Growth and expression level of growth hormone in transgenic mutiara catfish second generation

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Received: April 29, 2019; **accepted:** May 22, 2019.

Growth improvement through a selection program on African catfish has been conducted in Indonesia but the genetic gain achieved was still low. This study was aimed to evaluate the growth and the expression level of African catfish growth hormone (*CgGH*) gene in transgenic Mutiara catfish second generation (G2). Three G1 transgenic Mutiara broodstock were selected based on *CgGH* expression level and spawned to produce G2. A pair of non-transgenic was provided as control. The identification of G2 transgenic fish was performed using the PCR method, while the analysis of *CgGH* expression level was done by using the semi-quantitative RT-PCR method. The results showed that one of the three G1 transgenic fish transmitted the *CgGH* transgene following the Mendelian segregation pattern. The level of *CgGH* expression in caudal fin, muscle, and liver tissues of G2 fish varies among crossing and individuals from the same G1 broodstock. The growth of G2 transgenic Mutiara catfish was 3-4 times higher than non-transgenic fish ($p < 0.05$). The feed conversion ratio of G2 transgenic fish were 50.3-64.5% lower than that of non-transgenic fish. Thus, this *CgGH* transgenic Mutiara catfish is most likely potential to be cultured to increase productivity and efficiency of cultivation.

Keywords: mutiara catfish; *CgGH*; over-expression; Inheritance; second generation.

Abbreviations: RT-PCR: reverse transcription polymerase chain reaction. GH: growth hormone. *CgGH*: *Clarias gariepinus* growth hormone. FCR: feed conversion ratio. pCMV-CgGH: plasmid Cytomegalovirus-*Clarias gariepinus* growth hormone.

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Introduction

Efforts to improve the growth of African catfish (*Clarias gariepinus*) in Indonesia have been carried out using traditional selection methods in order to produce superior strains, but the genetic gain was relatively small [1, 2]. The achievement in growth stimulation using transgenesis method was higher than selective breeding in previous studies [3, 4]. Growth rate can drastically be accelerated by over-expressing of growth hormone (GH) gene such as reported in transgenic coho salmon [5], mud loach [6], and Nile tilapia [7].

The fast-growing character due to over-expression of GH needs to be inherited biologically to the offspring through broodstock sexual reproduction. It is expected that *Clarias gariepinus* growth hormone (*CgGH*) gene can be inherited to mutiara catfish transgenic with rapid growth characteristic in offspring. The stability of the exogenous gene inheritance depends on its integration pattern in the host genome. It was

known previously that exogenous GH genes integrated in broodstock germ cells was capable to be inherited and expressed efficiently to their offspring [8].

The exogenous DNA could be integrated on more than one site of chromosome hence causing variations in transgene expression, as shown by variations in body weight of transgenic carp [9] and transgenic mice [10]. The growth polymorphism phenomena were caused by the integration of transgenes on chromosomes in the gonadal cells, causing transgenes to be activated when integrated on homologous chromosomes or, on the other hand, inactivated by sex chromosome transcription machines when integrated on non-homologous chromosomes [10].

The crossing of female or transgenic males with non-transgenic fish was known to cause differences in the variation of transgene expression on certain tissues [11]. Expression of transgenes in offspring was more likely to be stable if the female broodstock is in homozygous transgenic status [12]. Conversely, the hemizygous transgenic broodstock may produce low or even absence of expression in the offspring while the transgene can also be expressed only on one particular tissue [10, 13].

Production of G2 transgenic mutiara catfish that involves the female or male transgenic in fish breeding determines the growth diversity because of the correlation between transgene expression and variation in G2 fish body weight. Evaluation of growth phenotypes in terms of fish weight, feed conversion ratio (FCR), and analysis of *CgGH* mRNA expression in the offspring of G2 transgenic catfish was needed to determine the stability of transgenic inheritance. Thus, the target for this research was increased production in transgenic mutiara catfish aquaculture with stable inheritance of growth characteristics through our proposed transgenesis study.

Materials and Methods

Screening of transgenic G1 broodstock

The first generation (G1) of transgenic mutiara catfish (one year old, body weight 1-1.6 kg) was selected by using the reverse transcription PCR method. The total RNA extraction was done from 20-30 mg of fish caudal fin tissue using High pure RNA tissue kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

The cDNA synthesis was performed using MyTaq one-step RT-PCR (Bioline, London, UK). PCR amplification was carried out using GoTaq® Green Master Mix (Promega, Madison, WI, USA) with the following program: first denaturation 95°C for 5 min; then 30 cycles with denaturation 95°C for 30 s, annealing 54°C for 30 s, extension 72°C for 1 min; and final extension at 72°C for 7 min. The GH primer sequence used was GH-F (5'-ATGGCTCGAGTTTTGGTGCTGCT-3') and GH-R (5'-CTACAGAGTGCAGTTGGAATCCAGGG-3') [14], while the β-actin gene (βAct-F: 5'-ACCGGAGTC CATCACAATACCAGt-3' and βAct-R: 5'-GAGCTG CGTGTTGCCCCTGAC-3') was used as an internal control [15]. The products of amplification were separated by 1% agarose gel electrophoresis method. The G1 transgenic fish should have the PCR product of 600 bp.

Production of G2 transgenic catfish and analysis of gene transmission

Three out of 10 G1 transgenic fish were selected and used to produce G2 transgenic fish based on the level of gonadal maturity. The broodstock of G1 fish was mated with four different cross treatments including A: female transgenic 1 × male non-transgenic 1; B: female transgenic 2 × male non-transgenic 2; C: female non-transgenic 3 × male transgenic 3; and D: female non-transgenic 4 × male non-transgenic 4 as the control.

Spermiation of male and ovulation of female were induced by hormonal treatment using Ovaprim (Syndel Laboratories Ltd., British Columbia, Canada) at a dose of 0.2 and 0.5 mL/kg for male and female fish, respectively. Each cross

was spawned in a round fiberglass (2 x 1.5 x 1 m^3) containing 2-3 *kakabans* (egg-sticking substrate). Water temperature was set at 28 ± 1°C using water heater with photoperiods of 12 hours light vs. 12 hours dark while aeration was provided to maintain dissolved oxygen level in water. Furthermore, *kakabans* containing eggs were transferred into the glass aquarium (80 x 60 x 50 cm^3) until the eggs hatched. Two days after hatching, the G2 larvae were transferred into another aquarium. The larvae were fed with Artemia nauplii (Mackay, Great Salt Lake, Utah, USA) until 10 days old. The juveniles were reared until one month old in a cylindrical fiberglass and fed on Tubifex worm. 50% water exchange was done once a week.

Ten fish from each cross were randomly taken for analysis of *CgGH* transgene transmission by PCR method. DNA was extracted from caudal fin clipped from one-month-old juvenile. The DNA extraction was conducted using the Wizard® Genomic Purification Kit (Promega, Madison, WI, USA) in accordance with the kit instructions. The mixture of PCR reactions for DNA amplification was carried out in the final volume of 25 µL containing 12.5 µL Go-Taq Green Master Mix (Promega, Madison, WI, USA), 1.5 µL of GH-F primer (10 µM), 1.5 µL of GH-R primer (10 µM), 2.5 µL of template DNA, and 7 µL of nuclease free water with the same PCR program as in G1 screening. PCR amplification product was separated using 1% agarose gel electrophoresis.

Analysis of *CgGH* mRNA expression level

At 2.5 months old, three transgenic G2 fish were taken from each cross for analysis of *CgGH* mRNA expression level. Total RNA was extracted from caudal fin, liver, and muscle tissue by the same method used in the G1 screening. The expression level was determined by semi-quantitative RT-PCR method [16] with the help of Image J software (version 1.33) (NIH, Bethesda, Maryland, USA). β-actin was used as internal control. The maximum gene expression of *CgGH* and β-actin were obtained after the amplification process reached the exponential phase (total cycle of 20 – 30) and after the optimal cycle (before reaching the plateau phase). Once the optimum cycle was determined, PCR was conducted with three different cycle for *CgGH* and β-actin. The determination of the optimization cycle was the one that produced the densest DNA band. The cycle number of amplifications were 30 and 25 for GH and actin genes obtained from the previous optimization, respectively.

Growth and feed conversion ratio of G2 transgenic fish

Transgenic and non-transgenic fishes with body weight of 6.61 ± 1.43 g were randomly taken from each cross (n = 10, one month old), and then reared for 35 days in 50 × 40 × 30 cm^3 glass aquaria at initial density of 10 fishes. Three aquaria were provided for each cross as replication. Fishes were fed with commercial diet with 33% crude protein content, twice a day with an amount of 5% fish biomass. Body weight was measured every week. Total water change was carried out once a week. Water temperature was maintained at 27 ± 1°C using a heater. The photoperiod was set to 12 hours light vs. 12 hours dark. Feed conversion ratio was calculated at the end of experiment.

Statistical analysis

Data were analyzed for the normality and homogeneity prior to statistical analysis. One-way ANOVA and Duncan's multiple distance test at p = 0.05 was used to determine statistically difference in growth and feed conversion ratio between transgenic and non-transgenic catfish.

Results

The *CgGH* gene transmission and mRNA expression

Three G1 fishes having *CgGH* expression in caudal fin tissue were selected to produce G2 transgenic fish (Figure 1). Using a specific primer set for *CgGH*, the percentage of G2 progenies carrying the *CgGH* transgene was detected. However, the proportion of transgenic progenies ranged from 20 to 50% among treatments

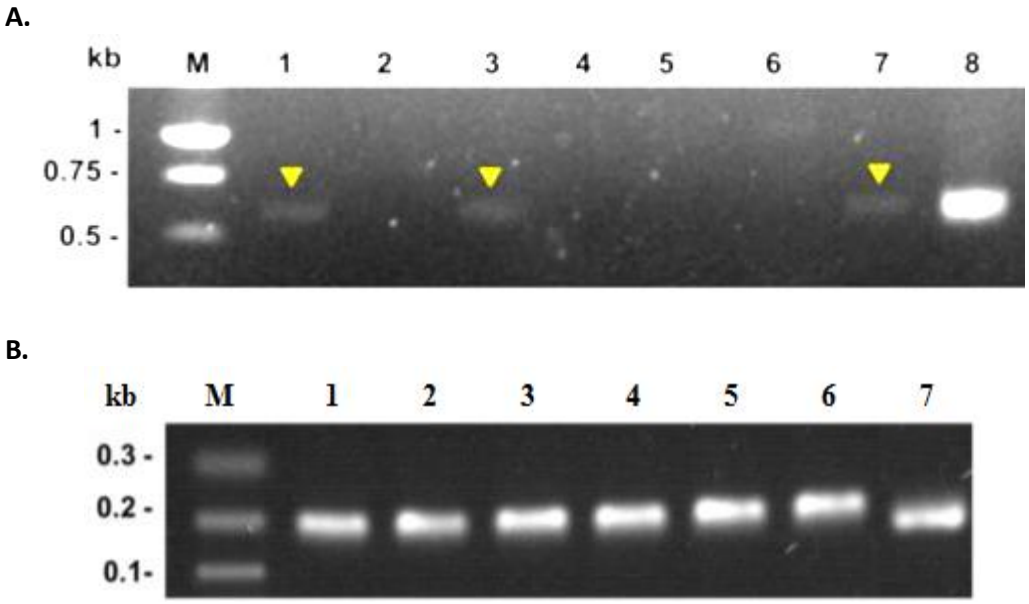

Figure 1. Electropherogram result from identification of transgenic broodstock. (A) *CgGH* (marked with an arrow). (B) β-actin of *C. gariepinus*. Lanes 1-4: broodstock of female; Lanes 5-7: broodstock of male; Lane 8: pCMV-CgGH; M: DNA ladder.

Table 1. The *CgGH* gene transmission and expression level in transgenic Mutiara catfish of second generation.

Crosses	Transmission (%)	Relative expression level of the *CgGH*/β-actin gene								
		Fin			Muscle			Liver		
		1	2	3	1	2	3	1	2	3
A	50	1.11	1.1	1.12	1.11	1.11	1.11	1.13	1.08	1.35
B	20	1.37	0.96	0	1.13	1.33	0	1.23	0	0
C	22	1.01	0	0.96	1.05	0	0	0.96	0	1.4
D	0	0	0	0	0	0	0	0	0	0

A: female-1 transgenic × male-1 non-transgenic; B: female-2 transgenic × male-2 non-transgenic; C: female-3 non-transgenic × male-3 transgenic; D: female non-transgenic × male non-transgenic; 1,2,3: fish number.

(crosses) while no DNA product of 600 bp was found in non-transgenic fish. The level of transgene expression among crosses was shown in table 1. One G1 broodstock (cross A) transmitted the transgene following the Mendelian segregation pattern with 50% of the progenies carried the *CgGH* transgene.

Furthermore, the progenies of A crossing have the similar transgene expression level among individuals in the three organs observed, while in crosses of B and C there were individuals with no expression on certain organs. In addition, there were no expression in the liver of fish number 2 in the B cross and in muscle of fish number 3 on the C cross (Figure 2).

Growth and feed conversion ratio

As shown in Figure 3, the average body weight of G2 transgenic fish was 3-4 times higher than that of non-transgenic control ($p < 0.05$). The average body weight of fish progeny from cross A was the same as cross C and cross B. In addition, the coefficient of variation (CV) of the body weight from the progeny of cross A (3.3%) was lower than that of B (19.9%) and C (10.4%). Furthermore, transgenic fish also had higher feed efficiency. Feed conversion ratio of

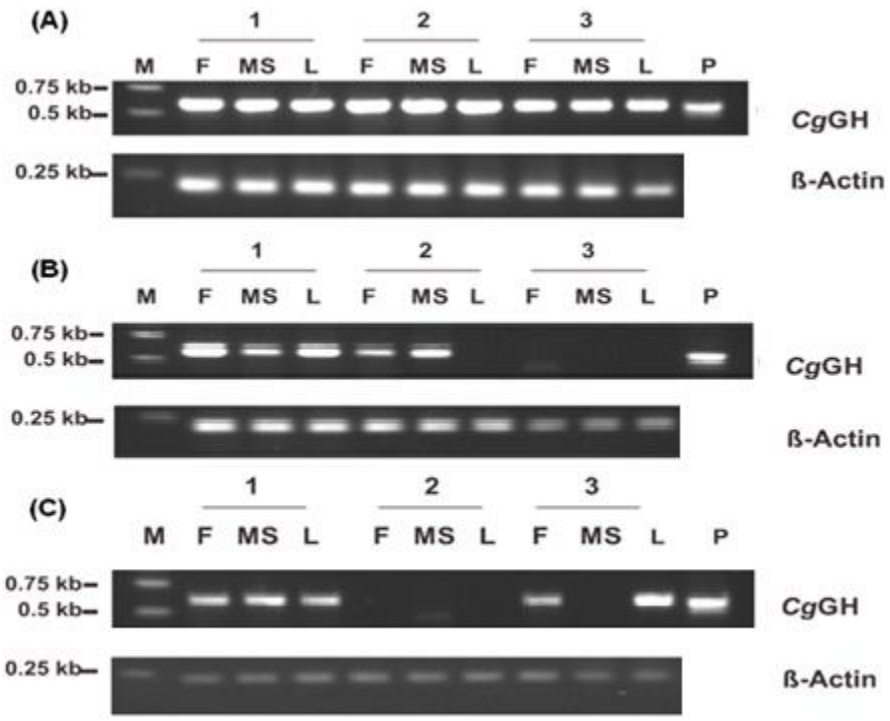

Figure 2. Electropherogram of *CgGH* gene amplification in various fish organ of G2 and β-actin *C. gariepinus*; M: DNA ladder; A, B, C: G1 transgenic crossing; F: fin; MS: muscle; L: liver; P: pCMV-CgGH; No. 1-3: fish sample number.

transgenic fish was 50.3 - 64.5% lower than that of the non-transgenic fish (cross D).

Discussion

The results of the PCR analysis showed that the *CgGH* gene was mainly expressed on the tissue (Figure 2). The percentage of *CgGH* gene transmission in the G2 mutiara catfish varied among crosses ranging from 20 - 50%. These variations showed different patterns of integration among crosses. The *CgGH* gene on different tissues also had different expression levels among crosses (table 1).

Inheritance of the *CgGH* gene

The *CgGH* transgene can be inherited in G1 and G2 progenies, which indicates that the transgene has highly integrated into the genome of mutiara catfish. However, there were variations of transgene transmission rate in G2 transgenic fish, which indicated a different pattern of integration among crosses. Mendelian inheritance pattern is common in transgenic fish generation [17-20]. However, in this study Mendelian inheritance pattern was only found in A cross fish. The low transgene transmission at B and C crosses may be related to the extrachromosomal condition. This case has also been reported by Chen et al. [21] when the insertion of the GH gene of transgenic carp on extrachromosomal causes failure on the transmission of transgenes to the offspring.

CgGH transgene expression

First transgenic generation having a high transgene expression level should be selected to produce a stable transgenic line [22]. In this study, we selected three lines that expressed the transgene in their caudal fin tissue to produce the G2 generations. In G2 generation, the transgene was expressed in all the examined tissues of A cross progenies, while there were exceptions in certain tissues of B and C cross fish progenies. The absence of transgenic expression

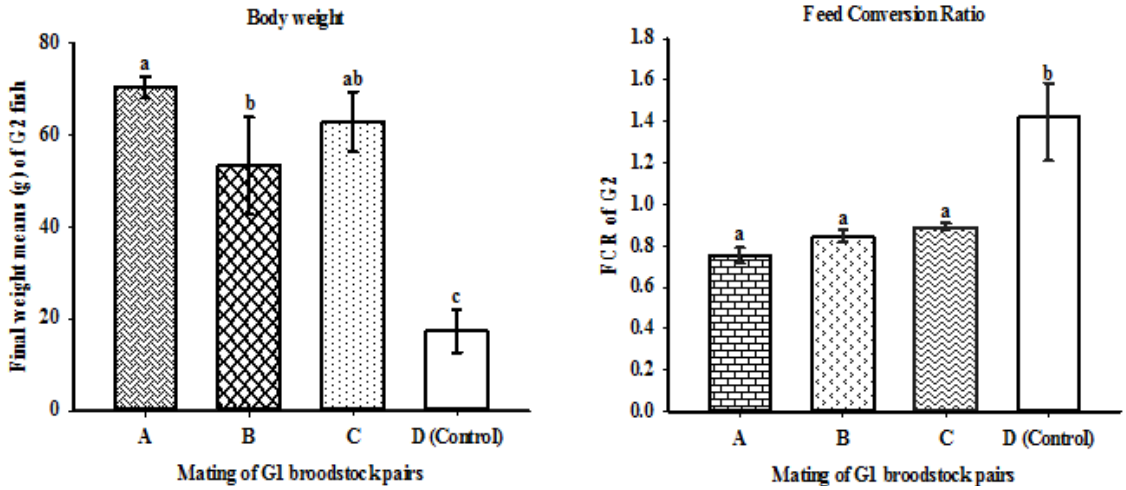

Figure 3. Body weight (left) and feed conversion ratio (right) of G2 transgenic mutiara catfish after 35 days of rearing. Data is presented as a mean ± standard error (replication = 3); Groups with different letters above the bars show significant difference ($p < 0.05$).

in certain tissues has also been reported in several transgenic fish species such as northern pike [23], mud loach [24], and zebrafish [17].The possible factor is due to the positional effect integration in the chromosome site that suppresses its expression [22, 25, 26]. In addition, the transgene integrated in heterochromatin regions can cause loss of expression or low (even absence) transmission of transgene to the offspring [21, 27].

Growth performance

In order to ascertain the benefit of the successful expression of the *CgGH* transgene, the growth of both transgenic and non-transgenic fishes for 35 days of rearing were examined. The results showed that the growth of transgenic was 3–4 times higher than that of non-transgenic fish ($p < 0.05$). The higher growth increment was found in A cross progeny, while the coefficient of variation of body weight (3.3%) was lower than that of the two other progenies (19.86% for B, and 10.40% for C progenies). Variations of transgene expression level in tissues (table 1, figure 2) were thought to be the cause of high CV value in body weight of B and C cross progenies. In addition, it was reported that the inheritance of exogenous GH genes of transgenic carp offspring produced higher body weight with equally distributed size due to integrated transgenes at the homologous chromosome loci [12]. On the other hand, a high variation in body weight of B and C progenies was probably due to the effects of polymorphism of the transgene inheritance originating from hemizygous transgenic broodstock [28].

FCR in transgenic mutiara catfish was lower than that of non-transgenic fish (Figure 3). Decreased FCR in transgenic fish has been reported in some transgenic fish species [11, 29-32]. Lower FCR is as improvement in feed conversion into body proteins of the GH transgenic fish [11, 33]. In addition, the transgenic-GH fish efficiently converts feed fats and carbohydrates into the source of energy for routine metabolic activities (sparring action proteins), which maintains the sufficiency of metabolic energy requirement.

According to the Mendelian inheritance pattern, higher growth and feed efficiency of G2 A cross can be used to produce a homozygous transgenic generation. The homozygous fish is useful for mass transgenic production. In fact, the homozygous transgenic has been produced for fish species of tilapia [19], trout [34], common carp [11], and zebrafish [35].

Conclusion

The *CgGH* transgene can be inherited to the second generation of transgenic fish, although transmission rate different among crosses. Over-expression of *CgGH* gene is able to improve the growth and feed efficiency in the G2 transgenic mutiara catfish.

Acknowledgement

The authors would like to thank the Ministry of Research, Technology, and Higher Education, Republic of Indonesia (Kemenristekdikti) (Grant number: 01/E/KPT/2018). Acknowledgments are also addressed to the Directorate of Research and Community Service of Padjadjaran University for the financial support through DIPA BLU (Contract number: 598/UN6.M/LT/2018). Thanks to Mr. Rosadi, Mr. Udin, Ms. Nisa, and Ms. Silvy to help in the rearing of the fish and the data analyses.

References

1. Dewi SRRPS and Iswanto B. 2016. Productivity and profitability of selection of catfish (Clarias gariepinus) aquaculture in rearing in soil ponds. Media Akuakultur11(1): 11–17.
2. Gusrina, Alimuddin, Sumantadinata K, Widyastuti U. 2009. Transfer of gene encoding tilapia growth hormone (mBPtiGH) in catfish (Clarias sp.) by microinjection method. J. Ris. Akuakultur. 4:333-340.
3. Devlin RH, Yesaki TY, Biagi CA, Donaldson EM, Shan RM. 1994. Extraordinary salmon growth. Nature 371:209– 210.
4. Hew CL and Fletcher GL. 2000. The role of aquatic biotechnology in aquaculture. Aquaculture 197:191– 204.
5. Mori T and Devlin RH. 1999. Transgene and host growth hormone gene expression in pituitary and nonpituitary tissues of normal and growth hormone transgenic salmon. Molecular and Cellular Endocrinology 149:129–139.
6. Nam YK, Cho HY, Cho YS, No, JK, Kim CG, Kim DS. 2001. Dramatically accelerated growth and extraordinary gigantism of transgenic mud loach Misgurnus mizolepis. Transgenic Research 10:353–362.
7. Kobayashi SI, Alimuddin, Morita T, Miwa M, Lu J, Endo M, Takeuchi T, Yoshizaki G. 2007. Transgenic Nile tilapia (Oreochromis niloticus) over-expressing growth hormone show reduced ammonia excretion. Aquaculture 270:427–435.
8. Phillips RB and Devlin RH. 2010. Integration of growth hormone gene contructs in transgenic strains of coho salmon (Oncorhynchus kisutch) at centromeric or telomeric sites. Genome 53:79-82.
9. Uh M, Khattra J, Devlin RH. 2006. Transgene constructs in coho salmon (Oncorhynchus kisutch) are repeated in a head-to-tail fashion and can be integrated adjacent to horizontally-transmitted parasite DNA. Transgenic research 15:7-11.
10. Chang S-P, Margaret L, Opsahl, Bruce AC, Whitelaw, Morley SD, West JD. 2013. Relative transgene expression frequencies in homozygous versus hemizygous transgenic mice. Transgenic Res. 22:1143-1154.
11. Zhong CY. Song Y, Wang Y, Li Y, Liao L, Xie S, Zhu Z, Hu W. 2012. Growth hormone transgene effects on growth performance are inconsistent among offspring derived from different homozygous transgenic common carp (*Cyprinus carpio* L.). Aquaculture 356-357:404-411.
12. Wang Y, Wei H, Gang W, Yonghua S, Shangping C, Fuying Z, Zouyan Z, Jianxin F, Xirul Z. 2001. Genetic analysis of "all-fish" growth hormone gene trans, ferred carp (*Cyprinus carpio* L.) and its F1 generation. Chinese Science Bulletin. 46:1-4.
13. Svitashev S, Ananiev E, Pawlowski WP, Somers DA. 2000. Association of transgene integration sites with chromosome rearrangements in hexaploid oat. Theor Appl Genet. 100:872-880.
14. Zhang Mao-qun, Chen Cheng-xun, Guo Y, Jing GW, Xiao-mei. 2009. Cloning and sequence analysis of full-length growth hormone cDNA from Clarias gariepinus. ACTA Agricultural Boreal-Sinica. 24:27–32.
15. Raghuveer K and Senthilkumaran B. 2010. Cloning and differential expression pattern of vasa in the developing and recrudescing gonads of catfish, Clarias gariepinus. Comparative Biochemistry and Physiology, Part A 157:79-85.
16. Laing KJ, Holland J, Bonilla S, Cunningham C, Secombes CJ. 2001. Cloning and sequencing of caspase 6 in rainbow trout Onchorhynchus mykiss, and analysis of its expression under conditions known to induce apoptosis. Development and Comparative Immunology. 25:303-312.
17. Alimuddin, Kiron V, Satoh S, Takeuchi T. 2005. Enhancement of EPA and DHA biosynthesis by over-expression of masu salmon Δ6-desaturase-like gene in zebrafish. Transgenic Res. 14:159-165.
18. Devlin RH, Biagi CA,Yesaki TY. 2004. Growth, viability and genetic characteristics of GH transgenic coho salmon strains. Aquaculture. 236(1–4):607–632.
19. Martinez RMP, Estrada J, Berlanga I, Guillen O, Hernandez E, Cabrera R, Morales, A, Morales P, Melamed R, Leionart, Fuente, J de la. 1996. Growth enhancement in transgenic tilapia by ectopic expression of tilapia growth hormone. Molecular Marine Biology and Biotechnology. 5(1):62 – 70.
20. Nam YK, Cho YS, Chang YJ, Jo I-Y, Kim DS. 2000. Generation of transgenic homozygous lines carrying the CAT gene in mud loach (*Misgurnus mizolepis*). Fish. Sci. 66:58-62.
21. Chen J, Luo Q, Bao H, Liao L, Li Y, Zhu Z, Wang Y, Hu, W. 2015. The integration characteristic of the exogenous growth hormone gene in a transgene common carp (*Cyprinus carpio* L.) Sci. Bull. 60(19):1654-1660.

22. Alimuddin, Kiron V, Satoh S, Takeuchi T, Yoshizaki G. 2008. Cloning and over-expression of a masu salmon (Onchorhynchus masou) fatty acid elongase-like gene in zebrafish. Aquaculture. 282:13-18.
23. Gross ML, Schneider JF, Moav B, Alvarez C, Myster SH, Liu Z, Hallerman EM, Hackett PB, Guise KS, Faras AJ, Kaspuscinski AR. 1992. Molecular analysis and growth evaluation of northern pike (*Esox lucius*) microinjencted with growth hormones genes. Aquaculture. 103:253-273.
24. Nam YK, Noh CH, Kim DS. 1999. Transmission and expression of an integrated reporter construct in three generations of transgenic mud loach (*Misgurnus mizolepis*). Aquaculture. 172:229-245.
25. Dobie KW, Lee M, Fantes JA, Graham E, Clark AJ, Springbett A, Lathie R, McClenaghan M. 1996. Variegated transgene expression in mouse mammary gland is determined by the transgene integration locus. Proc Natl Acad Sci USA. 93:6659-6664.
26. Iyengar A, Muller F, Maclean N. 1996. Regulation and expression of transgenes in fish - a review. Transgenic Research. 5:147-166.
27. Reed KM, Dorschner MO, Phillips RB. 1997. Characteristics of two salmonid repetitive DNA families in rainbow trout, Oncorhynchus mykiss. Cytogenetics and Cell Genetics. 79:184-187.
28. Wu B, Sun YH, Wang YW, Wang YP, Zhu ZY. 2005. Characterization of transgene integration pattern in F4 h-GH transgenic common carp (Cyprinus carpio L.). Cell Research 15:447-454.
29. Faridah N, Yoshizaki G, Nuryati S, Setiawati M. 2016. Growth, survival, and body composition of transgenic common carp Cyprinus carpio 3rd generation expressing tilapia growth hormone cDNA. Hayati Journal of Biosciences 23:150-154.
30. Liu S, Zhang X, Zang X, Liu B, Arunakumara KKIU, Xu D, Zhang X. 2008. Growth, feed efficiency, body muscle composition, and histology of flounder (Paralichthys olivaceus) fed GH transgenic Synechocystis. Aquaculture 277(1-2):78-82.
31. Lubis RA, Alimuddin Utomo NBP. 2018. Enrichment of recombinant growth hormone in diet containing different levels of protein enhanced growth and meat quality of striped catfish (Pangasionodon hypophthalmus). Biotropia-The Southeast Asian Journal of Tropical Biology 26:1-8.
32. Tibbetts SM, Wall CL, Barbosa-Solomieu V, Bryenton MD, Plouffe DA, Buchanan JT, Lall SP. 2013. Effects of combined 'all-fish' growth hormone transgenics and triploidy on growth and nutrient utilization of Atlantic salmon (Salmo salar L.) fed a practical grower diet of known composition. Aquaculture 406:141-152.
33. Raven PA, Devlin RH, Higgs DA. 2006. Influence of dietary digestible energy content on growth, protein and energy utilization and body composition of growth hormone transgenic and non-transgenic coho salmon (Oncorrhynchus kisutch). Aquaculture. 254:730-747.
34. Tewari R, Michard-Vanhee C, Perrot E, Chourrout D. 1992. Mendelian transmission, structure and expression of transgenes following their injection into the cytoplasm of trout eggs. Transgenic Research 1:250-260.
35. Rosa CED, Figueiredo MDA, Lanes CFC, Almeida DV, Monserrat JM, Marins LF. 2008. Metabolic rate and reactive oxygen species production in different genotypes of GH-transgenic zebrafish. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 149:209-214.