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Comparative proteome and transcriptome analyses suggest the regulation of starch and sucrose metabolism and rubber biosynthesis pathways in the recovery of tapping panel dryness in rubber tree

Kun Yuan[1], Chengtian Feng[1], Qiguang He[1], Yiyu Hu[1], Hui Liu[1*] and Zhenhui Wang[1*]

Abstract

Background Tapping panel dryness (TPD) in rubber tree has become the most severe restricting factor of natural rubber production. To date, there is no effective measures to prevent and control TPD. Previous studies primarily focused on analyzing the molecular mechanism underlying TPD occurrence. However, there is no research on the molecular mechanism of TPD recovery.

Results In this study, the TPD trees were recovered by treatment with TPD rehabilitation nutrient agents that could promote the recovery of latex flow on the tapping panel of TPD trees. The genes and proteins involved in TPD recovery were first identified by employing integrated transcriptomics and proteomics analyses. In total, 2029 differentially expressed genes (DEGs) and 951 differentially expressed proteins (DEPs) were detected in the bark of recovery trees compared to that of TPD trees. Among them, 19 DEPs and 11 DEGs were found to be involved in the starch and sucrose metabolism pathway, suggesting their important roles in regulating the syntheses of sucrose and D-glucose, which were the key precursors of natural rubber biosynthesis. Furthermore, 16 DEPs and 15 DEGs were identified in the rubber biosynthesis pathway. Interestingly, almost all the DEPs and DEGs related to rubber biosynthesis exhibited significantly up-regulated expressions in the recovery trees, indicating that latex biosynthesis were probably markedly enhanced during TPD recovery.

Conclusions These results provide new insights into the molecular mechanisms underlying TPD recovery, as well as excellent supplements to the mechanisms of TPD occurrence, which will contribute to the development of more effective agents for the prevention and treatment of TPD in the future.

Keywords Rubber tree, Tapping panel dryness (TPD), TPD recovery, Starch and sucrose metabolism, Rubber biosynthesis

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Background

Tapping panel dryness (TPD) is a key factor restricting latex production in rubber tree (*Hevea brasiliensis* Muell. Arg.), which is the main source of natural rubber [1–2]. The TPD symptom displays a partial or complete stoppage of latex flow on the tapping panel of the rubber tree trunk [3]. Generally, TPD is recognized as a complicated physiological disorder that is caused by excessive tapping or overstimulation with ethephon [4–5]. Although a large number of researches on TPD have been carried out, the mechanism underlying TPD remains unclear.

So far, many works have been focused on studying the molecular mechanisms of TPD occurrence and lots of genes and noncoding RNAs (ncRNAs) related to TPD occurrence have been identified by transcriptomic sequencing analysis [6-10]. Liu et al. [6] identified 22,577 differentially expressed genes (DEGs) between the healthy and TPD-affected rubber tree barks by comparative transcriptome analysis and proposed that the significant down-regulation of the genes involved in the rubber biosynthesis and jasmonate pathways was possibly the cause of TPD occurrence. Yue et al. [10] identified 8607 DEGs in the bark of TPD trees compared with that of healthy ones and the DEGs were involved in the terpenoid backbone biosynthesis, carbon metabolism, starch and sucrose metabolism, plant hormone signal transduction, MAPK signaling pathway, etc. Moreover, the whole transcriptome analyses of latex and bark tissues from healthy and TPD trees were carried out, and the competitive endogenous RNA (ceRNA) regulatory networks of lncRNA-miRNA-mRNA associated with TPD were constructed [8–9]. Recently, the proteins related to TPD were identified by proteomics technology [11–12]. Using iTRAQ (isobaric tags for relative and absolute quantification)-based quantitative proteomic analysis, 272 differentially expressed proteins (DEPs) were identified in the latex of TPD trees, which were significantly enriched in 16 KEGG pathways associated with the carbohydrate metabolism (ko00010, ko00020, and ko01200), nucleotide/aminoacid metabolism (ko00240, ko00270, ko00350, and ko00460), lipid metabolism (ko00062, ko00564, ko00565, and ko00590), energy metabolism (ko00010, ko00710, and ko01200), programmed cell death (ko03050 and ko04141), ROS scavenging (ko00270 and ko00480), and signal transduction/information processing (ko03050, ko04141, and ko04144) [11]. Additionally, through tandem mass tag (TMT)-based quantitative proteomics, 671 DEPs were identified in the bark of TPD trees. It was found that plant hormone signaling and rubber biosynthesis pathways might play crucial roles in TPD occurrence [12]. Although numerous genes and proteins related to TPD have been identified, only a small number of them have been functionally characterized, such as *HbMyb1*, *HbMC1*, and *HbFPS1* [11, 13–14]. It was reported that the expression of *HbMyb1* was largely decreased in the bark of TPD-affected rubber trees [13], and overexpression of *HbMyb1* in tobacco significantly suppressed cell death-induced by different stresses [15]. Liu et al. [14] identified a TPD-related gene *HbMC1* and found that *HbMC1* expression was positively correlated with the severity of TPD in rubber tree. Further analysis showed that overexpressing *HbMC1* in tobacco enhanced cell death under oxidative stress [14].

TPD-affected trees can sometimes be cured by scraping the bark or applying chemicals to promote bark renewal [16–17]. Dynamic analysis of TPD in *Hevea brasiliensis* reveals new insights on this physiological syndrome affecting latex production. In our previous study, it was demonstrated that 1-methylcyclopropene could promote the recovery of latex flow of TPD trees caused by a high concentration ethephon stimulation [16]. During the recovery process of TPD, the genes and enzymes associated with reactive oxygen species (ROS) scavenging underwent significant changes, suggesting their important functions in promoting TPD recovery [16]. In addition, the latex thiol content, sucrose content, inorganic phosphorus content, and lutoid bursting index gradually returned to the normal levels during the recovery process of TPD trees treated with the rehabilitation nutrient agents [17]. TPD recovery is the reverse process of TPD occurrence. Exploring the molecular basis of TPD recovery is an important supplement to TPD occurrence mechanism and will provide a theoretical basis for the development of effective agents for preventing and treating TPD. After years of researches, we have gained a certain understanding of the molecular mechanism of TPD. However, the molecular mechanisms underlying the recovery of TPD are currently unknown. In the current study, the genes and proteins associated with TPD recovery were identified by RNA-sequencing and TMT-based quantitative proteomics. Our Results indicated that the starch and sucrose metabolism and rubber biosynthesis pathways have potentially important roles in TPD recovery in rubber tree.

Methods

Plant materials

The *Hevea brasiliensis* clone Reyan 73397, was planted at the experimental field of the Chinese Academy of Tropical Agricultural Sciences in Danzhou of Hainan (China) and selected as the experimental materials in this study. The rubber trees had been regularly tapped for eight years. The TPD-affected trees displaying incomplete latex flow (Fig. S1a), with the dry cut length (DCL) on the tapping panel being 60–80% of total cut length (TCL), were selected and divided into two groups. One group was regarded as the TPD group without any treatments. The other group of TPD trees were treated by

the TPD rehabilitation nutrient agents developed by our team according to the method as described by Liu et al. [17]. The TPD rehabilitation nutrient agents consists of the colloidal and aqueous agents. The colloidal agents were evenly applied to the tapping panel of TPD trees by using a brush once every 10 days for two consecutive months. Meanwhile, the aqueous agents were diluted and the diluent was sprayed onto the trunk 1.8 m below by using a sprayer once every seven days for five consecutive months. These agents can partially or even fully promote the recovery of latex flow on the tapping panel of TPD trees. Eventually, the trees with the ratio of the restored latex flow length to DCL above 70% were considered as the recovery ones (Fig. S1b). Finally, 15 TPD recovery trees, 15 TPD trees, and 15 healthy trees were selected. Before sample collection, the latex yield of each individual tree was measured. After tapping, the barks from the tapping panel of each TPD recovery tree was harvested, and equal amounts of barks from five trees were pooled to form one biological replicate. Three biological replicates were performed, labeled as RB_1, RB_2, and RB_3. The bark samples from TPD trees (designated as TB) and healthy trees (designated as HB) were identical to those used in our previous research [12], which were collected simultaneously with the RB samples.

TMT-based quantitative proteomic analysis

Each bark sample was thoroughly ground into powder with the liquid nitrogen. Bark proteins were extracted according to the method of Niu et al. [18], followed by trypsin digestion and TMT analysis. Peptides were labelled with TMT tags as follows: RB_1 (132 N), RB_2 (133 N), RB_3 (134 N), TB_1 (129 N), TB_2 (130 N), and TB_3 (131 C) according to the manufacturer's protocol. Fractionation of the labeled peptides was conducted using a C18 column (Waters BEH C18, 4.6 × 250 mm, 5 μm) on a Rigol L3000 HPLC system, the column oven was set as 45 °C. And then the collected fractions were analyzed using a Q Exactive™ HF-X mass spectrometer (Thermo Fisher Scientific) [19]. The software of Proteome Discoverer 2.2 (Thermo Fisher Scientific) was applied to analyze MS/MS spectra. The obtained peptides were further subjected to searching against the reference genome of rubber tree (accession no. PRJNA394253). The searched parameters were set as follows: mass tolerance for precursor ion was 10 ppm and mass tolerance for product ion was 0.02 Da. Carbamidomethyl was specified as fixed modifications, Oxidation of methionine (M) and TMT plex were specified as dynamic modification, acetylation and TMT plex were specified as N-Terminal modification. A maximum of two miscleavage sites were allowed. The DEPs between RB and TB were identified using the criteria of *P*-value < 0.05 and FC < 0.83 (significantly down-regulated) or FC > 1.2 (significantly up-regulated).

Transcriptome analysis

The total RNA of each bark sample was isolated using the plant RNA kit (QIAGEN, Germany) according to the manufacturer's manual. The integrity of RNA samples was examined by a Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The complementary DNA (cDNA) library of RNA-seq was constructed according to the previous method [20], and sequencing analysis was performed on an Illumina NovaSeq platform (Illumina, USA). The cleaned reads were mapped to the rubber tree reference genome (accession no. PRJNA394253) by Hisat2 (v2.0.5). The read numbers mapped to each gene were counted by the software of FeatureCounts (v1.5.0-p3). The gene expression was quantified via the FPKM (expected number of fragments per kilobase of transcript sequence per millions of base pairs sequenced) method [21]. The DESeq2 (v1.20.0) software was employed to identify the DEGs between RB and TB according to the criteria of false discovery rate (FDR) < 0.05 and the absolute value of $\log_2$ fold change (FC) > 1 ($|\log_2$ RB / TB$|$ > 1).

Functional analysis of DEPs and DEGs

GO (Gene Ontology) annotation of the identified DEPs and DEGs was carried out by the ClusterProfiler R package. KEGG (kyoto encyclopedia of genes and genomes) pathway enrichment analysis of the DEPs and DEGs was performed using the KEGG Orthology. The significantly enriched GO terms or KEGG pathways were defined as those having a *P*-value < 0.05.

qRT-PCR validation

A total of 20 DEGs were selected for quantitative real-time PCR (qRT-PCR) to validate the results of RNA-seq. The software of Primer Premier 5.0 was used to design specific primers of the selected genes (Table S8). The *UBC4* gene from the rubber tree (NCBI Reference Sequence: HQ323249.1) was used as the internal reference gene for normalization [8]. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, in which Ct denotes cycle threshold. $\Delta\Delta Ct = (Ct_{Target\ gene} - Ct_{UBC4})_{Target\ group} - (Ct_{Target\ gene} - Ct_{UBC4})_{TB\ group}$. Subsequently, the gene expression fold change of RB/TB was log2-transformed for comparison with data obtained from RNA-seq.

Results

Identification of DEPs involved in TPD recovery

To uncover the molecular mechanism underlying the recovery of TPD in rubber tree, the TPD rehabilitation nutrient agents were used to treat TPD-affected trees to promote their recovery. Subsequently, the trees with

better recovery were selected. Meanwhile, TPD trees with symptoms similar to those prior to treatment were selected from the TPD group. Compared with the TPD trees, the latex yields of TPD recovery trees increased significantly and were nearly at the healthy levels (Fig. S2), suggesting that the TPD rehabilitation nutrient agents promoted the recovery of latex flow on the tapping panel of TPD trees. The barks of TPD recovery trees (recovered barks, RB) as well as those of TPD trees (TPD barks, TB) were harvested, and the differences in protein expression between RB and TB were compared via TMT-based quantitative proteomic analysis. In total, 951 proteins showed differential expressions in the RB group compared with the TB group, containing 398 up-regulated proteins and 553 down-regulated ones (Fig. 1a, Table S1). In our previous study, 671 proteins were found to be differentially expressed in TB compared with the barks of healthy trees (healthy barks, HB), comprising 231 down-regulated proteins and 440 up-regulated ones [12]. We further analyzed the differentiation in protein expression between TPD recovery and occurrence. A total of 201 DEPs exhibited opposite expression changes during the occurrence and recovery of TPD (Fig. 1b). Among them, 108 DEPs were remarkably up-regulated in RB vs TB, but were significantly down-regulated in TB vs HB, such as peroxidase 15 (scaffold0471_737858), glucan endo-1,3-beta-glucosidase (scaffold0316_90317), farnesyl pyrophosphate synthase 1 (HbFPS1, scaffold0157_1428529), etc. The expression trends of these 108 DEPs are consistent with the changes in latex yield during the occurrence and recovery of TPD (Fig. S2), suggesting that these genes may play a positive regulatory role in latex yield. Moreover, 93 DEPs showed significant down-regulation in RB vs TB, but significant up-regulation in TB vs HB, such as inositol-3-phosphate synthase (scaffold0478_893335), 1,4-alpha-glucan-branching

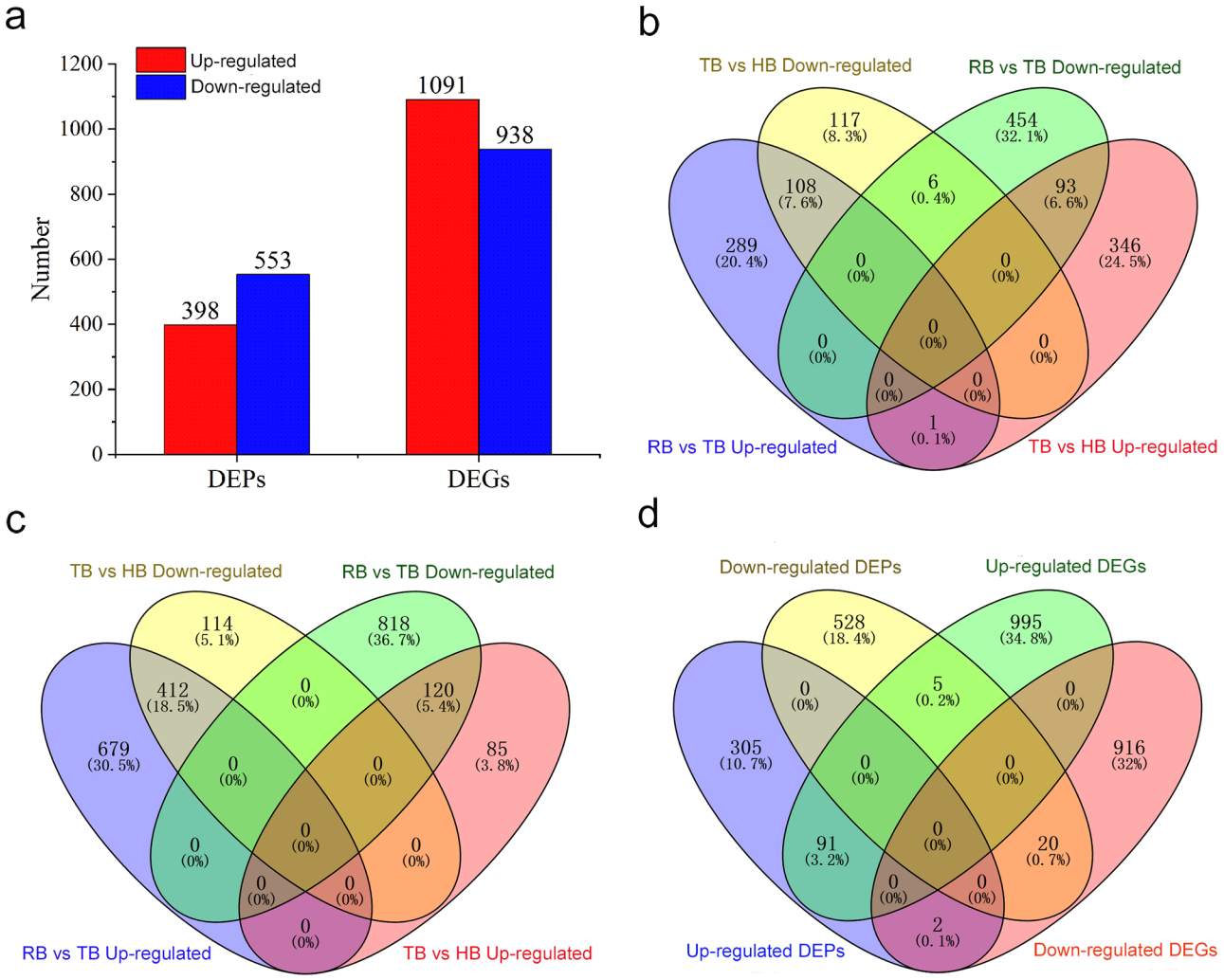

Fig. 1 DEPs and DEGs between RB and TB. (**a**) The number of DEPs and DEGs between RB and TB. (**b**) Venn diagram denotes the overlapped DEPs between TPD recovery (RB vs TB) and occurrence (TB vs HB). (**c**) Venn diagram denotes the overlapped DEGs between TPD recovery (RB vs TB) and occurrence (TB vs HB). (**d**) Venn diagram denotes up- and down-regulated shared DEPs and DEGs between RB and TB

enzyme 1 (GBE1, scaffold0073_1841869), mitochondrial Rho GTPase 1 (scaffold1192_104593), etc. The expression changes of these 93 DEPs display an opposite trend to the changes in latex yield during TPD occurrence and recovery (Fig. S2), implying that these DEPs may negatively regulate latex yield. In addition, only 7 DEPs had the same expression trends during the occurrence and recovery of TPD. These DEPs that exhibited opposite expression changes during the occurrence and recovery of TPD might play critical roles in regulating TPD.

Identification of DEGs involved in TPD recovery

In total, 254,644,190 quality reads were obtained from RB and TB cDNA libraries. Of them, 238,376,531 reads were mapped to the rubber tree reference genome. In all, 34,226 unique genes were detected in the barks of TPD and recovery trees. Subsequently, 2029 DEGs were identified in the RB group compared with the TB group, of which, 1091 and 938 genes were up-regulated and down-regulated, respectively (Fig. 1a, Table S2). Compared to the DEGs previously identified in TB vs HB [12], 532 DEGs showed opposite expression changes during the occurrence and recovery of TPD (Fig. 1c). Among them, 412 DEGs showed significant up-regulation in RB vs TB, but remarkable down-regulation in TB vs HB, such as GATA transcription factor 5 (scaffold1368_52032), rubber elongation factor protein (REF1, scaffold1222_136753), beta-amylase 1 (BAM, scaffold0588_510388), etc. The expression changes of these DEGs are similar to the trend of changes in latex yield during TPD occurrence and recovery, implying that these DEGs may positively regulate latex yield. In addition, 120 DEGs were remarkably down-regulated in RB vs TB, but were significantly up-regulated in TB vs HB, such as granule-bound starch synthase 1 (GBSS, scaffold0023_1355832), 2-oxoglutarate-dependent dioxygenase 19 (scaffold0675_91444), strigolactone esterase (scaffold0532_741373), etc. The expression trends of these 120 genes are opposite to the changes in latex yield during TPD occurrence and recovery, suggesting that these genes may play a negative regulatory role in latex yield. These DEGs with opposite expression changes during the occurrence and recovery of TPD may play key roles in regulating TPD.

To validate the RNA-seq results, 20 DEGs were selected to validate their expressions via qRT-PCR. The results showed that 17 genes had similar expression trends between qRT-PCR and RNA-Seq analyses (Fig. 2, Table S8). The above findings demonstrated that RNA-Seq analysis could effectively assess the gene expression in rubber tree.

Comparison of proteome and transcriptome data

To investigate the relevance between the proteome and transcriptome data, the 951 DEPs were compared with the 2029 DEGs. In all, 118 proteins, such as REF1 (scaffold1222_136753), caffeoyl-CoA O-methyltransferase (scaffold0635_104732), and starch synthase (scaffold0023_1355832), were found to show differential expressions at both the proteome and transcriptome levels (Fig. 1d, Table S3). Of these 118 shared DEPs and DEGs, 111 proteins showed the same expression changes at both the protein and transcript levels (91 up-regulated and 20 down-regulated), while seven ones displayed opposite expression changes at the protein and transcriptional levels. A correlation analysis of Pearson between the proteome and transcriptome was conducted. The results showed that the expression profiles of the quantified proteins had a certain correlation with their corresponding transcripts ($r = 0.349$).

GO annotations of DEPs and DEGs

The identified DEPs and DEGs were annotated by GO classification with the ClusterProfiler R package. Three categories, namely biological process (BP), cellular component (CC), and molecular function (MF), were assigned. The 951 DEPs were categorized into 301 GO terms, including 117 BP terms, 31 CC terms, and 153 MF terms (Table S4). Of them, 21 in BP, seven in CC, and 23 in MF were significantly enriched ($P < 0.05$). The top two significant BP, CC and MF terms were response to stimulus (GO:0050896) and response to stress (GO:0006950), membrane protein complex (GO:0098796) and photosystem (GO:0009521), and cation binding (GO:0043169) and metal ion binding (GO:0046872), respectively (Fig. 3a, Table S4). The 2029 DEGs were classified into 688 GO terms, including 409 BP terms, 49 CC terms and 230 MF terms (Table S4). Among them, 35 in BP, nine in CC, and 37 in MF were significantly enriched ($P < 0.05$). The top two significant GO terms for each category were response to stress (GO:0006950) and defense response (GO:0006952) in BP, cell periphery (GO:0071944) and extracellular region (GO:0005576) in CC, and transcription regulator activity (GO:0140110) and tetrapyrrole binding (GO:0046906) in MF (Fig. 3b, Table S4).

It was noteworthy that there were respectively 63, seven and 61 common GO terms under BP, CC and MF categories detected at both the proteome and transcriptome levels, such as the response to biotic stimulus (GO:0009607) and defense response (GO:0006952) in BP, the membrane protein complex (GO:0098796) and cell wall (GO:0005618) in CC, and the terpene synthase activity (GO:0010333) and actin binding (GO:0003779) in MF. Further comparison of the significantly enriched GO terms of DEPs and DEGs, we found that there were respectively four, one and five common GO terms

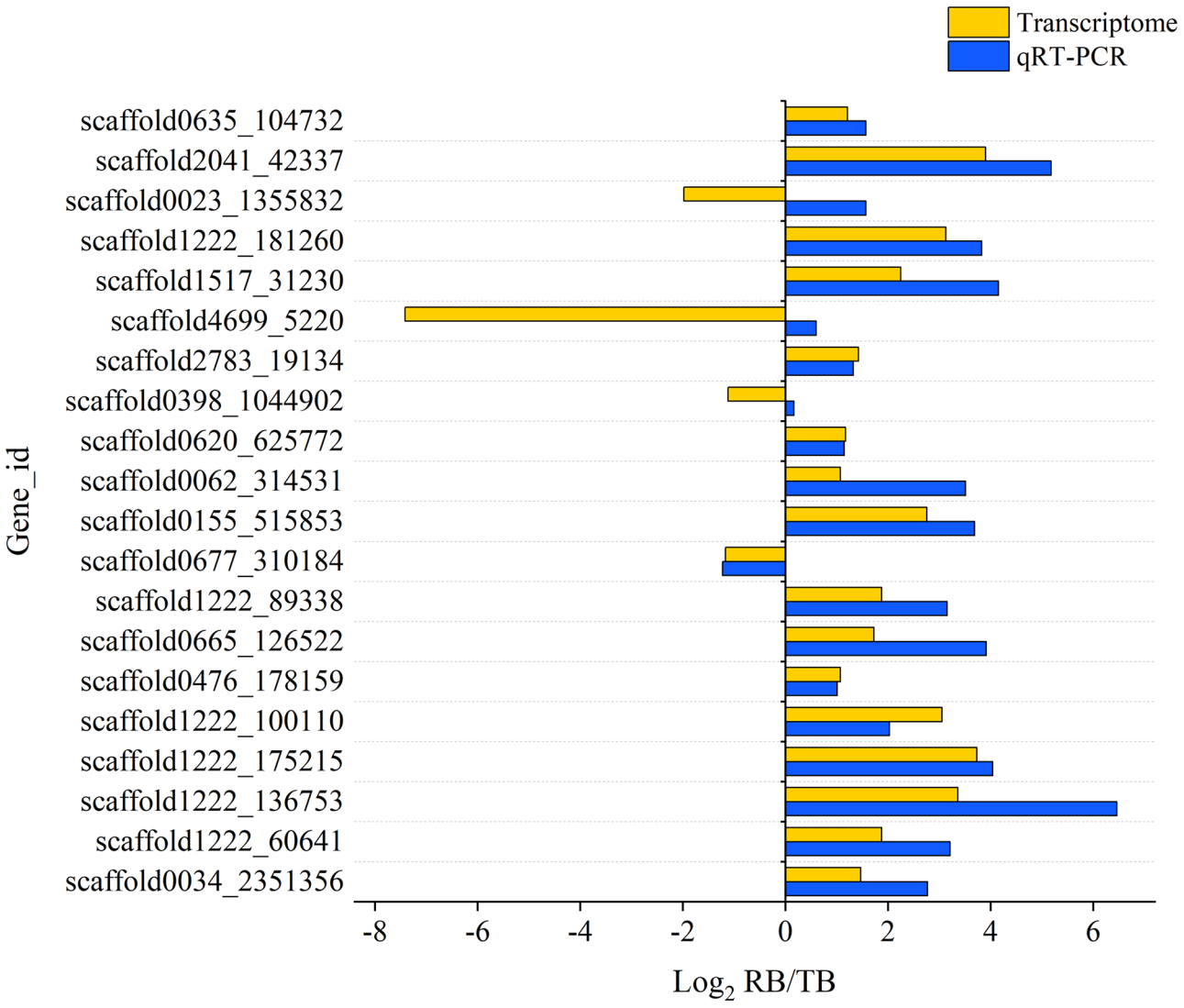

Fig. 2 Comparision of the expression changes of the 20 selected DEGs detected by qRT-PCR and transcriptome in RB/TB

in the BP, CC and MF categories at both the proteome and transcriptome levels, including the response to biotic stimulus (GO:0009607), defense response (GO:0006952), response to stress (GO:0006950) and isoprenoid biosynthetic process (GO:0008299) in BP, the cell wall (GO:0005618) in CC, and the terpene synthase activity (GO:0010333), carbon-oxygen lyase activity (GO:0016835), tetrapyrrole binding (GO:0046906), calcium ion binding (GO:0005509) and carbon-oxygen lyase activity, acting on phosphates (GO:0016838) in MF.

KEGG pathway annotations of DEPs and DEGs

KEGG analysis indicated that the identified DEPs and DEGs were enriched in many different KEGG pathways. The 951 DEPs were enriched in 43 KEGG pathways, including the ether lipid metabolism (map00565), glycerophospholipid metabolism (map00564), sesquiterpenoid and triterpenoid biosynthesis (map00909) and so on, while 2029 DEGs were enriched in 106 pathways, including the linoleic acid metabolism (hbr00591), terpenoid backbone biosynthesis (hbr00900), cysteine and methionine metabolism (hbr00270), etc. (Table S5). In total, five pathways were significantly enriched at the proteome level, containing the ether lipid metabolism (map00565), diterpenoid biosynthesis (map00904), glycerophospholipid metabolism (map00564), photosynthesis (map00195) and photosynthesis -antenna proteins (map00196), and 16 ones at the transcriptome level ($P<0.05$), such as the MAPK signaling pathway-plant (hbr04016), fatty acid elongation (hbr00062) and plant-pathogen interaction (hbr04626) (Table S5). Moreover, 118 shared DEPs were enriched in 23 pathways, of which, there were six significant enrichment pathways ($P<0.05$), including the amino sugar and

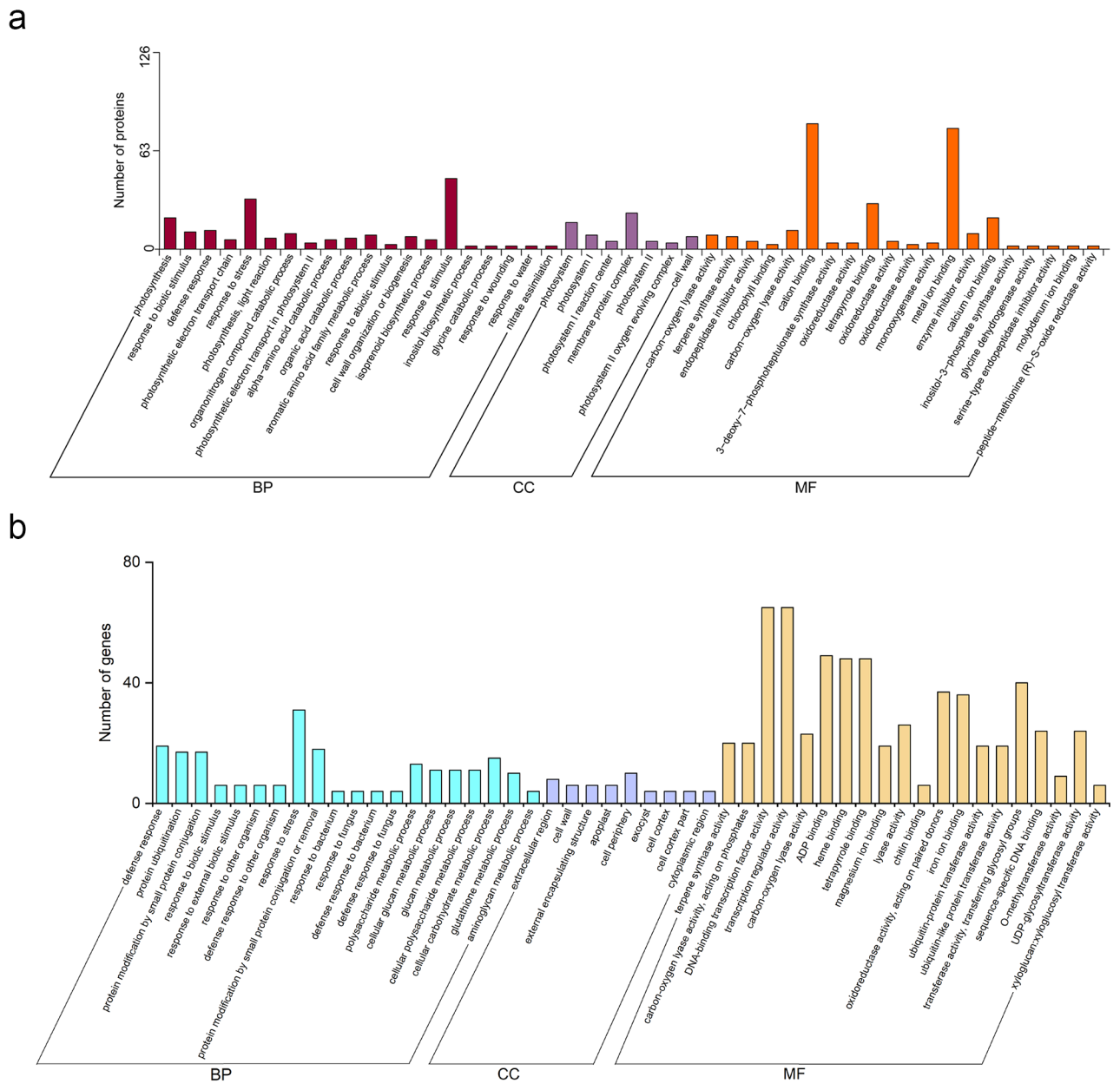

Fig. 3 GO classification of DEPs and DEGs between RB and TB. (**a**) The top 20 significantly enriched GO terms of DEPs ($P < 0.05$). (**b**) The top 20 significantly enriched GO terms of DEGs ($P < 0.05$). BP, CC and MF represents the biological process (BP), cellular component (CC), and molecular function (MF), respectively

nucleotide sugar metabolism (map00520), terpenoid backbone biosynthesis (map00900), nitrogen metabolism (map00910), sesquiterpenoid and triterpenoid biosynthesis (map00909), diterpenoid biosynthesis (map00904), and photosynthesis-antenna proteins (map00196) (Fig. 4).

Further analysis indicated that the up-regulated DEPs were significantly enriched in 11 pathways ($P < 0.05$), such as the diterpenoid biosynthesis (map00904), glutathione metabolism (map 00480), and starch and sucrose metabolism (map00500) (Fig. 5a), while the down-regulated DEPs enriched in three pathways, such as the ether lipid metabolism (map00565), photosynthesis (map00195), and photosynthesis-antenna proteins (map00196) (Fig. 5b). Moreover, the up-regulated DEGs were significantly enriched in 13 pathways, such as the MAPK signaling pathway-plant (hbr04016), plant-pathogen interaction (hbr04626), and plant hormone signal transduction (hbr04075) (Fig. 5c), while the down-regulated DEGs enriched in 11 pathways, such as the glucosinolate biosynthesis (hbr00966), fatty acid elongation (hbr00062), and cyanoamino acid metabolism

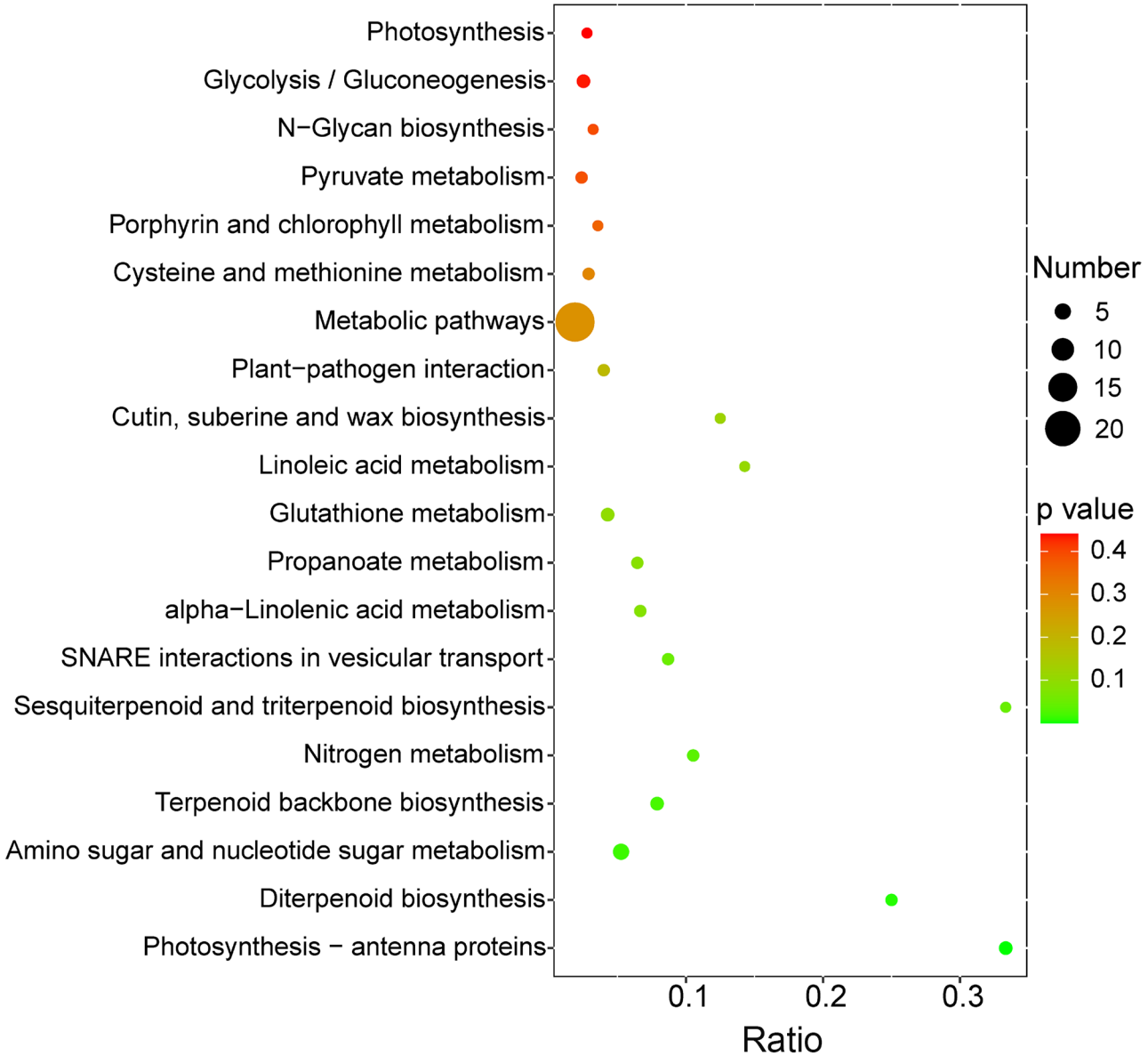

Fig. 4 The top 20 enriched KEGG pathways of 118 shared DEPs at both the transcriptome and proteome levels

(hbr00460) (Fig. 5d). Obviously, there were five common pathways significantly enriched between the up-regulated DEPs and DEGs, including the diterpenoid biosynthesis (map00904), terpenoid backbone biosynthesis (map00900), phenylpropanoid biosynthesis (map00940), nitrogen metabolism (map00910), and glutathione metabolism (map00480). However, there were no common pathways significantly enriched between the down-regulated DEPs and DEGs. Additionally, there were 35 common pathways detected at both the proteome and transcriptome levels, such as the tyrosine metabolism (map00350), starch and sucrose metabolism (map00500), diterpenoid biosynthesis (map00904), galactose metabolism (map00052), and inositol phosphate metabolism (map00562) (Fig. 6).

Modulation of starch and sucrose metabolism pathway during TPD recovery

The D-glucose, which is synthesized by the starch and sucrose metabolism pathway, is a key precursor for the synthesis of natural rubber. In our study, it was observed that the expressions of numerous genes and proteins related to the starch and sucrose metabolism pathway underwent changes after TPD recovery. A total of 11 DEGs (seven up-regulated and four down-regulated) and 19 DEPs (nine up-regulated and 10 down-regulated) involved in the synthesis of D-glucose in this pathway showed differential expressions in the RB group compared with the TB group (Fig. 7, Table S6). Of these DEGs and DEPs, only the *HbGBSS* gene (scaffold0023_1355832), encoding the granule-bound starch

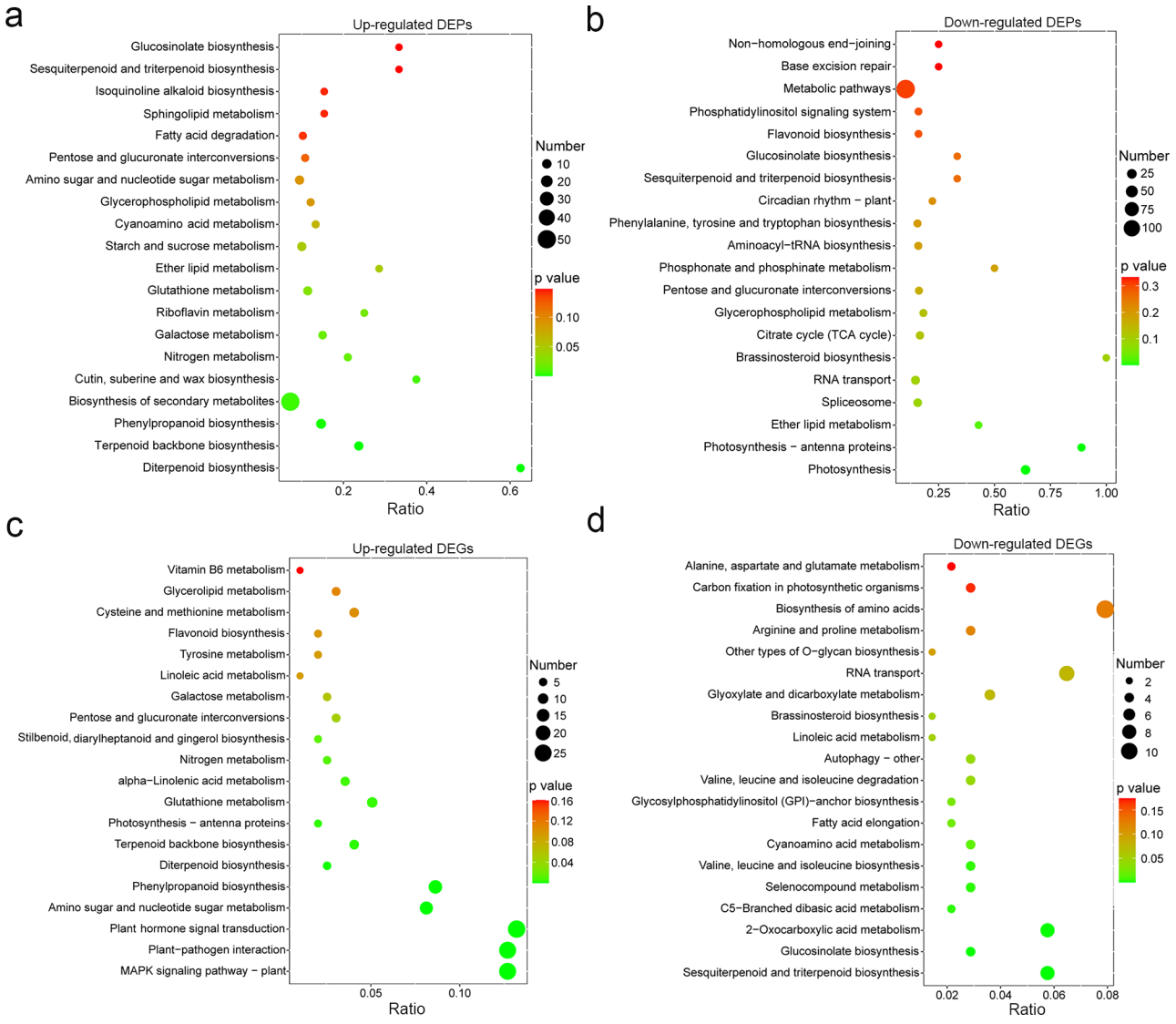

Fig. 5 KEGG enrichment analysis of DEPs and DEGs between RB and TB. (**a**) The top 20 enriched KEGG pathways of up-regulated DEPs. (**b**) The top 20 enriched KEGG pathways of down-regulated DEPs. (**c**) The top 20 enriched KEGG pathways of up-regulated DEGs. (**d**) The top 20 enriched KEGG pathways of down-regulated DEGs

synthase 1, was found to be down-regulated in the RB group at both the transcriptome and proteome levels. It was noteworthy that one *BG* gene (*HbBG42*, scaffold1068_231968) encoding the beta-glucosidase and three BG proteins, namely BG11 (scaffold0007_2197548), BG15 (scaffold0420_543762) and BG18 (scaffold0861_409046), were differentially expressed between the RB and TB groups.

At the transcriptome level, the expressions of most of DEGs related to D-glucose synthesis of the starch and sucrose metabolism pathway were significantly enhanced in the RB group, such as the *HbBAM* (beta-amylase 1, scaffold0588_510388), *HbFEH* (fructan 6-exohydrolase, scaffold1040_196250), and *HbAMYA* (alpha-amylase, scaffold1127_73543) (Fig. 7, Table S6). Obviously, two *HbTPPD* genes (*HbTPPD1*/scaffold0649_424941 and *HbTPPD2*/scaffold0030_911593), encoding the probable trehalose-phosphate phosphatase D, were also significantly up-regulated in the RB group. Whereas, the expressions of the *HbSSS* (soluble starch synthase 3, novel.1438), *HbEG* (endoglucanase 25, scaffold0754_179815), *HbGBSS*, and *HbBG42* genes were all significantly inhibited in the RB group compared to the TB group.

At the proteome level, more DEPs involved in the starch and sucrose metabolism pathway were detected compared to DEGs after TPD recovery (Fig. 7, Table S6). Two HKs (Hexokinase, HbHK1/scaffold0859_388294 and HbHK2/scaffold0016_3377839) proteins displayed a significantly up-regulated expression in the RB group, while two TPSs

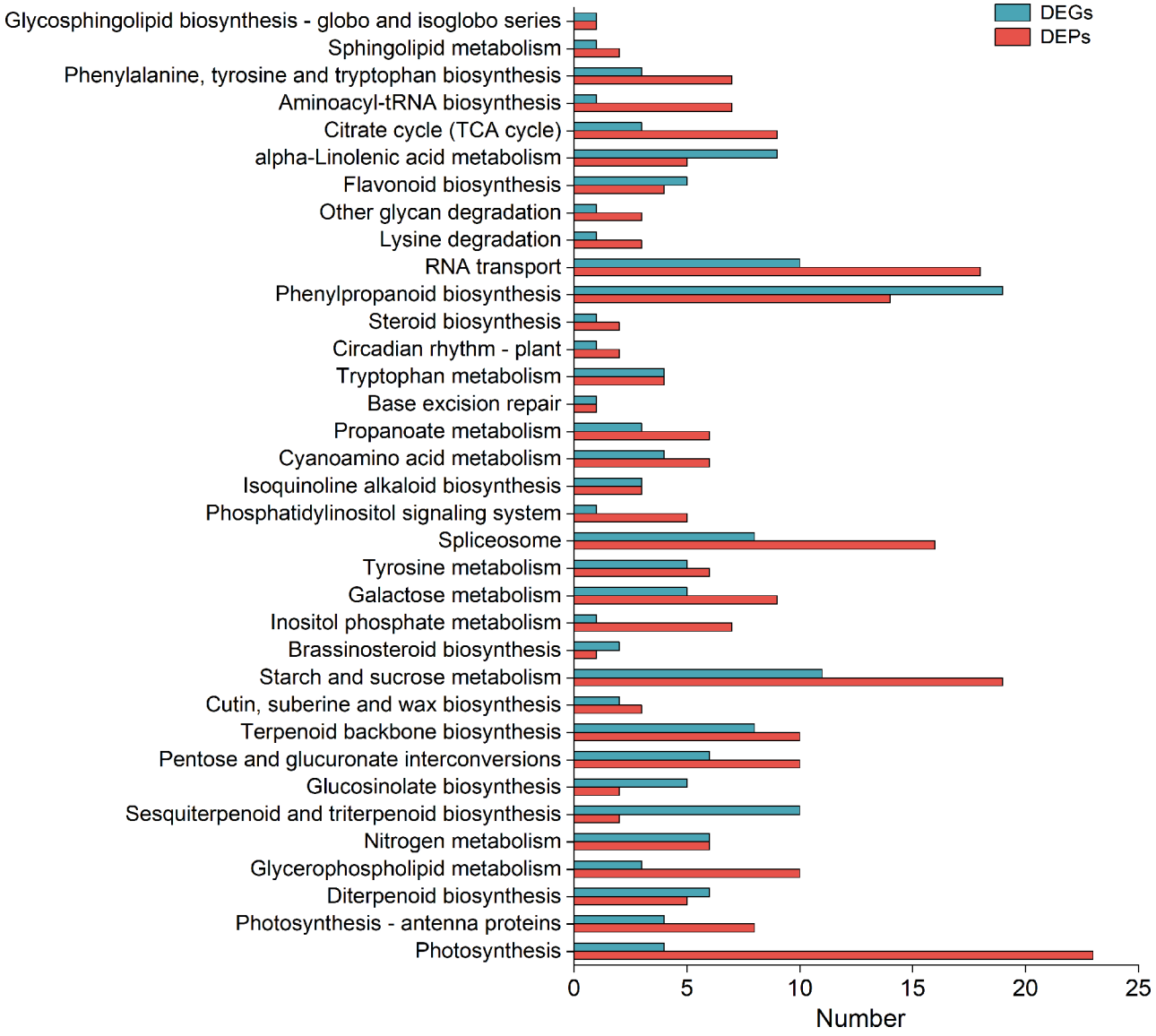

Fig. 6 Common KEGG pathways between DEPs and DEGs

(alpha, alpha-trehalose-phosphate synthase, HbTPS5/scaffold0282_458626 and HbTPS7/scaffold0050_2266950) proteins and two GBE (HbGBE1/scaffold0073_1841869 and HbGBE2/scaffold1611_35064) proteins had a significantly down-regulated expression. Moreover, some proteins, such as the sucrose synthase (HbSUS, scaffold1195_98055), ectonucleotide pyrophosphatase/phosphodiesterase family member 3 (HbENPP, scaffold0894_353573), alpha-amylase (HbAAS, scaffold0639_641173) and alpha-glucosidase (HbAG, scaffold0480_526095), were obviously increased in the RB group, while the other proteins, such as the HbGPI (alpha-1,4 glucan phosphorylase L-1 isozyme, scaffold0137_936551), HbGTS (4-alpha-glucanotransferase DPE2, scaffold0378_203578), and HbSPS (probable sucrose-phosphate synthase 1, scaffold2377_28340), were significantly decreased.

Modulation of natural rubber biosynthesis pathway during TPD recovery

The synthesis of natural rubber contains two pathways, namely MVA (mevalonic acid) and MEP (methylerythritol phosphate). The MVA is the main pathway of natural rubber synthesis [22]. KEEG enrichment analysis revealed that the terpenoid backbone biosynthesis pathway containing the MVA and MEP pathways was significantly enriched in both the up-regulated DEPs and DEGs (Fig. 5a and c). In this study, it was found that a number of genes and proteins participated in the rubber biosynthesis pathway showed differential expressions between the RB and TB groups (Fig. 8, Table S7). In total, 17 DEPs and 15 DEGs in the MVA and MEP pathways were identified. Notably, all the 15 DEGs and 16 DEPs except for the HbGGPS3 (geranylgeranyl pyrophosphate synthase,

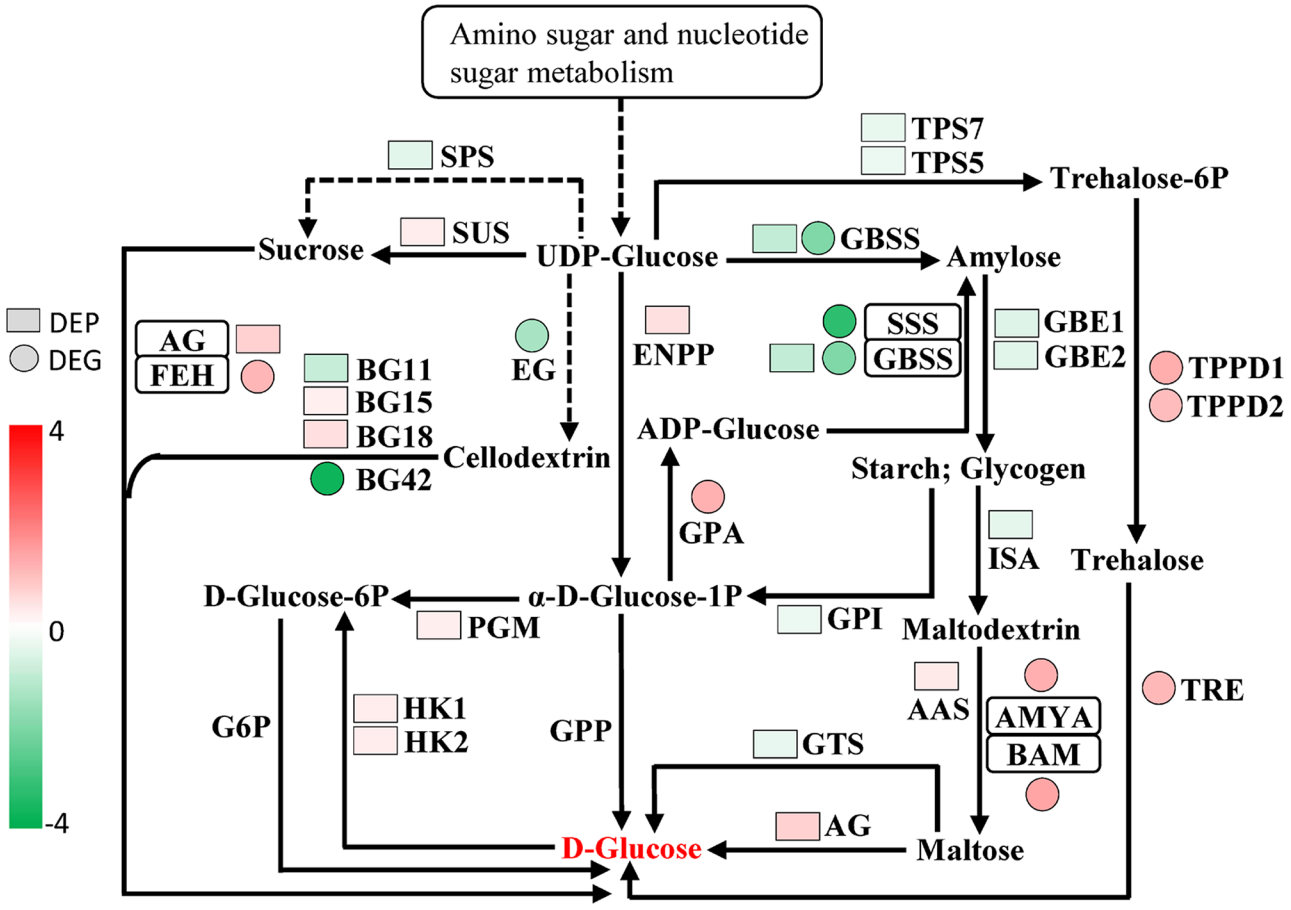

Fig. 7 The DEPs and DEGs related to starch and sucrose metabolism. The schematic diagram was modified based on the KEGG pathway of starch and sucrose metabolism. Refer to Supplemental Table S6 for detailed information

scaffold0916_196671) protein displayed a significantly up-regulated expression, suggesting the enhanced natural rubber synthesis in the RB group. For the synthesis of IPP (isopentenyl pyrophosphate), one HbACAT1 (acetyl-CoA acetyltransferase, scaffold0992_72818) protein and one *HbHMGR4* (3-hydroxy-3-methylglutaryl-coenzyme A reductase 1, scaffold0592_226611) gene in the MVA pathway, and one HbDXS9 (probable 1-deoxy-D-xylulose-5-phosphate synthase 2, scaffold0007_189167) protein and two *HbDXS* (*HbDXS5*/scaffold0155_1933487 and *HbDXS10*/scaffold0615_634198) genes in the MEP pathway were all up-regulated in the RB group. From IPP to *cis*-polyisoprene synthesis, one HbGPS4 (solanesyl diphosphate synthase 3, scaffold1292_61015) protein and one *HbGPS1*(scaffold0221_308815) gene were found to have an up-regulated expression. Only one HbFPS1 protein showed a differential expression in the RB group. Two *HbGGPS* genes, *HbGGPS1* (scaffold0476_178159) and *HbGGPS2* (scaffold0724_385204), encoding the heterodimeric geranylgeranyl pyrophosphate synthase large subunit 1, were largely up-regulated after TPD recovery at both the transcriptome and proteome levels. Obviously, five *REF* (*HbREF1*/scaffold1222_136753, *HbREF3*/scaffold1222_100110, *HbREF5*/scaffold1222_181260, *HbREF7*/scaffold1222_89338, and *HbREF8*/scaffold1222_175215) genes exhibited up-regulation in the RB group at both the transcriptome and proteome levels. Moreover, *HbREF2* (scaffold1222_121702) was only up-regulated at the transcriptome level. Three HbSRPP proteins (small rubber particle protein, HbSRPP1/scaffold1222_60641, HbSRPP5/scaffold1222_196376, and HbSRPP9/scaffold1222_37173) had an enhanced expression after TPD recovery at the proteome level, and the *HbSRPP1* gene was also found to be up-regulated at the transcriptome level. In addition, the key gene of rubber *cis*-polyprenyltransferase HRT2 (*HbCPT7*, scaffold1517_31230) involved in latex production had a differential expression at both the transcriptome and proteome levels, and *HbCPT8* (scaffold0385_30655) was only differentially expressed at the transcriptome level.

Discussion

TPD has a severe influence on the natural rubber yield, resulting in 10–40% latex losses annually [1, 7]. Nevertheless, there are no effective methods to preventing

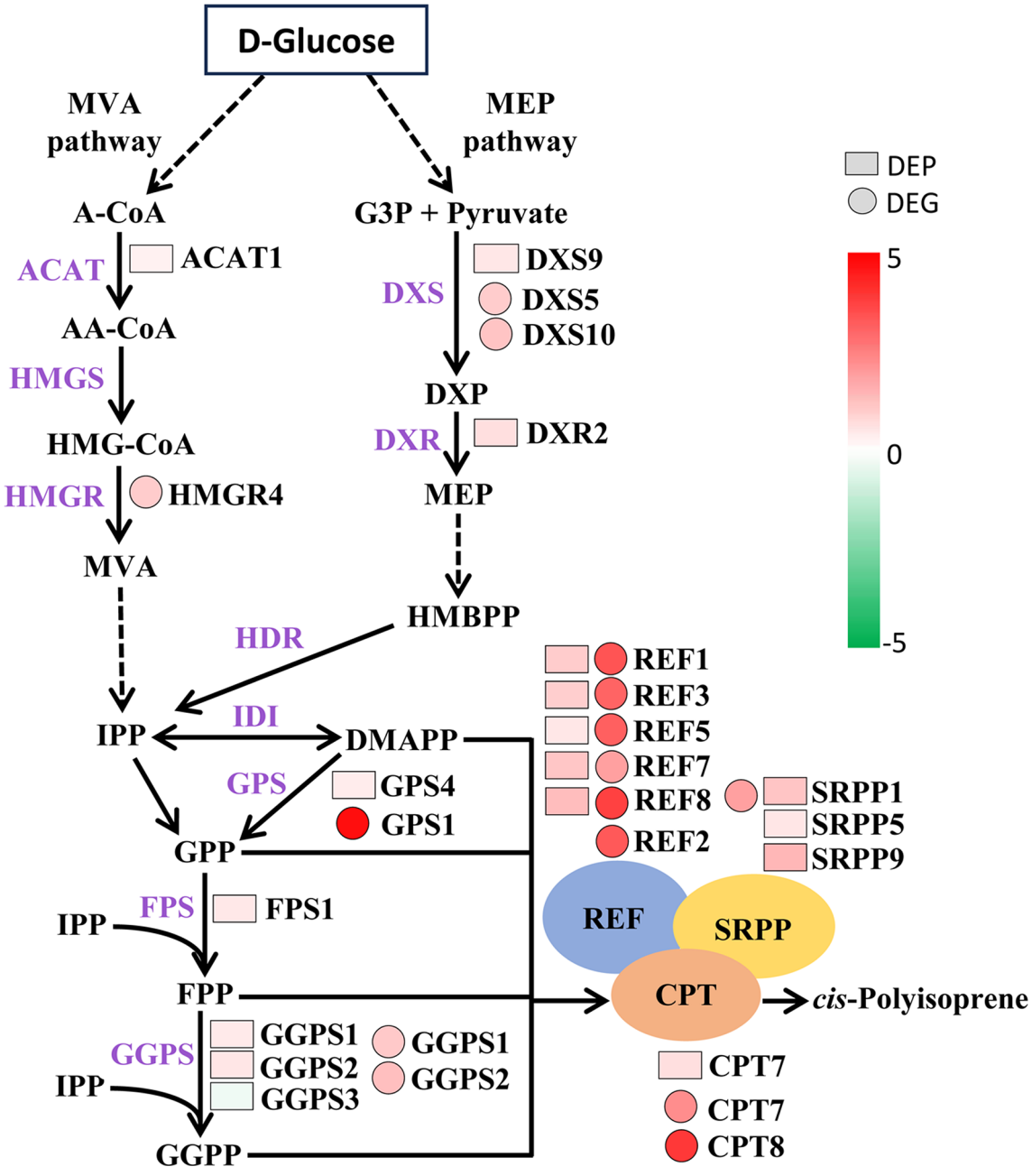

Fig. 8 The DEPs and DEGs related to rubber biosynthesis. The schematic diagram was drawn referring to the previous study (Nie et al., 2023). Abbreviations are in accordance with the article of Nie et al. (2023). Refer to Supplemental Table S7 for detailed information

and treating TPD in rubber trees at present. Previous researches were mainly focused on studying the molecular mechanism of TPD occurrence [8–9, 15, 23]. However, the studies on the mechanism of TPD recovery, which would be conducive to better understanding the TPD occurrence mechanism and developing effective agents to treating TPD, was extremely scarce [16–17]. On this basis, the genes and proteins involved in TPD recovery were identified to enhance our understanding of TPD recovery mechanism by transcriptomic sequencing and TMT proteomics methods. TPD-affected trees were induced to partial or even complete restoration of latex flow by the TPD rehabilitation nutrient agents developed by our team according to the previous method [17]. In this study, significant changes of the bark proteome and transcriptome profiles were found after TPD recovery. A total of 951 DEPs and 2029 DEGs were identified in the RB group compared with the TB group, suggesting that these proteins and genes might regulate the recovery of TPD. It was worth noting that 118 genes showed significant changes

in RB vs TB at both the protein and transcriptional levels, which may be crucial for the recovery of TPD. GO classification and KEGG analysis showed that the 951 DEPs and 2029 DEGs between RB and TB were enriched in various GO terms and KEGG pathways. Furthermore, 35 pathways were enriched at both the proteome and transcriptome levels, including diterpenoid biosynthesis, starch and sucrose metabolism, tyrosine metabolism pathways, etc., suggesting their essential functions in regulating TPD recovery. By comparing the DEPs or DEGs between TPD recovery and occurrence (Fig. 1b and c), we found that almost all DEPs (201) detected at both the TPD occurrence and recovery had opposite expressions in the two processes and all 532 common DEGs were also identical, indicating that these DEPs or DEGs played vital roles in regulating the occurrence and recovery of TPD. Their functions need to be deeply studied in the future.

Notably, the up-regulated DEPs in the barks of recovery trees were significantly enriched in the starch and sucrose metabolism pathway. A total of 19 DEPs and 11 DEGs in the starch and sucrose metabolism pathway were detected after TPD recovery. These DEPs and DEGs participated in responses to TPD recovery. In rubber tree, sucrose acts as the key precursor of rubber biosynthesis and supplies the carbon and energy demands for the latex regeneration [24, 25, 26]. Furthermore, SUS or SPS catalyzes the UDP-glucose into sucrose, which is further transformed into D-glucose (Fig. 7). In this study, the HbSUS protein was significantly up-regulated and the HbSPS protein was significantly down-regulated after TPD recovery (Fig. 7, Table S6), suggesting their important roles in controlling the syntheses of sucrose and D-glucose, which could provide the raw materials for the following rubber biosynthesis during the recovery of TPD. Previously, SUS and SPS were also reported to be involved in leaf development in rubber tree [27]. In other plants, the cucumber *CsSUS4* gene was found to regulate the growth and development of flowers and fruits [28]. Additionally, SUS and SPS participated in responding to the cold stress in *Zea mays* [29]. Apart from SUS and SPS, TPS is also a vital enzyme for D-glucose synthesis. In the present study, two TPS proteins, HbTPS5 and HbTPS7, showed down-regulated expressions in the RB group compared to TB group (Fig. 7, Table S6), implying that they might be involved in the response to TPD recovery by controlling the synthesis of D-glucose. In *Hevea* genome, a total of 14 TPS genes were identified [30]. Zhou et al. [31] found that the expressions of two rubber tree TPS genes, *HbTPS1* and *HbTPS2*, were influenced by multiple treatments, containing ethephon, different hormones, heat, and cold, and the results suggested possible functions of TPS genes in controlling latex production. The rice genome has 11 TPS genes, and overexpressing the *OsTPS1* gene enhanced the tolerance of rice to various abiotic stresses by raising the levels of trehalose and proline and up-regulating the expressions of stress-related genes [32]. Additionally, it was reported that the expression of the cotton *TPS* gene was increased in the roots and leaves under drought stress [33], and the *Ginkgo biloba GbTPS* gene was induced by multiple stresses [34]. The above results demonstrated that the SUS, SPS, and TPS played essential roles in plant growth and development and in responding to various environment factors.

In particular, 16 DEPs and 15 DEGs related to natural rubber biosynthesis in the two pathways of MVA and MEP were found to be differentially expressed between RB and TB groups, and almost all of these DEPs or DEGs showed markedly increased expressions after TPD recovery (Fig. 8, Table S7), indicating that the treatment of nutrient agents might improve natural rubber biosynthesis by regulating the expression levels of these genes or proteins. Furthermore, a total of nine genes, namely *HbGGPS1*, *HbGGPS2*, *HbSRPP1*, *HbCPT7*, *HbREF1*, *HbREF3*, *HbREF5*, *HbREF7*, and *HbREF8*, were all up-regulated at both the transcriptome and proteome levels (Fig. 8), suggesting their vital functions in promoting the recovery of TPD trees. In previous studies, the expressions of most of the genes or proteins participated in natural rubber biosynthesis were reported to be repressed in the TPD trees compared with the healthy ones [5–6, 10, 12], while they were found to be up-regulated in the recovery trees compared with the TPD ones in the present study. These genes or proteins showed opposite expression trends during the processes of TPD occurrence and recovery, demonstrating that the down-regulated or up-regulated expressions of them might inhibit or promote latex biosynthesis, ultimately leading to the occurrence or recovery of TPD. HMGR is a key enzyme for the biosynthesis of IPP in the MVA pathway [35, 36, 37]. In this study, the *HMGR4* gene had an obviously up-regulated expression after TPD recovery (Fig. 8), implying an increased IPP supply in the recovery trees. The *HbFPS1* gene, which regulates the FPP synthesis, was reported to have a positive correlation with rubber biosynthesis, and the down-regulated expression of this gene led to the declined activity of rubber biosynthesis in the latex of TPD trees [11, 22]. In our previous research, the HbFPS1 protein was found to be significantly repressed in the barks of TPD trees [12], while HbFPS1 protein increased in the TPD recovery trees in the present study (Fig. 8). We speculated that the up-regulation of HbFPS1 protein increased the rubber biosynthesis activity after the treatment of nutrient agents and then promoted the recovery of latex flow on the tapping panel of TPD trees. In total, 11 *CPT* genes were found in the *Hevea* genome [30]. CPT initiates the synthesis of rubber molecular and uses IPP monomers to produce the polymer (*cis*-polyisoprene). The *CPT7* gene showed a significantly up-regulated expression in the barks at both the transcriptome

and proteome levels after TPD recovery (Fig. 8), while this gene had a contrary expression trend (down-regulated) after TPD occurrence [10, 12], indicating that *CPT7* gene probably enhanced the production of rubber polymer and thus induced the recovery of TPD trees. Particularly, nine out of 18 members (HbREF1, HbREF2, HbREF3, HbREF5, HbREF7, HbREF8, HbSRPP1, HbSRPP5, and HbSRPP9) of REF/SRPP family in the rubber tree were all increased after TPD recovery in this study (Fig. 8). Among them, the five genes of *HbREF3*, *HbREF5*, *HbREF7*, *HbREF8*, and *HbSRPP1* were found to decrease after TPD occurrence [10, 12]. Previously, REF/SRPP was considered to have a positive role in the biosynthesis of natural rubber [38, 39, 40]. It was speculated that the nine REF/SRPP members identified in this study played essential functions in improving latex regeneration of TPD trees.

Conclusions

In conclusion, we first identified the key genes and proteins involved in TPD recovery by using the integrated transcriptomics and proteomics methods. A total of 2029 DEGs and 951 DEPs were identified in the barks of TPD recovery trees compared with those of TPD trees. Further analysis showed that 19 DEPs and 11 DEGs were associated with starch and sucrose metabolism, and 16 DEPs and 15 DEGs participated in rubber biosynthesis. These DEPs and DEGs in the two pathways might play vital roles in regulating the recovery of latex flow on the tapping panel of TPD trees. Moreover, it was suggested that the TPD rehabilitation nutrient agents used in the present research could promote the recovery of TPD trees by modulating the expressions of these DEPs and DEGs. TPD rehabilitation nutrient agents treatment can be utilized as a measure to prevent and treat TPD in the rubber tree plantations. In summary, the current results provide a base for further revealing the mechanism of TPD recovery and is also a good supplement to the mechanism of TPD occurrence. It will facilitate the development of more effective agents for preventing and treating TPD in rubber tree in the future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06254-x.

Supplementary Material 1: Fig. S1. The symptoms of tapping panel of TPD-affected trees (a) and TPD recovery trees (b).

Supplementary Material 2: Fig. S2. The comparison of the latex yield of TPD recovery trees, TPD trees and healthy trees.

Supplementary Material 3: Table S1. The characteristics of DEPs between RB and TB.

Supplementary Material 4: Table S2. The characteristics of DEGs between RB and TB.

Supplementary Material 5: Table S3. The shared DEPs and DEGs.

Supplementary Material 6: Table S4. The enriched GO terms of DEPs and DEGs between RB and TB.

Supplementary Material 7: Table S5. The enriched KEGG pathways of DEPs and DEGs between RB and TB.

Supplementary Material 8: Table S6. The DEPs and DEGs related to starch and sucrose metabolism.

Supplementary Material 9: Table S7. The DEPs and DEGs related to natural rubber biosynthesis.

Supplementary Material 10: Table S8. List of primers used in this study

Author contributions

H.L. and ZH.W. designed the project. CT.F. and YY.H. collected the experimental materials. QG.H. performed the experiments. K.Y. and H.L. analyzed data and wrote the manuscript. All the authors have read and contributed to the final manuscript.

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Data availability

The mass spectrometry data have been submitted to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository with the dataset identifier PXD057557. The raw RNA-Seq data have been deposited to the Genome Sequence Archive (GSA) of China National Center for Bioinformation (https://www.cncb.ac.cn/) with the accession number CRA012789.

Declarations

Ethics approval and consent to participate

We declare that the plant material (rubber tree clone Reyan73397) used in this study were bred by Huasun Huang's group at Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences and have the right to use it. The clone of Reyan7-33-97 is commercial cultivar, not wild. A voucher specimen of this material has been deposited in the National Rubber Tree Germplasm Repository (NRTGR). All experiments involving the plant and its material complied with relevant institutional, national, and international guidelines and legislation, and the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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