesA1*, *CesA3*, *CesA5*, and *Cx* was down-regulated.  
673 Shading of 100% decreased the content of cellulose at the young fruit stage (30 DAA), and increased the  
674 expression of *CesA5*, while it significantly increased the content of cellulose at the mature stage (90 DAA),  
675 indicating that *CesA5* may play an important role in the accumulation of cellulose under shading treatment (Fig.  
676 5).

677 Of the genes involved in the hemicellulose biosynthetic pathway, *ARAD1* expression first increased and  
678 then decreased, while that of *ARAD2* and *IRX* had a downward trend across fruit development, which  
679 correlated with the trend of hemicellulose content. Shading had no obvious effect on the accumulation of  
680 hemicellulose; however, the relative gene expression identified that the trend of hemicellulose content

681 correlated strongly with the expression of *IRX* under 50% shading, suggesting that *IRX* may influence  
682 hemicellulose accumulation (Fig. 5).

683 cDNA sequences of lignin synthesis pathway genes were identified from previous transcriptome data and  
684 further explored by qRT-PCR to analyze the expression level through fruit development and their  
685 response to shading. These included phenylpropanoid pathway genes (*4CL1*, *4CL2*, and *4CL3*), special  
686 pathway genes (*HCT*, *CCR1*, *CCR2*, *CCR3*, *CCR4*, *C3'H*, *CAD*, *COMT1*, *COMT2*, and *CcoAOMT*), and  
687 those involved in lignin monomer polymerization (*POD1*, *POD2*, *POD3*, *POD4*, *POD5*, and *POD6*). The  
688 expression levels of *4CL1*, *4CL2*, *4CL3*, *HCT*, *CCR3*, *CCR4*, *C3'H*, *COMT1*, *POD1*, *POD2*, *POD3*,  
689 *POD4*, and *POD5* showed a downward trend throughout development, while *CCR2*, *COMT2*, *CcoAOMT*,  
690 and *CAD* increased in expression throughout development. *CCR1* and *POD6* expression first increased  
691 and then decreased towards the latter stages of development, which was consistent with the trend of lignin  
692 content, suggesting that *CCR1* and *POD6* may influence lignin accumulation. Shading treatment (50%)  
693 down-regulated the expression of *POD3*, *POD5*, and *HCT* before 60 DAA. However, at 90 DAA, the  
694 expression of *HCT*, *POD3*, and *CCR2* was up-regulated. This suggests that *HCT* and *POD3* expression  
695 promoted lignin accumulation. Likewise, the 100% shading treatment down-regulated the expression of  
696 *POD2*, *POD4*, *POD5*, *CcoAOMT*, *CCR1*, *CCR2*, *CCR4*, *HCT*, *COMT1*, and *4CL1* before 60 DAA.  
697 However, at 90 DAA, the expression of *CCR1*, *CCR3*, and *CAD* was up-regulated. This suggests that  
698 *CCR1*, *CCR3*, and *CAD* may regulate lignin accumulation under shading treatment (Fig. 5).

699 The mechanism of pectin synthesis in *R. roxburghii* fruit was similarly studied. The expression of *GAUT1*,  
700 *GAUT5*, and *GAUT2* showed a trend of first increasing and then decreasing, whereas *PME* and *GAUT4*  
701 expression declined, and *GAUT3* expression initially decreased before increasing, which was consistent  
702 with the pectin contents, suggesting that *GAUT3* may influence pectin accumulation throughout fruit  
703 development. However, the effect of shading on the expression of pectin-related genes varied across fruit  
704 developmental stages. 50% shading increased the expression of *PME*, *GAUT1*, and *GAUT5*, and  
705 down-regulated the expression of *GAUT3* and *GAUT4* at 30 DAA. However, at 60 DAA, the expression  
706 of *PME* and *GAUT4* was up-regulated, and the expression of *GAUT1*, *GAUT2*, and *GAUT5* was  
707 down-regulated. Furthermore, at 90 DAA, the expression of *PME* and *GAUT4* was up-regulated, while  
708 the expression of *GAUT3* was down-regulated, which overall suggests that *PME* and *GAUT1* may  
709 regulate pectin accumulation in fruit grown under 50% shading. On the other hand, 100% shading  
710 up-regulated the expression of only *GAUT1* and *GAUT5*, and down-regulated the expression of *GAUT2*  
711 and *GAUT4* at 30 DAA. At 60 DAA, the expression of *GAUT4* was up-regulated, while the expression of  
712 *GAUT1*, *GAUT2*, and *GAUT3* was down-regulated. At 90 DAA, the expression of *PME*, *GAUT3*, and

713 *GAUT4* was down-regulated. Here, *GAUT3* expression positively correlated with pectin accumulation  
714 under 100% shading (Fig. 5).

### 715 **3.6 Correlation analysis of dietary fiber components content and gene expression**

716 Further correlation analysis was conducted between the dietary fiber components content and the  
717 expression levels of related genes, and indicated that there was a significant correlation between the  
718 cellulose content of fruit and *CesA1*, *CesA2*, and *CesA3* expression. Hemicellulose content was  
719 significantly correlated with *IRX* and *ARAD2* expression. The expression levels of *CAD* genes were  
720 significantly positively correlated with lignin content. The expression levels of *POD1*, *POD2*, *POD3*,  
721 *POD4*, *POD5*, *CcoAOMT*, *4CL1*, *4CL2*, and *4CL3* were significantly negatively correlated with lignin  
722 content. The relative expression level of *GAUT3* in fruit was significantly positively correlated with the  
723 total pectin and insoluble pectin content (Table 1).

## 724 **4 Discussion**

725 Cellulose, hemicellulose, and pectin are plant cell wall polysaccharides, usually studied as cellular  
726 structural substances (Guillon et al. 2017; Xu et al. 2016). They are also predominant components of  
727 fibers, but their metabolic patterns have not received much attention, especially in fruit organs. Dong et al.  
728 (2009) researched the dietary fiber metabolism in orange but focused on genes of the decomposition  
729 process. However, the accumulation of dietary fiber is the result of synthesis and decomposition, which is  
730 a dynamic process. Here, we show that from 30 to 90 DAA, the content of cellulose, hemicellulose, and  
731 total pectin decreased steadily. In addition, gene expression clusters 5, 10, 13, and 14 seemed to behave  
732 similarly. After conducting correlation analysis, 18 related genes were identified that significantly  
733 correlated with fiber content, mainly in clusters 5 and 13. Nine unigenes were present in cluster 5, 6 and  
734 13, all of which were involved in cellulose, hemicellulose, and pectin biosynthesis, including *CesA*, *IRX*,  
735 *ARAD*, and *GAUT*. The CSL proteins are regarded as intrinsic for hemicellulose biosynthesis in  
736 Arabidopsis (Richmond & Somerville 2000, 2001), rice (Hazen et al. 2002), and other plants. However,  
737 here we identified only one differentially expressed unigene annotated as CSL. Furthermore, no  
738 significant correlation was detected between its expression abundance and the content of total dietary  
739 fiber, cellulose, hemicellulose, and total pectin, suggesting its limited role in *R. roxburghii* fruit  
740 maturation-related processes. The same findings and supposition are valid for the enzymes related to the  
741 degradation of hemicellulose. *Cx* and *PME* were placed in cluster 11 and 4, respectively, which displayed  
742 a negative correlation to the content of total dietary fiber, cellulose, hemicellulose, and total pectin, and

743 are responsible for cellulose and pectin degradation. These results suggest that fiber accumulation is  
744 dependent on both biosynthesis and degradation in a dynamic process.

745 The expression levels of *RrCesA1*, *RrCesA2*, *RrCesA3*, and *RrCesA5* were significantly correlated with  
746 the contents of cellulose, hemicellulose, and pectin in fruit of *R. roxburghii*. In Arabidopsis, *CesA1* and  
747 *CesA3* are necessary for the synthesis of primary wall cellulose, while *CesA2* and *CesA5* have some  
748 functional redundancy to *CesA6* (Song et al. 2018). In apple fruit, there are no *CesA2* or *CesA5* genes, but  
749 only 3 *CesA6* genes (Guerriero et al. 2014). Contrastingly, here we did not find any *CesA6s* in the fruit of  
750 *R. roxburghii*, but we found *CesA2* and *CesA5*. This might be because *R. roxburghii* is a high-fiber fruit  
751 compared to apple, and the high content of cellulose requires more *CesAs* to work synergistically. In this  
752 study, the cellulose content decreased through maturation, which is consistent with research on apple  
753 (Guerriero et al. 2014). Therefore, *CesA1*, *CesA2*, *CesA5*, and *Cx* may play an important role in cellulose  
754 accumulation. In Arabidopsis, soybean (*Glycine max*), and other dicotyledonous plants and citrus fruits,  
755 the expression of *CesA* positively correlated with cellulose content (Nawaz et al. 2019; Li et al. 2016).  
756 Furthermore, previous studies have shown that shading reduced the cellulose content in cotton (Chen et al.  
757 2014), but this is inconsistent with our results. This difference may be linked to the abundance of  
758 trichomes on the surface of *R. roxburghii*, which are not present in Cotton (Wang et al. 2019). In this  
759 study, *CesA1*, *CesA2*, and *CesA3* appear to play an important role in the accumulation of cellulose under  
760 shading treatment. Research by Joshi (2003) identified all the conserved features of typical plant *CesA*  
761 proteins, namely a zinc-binding domain, eight transmembrane domains, two hypervariable regions, and  
762 processive glycosyltransferases' signature motif D-D-D-QXXRW. Furthermore, the first of these two  
763 hypervariable regions, HVR1, of *CesA5* was shown to have low homology with *CesA1* and *CesA2*  
764 (Kalluri et al. 2003). Therefore, we infer that these differences may have led to the apparent  
765 responsiveness of *CesA1*, *CesA2*, and *CesA3* expression to shading.

766 Hemicellulose content decreased in fruit throughout maturation, and the expression of *IRX* and *ARAD2*  
767 strongly correlated with this change. In Arabidopsis, *IRX* is involved in the synthesis of the xylan  
768 backbone and is largely expressed during the synthesis of secondary cell walls (Ren et al. 2014). Studies  
769 in tobacco (*Nicotiana tabacum*) and other plants also show that *IRX* is involved in the synthesis of the  
770 xylan backbone (Lee et al. 2012; Pauly et al. 2010). Therefore, it can be inferred that xylan is the main  
771 component of hemicellulose in *R. roxburghii*, which is consistent with the results of studies in  
772 Arabidopsis and tobacco. The effect of shading on hemicellulose accumulation has rarely been reported.  
773 Here, shading had no obvious effect on the accumulation of hemicellulose, which is consistent with our  
774 previous study (Zhang et al. 2020). However, our expression analysis has further demonstrated that *IRX*  
775 likely plays an important role in hemicellulose accumulation under shading.

776 Lignin can bind with cellulose to increase the rigidity and strength of plant cell walls. Changes in its  
777 content can change the physical properties of plant tissues and affect the development of tissue structure,  
778 for instance, to confer lodging or disease resistance to crops (Ralph et al. 2004). Moreover, previous  
779 studies have shown that there is a high lignin content in *R. roxburghii* fruit, especially in the epidermal  
780 prickles, which are modified trichomes (Lu et al. 2020). Lignin mainly accumulates in the prickly skin  
781 part of the *R. roxburghii* fruit (Liu et al. 2015a). In our study, during the development of *R. roxburghii*  
782 fruit, lignin content first increased and then decreased, which is consistent with the change in leaves of  
783 tobacco, corn (*Zea mays*), and other plants during maturation (Yu et al. 2013). Correlation analysis  
784 showed that *4CLI*, *4CL2*, *HCT*, *C3'H*, *CCoAOMT*, *COMT1*, *CAD2*, and *CCRI* play important regulatory  
785 roles in lignin synthesis (Tomotaka et al. 2016), and their expression levels are closely related to the  
786 lignin content (Lu et al. 2020). POD is one of the key enzymes of enzymatic browning (Oliveira et al.  
787 2016), which can reduce H<sub>2</sub>O<sub>2</sub> in cells to H<sub>2</sub>O and remove free radicals in fruits. Therefore, a decline in  
788 POD activity accelerates the senescence of fruits (Han et al. 2017), which is consistent with our findings.  
789 4CL is a key enzyme in the biosynthesis of phenylpropane derivatives, such as lignin and flavonoids.  
790 Different species of plants contain varying numbers of lignin biosynthetic gene families, and there is also  
791 a degree of variation in their structure and function (Meng et al. 2017). Some studies have shown that the  
792 evolutionary relationship of 4CL is generally divided into class I and class II, involved in the synthesis of  
793 lignin and flavonoids, respectively (Yuan et al. 2014). In *japonica* rice, under shade conditions, *OsPAL*,  
794 *OsCOMT*, *OsCcoAMT*, *OsCCR*, and *OsCAD2* expression decreased significantly (Wu et al. 2017). Here,  
795 the expression of *CCRI*, *POD2*, *POD3*, *POD4*, *POD5*, *HCT*, *4CLI*, *4CL2*, and *4CL3* also decreased  
796 significantly; the possible reason may be that there is a functional element ERF related to stress or GT-1  
797 motif, ASF-1 motif, GATA-box, or I-box elements associated with light regulation in the promoter  
798 sequence (Hu et al. 2020).

799 Pectin is produced in the cell wall in a highly methylesterified arrangement and *PME* genes subsequently  
800 de-esterify it (Zega et al. 2016). PMEs may also influence the extent to which demethylated  
801 polygalacturonans are available by PGs for degradation, releasing galacturonic acid or oligogalacturonate,  
802 and the availability of carboxylic groups of homogalacturonan for calcium (Ca<sup>2+</sup>) binding, leading to the  
803 formation of supramolecular assemblies and gels. These gels are believed to affect the mechanical  
804 characteristics of the cell wall, increasing firmness (Wang et al. 2018). In most fruits, *PME* is expressed  
805 before ripening and has a minor role in fruit softening, but it does affect the integrity of tissues (Kalia et al.  
806 2015). *PME* expression increases during fruit development, which is consistent with the findings in  
807 pomelo (*Citrus maxima* (Burm.) Merr) (Liu et al. 2015b). Galacturontransferase genes *GAUT1*, *GAUT2*,  
808 and *GAUT5* initially increased in expression before decreasing towards the end of fruit maturation,

809 indicating that these genes were highly expressed during the fruit expansion process, in which it was  
810 speculated to affect fiber length. *GAUT3* and *GAUT4* decreased in expression throughout fruit  
811 development, and these genes may play a role in the initial stage of fiber development. In melon, low  
812 light intensity decreased the pectin content (Toshiyuki et al. 2006). Wei (2015) researched shading  
813 treatment in jujube (*Ziziphus jujube*) cv. Jun zao, and found that shading increased the pectin content and  
814 improved the taste of fruit. Here, at the mature fruit stage, under 50% and 100% shading the soluble  
815 pectin content increased by 16.39% and 18.79%, respectively, while only 100% shading promoted the  
816 accumulation of total pectin and insoluble pectin. Analysis of the expression of related genes showed that  
817 *GAUT3* correlates with pectin accumulation under shading, suggesting that the *GAUT3* promoter  
818 sequence has a GT-1 motif, ASF-1 motif, GATA-box, or I-box elements associated with light regulation  
819 (Gangappa et al. 2013). In this study, the increase in soluble pectin content was much greater than that of  
820 cellulose or lignin under 50% shading. The high expression of *PME* may transform large amounts of  
821 insoluble pectin into soluble pectin, thereby improving the taste of the fruit. Although soluble pectin  
822 levels also increased under 100% shade, it was accompanied by a marked increase in the content of lignin,  
823 cellulose, and insoluble pectin, and as such, would not improve the taste of the fruit.

824 Light intensity significantly affects fruit color. Reduced light intensity promotes the degradation of  
825 chlorophyll, thus expediting the color transformation of fruit (Bárcena et al. 2020). Some studies,  
826 however, have shown that shading can deleteriously affect the color of the fruit by altering the ratio of  
827 carotenoids through changing gene expression (Chen et al. 2017). Here, the fruit color became paler in the  
828 earlier stages of fruit development, then developed orange and yellow hues in the middle and late stages  
829 of fruit development. The pale color early in development is likely attributed to reduced chlorophyll  
830 levels, and it is hypothesized that in the later stages, shading treatment may promote the accumulation of  
831 carotenoids and flavonoids, resulting in the orange color of the fruit. Studies have shown that under shade  
832 conditions, the low far-red ratio inhibited the synthesis of plant pigments and increased the activity of  
833 gibberellin (Gommers et al. 2013). While increased gibberellin activity may have been expected to  
834 promote heavier fruits, in our study, shade treatments reduced fruit weight, which may have been a result  
835 of reduced photosynthetic capacity. Future work to determine the content of flavonoids and carotenoids in  
836 fruit after shading treatment would further elucidate the causes of fruit color change in response to  
837 shading.

## 838 **5 Conclusion**

839 Short-read Illumina and long-read SMRT sequencing were used in conjunction to construct a  
840 transcriptome of *R. roxburghii* during fruit development. Using functional enrichment and KEGG

841 analysis of DEGs, we identified candidate genes involved in dietary fiber metabolism, including *CesA1*,  
842 *CesA2*, *CesA3*, *CesA5*, *IRX*, *ARAD2*, *GAUT*, *Cx*, and *PME*. To our knowledge, this is the first time that  
843 *CesA2* and *CesA5* have been identified from the edible fruit. The accumulation of cellulose, lignin, and  
844 pectin was increased by shading treatment at the maturation stage of fruit development. Analysis of the  
845 expression of related genes showed that *CesA1*, *CesA2*, *CesA3*, *IRX*, *ARAD2*, *GAUT3*, and *CAD* play an  
846 important role in dietary fiber metabolism with shading treatment. Light is an important environmental  
847 factor affecting the accumulation of dietary fiber in *R. roxburghii* fruit, with 50% shading offering a  
848 potential to redress the imbalance between soluble pectin and lignin and thus improve fruit quality. These  
849 results provide a basis for understanding the molecular mechanisms of dietary fiber metabolism in *R.*  
850 *roxburghii* fruit and provide a framework for future crop improvement.

851 **Table S1.** Primer sequences for qRT-PCR analysis

Gene ID	Gene name	Primer sequence 5'-3'	
3-6k.c21066/11/3801	<i>CesA1</i>	TTGCCTGTAATGAGTGTGCCTTCC	TTGCGGACAAGCCTGGTTGC
3-6k.c17146/1/4436	<i>CesA2</i>	TGGTGAAGCACGAAGGAGGAATTG	AATAGGCCAAGAACC GAAGACG
2-3k.c53599/4/2184	<i>CesA3</i>	AGTGGTGTGGAATTGACGAGTGG	CCTTGGAGGTGACAGTGAAGTTGG
1-2k.c16341/1/1287	<i>CesA5</i>	GTCTGCCTGCCATCTGTCTTCTG	TGCGATGAAGCACCTCCAATTACC
2-3k.c56230/7/2136	<i>Cx</i>	TGGTCGGTAGCAGAAGGATGAGG	TTGGTCTTACGTCTCACTGTTGGC
1-2k.c25067/1/1220	<i>ARAD1</i>	GAACACTGAGGCTTGAAGAGGTC	TCTGACATGCCACATTGCGACTG
2-3k.c54088/9/2163	<i>ARAD2</i>	ACAAGAGGTTGCAGGACAAGTTGG	GCAAGCACGAACATGGCAGAAC
1-2k.c22164/1/1427	<i>IRX</i>	TGCTGCCTTGGTGTGGAGATTG	CCAAGACATCACCAGCACTACCTG
2-3k.c28853/1/2146	<i>CCR1</i>	TCCGCCTTCTCTCTCCAGTTC	GCCTCGTTCTAAGCAGCAAGACTC
1-2k.c5667/2/1269	<i>CCR2</i>	CATCGATCAGCGACCCACAG	AACTGCCCCATGGAAGATG
1-2k.c12160/5/1395	<i>CCR3</i>	TCTGTGCAAGTTCAAGCTATGGC	GCACGCCGTCTTACATATCTCC
1-2k.c54650/6/1411	<i>CCR4</i>	TGACTCGGAAGAGAAGCTCGTCTG	CGGAGGAGGAGGCGGTTGAC
3-6k.c9867/1/4805.2	<i>HCT</i>	ATCCTGATGACGCTGCTGAAGTTC	CAGAGCCGTAGCAACAGCCTTAG
1-2k.c17541/21/1413	<i>COMT1</i>	GCTGACCCTCCACCATTACCATG	GTGCCGCTCCGACATCAAC
1-2k.c10057/3/1300	<i>COMT2</i>	GCCATAGAACTTGGTGTGCTCGAC	GCAGACAGAAGGCGAAGCATAACG
1-2k.c44996/1/1611	<i>CAD</i>	TCGTTGGTGAAGCGACATTGG	AGCCAAGACTACTGAGACGAGGAG
1-2k.c9880/6/1245	<i>POD1</i>	CTCTCCTTCGCCTTCACTTCCATG	GACCTGTGTCTTCTCACCAGTG
1-2k.c15601/2/1502	<i>POD2</i>	GAGCAATGAGGAAGAGCCAGGTC	TGGACAGCACAGTCATGGAAGATG
1-2k.c54417/2/1276	<i>POD3</i>	TCTGCTCAGCTTAGGACGACTTC	GAAGAGTCGAAGAGTGGCTGGAAC
1-2k.c51022/1/1295	<i>POD4</i>	TTCTTGGCGTGCTTCTACTGTTGG	GCCTGTGCTAAGGTTGGATCAG
1-2k.c54627/3/1218	<i>POD5</i>	GCTTCATGTCCTGGTGTGGTCTC	AGGTTGGTGCTGTAATTGTCTGG
1-2k.c32151/2/1389	<i>POD6</i>	AGCAGGTTCCGGTAGTCGGATCG	TTCGGACACTGTTGTTGCAGCTC
1-2k.c25768/1/1004	<i>CcoAOMT</i>	ATCTTCGTTGACGCAGACAAGGAC	GTGCCACCACAGAGCCGTTT
1-2k.c47100/1/1669	<i>C3'H</i>	AAGTTCAGCAAGGACGGTCAAGAC	TGGCGGTGACCTCGTCTTCC
2-3k.c26005/8/1941	<i>4CL1</i>	CGAGCGTGTCTCAGCAGGTTG	CCACATAGCAAGACCGAGTTCAGC
2-3k.c2539/43/2037	<i>4CL2</i>	GCCACGTCATCTCTCTCTCTC	CCGAGTTGTGAAGCGGAGAACG
2-3k.c12242/4/1959	<i>4CL3</i>	TATGTTACGCTGCCGCTGTTT	CGACTTGGTCAGAGCCACAATCAG
2-3k.c45874/2/1923	<i>PME</i>	TCTCAGCCGAATCCGTAACAATGC	CAGGCGACGCTCAAGGAAGTTC
1-2k.c21559/1/1082	<i>GAUT1</i>	CATGGCTGGAGTTGGCGATGAC	AGGAGCAGGTGAACGAGACAGAG



2-3k.c26947/2/2489	<i>GAUT2</i>	TGATGGTGAGGAATGTGGTGATGC	AAGAGGAGGAGGAGGAGGAGGAG
1-2k.c54278/1/1356	<i>GAUT3</i>	CAGGTGTGATGGTGATGGACTTGG	AGCCTCTACATCTCCACCGAAGAC
2-3k.c50854/1/2501	<i>GAUT4</i>	CTGCCGCTGCCTGTTCTTCC	TCCTGGACAAGCTGGTTGAATGAC
2-3k.c28445/1/4160	<i>GAUT5</i>	TCCTGGACAAGCTGGTTGAATGAC	GGAGTGACGCATCAGTTCTCAGAG
	<i>UBQ</i>	ATGCAGATTTTGTGAAGAC	ACCACCACGRAGACGGAG

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**Table S2.** Differentially expressed genes possibly related to dietary fiber metabolism

gene ID	Annotation	correlation coefficient				cluster
		Total dietary fiber	Cellulose	hemicellulose	Total pectin	
3-6k.c21066/11/3801	<i>CesA1</i>	0.99993	0.98690	0.99960	0.96962	7
3-6k.c17146/1/4436	<i>CesA2</i>	0.98556	0.94913	0.99158	0.99606	5
2-3k.c13506/4/2290	<i>CesA2</i>	0.96868	0.92063	0.97790	0.99997	5
2-3k.c53599/4/2184	<i>CesA3</i>	0.99845	0.97890	0.99988	0.97939	13
3-6k.c2764/2/3959	<i>CesA3</i>	0.96941	0.92178	0.97851	0.99994	5
3-6k.c6339/1/3496	<i>CesA3</i>	0.93802	0.87561	0.95121	0.99550	13
1-2k.c16341/1/1287	<i>CesA5</i>	0.98114	0.94118	0.98813	0.99792	5
1-2k.c22164/1/1427	<i>IRX</i>	0.97192	0.92578	0.98061	0.99977	5
1-2k.c25067/1/1220	<i>ARAD</i>	0.93746	0.87485	0.95072	0.99534	13
2-3k.c54088/9/2163	<i>ARAD</i>	0.90395	0.82981	0.92044	0.98332	13
1-2k.c21559/1/1082	<i>GAUT</i>	0.96161	0.90974	0.97189	0.99982	13
2-3k.c26947/2/2489	<i>GAUT</i>	0.93202	0.86732	0.94586	0.99375	13
1-2k.c54278/1/1356	<i>GAUT</i>	0.92691	0.86034	0.94128	0.99211	5
2-3k.c2467/1/2261	<i>GAUT</i>	0.91507	0.84444	0.93057	0.98784	13
2-3k.c50854/1/2501	<i>GAUT</i>	0.89836	0.82254	0.91533	0.98090	13
2-3k.c28445/1/4160	<i>GAUT</i>	0.86279	0.77745	0.88245	0.96349	13
2-3k.c56230/7/2136	<i>Cx</i>	-0.99605	-0.97155	-0.99882	-0.98558	11
2-3k.c45874/2/1923	<i>PME</i>	-0.87641	-0.79451	-0.89509	-0.97051	4

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**Author Contributions:** This study was conceived by X.Z., M.L., and H.A. The plant material preparations were carried out by X.Z. X.Z., M.L., and W.M. performed the laboratory experiments and analyses. Z.X. and M.L. drafted the manuscript. M.L., H.A., and R.L. revised the manuscript. All authors read and approved the final manuscript.

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867 **Research involving human participants and/or animals:** This article does not contain any studies with  
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**Table 1.** The correlations between cellulose, hemicellulose, lignin, and pectin content and relative gene expression levels in fruit during fruit development in *R. roxburghii*.

	Cellulose	Hemicellulose	Total Pectin	insoluble pectin	Soluble pectin	Lignin
<i>CesA1</i>	0.602**					
<i>CesA2</i>	0.534**					
<i>CesA3</i>	0.427*					
<i>CesA5</i>	0.194					
<i>Cx</i>	-0.345					
<i>IRX</i>		0.758**				
<i>ARAD1</i>		-0.026				
<i>ARAD2</i>		0.641**				
<i>PME</i>			-0.266	-0.304	-0.091	
<i>GAUT1</i>			0.085	0.058	0.182	
<i>GAUT2</i>			0.061	0.064	0.041	
<i>GAUT3</i>			0.379*	0.388*	0.310	
<i>GAUT4</i>			0.115	0.125	0.068	
<i>GAUT5</i>			-0.015	-0.005	-0.053	
<i>CCR1</i>						-0.041
<i>CCR2</i>						0.225
<i>CCR3</i>						-0.085
<i>CCR4</i>						-0.204
<i>HCT</i>						-0.152
<i>COMT1</i>						-0.202
<i>COMT2</i>						-0.186
<i>POD1</i>						-0.349*
<i>POD2</i>						-0.354*
<i>POD3</i>						-0.406*
<i>POD4</i>						-0.209*
<i>POD5</i>						-0.338*
<i>POD6</i>						0.088
<i>CcoAOMT</i>						-0.332*
<i>C3'H</i>						-0.147
<i>4CL1</i>						-0.415
<i>4CL2</i>						-0.419*
<i>4CL3</i>						-0.499*
<i>CAD</i>						0.608**

1033 \*\* Significant correlation at the 0.01 level (bilateral), \* Significant correlation at the 0.05 level (bilateral). *CesA*: cellulose  
1034 synthase; *Cx*: hydrolase-cellulase; *IRX*:  $\alpha$ -1,4-xylosyltransferase; *ARAD*: arabinosyltransferase; *PME*: pectin methylesterase;  
1035 *GAUT*: galacturonosyltransferase; *CCR*: cinnamoyl-CoA reductase; *HCT*: O-hydroxycinnamoyltransferase; *COMT*: caffeic acid  
1036 Omethyltransferase; *POD*: peroxidase; *CcoAOMT*: caffeoyl-CoA O-methyltransferase; *C3'H*: coumaroylquininate  
1037 (coumaroylshikimate) 3'-monoxygenase; *4CL*: 4-coumaroyl/CoAligase.

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1040 **FIG LEGENDS:**

1041 **Fig 1.** Effect of shading on the appearance and morphology of fruit of *R. roxburghii* at different  
1042 developmental periods. White bar = 2 cm.

1043 **Fig 2.** Effects of shading on the accumulation of dietary fiber components in *R. roxburghii* fruit. All  
1044 experiments were conducted in triplicate. Values represent mean  $\pm$  standard deviation, and the error bars  
1045 are standard deviations. Different letters (a-i) in the same column indicate significant differences at  $p <$   
1046 0.05 determined by ANOVA. In a, b, and c, % represents the proportion of each component content in dry  
1047 weight, while in d, e, and f, % represents the proportion of each component content in fresh weight.

1048 **Fig 3.** Cluster analysis of the differentially expressed genes (DEGs) in each comparison. DEGs were  
1049 categorized into 18 clusters depending on their expression during fruit growth and maturation.

1050 **Fig 4.** Heat map depicting the expression profile of dietary fiber metabolism-related genes in *R.*  
1051 *roxburghii* during fruit development. The values used for heat map construction were the mean from the  
1052 transcriptome data of triplicate experiments. The gene name and corresponding candidate gene are  
1053 presented on the right side of the heat map. The expression level is represented by a color scale ranging  
1054 from saturated green for RPKM = 0 to saturated red for RPKM = 23.67.

1055 **Fig 5.** Heatmap of the 33 identified differentially expressed genes in the 50% shading (B) and 100%  
1056 shading treatments (C) compared to the control (A) in *R. roxburghii* during fruit development.

1057 **Fig S1.** Expression analysis of 9 differentially expressed genes related to dietary fiber metabolism in *R.*  
1058 *roxburghii* during fruit development. *UBQ* was used as the internal control. The error bars represent the  
1059 standard error of three biological replicates. The numbers above the graphics correspond to values  
1060 obtained with the Pearson correlation. Pearson correlation between the RNA-seq data and qRT-PCR data  
1061 was calculated using the value of FPKM and the relative expression level.