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Research Article

Polymorphism in the Growth Hormone Gene and Its Association With Growth-Related Traits in Common Carp, *Cyprinus carpio* (Linnaeus, 1778), in the Inland Saline Production System

Anjali Kumari,[1] **Mujahidkhan A. Pathan**,[2] **Raghul R.**,[1] **Nagaraja P. S.**,[1] **Susitharan V.**,[2] **Priyanka Sanwal**,[2] **and Angom Lenin Singh**[1]

[1]*Division of Fish Genetics and Biotechnology, ICAR-Central Institute of Fisheries Education, Panch Marg, Yari Road, Mumbai 400061, India*
[2]*Fish Genetics and Breeding, ICAR-Central Institute of Fisheries Education, Rohtak Centre, Rohtak 124411, Haryana, India*

Correspondence should be addressed to Mujahidkhan A. Pathan; mujahidkhan@cife.edu.in

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Identification of polymorphism in growth-related genes and their association with growth traits is an area of intense research. The growth rate is the most sought-after economic trait in the breeding program of fish. The significant association of polymorphism will aid in marker-assisted selection and speed up the genetic improvement program. The present study was performed in a population of common carp belonging to an ongoing selective breeding program at ICAR-CIFE, Rohtak Center. A total of 50 common carp based on the breeding value for body weight were selected and bifurcated into two groups: high and low performers. Each fish had three records (based on pond age) for growth-related traits, viz., body weight (***Bw***), body length (***Bl***), and body height (***Bh***). At 200 days of pond age, the mean ***Bw, Bl***, and ***Bh*** for high performers was 494.28 ± 52.12 g, 23.764 ± 0.63 cm, and 9.36 ± 0.35 cm, respectively, whereas the mean ***Bw***, ***Bl***, and ***Bh*** for low performers was found to be 165.72 ± 17.31 g, 16.20 ± 0.57 cm, and 6.40 ± 0.23 cm, respectively. The DNA was extracted from the fin clips of these fish. Five primers were used to amplify five distinct regions of the growth hormone gene covering five exons and four introns. The positive PCR products were purified and subjected to Sanger sequencing. The sequences with a Phred quality score above 20 were imported in Sequencher V for SNP hunting. Five polymorphic sites were detected, viz., C165 (amplicon 3), D60 (amplicon 4), D114 (amplicon 4), E90 (amplicon 5), and E185 loci (amplicon 5). The population genetic analysis was performed using GenAlex V. The D60 and E185 loci significantly deviated from the Hardy–Weinberg equilibrium ($p < 0.05$). Growth association was performed for the polymorphic loci using a linear model by invoking PROC GLM in SAS. The loci C165, D60, and E90 were significantly associated with the ***Bw***, ***Bl***, and ***Bh***. The results obtained in this study must be utilized in the ongoing selective breeding of common carp to speed up genetic improvement.

Keywords: body weight; exons; genetic variation

1. Introduction

Cyprinus carpio, Linnaeus 1758, commonly known as common carp, native to central and eastern Asia and some parts of Europe, is one of the world's oldest and most domesticated fish. Common carp is a freshwater species that is widely cultured due to its omnivore diet, rapid growth, and ability to reproduce easily in confined waters [1], making it a significant year-round food fish. It can tolerate low oxygen levels, high turbidity, moderate salinity

(up to 14%), and a broad range of temperatures (40°C). Ecologically, it contributes to nutrient cycling by accelerating nutrient availability, which in turn enhances photosynthesis and increases phytoplankton biomass [2]. Common carp is increasingly being considered for commercial aquaculture in Asia and Europe due to its extremely high adaptability to both climate and food [3]. It is the fourth most important fish species in the world's aquaculture production and third among the carp, with a global production of 4.18 million tons, contributing 7.03% of total finfish aquaculture production in 2021 [4].

Growth is a significant characteristic in the fish farming sector due to its direct correlation to fish production. Enhancing the growth rate offers advantages to aquaculture by reducing the time required for fish rearing at farm facilities, reducing expenses, and increasing harvest [5, 6]. Various growth-related traits, including body weight, total length, standard length, body depth, and body thickness, are quantitative traits influenced by environmental factors and multiple genes with relatively minor effects, as per the infinitesimal model [7]. Although all the genes that impact a polygenic trait, such as growth, remain unidentified, several potential candidate genes have been acknowledged. These candidate genes have been chosen based on an established relationship between physiological or biochemical processes, and a trait is subsequently assessed as potential quantitative trait loci (QTL) [8, 9].

Studies in humans, rodents, and other vertebrate species have unequivocally shown that the somatotropic axis hormones are the principal controller of skeletal growth and body size [10, 11]. The pituitary growth hormone (GH) plays an essential role in regulating growth and development by promoting cell division, differentiation, and enlargement [12]. The metabolic effects of GH include increased protein synthesis, increased use of fat for energy production, and decreased glucose utilization throughout the body [12]. The importance of GH as a potential growth-promoting agent has long been recognized in fish [13]. Furthermore, GH gene transfer has been described, and the technique was developed to enhance common carp production in China, firstly using human GH and later using grass carp GH fused to the common carp β-actin promoter [14]. The transgenic common carp showed higher growth performance and food conversion efficiency than the controls [15].

The GH has long been known to play a central role in vertebrate growth regulation. In teleosts, in addition to its growth-promoting activity, the hormone is involved in osmoregulation, electrolyte balance regulation, and many other metabolic functions. Therefore, it is worth studying for sequencing, cloning, and expression [8]. Polymorphisms associated with growth within the exon sequences of the piscine GH gene are very rare, with the majority of mutations in the GH gene occurring within introns [16]. Introns usually occupy the majority of a gene's sequence, and comparative mapping studies have recently shown that many introns remain remarkably conserved throughout evolutionary time in many species, suggesting that they are under selection and may play a role in gene regulation. The variation in these regions has the potential to induce up/down regulation of mRNA transcription [17]. Understanding how these genes of large effect impact trait variability and including this additional information into the overall breeding strategy has spawned a whole new field of the quantitative genetic theory, whereby simulations have demonstrated that incorporation of marker data can substantially improve the accuracy of estimated breeding value (EBV) estimation and thus genetic gains from a selection [17].

Common carp exhibits salinity and cold tolerance and is an attractive species for aquaculture in degraded soils. ICAR-CIFE is conducting a genetic improvement program for common carp in India. The genetic selection based on EBV is performed in common carp in an inland saline production system. The present study was conducted using a population whose EBVs were determined, and fish with extreme EBVs (high and low performers) were chosen. The objectives were to identify the polymorphism in the GH gene of fish and associate the polymorphism with growth-related traits, viz., body weight (***Bw***), body length (***Bl***), and body height (***Bh***).

2. Materials and Methods

2.1. Experimental Population. The experimental population consists of 50 common carp fish belonging to an ongoing selective breeding program of common carp in an inland saline production system at ICAR-CIFE, Rohtak Center, Haryana, India. Each fish had growth-related records of body weight (***Bw***), body length (***Bl***), and body height (***Bh***) at three pond ages viz., pond age 1 day (at tagging), pond age 110 days, and pond age 200 days. The selection criterion used was body weight (***Bw***) at 200 pond age. The population comprised two major groups based on EBVs: the high-performer and the low-performer groups. High performers consisted of 25 fish belonging to BLUP, ranking 1–104, and the other 25 fish were low performers belonging to BLUP, ranking 1000–1200.

2.2. Sample Collection and Genomic DNA Isolation. The part of the caudal fin was clipped from the fish, collected in a 2-mL microcentrifuge tube containing absolute alcohol, and stored at −20°C until DNA isolation. Genomic DNA was extracted using the phenol-chloroform method [18]. The quantification of DNA was performed using a maestro nano spectrophotometer (Maestrogen, Taiwan), and the purity was estimated using A260/A280. The integrity of total genomic DNA was checked by the 0.8% agarose gel electrophoresis.

2.3. PCR Amplification. The full length of the GH gene in common carp (accession no. X51969.1) is around 2838 bp, consisting of 5 exons and 4 introns, yielding a protein of 210 residues. A total of five reported primers were used to amplify the distinct region of the GH gene of common carp [19]. The amplicons 1, 2, 3, 4, and 5 covered the exon and intron 1 (genomic coordinates—SAUI01032737 626957 to 627178(+)), exon and intron 2 (genomic coordinates—SAUI01032737 627492 to 627679(+)), exon and

intron 3 (genomic coordinates—SAUI01032737 627935 to 628133(+)), and exon and intron 4 (genomic coordinates—SAUI01032737 628137 to 628360(+)), and exon 5 (genomic coordinates—SAUI01032737 627492 to 627679(+)) region of the GH gene, respectively. The primers were synthesized by Eurofins India Ltd., Bengaluru. The details of the primer used in this study are provided in Table 1.

The PCR amplification was conducted in a final volume of 50-μL reaction mixture consisting of 2 μL of DNA template (~50–100 ng/μL), 25 μL of 2X PCR master mix, 2 μL of forward primer (10pM), 2 μL of reverse primer (10pM), and 19 μL of nuclease-free water. The reaction was performed using the following PCR conditions in the gradient thermocycler (iGene, Labserve): One cycle of initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at primer-specific temperature for 30 s, and extension at 72°C for 30 s. Finally, one cycle of extension at 72°C for 6 min and a hold temperature set at 4°C for 10 min was carried out. The PCR products were purified using a GeneJet PCR Purification Kit and analyzed by electrophoresis on 2% agarose gel.

2.4. Sequencing and Bioinformatic Analysis. The purified PCR products were sequenced by Eurofins Pvt. Ltd., Bengaluru. The Sanger sequencing was performed, and the results were obtained in FASTA and ABI format. The sequence alignment was performed using BLAST (https://www.ensembl.org/Homo_sapiens/Tools/Blast; Ensemble browser 112) for each amplicon against the common carp huanghe genome sequence (accession no. ENSCCRG00015026164). The reading of the chromatogram (.abi) was performed with Flinch TV V.1.5.0 [20]. The sequences with the Phred quality score above 20 were imported in Sequencher V 5.4.6 [21, 22] for single nucleotide polymorphism (SNP) hunting.

2.5. SNP Hunting. The sequence file was imported in the software Sequencher V 5.4.6 [21, 22]. Quality trimming was performed to improve the sequence data. The post-trimmed sequences, along with the reference sequence (accession no. ENSCCRG00015026164), were further used to generate a contig. The contig was then subjected to multiple sequence alignment, and a variance table was generated. Finally, the difference in the chromatogram was checked manually to confirm the SNPs, and the genotype was determined.

2.6. Population Genetics Analysis. The genotype data were obtained and further used for diversity analysis. The population genetics analysis was performed using the GenAlex V 6.5 software [23]. The various parameters, including expected heterozygosity (He), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe), the Hardy–Weinberg equilibrium test (HWE), effective allele numbers (Ne), fixation index value (F value), allele and genotype frequency for the codominant marker data, were estimated. The Ne, Ho, He, uHe, and F value were calculated as follows (GenAlex V 6.5 software):

$$\begin{aligned}
\text{Ne (No. of Effective Alleles)} &= \frac{1}{\left(\text{Sum pi}^2\right)},\\
\text{Ho (Observed Heterozygosity)} &= \frac{\text{No. of Hets}}{N},\\
\text{He (Expected Heterozygosity)} &= 1 - \text{Sum pi}^2,\\
\text{uHe (Unbiased Expected Heterozygosity)} &= \left(\frac{2N}{(2N-1)}\right) * \text{He},\\
\text{F (Fixation Index)} &= \frac{(\text{He} - \text{Ho})}{\text{He}} = 1 - \left(\frac{\text{Ho}}{\text{He}}\right),
\end{aligned} \tag{1}$$

where pi = frequency of the i_{th} allele for the population and Sum pi^2 = sum of the squared population allele frequencies.

2.7. Statistical Analysis. The statistical analysis was performed using SAS OnDemand for Academics (https://www.sas.com/en_in/software/on-demand-for-academics.html). The descriptive statistics were performed using PROC MEANS and PROC SGPLOT procedures. The normality of growth-related data was tested using PROC UNIVARIATE. The log transformation was performed to make data conform to normality. The trait association was performed using the following statistical model by invoking PROC GLM:

$$\text{Yij} = \mu + \text{Gi} + \text{eij}, \tag{2}$$

where Yij = j_{th} measurement of the trait belonging to the i_{th} genotype, μ = total mean value, Gi = fixed effect of the i_{th} genotype, and eij = random residual error.

Table 1: Details of the primer used in this study.

Primer name	Primer sequence (5′-3′)	Start	Stop	% GC	Location	Expected amplicon size (bp)	Genomic coordinates
Amplicon 1 (A1)	F-AGTGCCAACATCATCCCAGAG (21) R-AGCCATTTCGCTCAGGGTAG (21)	9 278	29 259	52.3 55.0	Exon 1 and intron 1	270	SAUI01032737 626957 to 627178 (+)
Amplicon 2 (A2)	F-AATAACCCTTCTTTTCTTCT (20) R- GTGACTAATAATGTTTGCTCTAA (23)	541 779	560 757	30.0 30.0	Exon 2 and intron 2	239	SAUI01032737 627492 to 627679 (+)
Amplicon 3 (A3)	F- TCTTCCCTCTGTCTTTCTGC (20) R- AGAGGGACCTCATCCTAAAA (20)	977 1257	996 1238	50.0 45.0	Exon 3 and intron 3	281	SAUI01032737 627935 to 628133 (+)
Amplicon 4 (A4)	F-GTTTGGGAAGCACTTGGAAT (20) R- GCTTGTCTTTCTCACCTGGAT (21)	1515 1792	1534 1772	45.0 48.0	Exon 4 and intron 4	278	SAUI01032737 628137 to 628360 (+)
Amplicon 5 (A5)	F-TTCGTCTGCTGGCTTGCTTC (20) R- GCACTCCCAAATGAGAAGAA (20)	1987 2276	2006 2257	55.0 45.0	Exon 5	290	SAUI01032737 627492 to 627679 (+)

3. Results

3.1. Mean Values of Growth Related Traits in the Experimental Population at Different Pond Ages. The experimental population consists of 50 common carp, comprising the high performer and the low performer groups based on EBVs. The fish with positive BLUP values were categorized as the high performers, whereas the fish with negative BLUP values were categorized as the low performers. Each fish had three growth-related records, viz., pond age 1 day (at tagging), pond age 110 days, and pond age 200 days. The overall mean ***Bw***, ***Bl***, and ***Bh*** of selected fish at pond age 1 were 25.72 ± 3.18 g, 8.59 ± 0.34 cm, and 3.04 ± 0.13 cm, respectively; at pond age 110 were 295.52 ± 33.56 g, 19.49 ± 0.80 cm, and 7.20 ± 0.30 cm, respectively; and at pond age 200 were 330.00 ± 35.91 g, 19.98 ± 0.68 cm, and 7.88 ± 0.30 cm, respectively (Table 2).

3.2. SNP Hunting. Genomic DNA isolated from the caudal fin of common carp was amplified (Figures 1, 2, 3, 4, 5), and purified products were sequenced using the Sanger sequencing method. The five polymorphic loci in the GH gene were detected, viz., one polymorphic locus in the amplicon 3 region, two polymorphic loci in the amplicon 4 region, and two polymorphic loci in the amplicon 5 region. The amplicon 1 region was conserved, whereas two monomorphic loci were found in the amplicon 2 region.

3.2.1. Polymorphism in Amplicon 3. The genomic coordinates of amplicon 3, when aligned to the reference sequence, SAUI01032737 627935 to 628133(+) were found to be with an *E* value of 4.86e-103 and a percentage identity (% ID) of 99% (Table 3). One polymorphic locus was detected at the nucleotide positions of 165, named as the C165 locus, and the alleles identified were A/T. The allele frequencies of A and T were 0.565 and 0.435. The allocated genotypes were AA (upper homozygous), AT (heterozygous), and TT (lower homozygous) (Figure 6). The frequencies of AA, AT, and TT genotypes were 0.319, 0.491, and 0.189, respectively. The locus was in the HWE ($p < 0.05$). The observed heterozygosity and expected heterozygosity were 0.478 and 0.491, respectively (Table 4).

3.2.2. Polymorphism in Amplicon 4. The genomic coordinates of amplicon 4, when aligned to the reference sequence, SAUI01032737 628137 to 628360(+) were found to be with an *E* value of 3.83e-113 and a percentage identity (% ID) of 98.22% (Table 3). There were two polymorphic loci at the nucleotide position of 60 and 114 named as the D60 locus and the D114 locus. The alleles identified at the D60 locus were A/T. The allele frequencies of A and T were 0.525 and 0.475, respectively. The allocated genotypes were AA, AT, and TT (Figure 7), with frequencies of 0.275, 0.498, and 0.225, respectively. The locus significantly deviated from the HWE ($p < 0.05$). The observed heterozygosity and expected

Table 2: Mean and CV for all traits at different pond ages.

Pond age	*N*	Traits	Mean ± SE	CV (%)
1	50	BW (g)	25.72 ± 3.18	87.32
		SL (cm)	8.59 ± 0.34	27.72
		BD (cm)	3.04 ± 0.13	29.49
110	50	BW (g)	295.52 ± 33.56	73.60
		SL (cm)	19.49 ± 0.80	26.48
		BD (cm)	7.20 ± 0.30	27.21
200	50	BW (g)	330.00 ± 35.91	76.95
		SL (cm)	19.98 ± 0.68	24.23
		BD (cm)	7.88 ± 0.30	26.83

Abbreviations: CV, coefficient of variation; SE, standard error.

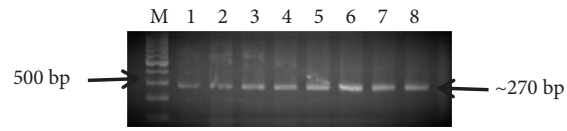

Figure 1: PCR amplification of amplicon 1. Lane M: 1 kb plus DNA ladder (gene ruler); Lane 1–8: PCR samples.

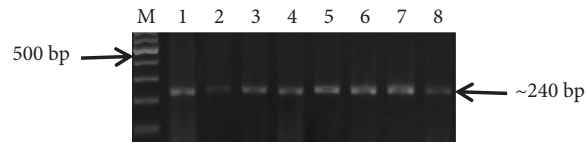

Figure 2: PCR amplification of amplicon 2. Lane M: 1 kb plus DNA ladder (gene ruler); Lane 1–8: PCR samples.

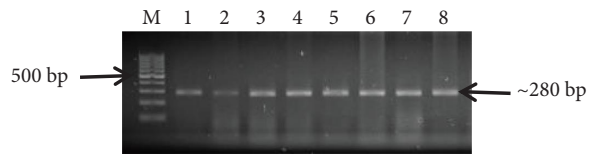

Figure 3: PCR amplification of amplicon 3. Lane M: 1 kb plus DNA ladder (gene ruler); Lane 1–8: PCR samples.

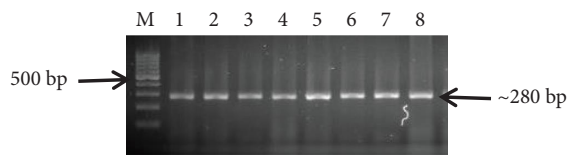

Figure 4: PCR amplification of amplicon 4. Lane M: 1 kb plus DNA ladder (gene ruler); Lane 1–8: PCR samples.

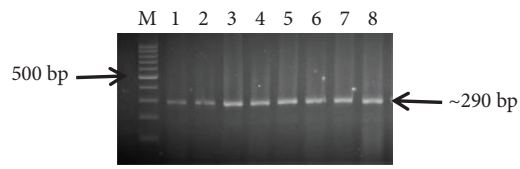

Figure 5: PCR amplification of amplicon 5. Lane M: 1 kb plus DNA ladder (gene ruler); Lane 1–8: PCR samples.

Table 3: Details of BLAST (Ensemble browser 112) for all the amplicons.

Query sequence	Genomic location	Overlapping gene(s)	Query start	Query end	Alignment length	Alignment score	*E* value	% ID
Amplicon 1	SAUI01032737 626957 to 627178 (+)	GH1	1	227	227	391	3.26e – 107	97.35
Amplicon 2	SAUI01032737 627492 to 627679 (+)	GH1	4	192	189	356	1.39e –96	98.94
Amplicon 3	SAUI01032737 627935 to 628133 (+)	GH1	4	203	200	378	4.86e –103	99.00
Amplicon 4	SAUI01032737 628137 to 628360 (+)	GH1	6	230	225	411	3.68e –113	98.22
Amplicon 5	SAUI01032737 627492 to 627679 (+)	GH1	15	235	221	411	3.83e – 113	98.64

Note: % ID ⟶ identity percentage.

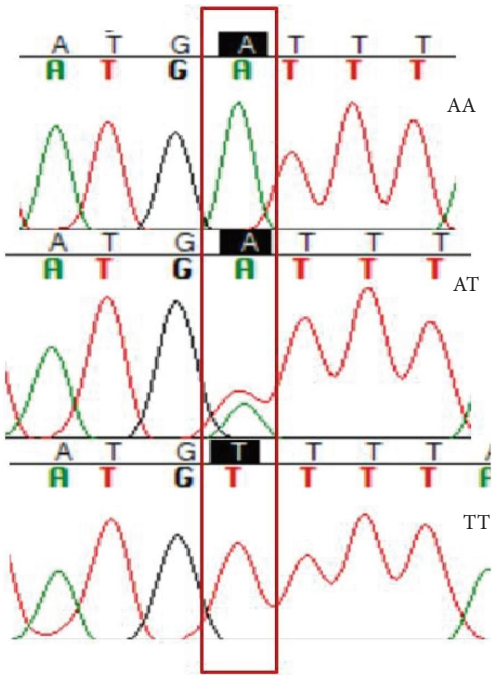

Figure 6: Chromatogram of the C165 locus.

heterozygosity were 0.313 and 0.464, respectively. The F value was 0.398 (Table 4). At the D114 locus, the alleles were C/T. The allele frequencies of C and T were 0.688 and 0.312, respectively. The allocated genotypes were CC, CT, and TT (Figure 8), with frequencies of 0.473, 0.430, and 0.097, respectively. The genotypic frequency at this locus was in the HWE ($p > 0.05$). The observed heterozygosity and expected heterozygosity were 0.325 and 0.430, respectively (Table 4).

3.2.3. Polymorphism in Amplicon 5. The genomic coordinates of amplicon 5, when aligned to the reference sequence, SAUI01032737 627492 to 627679(+) were found to be with an *E* value of 3.83e-113 and a percentage identity (% ID) of 98.64% (Table 3). There were two polymorphic loci at the nucleotide position of 90 and 185 named the E90 locus and the E185 locus. At the E90 locus, the identified alleles were A/C. The allele frequencies of A and C were 0.633 and 0.367, respectively. The allocated genotypes were AA, AC, and CC (Figure 9), with frequencies of 0.4, 0.464, and 0.134, respectively. The locus was in the HWE ($p > 0.05$). The observed heterozygosity and expected heterozygosity were 0.467 and 0.464, respectively (Table 4). The alleles identified at the E185 locus were C/A. The allele frequencies of A and C were 0.57 and 0.43, respectively. The allocated genotypes were CC, CA, and AA (Figure 10), with frequencies of 0.571, 0.368, and 0.059, respectively. The locus significantly deviated from the HWE ($p < 0.05$). The observed heterozygosity and expected heterozygosity were 0.209 and 0.369, respectively. The F value was 0.433(Table 4).

3.3. Trait Association. The ANOVA for growth-related traits at different polymorphic loci is provided in Table 5. The loci C165, D60, and E90 had significant associations with growth-related traits.

3.3.1. C165 Locus. The distribution of body weight, body length, and body height at the C165 locus exhibited variation across the genotypes (Figures 11, 12, 13). The genotypes TT and AA exhibited significantly highest lsmeans ***Bw***, viz., 135.63 ± 1.17 g and 125.21 ± 1.13 g, respectively, whereas the AT genotype had a lsmeans ***Bw*** of 82.26 ± 1.11 g. A significant difference was observed for the lsmeans ***Bl*** among the genotypes. The genotypes TT and AA exhibited significantly highest lsmeans ***Bl***, viz., 15.48 ± 1.05 cm and 15.48 ± 1.04 cm, respectively, whereas the AT genotype had a lsmeans ***Bl*** of 12.42 ± 1.03 cm. Similarly, the genotypes TT and AA exhibited significantly highest lsmeans ***Bh,*** viz., 5.98 ± 1.05 cm and 5.58 ± 1.04 cm, respectively, whereas the AT genotype had a lsmeans ***Bh*** of 4.66 ± 1.03 cm. The lsmeans for log ***Bw***, ***Bl***, and ***Bh*** are given in Table 6. Tukey–Kramer grouping for growth-related traits across genotypes at the C165 locus is given in Figures 14, 15, 16.

3.3.2. D60 Locus. The distribution of body weight, body length, and body height at the D60 locus exhibited differences across the genotypes (Figures 17, 18, 19). The genotypes TT and AA exhibited significantly highest lsmeans ***Bw***, viz., 131.63 ± 1.16 g and 106.69 ± 1.13 g, respectively, whereas the AT genotype had a lsmeans ***Bw*** of 55.14 ± 1.17 g. A significant difference was observed for the lsmeans ***Bl*** among

Table 4: Genetic diversity analysis of the polymorphic loci.

Loci	Genotype frequency	Allele frequency A	T	C	HWE[2] X^2	Ho	He	uHe	Ne	F value
C165	AA (0.319) AT (0.491) TT (0.189)	0.565	0.435	NA	0.033[ns]	0.478	0.491	0.497	1.96	0.027
D60	AA (0.275) AT (0.498) TT (0.225)	0.525	0.475	NA	6.352[*]	0.300	0.499	0.505	1.99	0.398
D114	CC (0.473) CT (0.430) TT (0.097)	NA	0.313	0.688	2.374[ns]	0.325	0.430	0.435	1.75	0.244
E90	AA (0.400) AC (0.464) CC (0.134)	0.633	NA	0.367	0.001[ns]	0.467	0.464	0.470	1.86	−0.005
E185	CC (0.571) CA (0.368) AA (0.059)	0.244	NA	0.756	8.061[*]	0.209	0.369	0.373	1.58	0.433

Note: Ho: observed heterozygosity, He: expected heterozygosity, uHe: unbiased expected heterozygosity, *F* value: fixation index value.
Abbreviation: ns, nonsignificant.
[*]significant.

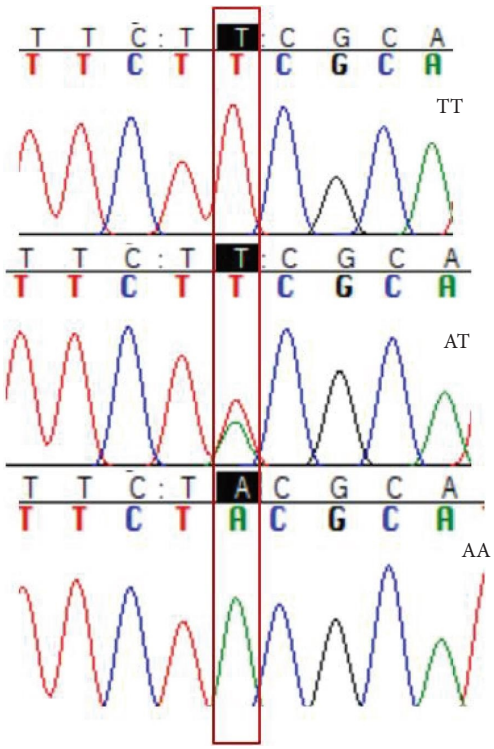

Figure 7: Chromatogram of the D60 locus.

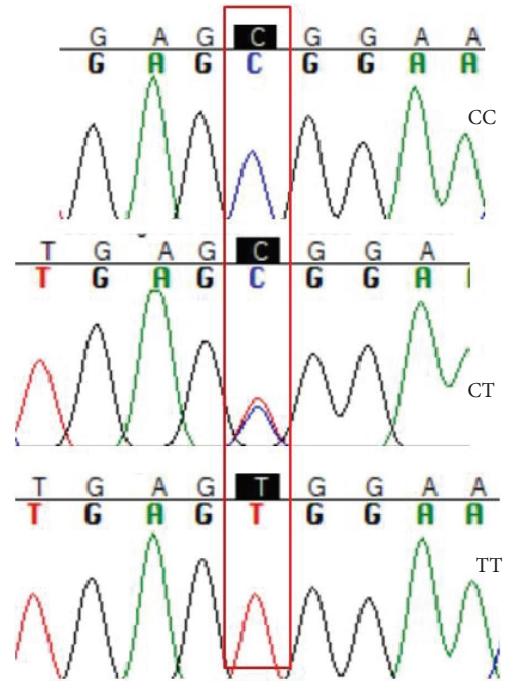

Figure 8: Chromatogram of the D114 locus.

the genotypes. The genotypes TT and AA showed significantly highest lsmeans ***Bl***, viz., 15.79 ± 1.04 cm and 14.58 ± 1.04 cm, respectively, whereas the AT genotype had lsmeans ***Bl*** of 11.82 ± 1.05 cm. Similarly, the genotypes TT and AA exhibited significantly the highest lsmeans ***Bh***, viz., 5.69 ± 1.05 cm and 5.47 ± 1.04 cm, respectively, whereas the AT genotype had lsmeans ***Bh*** of 4.39 ± 1.05 cm. The lsmeans for log ***Bw***, ***Bl***, and ***Bh*** are given in Table 6. Tukey–Kramer grouping for growth-related traits across genotypes at the D60 locus is given in Figures 20, 21, 22.

3.3.3. E90 Locus. The distribution of body weight, body length, and body height at the E90 locus exhibited differences across the genotypes (Figures 23, 24, 25). The genotypes CC and AA exhibited significantly highest lsmeans ***Bw***, viz., 146.93 ± 1.23 g and 121.51 ± 1.12 g, respectively, whereas the AC genotype had a lsmeans ***Bw*** of 61.55 ± 1.11 g. A significant difference was observed for the lsmeans ***Bl*** among

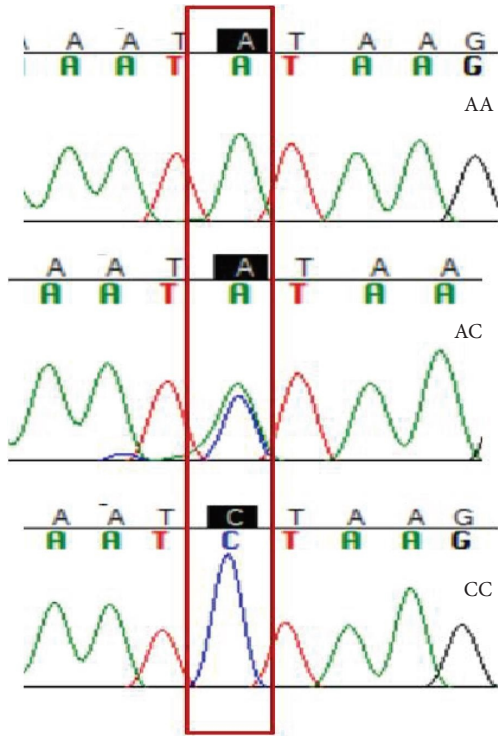

Figure 9: Chromatogram of the E90 locus.

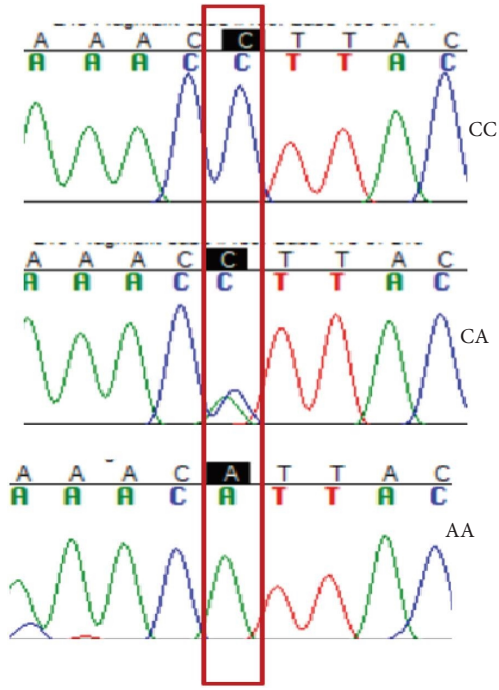

Figure 10: Chromatogram of the E185 locus.

the genotypes. The genotypes CC and AA showed significantly highest lsmeans ***Bl***, viz., 15.79 ± 1.07 cm and 15.48 ± 1.04 cm, respectively, whereas the AC genotype had lsmeans ***Bl*** of 12.30 ± 1.03 cm. Similarly, the genotypes CC and AA exhibited significantly the highest lsmeans ***Bh***, viz., 6.23 ± 1.07 cm and 5.52 ± 1.04 cm, respectively, whereas the AC genotype had lsmeans ***Bh*** of 4.66 ± 1.04 cm. The lsmeans for log ***Bw***, ***Bl***, and ***Bh*** are given in Table 6. Tukey–Kramer grouping for growth-related traits across genotypes at the E90 locus is given in Figures 26, 27, 28.

4. Discussion

Candidate gene markers can be utilized analogously to anonymous markers; however, they exhibit greater specificity and have the capability to identify genetic variation in or near genes that directly influence traits. Often selected from genes involved in metabolic pathways, candidate gene markers can provide more precise information about the genetic basis of a trait [24]. The most studied candidate genes in livestock and finfish for growth-regulating pathways are genes within the somatotropic axis and transforming growth factors [17, 25]. Numerous studies have explored the association between growth-related traits and genes within the somatotropic axis and transforming growth factor pathways, such as GH receptor (GHR) [26–28], GH gene [8, 29], insulin-like growth factors (IGF-I & II) [30–32], and myostatin [33–36]. Therefore, SNPs in these genes are likely to influence the growth-related traits in vertebrates.

The GH is a key hormone in regulating postnatal growth by promoting processes such as cell division, bone growth, and protein production. It also affects how the body utilizes energy by influencing fat burning, glucose uptake, and protein synthesis [37]. The GH is released into the bloodstream, where it binds to a specific GHR, primarily in the liver. It also stimulates the production of IGF-I, which is carried by insulin-like growth factor binding proteins to various cells, where it binds to IGF receptors. IGFs help regulate processes such as glucose uptake and are key intermediaries between the GH and muscle growth in vertebrates. The GH gene consists of several key regions: coding exons, a promoter that drives mRNA transcription, and noncoding intron sequences. Polymorphism in any of these regions has the potential to either alter the structure and activity of the resultant protein or affect the regulation of mRNA transcription, ultimately influencing growth-related traits [17].

In the present study, 50 common carp were selected, comprising high performers and low performers based on EBVs. Each fish had three records for growth-related traits based on pond age. The polymorphism in the GH gene of selected common carp was identified, and further investigation was performed for trait association. The present study is in line with those reported by various researchers, wherein the polymorphism in the GH gene was associated with growth in common carp [19, 38–40], in other fish species [41–43], and in livestock [44, 45].

The present study revealed five polymorphic sites in the GH gene of the common carp viz., the C165 locus (amplicon 3, genomic coordinates-SAUI01032737 627935 to 628133(+), gene location—exon and intron 3), the D60 locus (amplicon 4, genomic coordinates-SAUI01032737 628137 to 628360(+), gene location-exon 4 and intron 4), the D114 locus (amplicon 4, genomic coordinates- SAUI01032737 628137 to 628360(+), gene location-exon 4 and intron 4), the E90 locus (amplicon 5, genomic coordinates-SAUI01032737 627492 to 627679(+), gene location-exon 5), and the E185

Table 5: ANOVA for growth-related traits at different polymorphic loci.

Source of variation	DF	MSS		
		lbw	lbl	lbh
C165	2	8.44*	0.78*	0.76*
Pond age	1	0.0135* ± 0.0009	0.0043* ± 0.0003	0.004* ± 0.0003
Error	127	0.778	0.0859	0.093
R^2 (%)		64.99	63.53	66.88
D60	2	6.96*	0.798*	0.677*
Pond age	1	0.0135* ± 0.001	0.0043* ± 0.0003	0.004* ± 0.0003
Error	110	95.33	0.091	0.103
R^2 (%)		62.46	62.69	64.92
D114	2	6.96*	0.798*	0.677*
Pond age	1	0.0135* ± 0.001	0.0043* ± 0.0003	0.004* ± 0.0003
Error	110	95.33	0.091	0.103
R^2 (%)		62.46	62.69	64.92
E90	2	8.19*	0.938*	0.77*
Pond age	1	0.0132* ± 0.0009	0.0043* ± 0.0003	0.004* ± 0.0003
Error	123	0.829	0.089	0.096
R^2 (%)		63.01	62.62	65.48
E185	2	1.03	0.195	0.032
Pond age	1	0.0136* ± 0.0010	0.0044* ± 0.0003	0.004* ± 0.0003
Error	118	0.939	0.100	0.106
R^2 (%)		59.21	58.99	61.47

Abbreviations: lbh, log body height; lbl, log body length; lbw, log body weight.
* p value < 0.05.

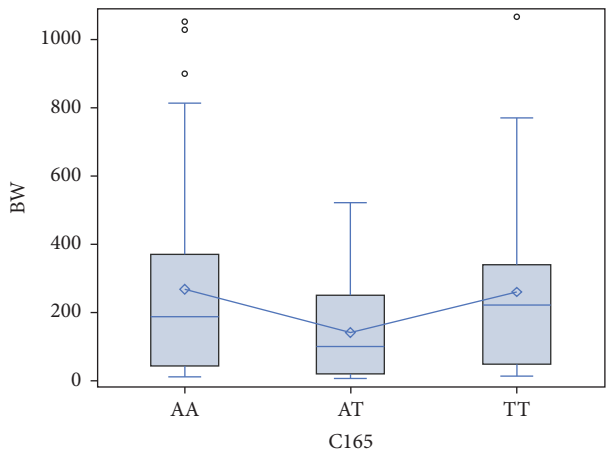

Figure 11: Distribution of body weight (*Bw*) across the genotypes at the C165 locus.

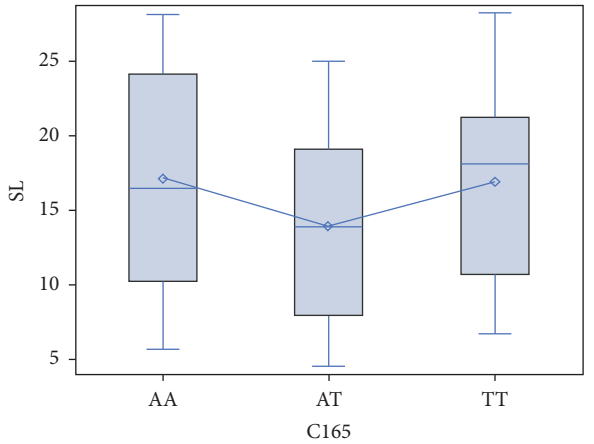

Figure 12: Distribution of body length (*Bl*) across the genotypes at the C165 locus.

locus (amplicon 5, genomic coordinates-SAUI01032737 627492 to 627679(+) location-exon 5). The amplicon 1 was conserved, and two monomorphic loci were identified in amplicon 2. Similarly, previous studies have found polymorphism in the GH gene of the common carp and other species. Six nucleotide mutations (G1041A, A1066T, G1120A, G1129C, G1151T, and A1200G) were identified in the P2 locus (exon and intron 3), whereas no sequencing polymorphism was detected in the other 4 pairs of exon or intron regions of the GH gene of common carp [19]. Additionally, a study identified two SNPs (A1132T and G1217T) in the third intron of the GH 1 gene of common carp [39]. The polymorphism in the exon and intron 4 and exon 5 region of the GH 1 gene of common carp was also detected [38]. Similarly, in other fish species, polymorphism in the exon 4 region of the GH gene of *Channa striata* in the form of insertion, deletion, and SNPs was found [42]. In yellow catfish, five SNPs were identified in the GH gene, with four SNPs (1674G > A, 2100A > G, 2154T > G, and 2285T > C) in intron 4 and one SNP (2822A > G) in the 3′ UTR region [41]. Additionally, in livestock, water buffalo showed polymorphism in the exon 5 region of the GH, and two SNPs (p.Leu153Val and p.Asn174His) were detected [44]. Further, the polymorphism in the GH of Wenshang Barred chickens is also reported [45].

In this study, the A/T substitution was found at the C165 locus (exon and intron 3). The allele frequencies of A and T

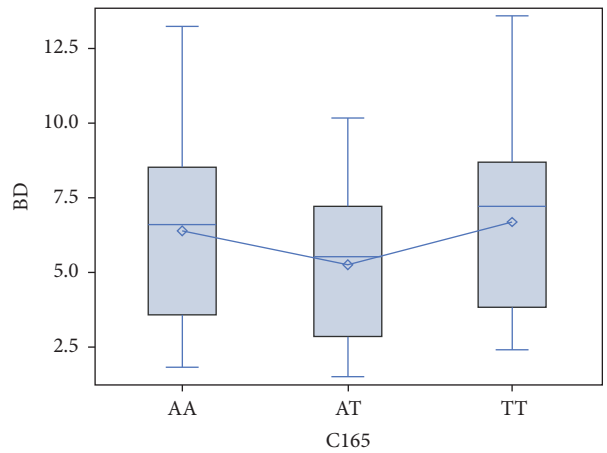

Figure 13: Distribution of body length (*Bl*) across the genotypes at the C165 locus.

Table 6: Least squares means for growth-related traits at different polymorphic loci.

Locus	Genotype	lbw	lbl	lbh
C165	AA	$4.83^{a} \pm 0.13$	$2.74^{a} \pm 0.04$	$1.72^{a} \pm 0.04$
	AT	$4.14^{b} \pm 0.11$	$2.52^{b} \pm 0.03$	$1.54^{b} \pm 0.03$
	TT	$4.91^{a} \pm 0.16$	$2.74^{a} \pm 0.05$	$1.79^{a} \pm 0.05$
D60	AA	$4.67^{a} \pm 0.13$	$2.68^{a} \pm 0.04$	$1.70^{a} \pm 0.04$
	AT	$4.01^{b} \pm 0.16$	$2.47^{b} \pm 0.05$	$1.48^{b} \pm 0.05$
	TT	$4.88^{a} \pm 0.15$	$2.76^{a} \pm 0.04$	$1.74^{a} \pm 0.05$
D114	CC	$4.68^{a} \pm 0.12$	$2.69^{a} \pm 0.04$	$1.68^{a} \pm 0.04$
	TC	$4.19^{a} \pm 0.16$	$2.53^{a} \pm 0.05$	$1.56^{a} \pm 0.05$
	TT	$4.84^{a} \pm 0.22$	$2.73^{a} \pm 0.07$	$1.75^{a} \pm 0.07$
E90	AA	$4.80^{a} \pm 0.12$	$2.74^{a} \pm 0.04$	$1.71^{a} \pm 0.04$
	AC	$4.12^{b} \pm 0.11$	$2.51^{b} \pm 0.03$	$1.54^{b} \pm 0.04$
	CC	$4.99^{a} \pm 0.21$	$2.76^{a} \pm 0.07$	$1.83^{a} \pm 0.07$
E185	AA	$4.76^{a} \pm 0.22$	$2.76^{a} \pm 0.07$	$1.71^{a} \pm 0.07$
	CA	$4.34^{a} \pm 0.20$	$2.57^{a} \pm 0.06$	$1.64^{a} \pm 0.06$
	CC	$4.50^{a} \pm 0.10$	$2.62^{a} \pm 0.03$	$1.64^{a} \pm 0.03$

Note: Means bearing the same superscript within a subgroup are not significantly different from one another ($p < 0.05$).
Abbreviations: lbh, log body height; lbl, log body length; lbw, log body weight.

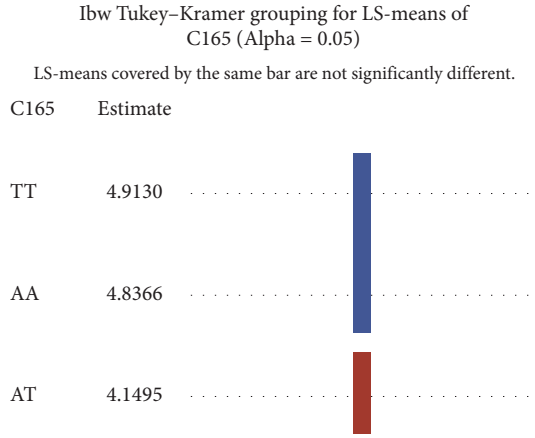

Figure 14: Tukey–Kramer grouping for *Bw* across genotypes at the C165 locus.

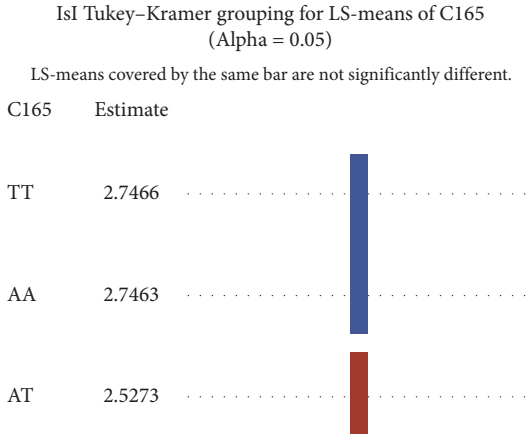

Figure 15: Tukey–Kramer grouping for *Bl* across genotypes at the C165 locus.

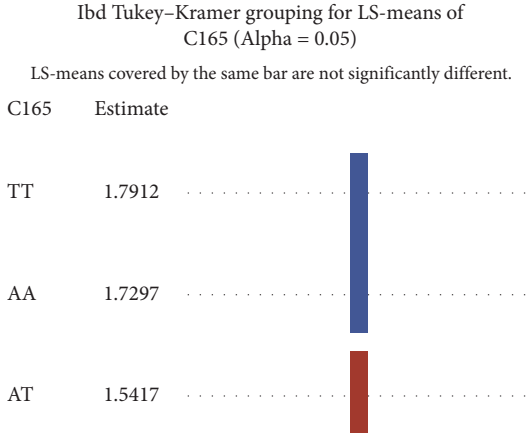

Figure 16: Tukey–Kramer grouping for *Bh* across genotypes at the C165 locus.

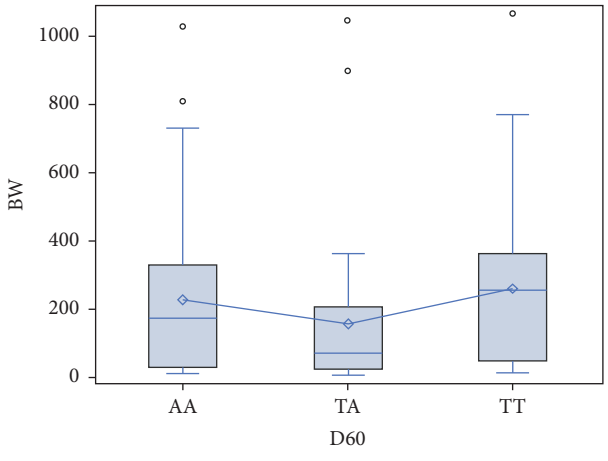

Figure 17: Distribution of body weight (*Bw*) across the genotypes at the D60 locus.

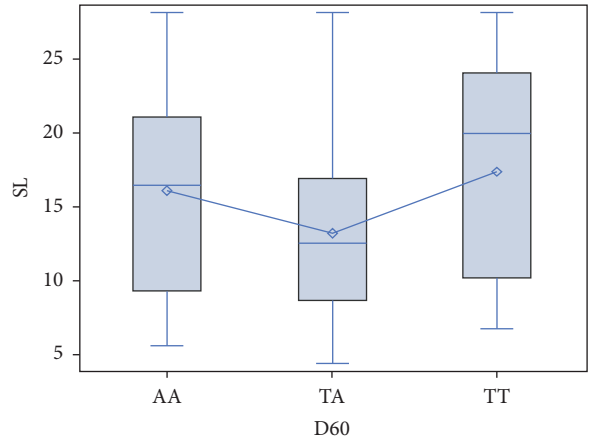

Figure 18: Distribution of body length (*Bl*) across the genotypes at the D60 locus.

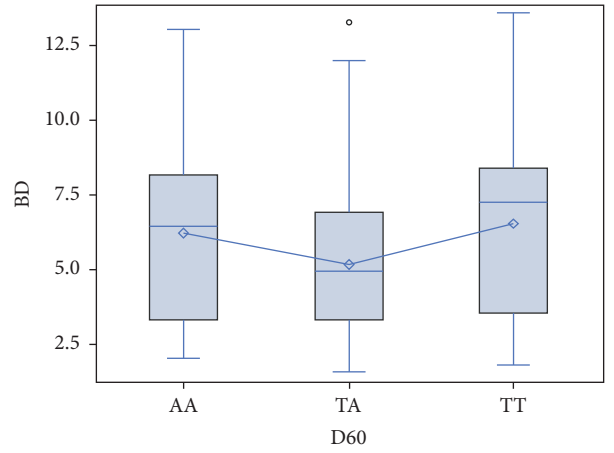

Figure 19: Distribution of body height (*Bh*) across the genotypes at the D60 locus.

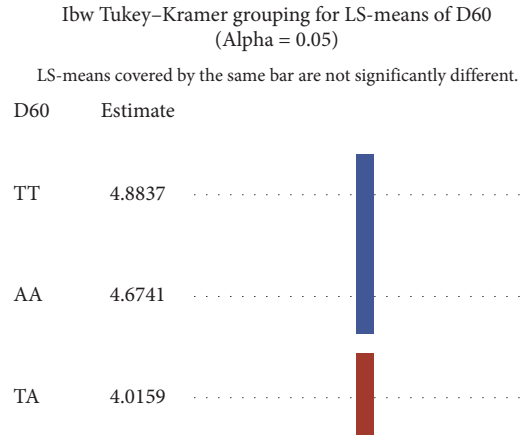

Figure 20: Tukey–Kramer grouping for *Bw* across genotypes at the D60 locus.

were 0.565 and 0.435, respectively. The frequencies of AA, AT, and TT genotypes were 0.319, 0.491, and 0.189, respectively. Similarly, A/T substitution at the A1132T site and G/T substitution at the G1217T site were found in the intron 3 region of the GH gene in common carp [39]. At the A1132T site, the allele frequencies of A and T were 0.68 and

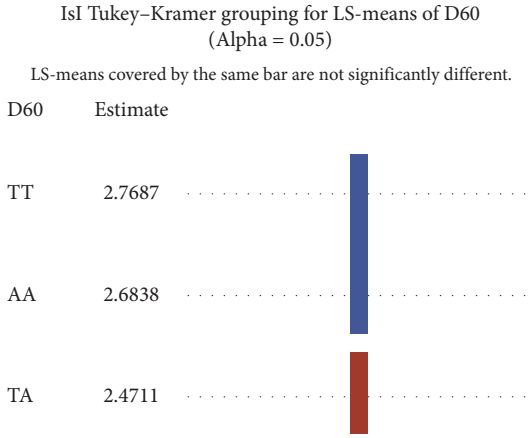

Figure 21: Tukey–Kramer grouping for *Bl* across genotypes at the D60 locus.

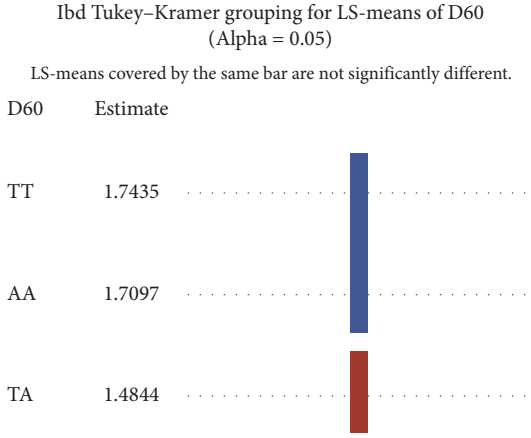

Figure 22: Tukey–Kramer grouping for *Bh* across genotypes at the D60 locus.

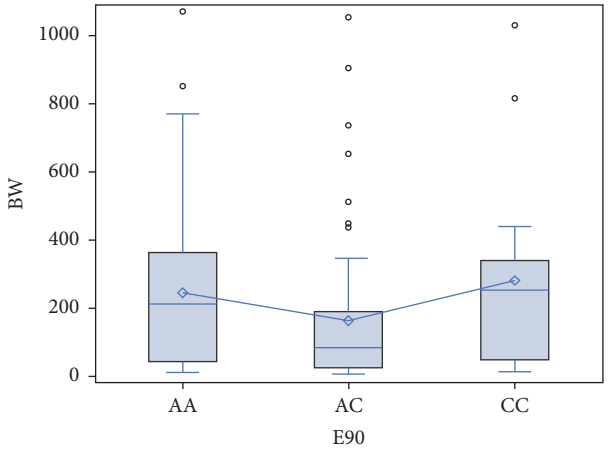

Figure 23: Distribution of body weight (*Bw*) across the genotypes at the E90 locus.

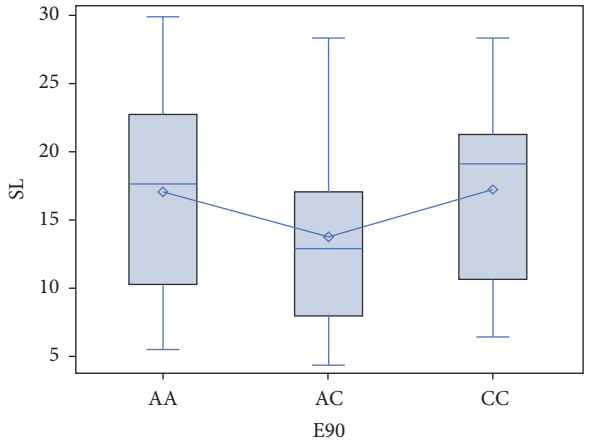

Figure 24: Distribution of body length (*Bl*) across the genotypes at the E90 locus.

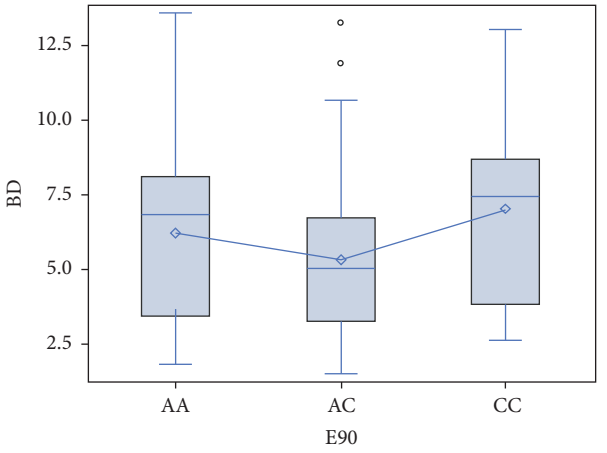

Figure 25: Distribution of body height (*Bh*) across the genotypes at the E90 locus.

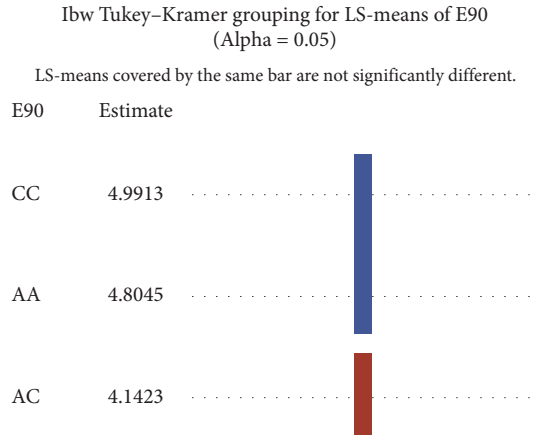

Figure 26: Tukey–Kramer grouping for *Bw* across genotypes at the E90 locus.

0.32, respectively. The frequencies of AA, AT, and TT were 0.425, 0.5, and 0.075, respectively. At the G1217T, the allele frequencies of G and T were 0.78 and 0.22, respectively. Two genotypes, GG and GT, were revealed, with frequencies of 0.55 and 0.45, respectively [39].

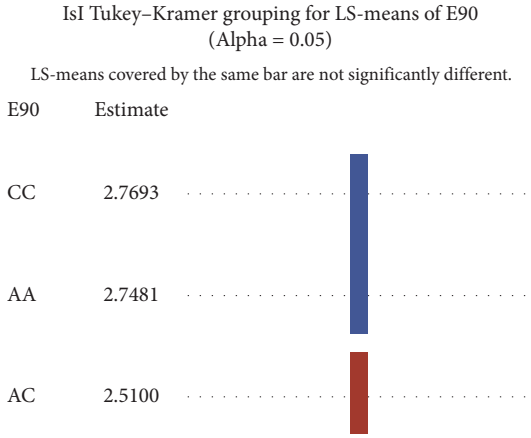

Figure 27: Tukey–Kramer grouping for *Bl* across genotypes at the E90 locus.

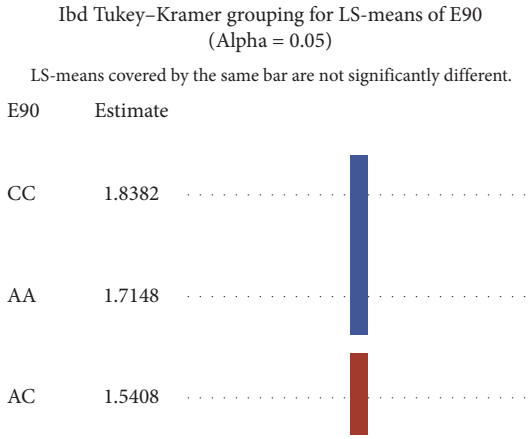

Figure 28: Tukey–Kramer grouping for *Bh* across genotypes at the E90 locus.

The present study revealed A/T substitution at the D60 locus (exon and intron 4), with the allele frequencies of A and T being 0.525 and 0.475, respectively. At the D114 locus, the identified alleles were C and T, with frequencies of 0.688 and 0.312, respectively. In contrast, genetic diversity in exon and intron four of the GH gene of common carp has not been previously reported. Additionally, this study revealed A/C substitution at the E90 locus and C/A substitution at the E185 locus in the amplicon 5 region (exon 5) of the GH gene. At the E90 locus, the allele frequencies of A and C were 0.633 and 0.367, respectively. The frequencies of AA, AC, and CC were 0.4, 0.464, and 0.134, respectively. At the E185 locus, the allele frequencies of C and A were 0.756 and 0.244, respectively. The frequencies of CC, CA, and AA genotypes were 0.571, 0.368, and 0.059, respectively. Similarly, in the water buffalo, C/G substitution at the C1529G site and A/C substitution at the A1592C site in the exon 5 region of the GH gene have been reported. The allele frequencies of C and G were 0.8 and 0.2, respectively. The frequencies of CC, CG, and GG genotypes were 0.63, 0.34, and 0.03, respectively, at the C1529G site. The allele frequencies of A and C were 0.57 and 0.43, respectively. The frequency of AA, AC, and CC genotypes were 0.35, 0.435, and 0.125, respectively [44].

In the current study, among the five polymorphic loci, C165 (exon and intron 3), D114 (exon and intron 4), and E90 (exon 5) loci were in the HWE ($p > 0.05$), whereas D60 (exon and intron 4) and E185 (exon 5) loci significantly deviated from the HWE ($p < 0.05$). Similarly, a study found that both the polymorphic loci (A1132T and G1217T) in the intron 3 region of the GH gene of common carp deviated from the HWE ($p < 0.05$) [23]. Additionally, in yellow catfish, four SNPs in intron 4 (1674G > A, 2100A > G, 2154T > G, and 2285T > C) and one in the 3′ UTR of the GH gene were detected. One locus (2822A > G) deviated significantly from the HWE ($p < 0.05$) [41]. In European seabass, a total of 10 novel SNPs were detected in the first partial intron, second exon, second intron, and third partial exon of the GH gene, in which four loci (g.1557A > T, g.1663C > G, g.1684T > C, and g.1799T > C) were in the HWE, whereas the three loci (g.1611T > C, g.1769T > C, and g.1857C > T) were not in the HWE [43]. Another study found that both the polymorphic loci (C1529G and A1592C) in the exon 5 region of the GH gene were consistent with the HWE ($p < 0.05$) in water buffalo [44].

In the present study, the trait association was performed for the polymorphic loci. The loci C165 (amplicon 3), D60 (amplicon 4), and E90 (amplicon 5) were significantly related to the ***Bw***, ***Bl***, and ***Bh*** of the common carp. The loci D114 and E185 had no significant relatedness with any of these growth-related traits. At the C165 locus (exon and intron 3), the genotypes TT and AA exhibited significantly higher least-square means for *Bw*, viz., 135.63 ± 1.17 g and 125.21 ± 1.13 g, respectively, compared to the AT genotype (82.26 ± 1.11 g). These results are consistent with previous studies that have associated the variation in the GH gene with the growth-related trait in common carp and other species. In common carp, the genotypes AA and AB at the P2 locus (intron 3) of the GH gene had a significant association with body weight, body length, body height, absolute growth rate (AGR), and specific growth rate (SGR). The AA genotype has the highest means for all the growth-related traits (body weight, body length, body height, AGR, and SGR) compared to the AB genotype [19]. Similarly, two SNPs (A1132T and G1217T) were detected in the intron 3 region of the GH gene of common carp. The SNP at the A1132T site showed a negative correlation with the growth trait (the AA genotype was significantly correlated with the growth trait [$p < 0.05$]). The G1217T site did not show any significant correlation with growth traits for any genotype [39]. The results of the present study are in accordance with the studies, wherein researchers observed 8 different banding patterns identified within the exon 4, intron 4, and exon 5 regions of the GH-1 gene in common carp using the PCR-SSCP technique. These banding patterns were found to be significantly associated with the body weight of common carp [38]. Similarly, in *Channa striata*, an insertion, deletion, and SNPs within the exon four of the GH gene were found to be associated with the standard length and head width [42]. Additionally, in yellow catfish, SNPs (1674G > A, 2100A > G, and 2154T > G) in the intron 4 of the GH gene showed an association with growth traits. Individuals with the GG

genotype at the position 2100A > G had significantly greater values for body thickness than did individuals with the AG genotype ($p < 0.01$) as well as the AA genotype ($p < 0.05$). The loci 1674G > A and 2100A > G had no significant difference in the genotypes [41]. Ten SNPs were reported in the first partial intron, second exon, second intron, and third partial exon of the GH gene of European seabass [43]. The two genotypes GH g.1611T > C locus and GH g.1557A > T were found to be significantly associated with growth-related traits viz., total weight, fillet weight, head length, preanal length, and abdominal length, respectively. Similarly, growth relatedness was performed with the two SNPs (p.Leu153Val and p.Asn174His) present in the exon 5 region of the GH of water buffalo. The p.Leu153Val SNP was significantly associated with growth traits, including body weight and average daily gain, unlike the p.Asn174His SNP, which showed no significant association with birth body weight (BW) and weaning body weight (WW) [44].

This study has newly identified the five polymorphic loci in the GH gene of the common carp: C165 (exon and intron 3), D60 (exon and intron 4), D114 (exon and intron 4), E90 (exon 5), and E185 (exon 5). The loci E90 and E185 provide new insight into exon 5 polymorphism. Unique allelic variations were observed, that is, A/T substitution at the C165 locus, A/T substitution at the D60 locus, C/T substitution at the D114 locus, A/C substitution at the E90 locus, and C/A substitution at the E185 locus. The D60 (exon and intron 4) and E185 (exon 5) loci significantly deviated from the HWE ($p < 0.05$), indicating potential selection pressure. Novel associations between GH polymorphisms and growth-related traits viz., ***Bw***, ***Bl***, and ***Bh***, particularly at C165, D60, and E90 loci, were identified. This study expanded the knowledge on the GH gene polymorphism in common carp.

SNPs represent genomic sequence variations that serve as potential genetic markers. SNP markers are more stable as compared to other genetic markers such as SSR, RFLP, AFLP, and RAPD [46]. Genomic selection aims to predict the EBV of individuals based on their genotype at a large number of markers spread over the genome, typically utilizing SNP arrays for comprehensive coverage [47]. SNPs also play a pivotal role in integrating multiomics data for crop improvement by acting as key genetic markers that link DNA variations to other molecular levels, such as gene expression (transcriptomics), protein abundance (proteomics), and metabolite profiles. By mapping SNPs to different omics layers, researchers can identify critical genes, pathways, and molecular interactions responsible for particular traits [48]. Thus, unraveling genome-wide SNPs has an important role in the identification of high-merit germplasm for ongoing as well as future breeding programs [46]. The SNPs of the present findings need to be explored in larger populations. Further research is needed to functionally validate these markers (gene expression analysis), explore additional growth-related loci, and develop predictive models for breeding applications.

5. Conclusion

In the present study, five polymorphic sites were identified in the amplicon 3 (C165 locus), amplicon 4 (D60 and D114 loci), and amplicon 5 (E90 and E185 loci) regions of the GH gene in the common carp. Amplicon 1 was conserved, whereas two monomorphic loci were found in the amplicon 2 region. The loci C165 (amplicon 3), D60 (amplicon 4), and E90 (amplicon 5) were significantly related to ***Bw***, ***Bl***, and ***Bh*** of the common carp. The loci D114 and E185 had no significant effect on any of these growth-related traits. The present study has identified and deciphered polymorphism in the GH gene of common carp and its growth relatedness. This needs to be explored further in a larger population, and the significant trait association could assist in the molecular breeding of common carp.

Data Availability Statement

The data supporting the findings of this study are accessible upon request from the corresponding author.

Ethics Statement

The study was approved by the Ethics Committee of ICAR-CIFE, Mumbai.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Anjali Kumari: conducted the experiments and writing – original draft; Raghul R., Nagaraja P. S., Sushitharan V., and Priyanka Sanwal: assistance in sample collection and molecular work; Angom Lenin Singh: overall guidance and supervision; Mujahidkhan A. Pathan: conceptualization, genetic data analysis, manuscript review and editing, supervision of research progress, and overall guidance and manuscript submission.

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