

Assessment of growth performance and transcriptome analysis of mechanisms underlying slow growth in triploid grass carp*

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Abstract Triploid induction has been widely used in aquaculture to increase somatic growth, induce reproductive sterility, and mitigate ecological risks from uncontrolled breeding. However, the differences in growth between triploids and diploids are species specific. Grass carp (*Ctenopharyngodon idella*) is a crucial species in China's freshwater aquaculture industry. A one-year growth trial was conducted, and the results reveal that triploids exhibited a slower growth rate than diploids did. Further analyses demonstrated that the number of erythrocytes and the size of their nuclei were significantly greater in triploids than in their diploid counterparts ($P < 0.001$), which was accompanied by a reduced packed cell volume (PCV) in triploids ($P < 0.05$). Histological analysis revealed that the triploid liver displayed features of metabolic stress. Liver transcriptome analysis identified 713 differentially expressed genes (DEGs), which showed primarily enrichment in metabolic processes, including hexose, lipid, and monosaccharide metabolism. This analysis also revealed significant downregulation of the growth hormone/insulin-like growth factor (GH/IGF) axis (*ghrb*, *igf1*, and *igfbp5a*) and downstream signaling pathways, namely, PI3K-AKT in triploids. The results of this study characterize the early growth performance of triploid grass carp and provide molecular insights into the growth retardation observed in triploid individuals.

Keyword: grass carp; triploidy; growth hormone/insulin-like growth factor (GH/IGF) axis; transcriptome

1 INTRODUCTION

The induction of triploidy is a valuable approach for genetic improvement in fish breeding. Compared with diploid fish, which expend considerable energy on gonadal development, triploid fish are considered to possess growth advantages because of their gonadal sterility (Tiwary et al., 2004). Several researchers have reported that triploid fish exhibit faster growth rates than their diploid counterparts. For instance, triploid Atlantic salmon (*Salmo salar*), yellow croaker (*Pseudosciaena crocea*), and Nile

tilapia (*Oreochromis niloticus*) outperform diploids in terms of growth performance (Oppedal et al., 2003; Xu et al., 2008; Soltan et al., 2017). However, other studies have indicated that the growth rates of triploid fish are comparable to those of diploid fish. For example, the growth rates of the triploid mud loach (*Misgurnus mizolepis*) and turbot (*Scophthalmus maximus*) are similar to those of their diploid

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counterparts, with no significant differences observed (Kim et al., 1994; Aydın et al., 2021). Furthermore, some studies have suggested that the growth rates of triploid fish may be slower than those of diploids. Notably, the growth performance of triploid blunt snout bream (*Megalobrama amblycephala*) is slower than that of diploids (Li et al., 2006). These studies provide evidence that the growth performance of triploid fish varies considerably across species and that the underlying mechanisms remain poorly understood.

It is well established that fish growth is regulated by the growth axis. The hypothalamus secretes growth hormone-releasing hormone (GHRH), which regulates the synthesis and release of growth hormone (GH) from the pituitary gland. GH is transported via the bloodstream to the liver, where it stimulates the production of insulin-like growth factor (IGF). IGF is then transported to peripheral tissues to promote cell growth and proliferation (Dai et al., 2015). Therefore, investigating the expression and regulatory patterns of growth-axis-related genes in triploid fish will help elucidation of the molecular mechanisms underlying their growth.

As a cornerstone of China's freshwater aquaculture industry, grass carp (*Ctenopharyngodon idella*) yields more than 5 million t annually (Li et al., 2023). However, its prolonged sexual maturation cycle of 4–5 years greatly limits the pace of genetic selection. Thus, there is a pressing need to develop additional breeding techniques to facilitate the genetic improvement of grass carp. We successfully induced a large population of triploid grass carp fry through cold shock treatment (E et al., 2025), and their growth potential remains to be evaluated. In this study, we conducted a 12-month monitoring period to assess the growth of diploid and triploid grass carp. Additionally, we examined erythrocyte size and conducted liver transcriptome analysis in 6-month-old grass carp to elucidate molecular mechanisms related to the growth hormone/insulin-like growth factor (GH/IGF) axis underlying the differences in growth between triploid and diploid fish.

2 MATERIAL AND METHOD

2.1 Assessment of fish rearing and growth performance

The grass carp used in this experiment were hatched in May 2024 and reared in earthen ponds (approximately 2 667 m², and average depth 1.5 m) in the Jianglong Shunjing Aquaculture Farm in

Zhongshan, China. Diploid and triploid fish (a total of 6 000 individuals) were co-reared and fed commercial feed daily at a ration equal to 2% of their body mass. The Animal Research and Ethics Committee of Sun Yat-sen University approved all the animal procedures (SYSU-IACUC-2025-B1553). Ploidy verification was performed prior to the experiments using flow cytometry, following described methods (E et al., 2025). Growth performance was evaluated periodically, with 30 individuals from each ploidy group randomly sampled for measurements of total length (cm) and body weight (g).

2.2 Morphological trait measurement

At 6 months of age, morphological measurements were taken from a random selection of 30 diploid and 30 triploid grass carp. The measurable traits included total length (ToL), body length (BL), head length (HL), trunk length (TrL), body height (BH), body width (BW), snout length (SL), eye diameter (ED), caudal peduncle length (CpL), and caudal peduncle height (CpH), and the following ratios were calculated: ToL/BL, ToL/HL, ToL/TrL, ToL/BH, ToL/BW, BH/BW, HL/SL, HL/ED, and CpL/CpH. The quantifiable characteristics included the fin ray counts in the dorsal, pectoral, ventral, anal, and caudal fins, along with the number of lateral line scales and scales situated above and below the lateral line.

2.3 Packed cell volume and blood smear

Five diploid and five triploid grass carp were randomly selected and anaesthetized with MS-222. A total of 100 μ L of anticoagulant ACD solution (Macklin, China) was drawn into a sterile syringe, after which 900 μ L of blood was collected via caudal vein puncture. Packed cell volume (PCV) was determined by collecting whole blood into capillary tubes, which were then centrifuged at 7 000 r/min for 5 min. After centrifugation, the height (in mm) of the erythrocyte layer and the total height of the blood column were measured. PCV was calculated as the ratio of the erythrocyte layer height to the total blood column height. Thereafter, blood smears were prepared using a described method (Jiang et al., 2024) and stained with Wright's stain following the manufacturer's instructions. Erythrocytes were captured using a Nikon Eclipse Ni-E microscope (Tokyo, Japan), and their major axis, minor axis, and area were measured with ImageJ software. The erythrocyte volume was determined using the following formula:

$$\text{Erythrocyte volume} = \frac{4}{3} \times \pi \times (\text{major axis}/2) \times (\text{minor axis}/2)^2.$$

2.4 Histological analysis of liver tissues

Liver tissues were obtained from diploid and triploid grass carp. After fixation in Bouin's solution, the tissues were dehydrated, permeabilized, embedded, sectioned into slices (4–5 μm), and stained with hematoxylin and eosin (H&E). Hepatic structure and hepatocyte morphology were examined under a ZEISS microscope (Carl Zeiss, Germany).

2.5 Transcriptomic sample preparation and sequencing

Liver tissues were collected from grass carp of both ploidy types ($n=3$), snap-frozen in liquid nitrogen, and subsequently used for total RNA extraction with TRIzol reagent (Invitrogen, USA). After quality assessment, mRNA was enriched and fragmented, followed by first- and second-strand cDNA synthesis, adaptor ligation, purification of ligated products, library amplification, and purification of amplified products using the Hieff NGS® Ultima Dual-mode mRNA Library Prep Kit (Yeasten, China). The resulting cDNA libraries were assessed using a DNA 1 000 assay kit (Agilent Technologies, USA) and sequenced on an Illumina NovaSeq X Plus instrument.

2.6 Transcriptomic analysis

Transcriptome data analysis was conducted following the methods described in our previous publication (E et al., 2025). After low-quality sequences and ribosomal RNA sequences were eliminated, the remaining clean reads were aligned to the grass carp reference genome (PRJNA745929). Gene expression levels were calculated as FPKMs using RSEM (Li and Dewey, 2011). Relationships among samples were assessed via principal component analysis (PCA) and correlation analysis using the gmodels package (v. 2.18.1) in R (<http://www.r-project.org/>). Differentially expressed genes (DEGs) between ploidy groups were identified with DESeq2 (v. 1.38.3) (Love et al., 2014). Genes were considered differentially expressed when they satisfied the threshold of $|\log_2(\text{fold change})| \geq 1$ and a false discovery rate (FDR)-adjusted $P < 0.05$. Functional annotation and enrichment analyses were performed separately for the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

2.7 Quantitative real-time polymerase chain reaction (qRT-PCR) validation

qRT-PCR was conducted to validate the transcriptome data. The expression levels of growth hormone receptor b (*ghrb*), insulin-like growth factor 1 (*igf1*), insulin-like growth factor binding proteins (*igfbp1a* and *igfbp5a*), v-akt murine thymoma viral oncogene homolog 1 (*akt1*), tyrosine-protein kinase JAK1 (*jak1*), phosphoinositide-3-kinase regulatory subunit 1 (*pik3r1*), protein phosphatase 1 regulatory subunit 3B (*ppp1r3b*), and eukaryotic translation initiation factor 4ea (*eif4ea*) were examined. The primer pairs used are listed in Supplementary Table S1. qRT-PCR analysis was performed with ChamQ Blue Universal SYBR qPCR Master Mix (Vazyme, China) on a LightCycler 480 II system (Roche, Basel, Switzerland). β -actin was the internal reference, and relative transcript levels were calculated according to the $2^{-\Delta\Delta C_t}$ method.

2.8 Statistical analysis

The data are expressed as the means \pm standard deviation (SD). Prior to analysis, data normality and homogeneity of variance were assessed. Statistical comparisons were conducted using unpaired Student's *t*-test or one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set as $P < 0.05$.

3 RESULT

3.1 Comparative characterization of morphological traits and growth performance in diploid and triploid grass carp

Growth performance, including total length and body weight, was assessed in both ploidy types at 2, 4, 6, and 12 months. The results revealed no significant difference in body weight between diploids and triploids at 2 months, although diploids had a significantly greater total length ($P < 0.05$). From 4 months of age onwards, triploid individuals exhibited significantly lower total length and body weight compared to diploid individuals ($P < 0.001$; Fig.1). Morphometric analysis revealed notable differences between diploid and triploid grass carp. Among the measurable traits, the ToL/BW, BH/BW, and CpL/CpH ratios were significantly lower in triploids than in diploids ($P < 0.05$; Table 1). In addition, countable traits such as the number of pectoral and caudal fin rays differed significantly between triploids and diploids ($P < 0.05$; Table 2).

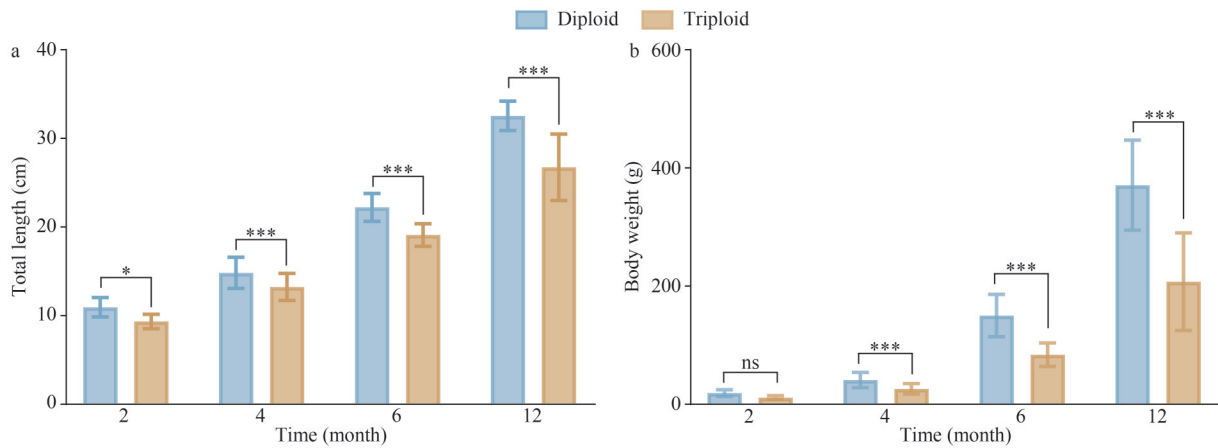


Fig.1 Growth performance of diploid and triploid grass carp at 2, 4, 6, and 12 months of age

a. total length (cm); b. body weight (g). ns: represents not significant; *: $P < 0.05$; ***: $P < 0.001$.

Table 1 Measurable characteristics of both ploidy types of grass carp

Group	ToL/BL	ToL/HL	ToL/TrL	ToL/BH	ToL/BW	BH/BW	HL/SL	HL/ED	CpL/CpH
Diploid	1.19±0.01	4.81±0.23	2.34±0.07	4.83±0.21	7.20±0.45 ^a	1.49±0.10 ^e	3.62±0.47	5.01±0.55	1.35±0.15 ^e
Triploid	1.19±0.02	4.72±0.22	2.31±0.10	4.97±0.36	6.38±0.39 ^b	1.29±0.08 ^d	3.38±0.32	5.16±0.58	1.27±0.11 ^f

ToL: total length; BL: body length; HL: head length; TrL: trunk length; BH: body height; BW: body width; SL: snout length; ED: eye diameter; CpL: caudal peduncle length; CpH: caudal peduncle height. Different superscript letters within the same column indicate significant differences ($P < 0.05$).

Table 2 Countable characteristics of both ploidy types of grass carp

Group	No. of dorsal fins	No. of pectoral fins	No. of ventral fins	No. of anal fins	No. of caudal fins	No. of lateral scales	No. of scales above the lateral line	No. of scales below the lateral line
Diploid	III-7.00±0	I-16.00±0 ^a	II-8.00±0	III-8.36±0.50	18.90±0.30 ^e	38.36±1.43	6.27±0.46	5.00±0
Triploid	III-7.10±0.31	I-17.70±0.67 ^b	II-7.70±0.48	III-8.10±0.31	18.37±0.52 ^d	39.25±1.76	6.50±0.52	4.87±0.31

Different superscript letters within the same column indicate significant differences ($P < 0.05$).

These results suggest that differences in ploidy could significantly affect the external body conformation and growth performance of grass carp.

3.2 Comparative assessment of packed cell volume and erythrocyte morphology in diploid and triploid grass carp

Significant morphological differences were observed in erythrocytes between triploid and diploid individuals (Fig.2a–b). PCV analysis revealed that compared with diploid individuals, triploid grass carp had a significantly lower ratio ($P < 0.05$; Fig.2c), indicating a reduced proportion of erythrocytes in whole blood. The mean lengths of the major and minor axes of triploid erythrocytes were 14.34 ± 0.94 and 7.52 ± 0.61 μm , respectively, whereas those of diploids were 11.02 ± 0.54 and 6.29 ± 0.56 μm , respectively. Furthermore, the area and volume of erythrocytes in triploids were 1.55-

and 1.84-fold greater than those in diploids, respectively. The nuclear volume of triploid erythrocytes was also 1.48-fold greater than that of diploids (Table 3; Fig.2d–e). Collectively, these results indicate that triploid grass carp have markedly larger erythrocytes than diploid grass carp.

3.3 Liver histology of diploid and triploid grass carp

Histological analysis revealed a normal overall hepatic architecture in both ploidy groups (Fig.3a & c). In the triploid liver tissue, the hepatocyte nuclei were enlarged with condensed chromatin, and the intercellular spaces were irregular. Moreover, triploid hepatocytes exhibited deepened cytoplasmic staining and cytoplasmic vacuolization (Fig.3d). These histological alterations suggest the presence of metabolic stress in the triploid liver.

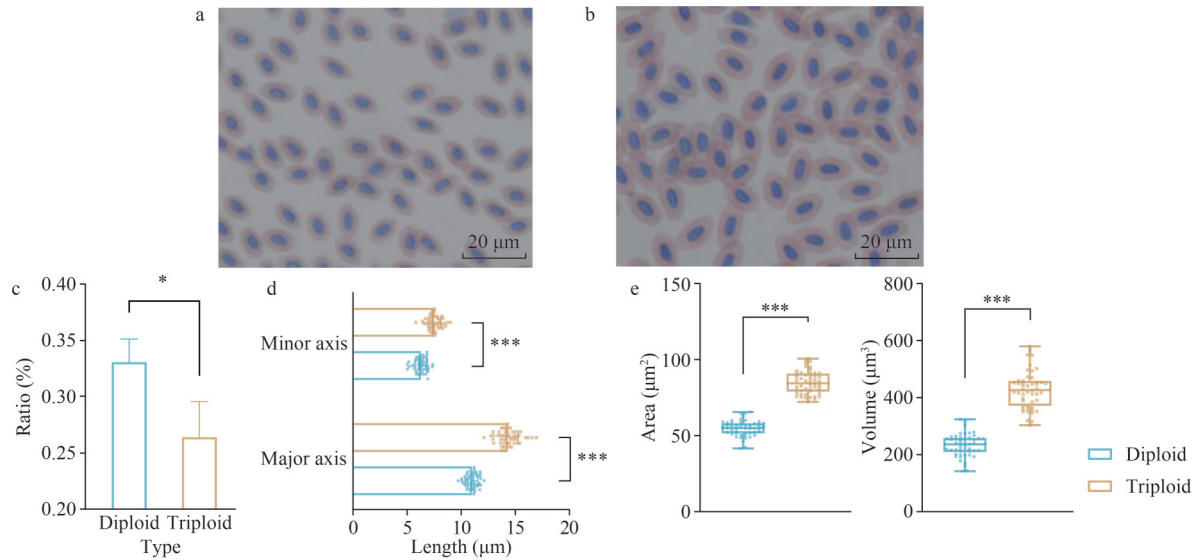


Fig.2 Morphological characteristics and hematological parameters of erythrocytes in both ploidy types of grass carp

a. blood smears of diploid grass carp; b. blood smears of triploid grass carp; c. packed cell volume (PCV) in diploid and triploid grass carp; d. comparison of erythrocyte major and minor axis lengths (μm) between diploid and triploid grass carp; e. comparison of erythrocyte area (μm^2) and volume (μm^3) between diploid and triploid grass carp. Scale bars represent 20 μm . Asterisks indicate significant differences between the diploid and triploid groups (*: $P<0.05$; ***: $P<0.001$).

Table 3 Comparative analysis of erythrocyte and nuclear morphological parameters in diploid and triploid grass carp

Group	Ploidy	Major axis (μm)	Minor axis (μm)	Area (μm^2)	Volume (μm^3)	Area ratio	Volume ratio
Erythrocyte	Diploid	11.02 \pm 0.54	6.29 \pm 0.56	54.47 \pm 5.16	230.87 \pm 39.87	1.55	1.84
	Triploid	14.34 \pm 0.96	7.52 \pm 0.61	84.65 \pm 7.42	426.73 \pm 66.72		
Nuclei	Diploid	5.08 \pm 0.35	2.43 \pm 0.26	9.71 \pm 1.24	15.95 \pm 3.52	1.36	1.48
	Triploid	6.32 \pm 0.19	2.66 \pm 0.22	13.25 \pm 1.28	23.76 \pm 4.24		

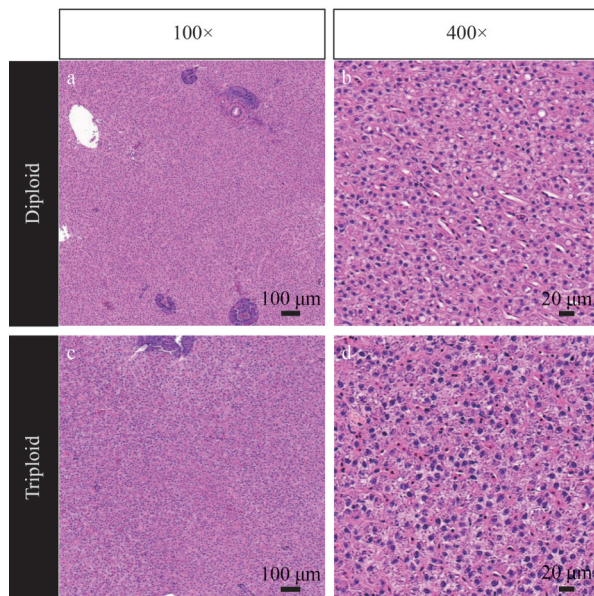


Fig.3 Liver sections of diploid (a and b) and triploid (c and d) grass carp

a & c. the overall liver architecture at 100 \times magnification; b & d. the morphology of hepatocytes at 400 \times magnification. Scale bars are in the lower right corner of each image.

3.4 Transcriptome sequencing and differentially expressed gene analysis

Six liver cDNA libraries were constructed and sequenced for diploid and triploid grass carp. After quality control, 41.07 Gb of clean data were obtained. The Q20 and Q30 exceeded 98% and 94%, respectively, and the GC content ranged from 45.92% to 47.13% (Supplementary Table S2). The raw data were submitted to NCBI (PRJNA1298407). After filtering, a total of 265 945 922 reads were obtained, with an average mapping rate of 96.64% to the *Ctenopharyngodon idella* reference genome (Supplementary Table S3). To evaluate the similarity among the samples, the Pearson correlation analysis and sample correlation heatmap were conducted. As shown in Fig.4a–b, biological replicates from the same ploidy and tissue group are generally clustered. These results indicate the reliability of the data for downstream analysis. A total of 713 DEGs were subsequently identified in the liver tissue between diploids and triploids, including 84 upregulated genes and 629 downregulated genes (Fig.4c).

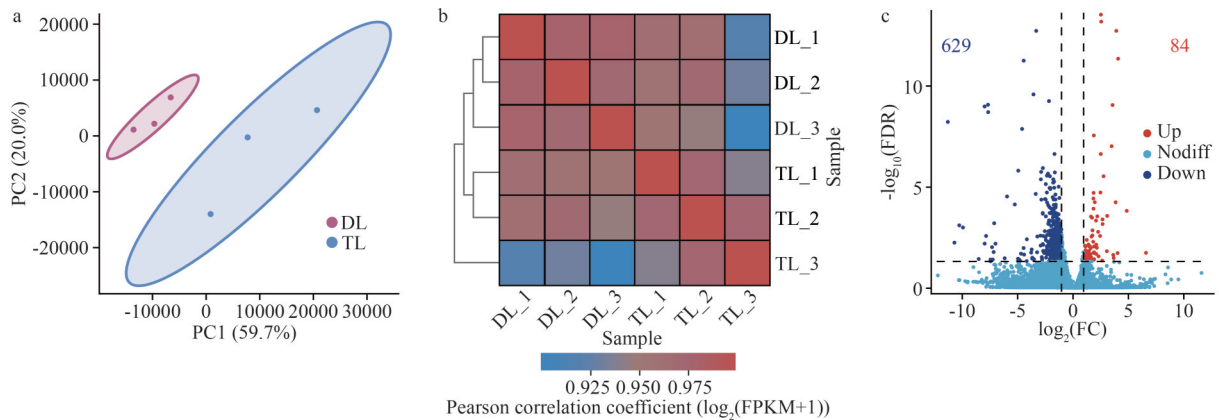


Fig.4 Analysis of the relationships among samples and DEGs in the livers of diploid and triploid grass carp

Diploid liver: DL_1, DL_2, DL_3; triploid liver: TL_1, TL_2, TL_3; nodiff: no difference.

3.5 Functional enrichment and expression profiling of DEGs

GO enrichment analysis of the DEGs revealed significant enrichment in GO terms associated with cytoplasm and vesicle related structures, such as the endomembrane system (GO: 0012505), cytoplasmic vesicle (GO: 0031410), and cytoplasmic part (GO: 0044444), as well as metabolic processes including hexose metabolism (GO: 0019318), lipid metabolism (GO: 0006629), and monosaccharide metabolism (GO: 0005996) (Fig.5a). Furthermore, KEGG

enrichment analysis revealed that the DEGs were enriched primarily in metabolism-related pathways, such as porphyrin metabolism (ko00960), cholesterol metabolism (ko04979), and purine metabolism (ko00230), as well as in signaling pathways involved in energy metabolism and cell proliferation, including the AMPK (ko04152), JAK-STAT (ko04630), and PPAR (ko03320) signaling pathways (Fig.5b).

To further explore the expression patterns of key genes involved in enriched pathways, expression profiling was conducted. Notably, key genes associated

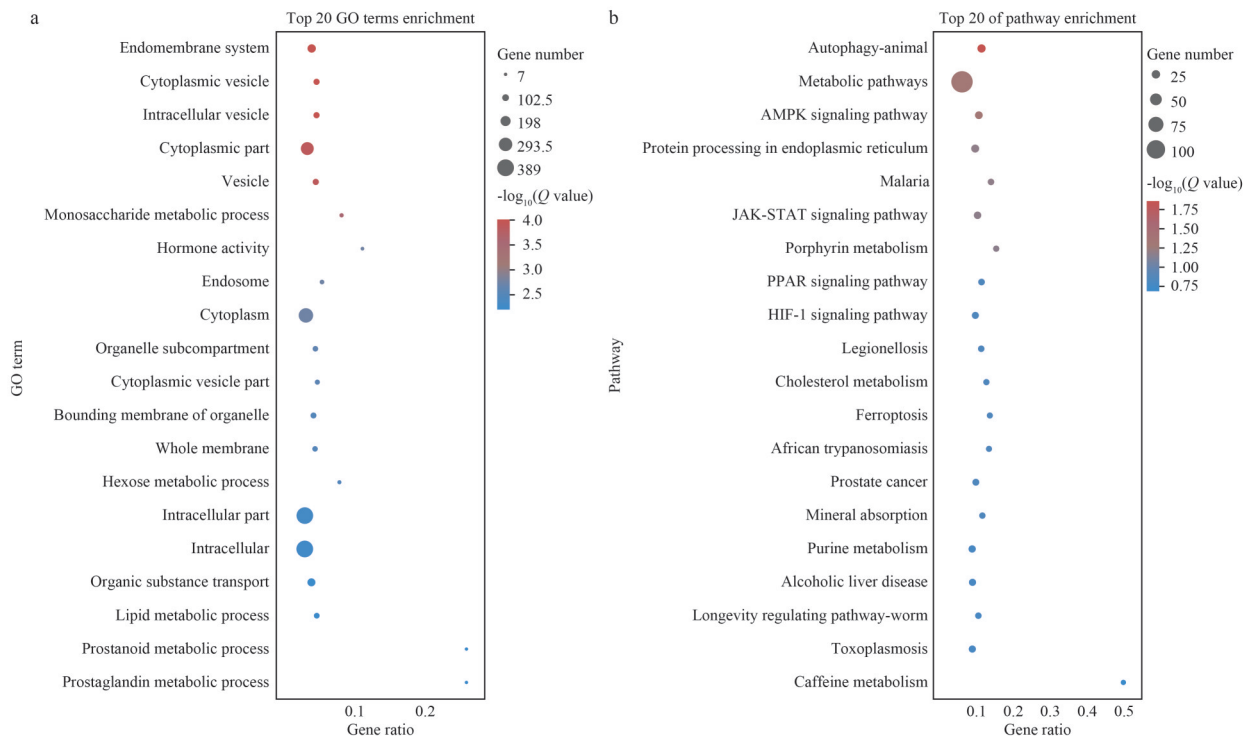


Fig.5 GO classifications and KEGG pathway enrichment of DEGs in the livers of diploid and triploid grass carp

a. top 20 significantly enriched GO terms; b. top 20 significantly enriched KEGG pathways.

with the PI3K-AKT signaling pathway, which regulate the cell cycle and cell survival, such as *akt1*, *pik3r1*, *sgk2a*, *epor*, *rps6kb1a*, *g6pc*, *lpar1*, *eif4ea*, *itgav*, and *ccnd1*, were significantly downregulated in the triploid liver. In addition, the expression of growth-related genes, including *igf1* and *igfbp5a*, was also significantly downregulated in the triploid liver (Fig.6a).

3.6 qRT-PCR confirmation of DEGs

The amplification efficiencies of the qRT-PCR primers were reliable, ranging from 90.5% to 108.9% (Supplementary Table S1). Furthermore, the qRT-PCR validation results demonstrated that the

expression patterns of the selected DEGs were consistent with those observed in the transcriptome data, thereby supporting the reliability of the transcriptomic results (Fig.6b).

4 DISCUSSION

In this study, the growth performance of triploid grass carp was monitored over a one-year period, and the results reveal that triploid individuals exhibited slower growth rates than their diploid counterparts, which is consistent with those of previous reports (Cassani and Caton, 1986), which

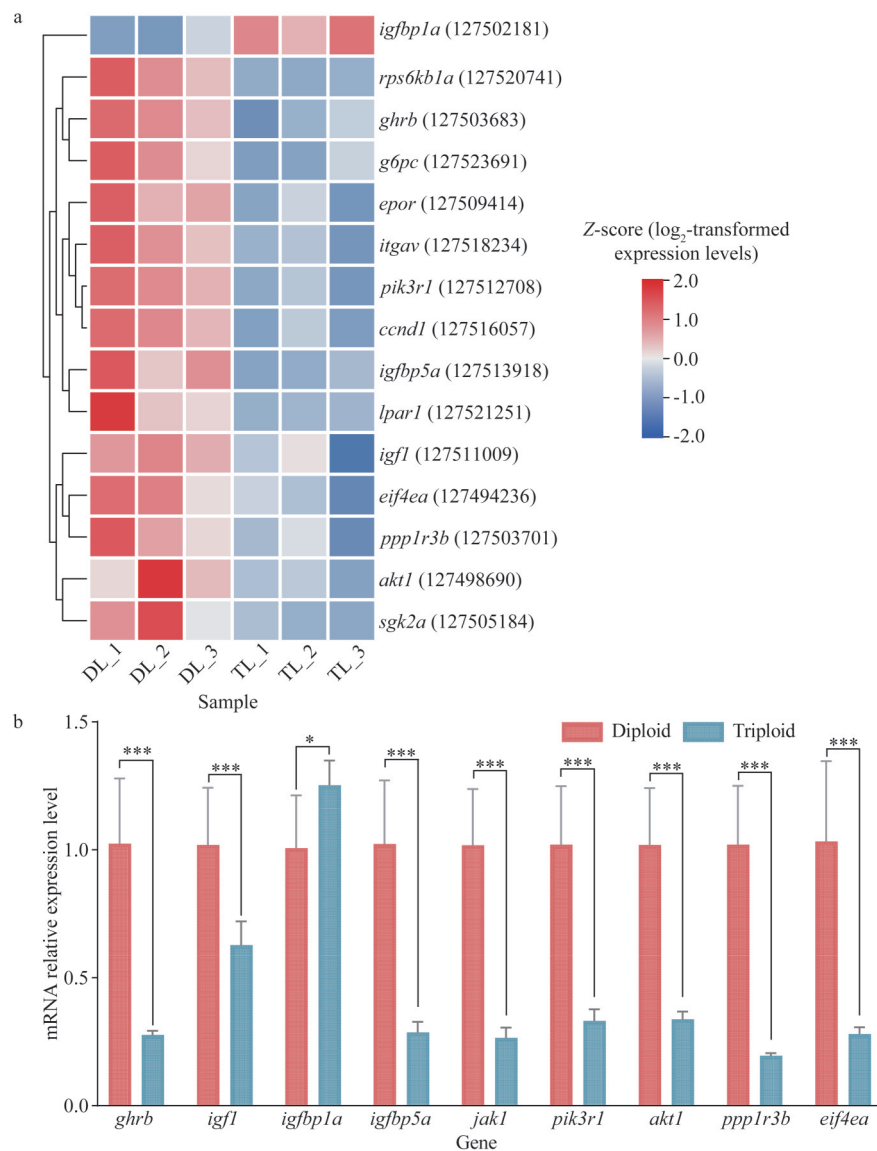


Fig.6 Differential expression of genes involved in the GH/GHR axis and downstream growth-regulating pathways, JAK-STAT and PI3K-AKT signaling pathways, between diploid and triploid grass carp

a. expression patterns of genes in the liver; b. qRT-PCR validation of selected DEGs. GH: growth hormone; GHR: growth hormone receptor. * and *** indicate $P < 0.05$ and $P < 0.001$, respectively.

have shown that when co-cultured with diploids, triploid grass carp display reduced growth rates; however, no significant difference is observed when the two ploidies are reared separately. This pattern suggests potential resource competition between ploidy types (Cassani and Caton, 1986). While we employed a co-culture system and found that triploid grass carp exhibited reduced erythrocyte counts compared with diploids, this reduction likely affected oxygen transport capacity, which may have influenced their feed intake. Nevertheless, this requires further investigation. Studies have also indicated that, prior to sexual maturity, triploid fish tend to grow more slowly than diploids do, whereas after maturity they often match or surpass diploids in terms of growth rate, potentially because of reproductive sterility (Guo et al., 1990; Fast et al., 1995; Felip et al., 2001). Given the prolonged reproductive cycle of grass carp, whether triploids develop a growth advantage during later life stages remains to be determined. In addition, although triploid and diploid grass carp are morphologically similar, measurable differences exist in certain traits (e.g., pectoral and caudal fin ray counts), indicating that triploidization affects growth and morphology.

To explore the potential mechanisms underlying the slower growth of triploids, we first examined erythrocyte morphology. Compared with diploids, triploid erythrocytes had significantly greater areas, cell volumes, nuclear areas, and nuclear volumes. This observation is consistent with the established principle that cells maintain a relatively constant nuclear-cytoplasmic ratio, with increased ploidy inducing proportional cell enlargement—a phenomenon also reported in triploid blunt snout bream and groupers (Zou et al., 2005; Shu et al., 2015). However, the packed cell volume (PCV) was reduced in the triploid grass carp, indicating a lower erythrocyte count. Given that erythrocytes are the primary oxygen carriers—transporting oxygen via hemoglobin to support aerobic metabolism (Ferguson et al., 1989; Helms et al., 2018)—this reduction could impair oxygen delivery, which might contribute to the observed slower growth by limiting aerobic metabolic capacity. Although we did not quantify cellular parameters across tissues, previous researches have reported significantly lower total cell counts in triploid grass carp larvae than in diploids (Cassani et al., 1990). It is hypothesized that alterations in cell number influence fundamental physiological processes, thereby reducing the overall performance of triploid fish (Benfey, 1999). Therefore, fewer cells would

reduce the total cellular surface area, potentially impairing metabolic exchange and further contributing to the growth retardation of triploid grass carp.

The liver is a pivotal organ in regulating fish growth, expressing growth-related growth hormone receptors (GHRs) and mediates GH signaling via IGFs and IGF-binding proteins (IGFBPs) (Sheridan, 2021). The liver is also essential in energy metabolism, influencing growth through metabolic processes (Roques et al., 2020). Histological analyses revealed that the hepatocytes of triploid grass carp possess larger nuclei and display disorganized cellular arrangements, suggesting a state of physiological stress. Whether these morphological changes directly contribute to growth retardation requires further investigation. To further elucidate the molecular mechanisms underlying growth regulation in triploid grass carp, transcriptome sequencing was conducted. Comparative analysis of hepatic transcriptomes between triploid and diploid individuals revealed only minimal differences, with a limited number of DEGs. The overall expression profile is similar to that of triploid bighead catfish (*Clarias macrocephalus*, order Siluriformes) and Atlantic salmon (*S. salar*, order Salmoniformes), two phylogenetically distant species that also exhibit few DEGs in hepatic tissue (Chatchaiphan et al., 2017; Odei et al., 2020). This similarity suggests that, despite the induction of triploidy, gene dosage effects are not prominently manifested; instead, phenomena akin to gene dosage compensation have been observed, indicating that gene expression may be subject to regulatory suppression to maintain genomic stability. Similar gene dosage compensation phenomena have been reported in other triploid fish species, such as coho salmon (*Oncorhynchus kisutch*) and hybrid grass carp (Ren et al., 2017; Christensen et al., 2019), suggesting a conserved genomic regulatory mechanism in triploid teleosts.

Notably, hepatic DEGs in triploids were predominantly downregulated. GO and KEGG analyses revealed significant enrichment in metabolic pathways, indicating hepatic metabolic dysregulation. Similar enriched signaling pathways have been observed in triploid bighead catfish (Chatchaiphan et al., 2017), supporting the notion that polyploidization may impact hepatic metabolic processes. In this study, particular emphasis was placed on the pathways related to growth axis regulation. Differential gene expression analysis revealed significant downregulation of several key genes involved in growth-related pathways, such as

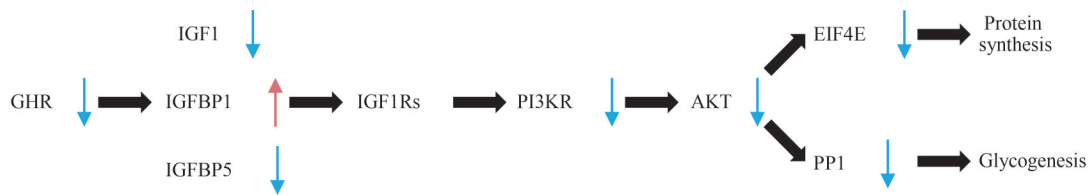


Fig.7 Predicted expression profiles of DEGs in triploid grass carp involved in the GH/GHR axis and its downstream pathways regulating growth

Blue arrows denote downregulation, and red arrows denote upregulation relative to their diploid counterparts.

ghrb, *igf1*, *igfbp5a*, and *akt1*. Furthermore, GHR mediates the cellular response to GH signaling, which modulates the expression of growth-related factors such as IGF via the JAK-STAT pathway (Dehkhoda et al., 2018). The observed decrease in *ghrb* expression suggests a weakened hepatic response to GH signals. Consequently, downstream genes, including *igf* and key components of the PI3K-AKT pathway (e.g., *akt1*), were also downregulated. Research has demonstrated that, through binding to its receptor, IGF1 activates pathways such as the PI3K-AKT, thereby stimulating metabolic processes, including protein and glycogen synthesis (Stitt et al., 2004). Therefore, the suppression of *igf1* and *akt1* expression indicates a reduced capacity for protein and glycogen synthesis in triploid grass carp. This is corroborated by the downregulation of critical genes involved in these processes, such as *eif4ea*—integral to protein synthesis (Kimball et al., 1999)—and other key metabolic regulators such as *ppp1r3b*, which is involved in metabolic conversion and energy storage, shifting from lipid to glycogen reserve accumulation (Creasy et al., 2025). The downregulation of these vital genes provides a plausible molecular explanation for the slow growth phenotype observed in triploid grass carp. Based on these data, we propose that in triploid grass carp, the GH/GHR axis modulates the IGF expression, which in turn influences protein and glycogen synthesis through downstream pathways such as the JAK-STAT and PI3K-AKT pathways (Fig.7).

5 CONCLUSION

This study demonstrates that triploid grass carp do not exhibit a phenotypic growth advantage during early development. Through hematological, histological, and transcriptomic analyses, we preliminarily revealed that their slow-growth phenotype is associated with reduced cell numbers and the downregulation of hepatic PI3K-AKT signaling pathway. These findings provide valuable

insights for polyploid breeding programs and future aquaculture applications of triploid grass carp.

6 DATA AVAILABILITY STATEMENT

All raw transcriptome sequencing reads have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1298407.

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Electronic supplementary material

Supplementary material (Supplementary Tables S1–S3) is available in the online version of this article at <https://doi.org/10.1007/s00343-025-5297-4>.