Comparative analysis of diet-associated responses in two rice planthopper species

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Abstract

Background: Host adaptation is the major determinant of insect diversification. However, knowledge of different host ranges in very close-related species remains scarce. The brown planthopper (*Nilaparvata lugens*, BPH) and small brown planthopper (*Laodelphax striatellus*, SBPH) are the most destructive insect pests belonging to Delphacidae. These two species differ in the host range (SBPH can well colonize on rice and wheat plants, while BPH survive on rice plants only), but the underlying mechanism still remains unknown. High-throughput sequencing technology provides a powerful approach for analyzing the association between gene expression changes and physiological responses of insects. Therefore, the gut transcriptomes were performed to elucidate the genes associated with host adaptation in planthoppers. Comparative analysis of planthoppers' responses to different diets would improve our knowledge of host adaptation regarding herbivores insects.

Results: In the present study, we analyzed the gene expression change of SBPH that transferred from rice plants to wheat plants in a short term (rSBPH vs tSBPH) and colonized on wheat plants for a long term (rSBPH vs wSBPH). The results showed that the majority of differentially expressed genes in SBPH showed similar expression change between short-term transfer and long-term colonization. Based on the comparative analysis of BPH and SBPH after transferring, genes associated with sugar transporters and heat shock proteins varied similarly. However, most of genes were differentially regulated between two species. The detoxification-related genes were upregulated in SBPH after transferring while downregulated in BPH under the same condition. Ribosomal-related genes were downregulated in SBPH after transferring while upregulated in BPH under the same condition.

Conclusion: The results of this study provided evidence that host plants played dominant roles in shaping the gene expression, and unfitness of BPH on wheat plants might be determined within 24 hours after transferring. This study deepens our understanding of different host ranges regarding two planthopper species, which may provide a potential strategy for pest management.

Background

Herbivorous insects and their host plants have been engaged in a tight co-evolutionary arm race, leading to specialization of most insects restricted on a narrow range of hosts, whereas other insects can be adapted to a wider host range [1]. Generally, successful host adaptation involves several essential traits of herbivorous insects, including sensing toward right plants, acquiring nutrients while avoiding intoxication, and overcoming plant defenses [2]. Changes in these essential features have been reported to cause failure of colonization or even new host specialization [3-6]. To date, studies of diet-associated responses have mainly focused on a specific insect that feeds on different diets [7]. The research findings showed that genes in association with detoxification, digestion, and transportation were significantly influenced after host transfer [7]. However, knowledge of the disparate host adaptation in different insect species was scarce, and most studies mainly focused on detoxification differences across species in response to plant secondary metabolites [8-10]. Comparative transcriptomic analysis of closely related

taxa provided an ideal approach for unveiling the distinctions in host adaptation across herbivorous species.

The brown planthopper (*Nilaparvata lugens*, BPH) and small brown planthopper (*Laodelphax striatellus*, SBPH) are closely related insect pests belonging to Delphacidae. Although they use rice plants as the primary food source, their host ranges differ. BPH is the monophagous insect pest restricted to rice plants, while SBPH is the oligophagous one that can feed on rice, wheat, and other gramineous plants [11]. Due to a wheat-rice rotation cultivation system, SBPH, which is able to overwinter in temperate zones, shifted between rice and wheat plants every year [12]. In contrast, the northern border overwintering areas of BPH is around 21–25°N. The migratory BPH seldom contacts with wheat plants in places where BPH overwintered [13]. Factors that are potentially implicated in different host range of planthoppers have been investigated for decades. Denno & Roderick presumed that it might be the chemical barrier instead of the physical one that prevented BPH feeding on non-adaptive plants, as the insects could still settle and insert their stylets into plant tissues [14]. SBPHs are better sustained on rice than wheat plants [15, 16]. Liu et al. found that feeding on different plants significantly influenced the activity of detoxification enzymes in SBPH, yet had little impact on BPH [17], which was in agreement with expanded gene families of cytochrome P450s and ABC transporters in the SBPH genome [18]. Additionally, the presence of endosymbionts, which provided essential amino acids, was also critical for planthoppers' colonization [19]. The sap compositions between rice and wheat plants were different, especially the amino acid concentration [20, 21]. It was interesting to investigate the influence of nutrition supplements on planthopper performance. So far, the genomic information of both planthoppers and their host plants (*Oryza sativa* and *Triticum aestivu*) became available [22], providing an opportunity to study the mechanism of different host adaptation in these non-model insects.

The gut is the main tissue where digestion and detoxification occurred, and exerts an important influence on insect feeding on specific plants. Through "Omics" technologies, changes of gut physiology in response to different host plants have been investigated in several herbivorous species, especially the Lepidoptera insects [7]. The expression patterns of gut-associated transcripts, including digestive and detoxifying enzymes, transporters, immune and peritrophic genes were reported to be substantially altered when larvae were exposed to novel diets [23-25]. For generalist larvae, there would be profound transcriptional variations in guts to overcome detrimental effects of plant secondary metabolites. Nevertheless, for specialist larvae, a specific detoxification system avoided activation of general stress responses and minimized the metabolic costs [23, 26], which might restrain a specialist from adapting to a novel phytotoxin [27]. Previously, genes associated with wheat adaptation were demonstrated to be dramatically changed in the gut compared with other tissues, especially the *CYP4DE1* [28]. It was intriguing to unveil the role of the gut in planthopper feeding on different hosts.

Gene regulation upon an initial exposure (short-term transfer) greatly influenced the subsequent adaptation of herbivores to a novel environment [29]. To unveil the successful colonization of SBPH on rice and wheat plants, we profiled the gut gene expression pattern of SBPH that colonizing rice plants (rSBPH), colonizing wheat plants (wSBPH), and transferring from rice plants to wheat plants (tSBPH).

Through comparing the differentially expressed genes (DEGs) of SBPH between short-term transfer (rSBPH vs tSBPH) and long-term colonization (rSBPH vs wSBPH), the quick response of SBPH to the changed host plant and the leading role of the host plant in shaping gene expression were found. Subsequently, to help elucidate the differences in host adaptation between BPH and SBPH, the gut transcriptomes of BPH that colonizing rice plants (rBPH) and transferring to wheat plants (tBPH) were also profiled. Based on comparative analysis of short-term transfer in BPH (rBPH vs tBPH) and SBPH (rSBPH vs tSBPH), the feasible mechanism of different host adaptation concerning two planthoppers was illustrated. The present study improved our knowledge of diet-associated responses in herbivores insects, which might provide a potential strategy for pest management.

Results

Performance of planthoppers on rice and wheat plants

Rice plants, but not wheat plants, can be well colonized by BPH, with only rice-colonized BPH strains (rBPH) generated in laboratory conditions. In contrast, SBPH could successfully colonize on both rice and wheat plants, and two SBPH strains (rSBPH and wSBPH) were maintained in our laboratory for over 30 generations. According to survival analysis, more than 90% of rBPH survived on rice plants for 12 days, which was significantly higher than that on wheat plants (Fig. 1A). Additionally, rBPH survived longer on wheat plants (LT_{50}=6.1 day) than that provided with water only (LT_{50}=3.3 day). These results indicated that BPH could uptake wheat sap and survive on wheat plants for a short time, but not for a long time. For SBPH, both rSBPH and wSBPH could survive on rice and wheat plants successfully (Fig. 1B) in accordance with previous reports [30].

Overview of RNA sequencing data

To explore the mechanism underlying different performances of two planthoppers, rice-colonized planthoppers were transferred to wheat plants for 24 hours (short-term transfer), or reared on wheat plants for over 30 generations (long-term colonization). Then, guts of planthoppers that colonized on rice plants (rSBPH and rBPH) or wheat plants (wSBPH), or transferred from rice plants to wheat plants (tSBPH and tBPH) were isolated and underwent Illumina HiSeq2500 sequencing. A total of 15 libraries were generated, with clean reads exceeding 45 million in each library. The clean reads were mapped to their reference genomes [18, 19], respectively. For BPH, there were 75% to 83% clean reads mapped to the reference genome. For SBPH, the percentage ranged from 60% to 66%. According to saturation analysis, the number of detected genes decreased as that of reads increased, and library capacity reached saturation when the number of sequence reads approached 20.0 million (Fig. S1, Supporting information). Furthermore, principal component analysis (PCA) demonstrated that the expression pattern of wSBPH and tSBPH was closely related, indicating that host plants exert a non-negligible influence on gene expression (Fig. S2, Supporting information).

Analysis of differentially expressed genes (DEGs)

Gene expression changes were analyzed via comparing rice-colony planthopper to transfer planthopper (tSBPH_vs_rSBPH and tBPH_vs_rBPH) and rice-colony planthopper to wheat-colony planthopper (wSBPH_vs_rSBPH) using a threshold of >2 fold change and an FDR adjusted p-value <0.05. For rice-colony planthoppers transferring to wheat plants, a total of 2,877 and 2,638 genes were differentially expressed in SBPH and BPH, respectively (Fig. 2). There were 2,372 genes upregulated and 505 genes downregulated when rSBPH was transferred to wheat hosts (Table S1). Among them, genes participating in signal transduction were remarkably upregulated. CYP4DE1, which mediated wheat adaptation and ethiprole tolerance [28] in SBPH was also significantly induced after transferring (Fig. S3, Supporting information). On the contrary, 71 genes related to ribosomal proteins and 48 genes in relation to oxidative phosphorylation were significantly downregulated, indicating a decreased protein production and energy metabolism (Fig. S4, Supporting information). In BPH, the amount of genes downregulated (2,171 genes) exceeded that of genes upregulated (467 genes) (Table S2). The majority of genes associated with intestinal mucins, serine proteinases, and sugar transporters were significantly downregulated. Besides, reduced expression was also found in detoxification-related genes (Fig. S3, Supporting information), which includes 9 ABC transporters, 8 P450s, 5 UGTs, and 1 GST. Contrary to SBPH, the majority of ribosomal proteins were upregulated in BPH (Fig. S4, Supporting information). Cuticular proteins, which formed the insect cuticle and participated in insect molting, were dramatically upregulated after BPH transferred to wheat (Table S2).

In the comparison of rSBPH and wSBPH, a total of 2,516 DEGs were identified (Fig. 2). Strikingly, 90.9% of DEGs (2,288 genes) showed higher expression in wSBPH than those in rSBPH (Table S3), with peroxisomal biogenesis factor, nucleotide exchange factor, peptide transporter, and CYP6FK1 exhibiting most dramatic changes. Similar to the patterns of rSBPH that transferred to wheat hosts, as many as 37 genes participating in signal transduction were significantly enriched. Among the 228 downregulated genes, there were the most dramatic changes in zinc metalloproteinase, UGT, and alpha-glucosidase. Other downregulated genes participating in chitin metabolism, carbohydrate derivative metabolism, starch and sucrose metabolism, oxidative phosphorylation were significantly enriched (Table S3).

Classification of SBPH genes associated with diet changes

To help elucidate the successful colonization of SBPH on rice and wheat plants, the DEGs of SBPH between short-term transfer (rSBPH vs tSBPH) and long-term colonization (rSBPH vs wSBPH) were analyzed. Based on the gene expression changes in response to different diets, DEGs of SBPH were classified into four types (Fig. 3): I) genes changed in the same direction between short-term transfer and long-term colonization, II) genes changed in the opposite direction between short-term transfer and long-term colonization, III) genes changed in short-term transfer, but not long-term colonization, and IV) genes changed in long-term colonization, but not short-term transfer .

The number of type I responsive genes (1,558 genes) ranked the first (Fig. 3; Table S4). Enrichment analysis showed that genes participating in signal transductions and immune systems were significantly overrepresented. Nevertheless, only 22 genes belonged to type II response (Fig. 3; Table S4). Four genes

were downregulated after transferring, but dramatically upregulated during colonization, while other 18 genes showed reciprocal expression patterns. There were 1,297 genes belonging to type III response (Fig. 3; Table S4). Enrichment analysis showed that ribosome pathway, oxidative phosphorylation pathway, and retrograde endocannabinoid signaling pathway were significantly overrepresented. It was noteworthy that the expression level of ribosome proteins was initially suppressed, but recovered when SBPH long-term colonized on wheat plants. A total of 936 genes belonged to type IV response (Fig. 3; Table S4). Nevertheless, we failed to find GO terms or KEGG terms that were significantly enriched. Genes such as integrin alpha-PS4-like, integumentary mucin, and proliferation-associated protein showed a higher expression level in wSBPH.

Comparative genomics of genes in response to host transfer

To comprehend the different diet-associated responses across species, 6139 gene families with only one ortholog in BPH and SBPH were selected and compared. A total of 1,995 gene families were differentially expressed in at least one planthopper species after transferring (Table S5), among which 370 genes were responsive to host transfer in both planthoppers. Interestingly, only 22 genes changed (14 genes upregulated and 8 genes downregulated) in the same direction, including heat shock protein, prophenoloxidase activating factor, MAP kinase-interacting serine/threonine-protein kinase, and small nuclear ribonucleoprotein. Nonetheless, other 348 genes showed different expression patterns between BPH and SBPH. Among them, 293 genes were upregulated in SBPH after transferring, but downregulated in BPH; 55 genes, including 25 ribosomal proteins, were downregulated in SBPH after transferring, but upregulated in BPH.

qPCR validation

To confirm the validity of transcriptomic data, 15 SBPH genes and 18 BPH genes were selected for qPCR analysis. Thirteen SBPH genes (Fig. 4) and 17 BPH genes (Fig. 5) showed concordant direction of change between qPCR and transcriptomic results, indicating the accuracy of DGE transcriptomic results. For heat shock proteins, they were significantly upregulated after BPH and SBPH transferring to wheat. For ABC transporters and cytochrome P450s, their expression levels were significantly increased after SBPH transferring to wheat, but were significantly decreased in BPH at the same condition. For ribosomal proteins, they were significantly downregulated after SBPH transferring to wheat, but were significantly upregulated in BPH after transferring. It was worth to mention that two trehalose transporters in BPH showed significantly different expression changes. The trehalose transporter of NLU013658.1 was dramatically downregulated after BPH transferring to wheat, but the NLU003716.1 was dramatically upregulated at the same condition. In SBPH, the nucleotide exchange factor, peroxisomal biogenesis factor, and peptide transporter were significantly upregulated after transferring, but the venom serine carboxypeptidase-like and maltase were significantly downregulated. In BPH, genes such as cryptopsoridial mucin, serine proteinase stubble, peptide methionine sulfoxide reductase were significantly downregulated after transferring, but the cuticle protein 16.5-like, chemosensory protein, and lipid storage droplets surface-binding protein were significantly upregulated.

Discussion

In the present study, the transcriptomic responses of two rice planthoppers on rice and wheat plants were investigated. We identified a total of 2877, 2638 and 2287 DEGs from gut transcriptome when comparing tSBPH to rSBPH, tBPH to rBPH, and wSBPH to rSBPH, respectively. After transferring from rice plants to wheat plants, BPH and SBPH showed distinct gene expression changes, which might account for their adaptive differences. Our study presents comprehensive and accurate gene expression profiles for herbivores adapting to different hosts.

The wSBPH and rSBPH used in this study have been reared under laboratory conditions for more than 30 generations, while the tSBPH was collected by transferring rSBPH to wheat plants within 24 hours. Surprisingly, the expression pattern of tSBPH was similar with wSBPH instead of rSBPH (Fig. S2, Supporting information), indicating the quick response of SBPH to the changed host plant and the leading role of the host plant in shaping gene expression. Additionally, transferring of rSBPH to wheat plants gave rise to broad transcriptional readjustments, and the majority of DEGs were changed in the same direction between short-term transfer and long-term colonization. SBPH shifted between rice and wheat plants every year, while BPH seldom contacts with wheat plants [12, 13]. Frequency in host transferring might help SBPH rapidly respond to new hosts, generating successful wheat adaptation. Similar phenomena were also reported in *Tetranychus urticae*, which was rapidly adapted to pre-exposed hosts [29]. In contrast, different expression patterns were revealed in 94.1% of homologous genes in BPH and SBPH when planthoppers transferred to wheat plants (Table S5). With the majority of genes changed in the “wrong” direction, the unfitness of BPH on wheat plants might be determined within 24 hours after transferring.

Genes involved in detoxification were significantly influenced when planthoppers transferred to wheat plants (Fig. S3, Supporting information). In previous work, CYP4DE1 was found to be responsible for wheat adaptation, and knockdown of CYP4DE1 significantly lowered the intestinal cell viability of SBPH reared on wheat plants [28]. In this study, we found CYP4DE1, as well as other P450 genes were significantly upregulated after rSBPH transferred to wheat plants, indicating that the induction of P450s might be critical. Similarly, 9 ABC transporters, which transport a large diversity of substrates across lipid membranes and out of cells, were also significantly upregulated in response to wheat plants. Involvement of ABC transporters in xenobiotic transport and insecticide resistance have been well documented [31]. These proteins were also reported to participate in host adaptation in *Manduca sexta* [32], *Aphis nerii* [33], and *Chrysomela populi* [34]. The increased expression of ABC transporters signified that SBPH might accelerate secretion of metabolites when feeding on wheat plants. Comparative analysis showed that the majority of differentially expressed detoxifying genes were upregulated when SBPH transferred to wheat plants. However, these genes showed downward expression in BPH (Fig. S3, Supporting information). We presumed that this might be associated with resource-based metabolic trade-off [35]. When coping with such adverse environment, it might become more critical for BPH to increase basic metabolism than the detoxification process. Further studies are needed to determine the detoxification variation between BPH and SBPH on wheat plants.

Large numbers of ribosomal proteins were differentially expressed when planthoppers transferred to wheat plants. However, the majority of these genes showed different expression change between SBPH and BPH (Fig. S4, Supporting information). Mostly associated with their standard roles in protein translation, ribosomal proteins are generally deemed to be stably expressed and used as housekeeping genes [36]. However, recent studies demonstrated that ribosomal proteins were differentially expressed in response to host transfers in *Bemisia tabaci* [37], *Helicoverpa armigera* [38], *Polygonia c-album* [39], and *Cryptolaemus montrouzieri* [40]. In an insect-plant model, regulations of ribosomal proteins are perceived to counteract the ribosome inactivating proteins (RIPs), which are produced by host plants and inhibit protein synthesis in insects [41]. In this study, the expression level of ribosomal proteins was significantly downregulated when rSBPH transferred to wheat, but upregulated in rBPH under the same condition. We presumed that the different expression changes of ribosomal proteins might be associated with different strategies of two planthoppers in dealing with RIPs in the food source. Additionally, ribosomal proteins played fundamental roles in the cellular process of translation [36]. The large-scale change of ribosomal proteins inevitably influenced the regulation of other genes, which might partially explain the distinct gene expression variation in two planthopper species.

Genes associated with amide/peptide biosynthetic process, amino sugar metabolic process, and aminoglycan metabolic process were significantly enriched in both SBPH and BPH after host transferring. Planthoppers are piercing-sucking herbivores that depend on phloem sap containing high concentration of sugar, amino acid and inorganic ions as their food sources [15, 39]. Previous work demonstrated that the sucrose concentration between rice phloem sap and wheat phloem sap was very similar, but the latter had higher concentration of total amino acids compared with the former [20, 21]. In addition, the predominance of amides between two phloem sap was also very different [20, 21]. In previous work, we found that the BPH genome lacks the ability to carry out *de novo* synthesis of some essential amino acids, requiring an additional supply from the yeast-like endosymbionts (YLS) or plant phloem sap [19]. Our presumption is that the alteration in phloem sap composition inevitably influenced the nutrition supplements, which might bring about the change of the basic metabolism (especially the amino acid metabolic process). This hypothesis has been partially verified by planthoppers reared on resistant rice, where the physiological adaptation of insects to novel rice variety was closely linked to a change in amino acid metabolism [42].

Sugar transporters were significantly differentially expressed when planthoppers transferred to/colonized on wheat plants (Fig. S5, Supporting information). Mediating the movement of sugars into and out of cells in a diverse array of organisms, sugar transporters are vital for utilization of ingested sugars as a nutritional resource and maintenance of osmotic balance in insects [43]. We found that the majority of differentially expressed sugar transporters belonged to facilitated trehalose transporter. Trehalose is the major hemolymph sugar in most insects [44]. Besides, its concentration in hemolymph was determined by the balance between discharge from the fat body and uptake by other tissues via facilitated trehalose transporter [45]. In *Anopheles gambiae*, suppression of trehalose transporter significantly diminished the hemolymph trehalose concentration, making the insect more sensitive to stress environment [46]. Similarly, the trehalose transporter from *Polypedilum vanderplanki* enhanced desiccation tolerance of cell

lines [47]. Involvement of trehalose transporter in host adaptation was only reported in a few insects [23]. However, increased evidence demonstrated that trehalose, the regulatory product of trehalose transporter, played key roles in insects feeding on different diets. For example, trehalose concentration in haemolymph affected the food preference and carbohydrate intake in *Heliothis zea* and *Manduca sexta* [48, 49]; trehalose homeostasis was critical for *Drosophila* adapting to unlike dietary conditions [50]. In this study, the majority of sugar transporters were suppressed in SBPH and BPH reared on wheat plants. Accordingly, we inferred that the two planthoppers might use similar strategy to keep sugar homeostasis. However, it was also worth to mention that two of the trehalose transporters in BPH were dramatically upregulated. BPH harbors a lot of sugar transporter genes, and they function differently [51]. Further studies are needed to illustrate the function of sugar transporters in planthoppers coping with different diets.

Heat shock proteins (HSPs) were prominently upregulated when two planthopper species transferred to/colonized on wheat plants (Fig. S5, Supporting information). HSPs are well-known stress proteins that respond to an array of stresses, including thermal hardening, oxidative hardening, chemical pesticides, and desiccation [52, 53]. Induced expression of these proteins, as a significant molecular chaperone, prevented irreversible denaturation of proteins, and promoted the coping capacity of insects in the face of stress environment [52]. Previously, the influence of diet quality on HSP expression has been unveiled in *Drosophila melanogaster*, and diet-induced HSPs increased heat and desiccation tolerance of flies [54]. Feeding on wheat was a biotic stress for BPH and SBPH [55], which might result in increased HSP expression. Interestingly, some SBPH overwintered in the wheat field after the rice-harvesting season [56]. Thus, further investigation concerning the influence of wheat-induced HSPs on SBPH cold tolerance is essential.

Conclusion

Overall, SBPH successfully coped with wheat hosts, with the majority of DEGs responded similarly between short-term transfer and long-term colonization. Compared with SBPH, BPH showed distinct gene expression changes after transferring to wheat plants. The different gene expression alteration between BPH and SBPH might account for their adaptive differences on wheat plants. Specifically, genes associated with sugar transporters and heat shock proteins showed similar expression trends between BPH and SBPH. Other genes in association with detoxification, ribosomal proteins, and amino acid metabolism were differently regulated between two planthoppers. Our work boosts our understanding of herbivore adapting to different food sources.

Methods

Insect strains

SBPHs and BPH populations used in the present study were originally collected from a rice field at Huajiachi Campus, Zhejiang University, Hangzhou, China. The rSBPHs and wSBPHs were generated by

rearing SBPHs on fresh rice (Xiushui 134) and wheat (Luyuan 502) plants for over 30 generations. The rBPHs have been reared on rice (Xiushui) 134 plants for more than 40 generations. All insects were reared in large cage, and more than 300 individuals were used for propagation in each generation.

Survival analysis

Survival analysis of rSBPH on different hosts has been done previously [30]. However, the performance of wSBPH, as well as rBPH, on wheat plants still remained unknown. For BPH, 3rd instar nymphs (rBPH) were reared on 4–5-leaf stage rice plants or transferred to 4–5-leaf stage wheat plants, and the rBPH that provided with water only were used as a control. For SBPH, rSBPH were reared on 4–5-leaf stage rice plants or transferred to 4–5-leaf stage wheat plants, and wSBPH were reared on their original hosts (4–5-leaf stage wheat plants). The survival rates of each treatment were recorded daily, and a group of 40–50 insects (approximately 4-5 insects per plant) were used in each replicates. Three biological replicates were conducted for each treatment. Statistical significance of survival distributions for each treatment group was determined using the log-rank test in SPSS 19.0 (SPSS, Chicago, IL, USA).

Transcriptomic sequencing

To compare the planthoppers' responses to different dies, transcriptomic sequencing of rice-colonized BPH and SBPH to rice and wheat plants, as well as wheat-colonized SBPH to wheat plants were performed. For rSBPH, wSBPH, and rBPH populations, newly emerged 5th instar nymphs were collected and continually reared on their original hosts for 24 h. For tSBPH and tBPH populations, newly emerged 5th instar nymphs (maintained on rice plants) were collected and transferred to wheat plants for 24 h. The guts of each nymph were dissected under a stereomicroscope, and total RNA was isolated using the TRIzol Total RNA Isolation Kit (Takara, Dalian, China). Three replicates (all replicates were collected from the same insect strain) were performed for each treatment, and each replicate contained a pool of 100 guts.

After determination of RNA integrity and quantity, poly (A) + RNA was purified from 20 μg pooled total RNA using oligo (dT) magnetic beads. Fragmentation was conducted in the presence of divalent cations at 94 °C for 5 min and then reverse transcribed to double-stranded cDNA (dscDNA) by N6 random primers. After end-repairing and adaptor ligation, the products were PCR-amplified and purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) to create a cDNA library. The library was sequenced on a BGISEQ-500 platform (Beijing Genomics Institute, Beijing, China) and the low quality reads were filtered using internal software. The clean reads from each cDNA library were aligned to reference genome sequences of *Nilaparvata lugens* (ftp://parrot.genomics.cn/gigadb/pub/10.5524/100001_101000/100139/) and *Laodelphax striatellus* (ftp://parrot.genomics.cn/gigadb/pub/10.5524/100001_101000/100361/) using Hierarchical Indexing for Spliced Alignment of Transcripts (HISAT). The clean data of rSBPH, rBPH, wSBPH, tSBPH, and tBPH have been submitted to the database of the NCBI Sequence Read Archive (http://trace.ncbi.nlm.nih.gov/Traces/sra).

For gene expression quantification, the mapped reads were calculated as FPKM using RESM software [57]. The differentially expressed genes were defined by the DEGseq [58, 59] using the criteria of log2-ratio >1and adjusted p value< 0.05. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were based on the Gene Ontology Database (http://www.geneontology.org/) and KEGG pathway database (http://www.genome.jp/kegg/), respectively.

Gene family identification

Previous work demonstrated that genes associated with detoxification, digestion, and transportation played critical roles in host transfer [7]. To comprehensively analyze these genes, gene families of interest were initially identified based on the gene annotations. To avoid possibly incomplete annotations, genes from *Drosophilamelanogaster*, *Acyrthosi phonpisum*, and *Bombyx mori* were used as queries to search against genomic databases using BLAST (ftp://ftp.ncbi.nlm.nih.gov/blast) with a significance cut-off of E-value < 10^{-5}. Gene annotations were further validated by aligning to the non-redundant (nr) National Center for Biotechnology Information (NCBI) protein database.

Comparative genomic analysis

The protein families of SBPH and BPH were analyzed using the ORTHOMCL software 2.0.3 (http://orthomcl.org/common/downloads/software/v2.0/). Briefly, the amino acid sequences derived from the coding sequences in genome were selected, and homologous pairs of sequences were found using the all-vs-all BLASTp algorithm with an E-value < 1e-5. OrthoMCL then converted the BLASTp result into a normalized similarity matrix that was analyzed by a Markov chain clustering (MCL) for clustering according to Li et al. recommendation [60]. An inflation factor of 1.5 was used to regulate cluster tightness.

Real-time quantitative PCR (qPCR) analysis

To confirm the validity of transcriptomic data, total RNA was extracted as described above. One μg RNA was reverse-transcribed in a 20-μl reaction system using HiScript II Q RT SuperMix (Vazyme, Nanjing, China). The primers used in qPCR were designed based on Primer Premier 6.0 (Table S6). The housekeeping genes for β-actin and GAPDH were used as internal control. Relative expression pattern was quantified by an ABI 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA) using the SYBR Green Supermix Kit (Yeasen, Shanghai, China). The first-strand cDNA and a no-reverse-transcription control were used as templates under the following reaction program: denaturation for 5 min at 95°C, followed by 40 cycles at 95°C for 10 s and 60°C for 30 s. A relative quantitative method ($2^{-\Delta\Delta Ct}$) was used to evaluate quantitative variation [61]. Three independent biological replicates were performed.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

We acknowledge all information is accurate to the best of our knowledge at the time of submission and not submitted for publication elsewhere. We give consent for BMC to publish this article.

Availability of data and materials

All sequencing data were submitted to the NCBI Sequence Read Archive (SRA) under accession number PRJNA564687. Other related data are available within the manuscript and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

H-J H and X-Y H planned and designed the research. J-R C and H-J H performed experiments and analyzed data. H-J H wrote the manuscript. All authors have read and approved the manuscript.

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Abbreviations

BPH: brown planthopper

DEG: differentially expressed gene

GST: glutathione S-transferase

HSP: heat shock protein

MCL: Markov chain clustering

PCA: Principal component analysis

RIP: ribosome inactivating protein

rBPH: brown planthopper that colony on rice

rSBPH: small brown planthopper that colony on rice

SBPH: small brown planthopper

tBPH: brown planthopper that transferred from rice to wheat

tSBPH: small brown planthopper that transferred from rice to wheat

UGT: UDP-glucosyltransferase

wSBPH: small brown planthopper that colonized on wheat

YLS: yeast-like endosymbiont

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Figures

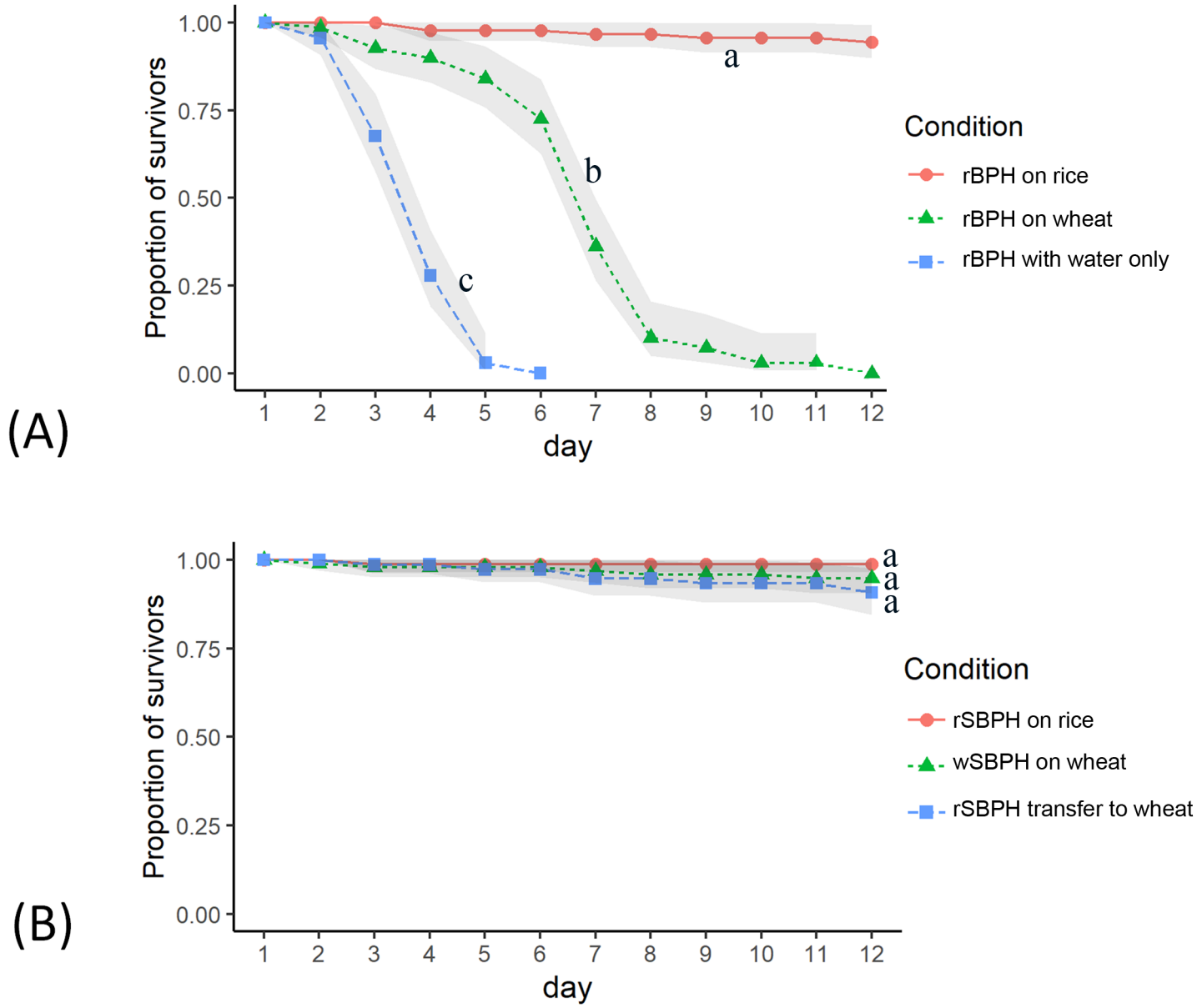

Figure 1

Survival analysis of planthoppers on rice and wheat plants. (A) The survival of rBPHs that colonized on rice plants, transferred to wheat plants, and provided with water only. (B) The survival of rSBPH that colonized on rice plants and transferred to wheat plants, and wSBPH that colonized on wheat plants. Light shades indicate 95% confidence intervals. Different letters signify significant different survival distributions among each treatment group at $P < 0.05$ according to the log-rank test.

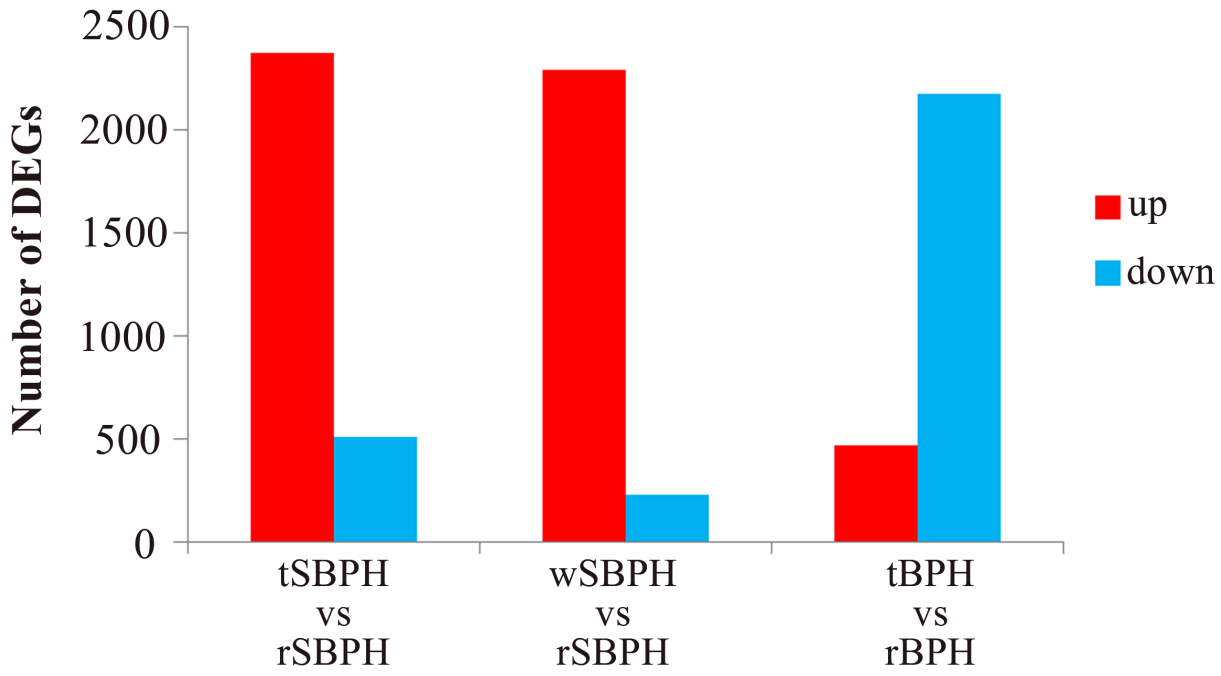

Figure 2

The number of differentially expressed genes in planthoppers that feed on different hosts. The differentially expressed genes were analyzed by comparing tSBPH to rSBPH, wSBPH to rSBPH, and tBPH to rBPH based on a threshold of >2 fold change and an FDR adjusted p-value <0.05.

Figure 3

Classification of DEGs in SBPH based on their expression patterns. The expression patterns of identified DEGs in SBPH can be classified into four types: Types I, genes changed in the same direction between short-term transfer and long-term colonization; Types II, genes changed in the opposite direction between short-term transfer and long-term colonization; Types III, genes changed in short-term transfer, but not long-term colonization; Types IV, genes changed in long-term colonization, but not short-term transfer. The number of genes belonging to each type was listed following the heat map. Red and green boxes represent genes up- and down-regulated in tSBPH and wSBPH relative to that of rSBPH. The white box represents genes which did not change in the comparison.

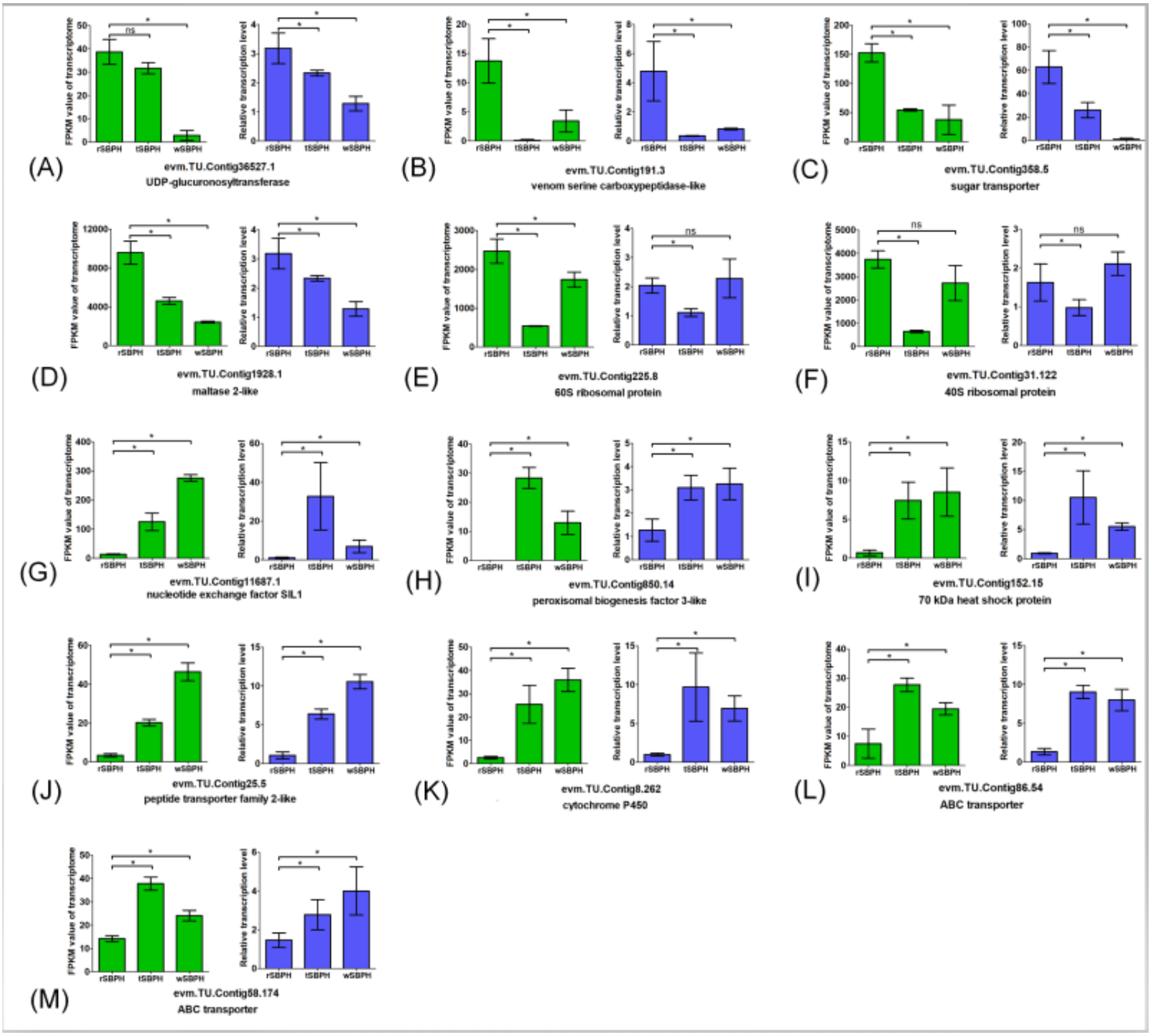

Figure 4

Correlation between transcriptomic data and qPCR results in SBPH. The relative expression level of each gene was determined by qPCR (blue) and compared with that of transcriptomic data (green).

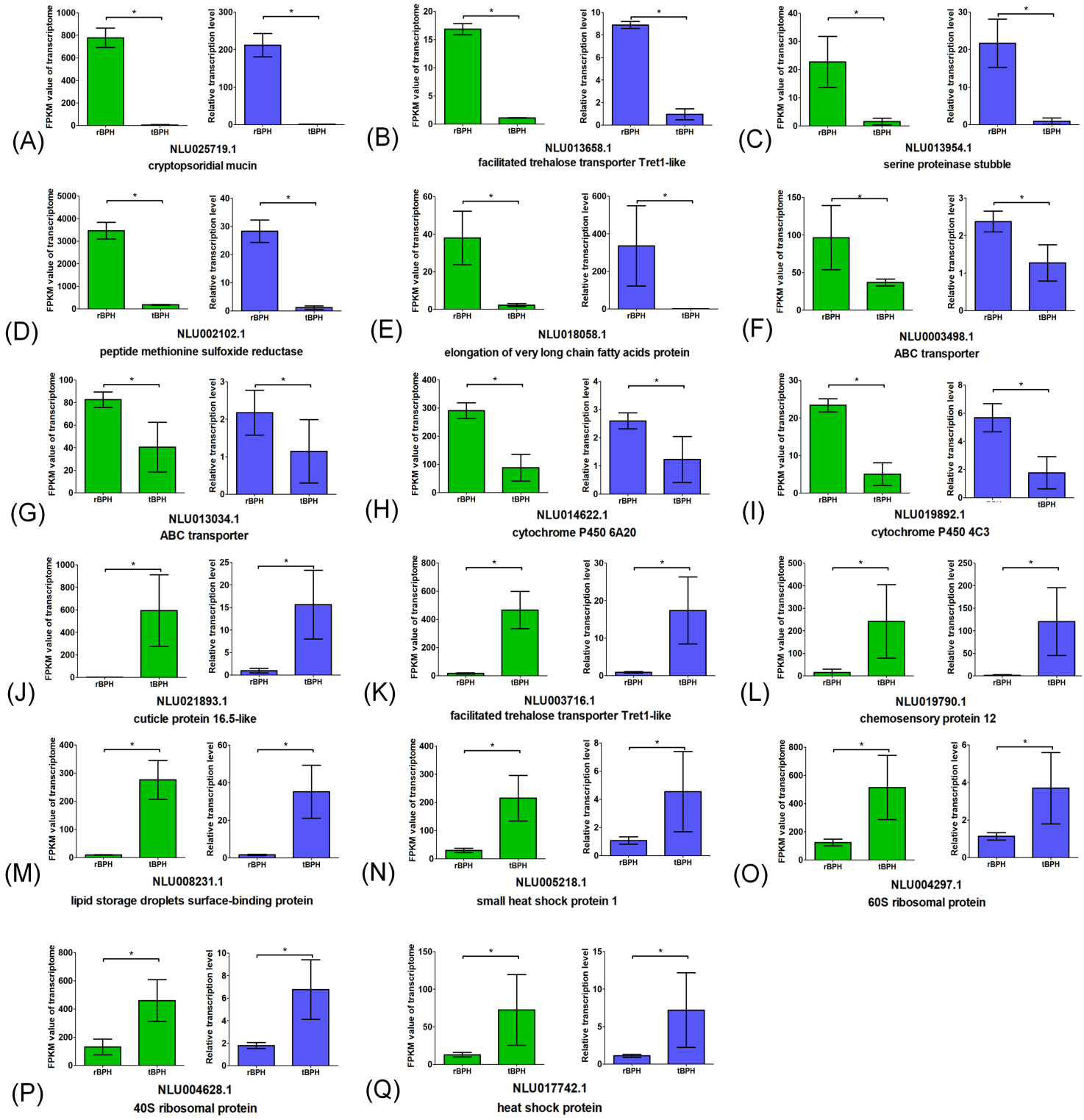

Figure 5

Correlation between transcriptomic data and qPCR results in BPH. The relative expression level of each gene was determined by qPCR (blue) and compared with that of transcriptomic data (green).

Supplementary Files

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