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# Comparative Transcriptome Analysis Depicts Candidate Genes Involved in Skin Color Differentiation in Red Tilapia

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**JH, PF and JQ presented the concept of the study and wrote the manuscript. YT and XL designed the study, interpreted the results, wrote and revised the manuscript. ZC performed the experiments while DZ helped him. HM helped in writing and revising the manuscript.**

**Key words**
**Comparative transcriptome analysis, Genetic molecular mechanism, Skin color differentiation, Red tilapia, Candidate genes for skin color differentiation, Tyrosine Kinase, HSP70, HSP30, Transcription factor Sp6, *Oreochromis* spp.**

## ABSTRACT

This study aimed to investigate the genetic molecular mechanism of body color differentiation and variation of red tilapia, selecting the main genes related to the variation and cultivating the pure and stable red tilapia variety. The effects of different temperature treatments on body color and survival of Guam red tilapia, pearl white red tilapia and Florida red tilapia were compared. Besides, comparative transcriptome analysis was used to screen the candidate genes linked to the skin color differentiation of pearl white red tilapia. Among them, the body color of Guam red tilapia changed when the water temperature dropped to 16-14°C, and continued to drop to about 11°C, it was discolored in a large area reaching above 90%. According to the differential analysis: Tyrosine Kinase STYK1, HSP70, HSP30 and Transcription factor Sp6 expressions were significantly increased in the low temperature group, while MC1R, Transcription factor (MafB, jun-D, AP-1, E2F5, ETV6, Sp9, Sp7, E2F1, Sp4) expressions were notably decreased. Further, the color variation of red tilapia at low temperature may result from the change of tyrosine activity in the body. The in-depth study of its regulatory mechanism contributes to understanding the genetic mechanism of red tilapia and improving the body shape.

## INTRODUCTION

Red Tilapia (genus *Oreochromis*), an essential tropical freshwater species is one of the most important edible fish in the world (Melo *et al*., 2014). It is widely cultured because of its rapid growth and tender meat (Noraini *et al*., 2013).

Fish belongs to poikilothermic animals whose body temperature varies with the environmental temperature (Yang *et al*., 2004), water temperature affects the survival, growth, metabolism, reproduction of fish (Ross *et al*., 2013). Tilapia is a warm-water fish with a temperature tolerance of 6-42 °C, but the tolerance to low temperature is influenced by water quality, fish age and health status (Nitzan *et al*., 2016). The extreme freezing weather in China always caused great economic loss to the tilapia farming industry (Luan, 2010). A larger number of red tilapia and wintering seedings that were ready for market were frozen and die under 8-10 °C of water temperature (Wang *et al*., 2018a). Additionally, the time of seedling release of the early spring red tilapia with low cold tolerance would be delayed, leading to its market price volatile (He *et al*., 2016). The supply and demand relationship of red tilapia was unbalanced, which had a major impact on the red tilapia breeding industry (El-Ebiary *et al*., 2013). Therefore, the physiological study on the cold tolerance of red tilapia can provide data supporting for the breeding of cold-resistant red tilapia in the future.

Differentiation during genetic selection and variation in body color has been a growing limitation to the commercial value of red tilapia (Wang *et al*., 2018b). The differentiation of body color in red tilapia is irreversible, while the change of skin color is reversible with the change of ambient temperature (Wang *et al*., 2018a). Fish pigment cells are mainly distributed in skin, and fins and scales are derivatives of the skin, and pigment cells are widely

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distributed. Previous study has shown that the two types of melanin produced by melanocytes extremely influence on body color phenotype, one of which is eumelanin being responsible for producing black and brown phenotypes, the other is brown melanin contributing to producing the yellow and red phenotypes (Kottler *et al.*, 2015). The formation of melanin is a complex process requiring the maturation of melanocytes, the synthesis and transportation of melanin, in which regulatory factors and signaling molecules are involved in each stage (Poletini *et al.*, 2016). The adenylate cyclase pathway, the protein kinase c pathway and the tyrosine pathway are three molecules and transduction pathways in melanocytes that synthesize melanin (Higanakamine *et al.*, 2015). The common genetic signaling pathways of two pigments have been discovered (Mandal *et al.*, 2010). The eumelanin is synthesized from tyrosine *in vivo*. Under the action of tyrosinase, tyrosine forms a eumelanin through a series of oxidation and catalytic polymerization (Chodurek *et al.*, 2013). It turns to the synthetic pathway of brown melanin after the reduction of tyrosinase activity (Chodurek *et al.*, 2013). Compared with other fish, little is known to the body color differentiation in red tilapia. Zhu *et al*. (2016) used comparative transcriptome sequencing to find the following genes related to skin color in red tilapia of three different colors: tyr, tyrp1, silv, sox10, slc24a5, CBS and slc7a11 (Zhu *et al*., 2016). The results showed that the miRNAs associated with red tilapia color mainly included slc7a11, mc1r and asip, predicating that miR-138-5p and miR-722 played an important role in the regulation of pigmentation. However, the molecular mechanism of body color differentiation remains unclear.

Thus, in this study, transcriptome sequencing was performed on the tissue samples of pearly white tilapia fin strips at low temperature and normal temperature to screen out the genes related to body color variation of red tilapia and provide a theoretical basis for the breeding of fine red tilapia strains.

## MATERIALS AND METHODS

*Sample collection*

Thirtysamples, each of Guam red tilapia (GR), pearl white red tilapia (WR) and Florida red tilapia (FR), which were bred in the same year and weighed 10g, 80g and 120g, respectively were selected for the experiment. The animal experiment complying with the ARRIVE guidelines was carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. Different red tilapia strains were placed in diverse aquarium for 5 days at 20 °C to adapt to the living environment of the aquarium for subsequent temperature culture test. Fish were fasted for 24h before the trial. The circulating water filtration system was adopted in this experiment, and feeding was conducted at 9:00 am and 4:00 pm, respectively every day. The laboratory temperature was artificially cooled, from 20 °C to 6 °C with gradient of 0.2 °C/2 h. During this period, water temperature was recorded every 2 h during the day and every 4 h at night. Meanwhile, data such as the deaths, the temperature at which the body color began to change, the location and degree of discoloration of red tilapia in each group were recorded. In the normal water temperature (20 °C) and cooling process (after 24 h of body color change), 3 fin tissues of WR were extracted for transcriptome sequencing respectively, stored in a -80 °C freezer before sequencing.

*RNA isolation and cDNA library construction*

Total RNA was extracted from tissue samples using MagZol Reagent. Then concentration and mass of each RNA sample was detected by nucleic acid protein detector (OD260: OD280) (Eppendorf, Germany) and agarose gel electrophoresis, respectively. The qualified RNA samples were enriched with magnetic beads with Oligo (dT). cDNA strand was synthesized with six base primers using enriched mRNA as template, and cDNA library was obtained by a series of modifications and PCR enrichment. The cDNA library was then quantitatively analyzed by Qbuit3.0, and the library insert size was detected by Agilent 2100 Bioanalyzer to ensure that the library effective concentration and quality meet the requirements of the RNA sequencing.

*Transcriptome sequencing and de novo assembly of transcriptome*

Each qualified cDNA library was sequenced using Illumina HiSeq2500 for 2 × 125 bp pair-end (PE) sequencing. Quality control of all raw reads was conducted by Fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) software. Raw data obtained from sequencing were filtered, and connector sequences and low-quality reads were removed to obtain high quality clean data. Gene assembly analysis was performed on high quality clean data after quality control. Trinity (v2.2.0) analysis software based on DE Bruijn graphs was used for subsequent analysis to assemble the transcriptome sequencing data from scratch. The sequences output from Trinity software can be used for further analysis.

*Assembled sequence annotation: Functional annotation*

Functional annotation of the assembled sequences was performed by homology searches against the Uni Prot-Swiss Prot (The Universal Protein Resource) database, the GO (Gene Ontology) database, the KEGG (Kyoto Encyclopedia of Genes and Genomes) database and the

TrEMBL protein database, and searches were conducted by the BLAST program.

*RT-PCR analysis*

Total RNA was isolated from samples by TRIzol method (Invitrogen, CA, USA), and cDNA was obtained by reverse transcription after DNA elimination. The results were calculated by ABI Prism 7500 RT-PCR system (Applied Biosystem, Foster City, CA, USA). Amplification was performed in a mixture (10 µl) containing 5 µl of 2X SYBR Green Master Mix, 1 µl of cDNA and 0.2 µl of each primer (10 µM). The thermal cycling curve consisted of an initial denaturation at 95°C for 5 min, followed by 40 cycles of 95 °C for 15 s, annealing/extension at 60 °C for 45s. Melting curve was generated by heating to 95 °C and cooling to 65 °C. All reactions were performed in triplicate and included a negative control without template. The value is based on identifying two biological replicates, each repeated 3 times. The gene expression level was normalized to β-actin expression in the same sample. A two-tailed t-test was used to compare expression levels.

## RESULTS

*Effect of temperature on apparent body color of red tilapia*

When the temperature dropped to 15 °C, body color of the PR and FR with the various body weight began to change; body color of the PR and FR changed over large areas when the temperature was decreased to 11 °C, and the discoloration ratio is all over 90%. When the temperature dropped to 10.2 °C, GR (10 g) began to change color, as temperature continued to reduce, the total body color change ratio was about 6%. However, for GR (80 g and 120 g), the decrease of temperature has no effect on the change of body color (Table I).

**Table I. Changes of body color of different species of red tilapia at different temperatures.**

| Weight | Species | $T_1$ (°C) | $T_2$ (°C) | Discoloration ratio (%) |
|---|---|---|---|---|
| 10 g | A | 10.2 | 0 | 6 |
| | B | 15~13 | 11~9 | 91 |
| | C | 14~13 | 11.5~9.6 | 95 |
| 80 g | A | 0 | 0 | 0 |
| | B | 15~14 | 11.6~9.8 | 94 |
| | C | 16~15 | 12.1~9.8 | 97 |
| 120 g | A | 0 | 0 | 0 |
| | B | 16~14 | 11.8~10.3 | 96 |
| | C | 16~15 | 11.8~10.0 | 97 |

T1, The water temperature starting to change color; T2, Water temperature when large areas of color changing. (A) Guam red tilapia; (B) Pearl white red tilapia; (C) Florida red tilapia.

*Transcriptome sequencing and assembly*

In the gradient cooling culture process, the apparent body color of WR changed obviously. Thus, 3 fin tissues of WR in the normal water temperature (20 °C) and cooling process (after 24 h of body color change) were selected for transcriptome sequencing. In total, a mean of 8, 194, 153 paired-end (PE) clean reads for each library were obtained after data filtering and the sequencing quality was high with Q30 ratio larger than 96% for all samples (Table II).

We pooled all the clean reads from six libraries and de novo assembled them using Trinity. A total of 117,609 contigs, corresponding to unique genes of which length ranged from 201 bp to 9,868 bp. There were 27,273 (23.19%) contigs longer than 1,000 bp. The detailed length distribution of transcripts from the red tilapia is shown in Figure 1.

**Table II. Transcriptome sequencing data of red tilapia.**

| Sample | Number of clean reads | Number of bases | Length (bp) | GC (%) | Q20 | Q30 |
|---|---|---|---|---|---|---|
| HLF3 | 8213990 | 1026748750 | 125 | 48.57 | 98.93 | 98.02 |
| HLF7 | 8174316 | 1021789500 | 125 | 48.58 | 97.89 | 96.26 |

Read_num, Number of sequences sequenced; Base_num (bp), Number of bases sequenced; Length (bp), Sequencing read length; GC%, Ratio of G base and C base to total base number; Q20, Ratio of the number of bases having a mass value greater than 20 (error rate less than 1%) to the total number of bases; Q30, Ratio of the number of bases whose mass value is greater than 30 (error rate less than 0.1%) to the total number of bases.

*GO annotation and analysis of DEGs*

Gene ontology (GO) annotation was then performed based on the Nr annotation and 34,542 contigs (29.37%) were assigned to GO term (Fig. 2). As shown in Figure 2, a total of 49 terms were assigned, including 23 (46.94%) biological process category, 13 (26.53%) cellular component terms and 13 (26.53%) molecular function terms. In the biological process category, cellular process was the most abundant term, followed by metabolic process biological regulation and pigmentation. For the cellular component category, cell and cell part were the predominant terms which were followed by organelle. For the molecular function category, binding was the main term, and it was followed by catalytic molecular transducer and transcription regulator (Fig. 2).

*Functional annotation*

All contigs were compared with four data bases including EMBL database, the UniProt-SwissProt database, KEGG database and GO database for functional annotation. There were 27997, 35370, 34542 assembled contigs that had significant hits against EMBL database, the UniProt-SwissProt database and GO database,

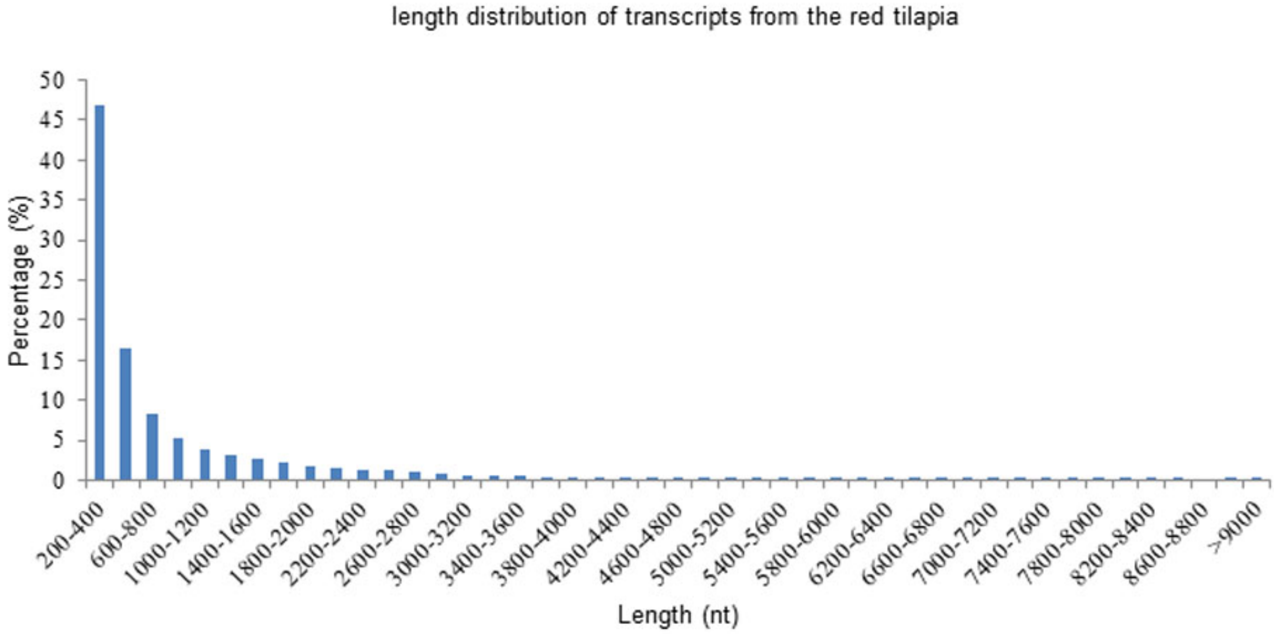


Fig. 1. Transcriptome sequencing and assembly. The detailed length distribution of transcripts from the red tilapia.

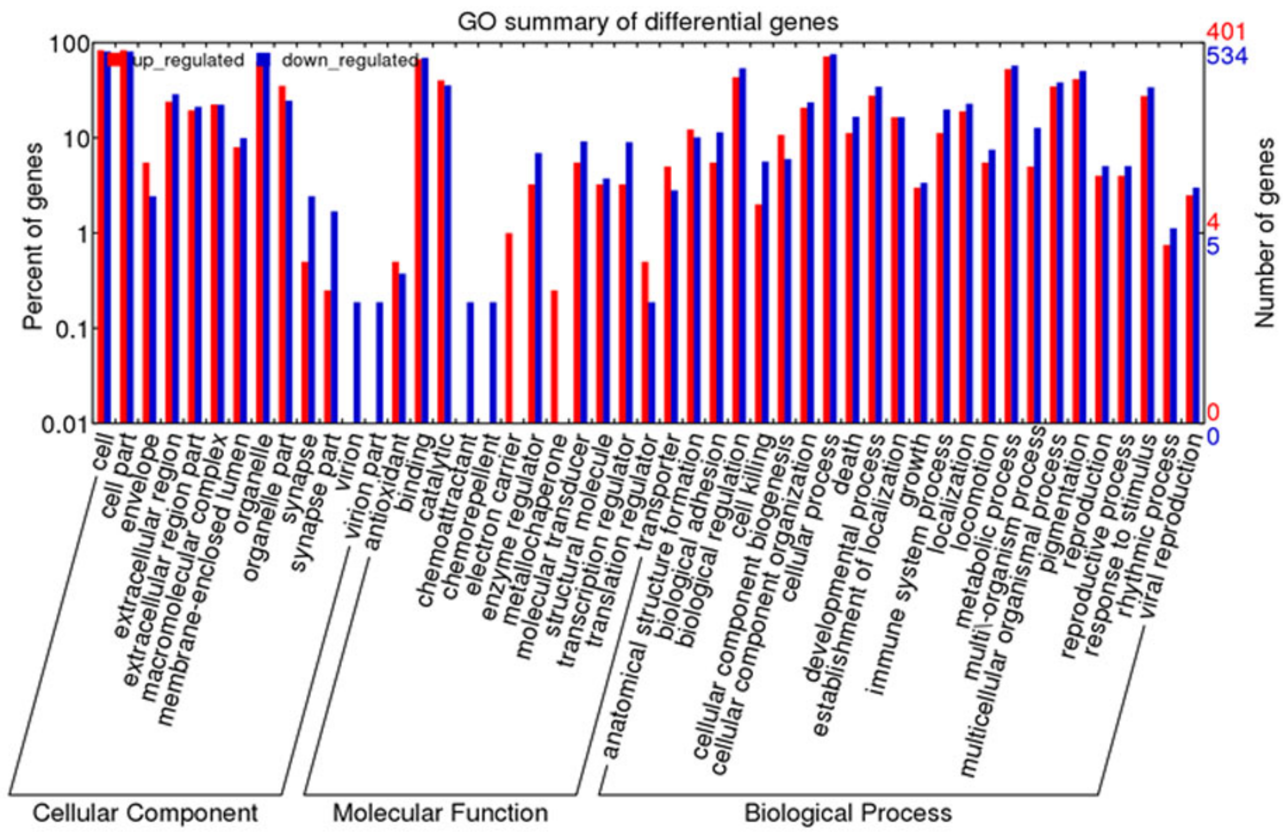


Fig. 2. GO annotation. Gene ontology classification of contigs.

respectively (Table III, Fig. 2). To identify the biological pathway in the red tilapia transcriptome, all contigs were mapped to the KEGG database and were associated with 216 pathways (Supplementary Table I).

**Table III. Statistics of function annotation.**

| Annotated database | Annotated number |
|---|---|
| Swiss-PROT | 35370 |
| EMBL | 27997 |
| GO | 34542 |
| All contigs | 117609 |

*Identifying candidate genes involved in skin color and pigmentation*

To show the differences in the skin color of red tilapia, we made a comparative analysis of two skin transcriptomes. Based on the standard that |logFC| ≥ 1 and FDR ≤ 0.5, 3, 228 differentially expressed transcripts were identified between HLF7 and HLF3, which include 1,327 up-regulated transcripts and 1,901 down-regulated transcripts in HLF7. Candidate gene enrichment analysis identified 49 candidate genes involved in the processes of skin color and pigmentation from the two transcriptome data sets. Interestingly, six genes were revealed to be specifically expressed in HLF7, while 16 genes were only found expressed in HLF3 (Table IV).

## DISCUSSION

We employed the transcriptome analysis to investigate the different gene expression of fin tissues of WR, indicating that the color change of red tilapia at low temperature arose from the co-regulation of multiple genes (tyrosine protein kinase *STYK1, HSP 70, HSP 30, HSP 90, MC1R* and transcription factor).

Heat shock protein (HSP70) is one of the most conservative and important proteins in HSPP family which is synthesized by the reaction of organism to the physical, chemical and biological stress agents in the environment (Shen *et al*., 2016). *HSP70* gene can produce abundant heat stress proteins, remove the denatured proteins, protect the cells from the damage of denatured proteins, and reduce the impact of external stress environment on the body of fish when the cells are under stress (González-Aravena *et al*., 2018). Studies have found that the HSP70 mRNA expression in the liver was significantly increased after 12 h of low-temperature stress on the fish (Ming *et al*., 2012). It has been demonstrated that both low temperature and high temperature caused the rapid expression of *HSP70* gene in the body cells of platy bream and the synthesis of heat stress protein (Ming *et al*., 2012). In our study, the expression of HSP70 in HLF7 cultured at low temperature was significantly higher than that in normal temperature group, suggesting that the low temperature environment outside led to the changes in the physiological function of red tilapia. Besides, it is worth noting that the fry that has not yet fully developed are more susceptible to temperature changes and change their body color in our experiments, such as GR. Consistently, studies have shown that huge genetic variation in body color can provide a good indication of its potential improvement, and the body length and width of red tilapia are negatively correlated with body color (-0.47 to -0.25) (Hamzah *et al*., 2017). However, the specific mechanism of this change still needs further study.

The biological basis of body color change in fish is the number, morphology and distribution of different pigment cells on skin and scales (Steffen *et al*., 2015). Compared with only a kind of pigment cell in the mammalian (melanocytes), bony fishes were found to have 6 kinds of pigment cells, including the melanocytes, red pigment cells, yellow pigment cells, rainbow, white and blue pigment cells, making the formation and changes of the fish body color more complicated and different red tilapia showing different body colors (Erickson, 2010). It has also been reported that *MC1R* gene, *ASIP* gene and *TYRP1* gene are the major candidate genes of fish body color formation (Jian-Xiong *et al*., 2014). *MC1R* gene, as a key gene to produce melanin, expressed slightly in HLF7 of the low temperature group, and it was not the main reason for the formation of black spots (San-Jose *et al*., 2015). It is speculated that the formation of melanin by *MC1R* gene was affected by the albino gene. Meanwhile, the *MC1R* gene, which was related to the synthesis of melanin, represents a critical role in the formation of fish body color. However, MC1R expression was low in HLF7 in black-spotted red tilapia of the low-temperature group in our study, probably because it was an acute regulator and made pigments significantly deposited in the short term. And hence the surface of the skin was colored with streaks, and then black markings or red markings, but the expression level was stable.

It has been revealed that the body color of Mozambique tilapia became black in the dark after 25 h, but there was no significant change in MC1R expression in the epidermis and the α-MSH level in the serum. Therefore, the *MC1R* gene could also act as an acute regulator in the regulation of fish pigmentation, but the pigment is significantly deposited in a short period sine this activity expression cannot be maintained for a long time (Moorman *et al*., 2015). So even if the red tilapia epidermis exhibited a certain color, there was no significant change in MC1R mRNA expression in fish tissues.

**Table IV. Expression and annotation of candidate genes involved in skin color and pigmentation between HLF7 and HLF3.**

| Gene_ID | FPKMHLF3 | FPKM HLF7 | Fold change (log2) | Annotation |
|---|---|---|---|---|
| TRINITY_DN24220_c0_g4 | 0 | 62.65 | 10.41823029 | Heat shock protein 30 |
| TRINITY_DN24220_c0_g1 | 0 | 62.17 | 10.0705945 | Heat shock protein 30 |
| TRINITY_DN45765_c0_g1 | 0 | 19.36 | 9.377526023 | Heat shock protein HSP 90-alpha |
| TRINITY_DN24220_c0_g2 | 0 | 61.72 | 8.461914888 | Heat shock protein 30 |
| TRINITY_DN24220_c0_g3 | 0 | 55.3 | 8.292501236 | Heat shock protein 30 |
| TRINITY_DN22422_c0_g1 | 0.49 | 50.84 | 8.0814764 | Heat shock 70 kDa protein 1 |
| TRINITY_DN2485_c0_g1 | 0 | 1.36 | 7.204597264 | Transcription factor Sp6 |
| TRINITY_DN40960_c0_g1 | 2.04 | 61.57 | 5.358205112 | Heat shock protein HSP 90-beta |
| TRINITY_DN55897_c0_g1 | 1.4 | 21.72 | 4.455123638 | Heat shock protein HSP 90-alpha |
| TRINITY_DN290_c0_g1 | 0.33 | 3.55 | 3.922387454 | Heat shock protein beta-8 |
| TRINITY_DN7424_c0_g1 | 0.45 | 4.57 | 3.839197418 | Myosin-binding protein C, fast-type |
| TRINITY_DN30976_c0_g1 | 4.41 | 38.63 | 3.665320161 | Heat shock protein HSP 90-alpha |
| TRINITY_DN45441_c0_g3 | 0.23 | 2.1 | 3.523276762 | Mitogen-activated protein kinase 8 |
| TRINITY_DN21271_c0_g1 | 2.58 | 12.4 | 2.880544153 | Tyrosine-protein kinase STYK1 |
| TRINITY_DN44078_c0_g1 | 2.11 | 10.63 | 2.861156424 | Heat shock protein HSP 90-alpha |
| TRINITY_DN20921_c0_g1 | 13.79 | 67.1 | 2.829661276 | Carbonic anhydrase 4 |
| TRINITY_DN12210_c0_g1 | 60.47 | 219.48 | 2.40838373 | Dual specificity protein phosphatase 5 |
| TRINITY_DN10113_c0_g1 | 1.69 | 7.21 | 2.37892893 | Cyclin-dependent kinase 16 |
| TRINITY_DN23272_c0_g2 | 5.5 | 18.25 | 2.278654253 | Heat shock 70 kDa protein 4 |
| TRINITY_DN11134_c0_g1 | 219.16 | 30.3 | -2.305170585 | Transcription factor jun-D |
| TRINITY_DN19725_c1_g2 | 14.92 | 1.89 | -2.426863715 | Fibroblast growth factor receptor 1-A |
| TRINITY_DN19383_c0_g1 | 0.48 | 0 | -2.558910746 | Sphingosine 1-phosphate receptor 1 |
| TRINITY_DN25429_c2_g1 | 7.02 | 0.74 | -2.644540997 | Calcium/calmodulin-dependent protein kinase type II subunit gamma |
| TRINITY_DN5260_c0_g1 | 21.2 | 2.24 | -2.686316481 | Protein kinase C beta type |
| TRINITY_DN22985_c0_g1 | 32.56 | 4.14 | -2.716853168 | Dual specificity protein phosphatase 2 |
| TRINITY_DN24675_c0_g2 | 11.08 | 1.16 | -2.731113656 | Myosin light chain kinase, smooth muscle |
| TRINITY_DN25683_c1_g3 | 5 | 0.37 | -2.789473925 | Heat shock 70 kDa protein 12A |
| TRINITY_DN15797_c0_g1 | 1.23 | 0 | -2.805596395 | Serine/threonine-protein kinase N1 |
| TRINITY_DN20404_c0_g1 | 4.67 | 0.36 | -3.138131195 | Mast/stem cell growth factor receptor Kit |
| TRINITY_DN5929_c0_g1 | 295.71 | 22.79 | -3.148401717 | Transcription factor jun-D |
| TRINITY_DN1493_c0_g1 | 4.86 | 0.36 | -3.168662628 | Epithelial discoidin domain-containing receptor 1 |
| TRINITY_DN2926_c0_g1 | 5.24 | 0.36 | -3.272375487 | Traf2 and NCK-interacting protein kinase |
| TRINITY_DN11441_c0_g1 | 196.91 | 9.25 | -3.862108956 | Transcription factor AP-1 |
| TRINITY_DN20977_c0_g1 | 5.43 | 0 | -4.447806609 | Transcription factor E2F5 |
| TRINITY_DN63906_c0_g1 | 4.11 | 0.11 | -4.585107644 | Transcription factor Sp7 |
| TRINITY_DN9373_c0_g1 | 6.33 | 0 | -6.800394984 | Hormonally up-regulated neu tumor-associated kinase homolog A |
| TRINITY_DN25415_c0_g1 | 1.25 | 0 | -7.019203017 | Collagen alpha-1(XII) chain |

*Table continued on next page .........................*

| Gene_ID | FPKMHLF3 | FPKM HLF7 | Fold change (log2) | Annotation |
|---|---|---|---|---|
| TRINITY_DN71751_c0_g1 | 2.12 | 0 | -7.09264739 | Transcription factor E2F1 |
| TRINITY_DN9015_c0_g1 | 6.62 | 0 | -7.09264739 | Transcription factor Sp4 |
| TRINITY_DN3355_c0_g1 | 1.84 | 0 | -7.09264739 | STE20-related kinase adapter protein beta |
| TRINITY_DN53770_c0_g1 | 3.26 | 0 | -7.09264739 | Ephrin type-A receptor 7 |
| TRINITY_DN8410_c1_g1 | 3.07 | 0 | -7.353919053 | Heat shock 70 kDa protein 12A |
| TRINITY_DN4370_c0_g1 | 10.21 | 0 | -7.468717718 | Heat shock 70 kDa protein 12B |
| TRINITY_DN25415_c0_g2 | 1.93 | 0 | -7.625416211 | Collagen alpha-1(XII) chain |
| TRINITY_DN25683_c0_g1 | 12.05 | 0 | -8.122847214 | Heat shock 70 kDa protein 12A |
| TRINITY_DN5021_c0_g1 | 4.83 | 0 | -8.670545959 | Transcription factor Sp9 |
| TRINITY_DN43769_c0_g1 | 100.52 | 0.14 | -8.913081177 | Neoverrucotoxin subunit alpha |
| TRINITY_DN24960_c0_g3 | 26.53 | 0 | -9.961230712 | Transcription factor ETV6 |
| TRINITY_DN1001_c0_g1 | 22.66 | 0 | -11.23238536 | Transcription factor MafB |

Tyrosinase is the rate-limiting enzyme of melanin synthesis vital for the rate and quantity of eumelanin and pheomelanin (Hirobe *et al*., 2011). During the formation of melanin, hormones, inorganic ions which are *in vivo* and external light, UV light physicochemical factors may affect the activity of TYR. Besides, the of the eumelanin to pheomelanin by *ASIP* gene was linked to the expression of the tyrosine family gene (*TYR, TYRP1, DCT*) and reduced its expression (Meng *et al*., 2014). Currently, the expression of gene related to the tyrosinase synthesis becomes an important indirect indicator of the energy of melanin formation in fish due to the absence of an effective method for determining the melanin content and body color index of fish (Chen *et al*., 2012). In addition, the coding transcription factors of pigment cells are also regulated by gene signal channels. It has been found that gene knockout can regulate the transcription factors of pigment cells in c-kit signal transduction, resulting in the loss of their function and pigmentation (Otsuki *et al*., 2020). Low temperature stress can also lead to changes in the expression of transcription factors. In our study, the expression of transcription factors decreased significantly at low temperature, but the related mechanism is unknown.

## CONCLUSION

In this study, the body color of the GR with a small body weight changed significantly with temperature, and the melanin synthesis ability was enhanced in the low temperature group HLF7. The result was consistent with the tendency of red tilapia to become darker as the temperature gradient decreased. The pathway of melanin synthesis is a complex process involving the participation and regulation of polygene. Nevertheless, the overall melanin content, the ratio of eumelanin to brown melanin and the molecular metabolic mechanism of red tilapia body color variation was still needed to be further studied.


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*Supplementary material*

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20200326090308

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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