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Monitoring of genetic variation in mono-sex tilapia stocks using randomly amplified polymorphic DNA markers in some private hatcheries of Bangladesh

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ABSTRACT

Tilapia (*Oreochromis niloticus*) is one of the most promising aquaculture species in Bangladesh, the culture of which is mostly dependent on the supply of hatchery-produced seed. Published information on genetic monitoring and genetic variability of the hatchery-reared tilapia population is rare in Bangladesh. Therefore, intra- and inter-population genetic variations were estimated among the five mono-sex tilapia populations collected from the matchday-kanon, khan, nancy, city, and brac hatcheries located at different districts in Bangladesh. Random amplified polymorphic DNA (RAPD) markers were amplified from tilapia's muscle DNA of each of the five populations using ten decamer random primers. The primers produced a total of 181 scorable bands of which 64.1% were found to be polymorphic ranged in molecular size from approximately 100bp to 2000bp. The brac population obtained the highest genetic variability (51%) of overall polymorphic loci and the lower index was estimated (55-62%) in that population with the four others in pair-wise similarity comparison. Moreover, a common pattern of distinctive genetic variation was observed in the brac hatchery population when the genetic differentiation (F_{ST}), gene flow (N_m) and genetic distance (D) had been analyzed. The distinctiveness was also supported from the unweighted pair group method of arithmetic mean (UPGMA) dendrogram analysis where the five populations aligned into two clusters- the brac alone belonged to one and the rest of the four grouped into another. Our overall findings reveal that a low level of genetic variation exists in the current tilapia populations of Bangladesh except in the brac hatchery population where broodstock is known to be well managed. This low level of genetic variation of the tilapia hatchery population will have a seriously detrimental effect on this species and its overall production if the genetic quality is not maintained.

INTRODUCTION

Bangladesh is sel-sufficient in fish production since 2018 with a total production of 4.27 million metric ton **(DoF, 2018)**. Of the total fish, tilapia (*Oreochromis niloticus*) production of more than 3.8 lakh metric ton has brought the country as the 4th largest tilapia producer in the world **(DoF, 2018)**. In fact, rapid growth rate, stress tolerance

ability and low production cost of this species have made it an important culture item for commercial purpose. Culture of tilapia is mostly dependent on the supply of hatchery reared seed (e.g. juvenile/hatchling/fry) from more than 400 public and private mono-sex (all male) tilapia hatcheries. To meet the supply of seed, the cumulative seed production of those hatcheries has increased to more than 60% during the last five years from 1.6 billion in 2014 to 2.7 billion in 2018 **(FRSS, 2016; DoF, 2018)**. Despite of the quantitative progress of seed production in recent years, maintenance of good quality brood stock and the production of good quality seeds in those hatcheries are considered to be the major challenges towards sustainable aquaculture practice **(Hussain *et al.*, 2013)**. The quality of tilapia seed is gradually deteriorating over the years mainly due to the genetic deterioration of brood stock through poor and similar hatchery management practice across the country **(Belton *et al.*, 2011; Mohamed Din and Subasinghe, 2017; Mekkawy *et al.*, 2017;)**.

Like carp, most of the tilapia hatcheries rear their own brood stock and generally do not recruit individuals from natural sources or exchange breeders between farms **(Hussain and Mazid, 2001; Hasan and Ahmed, 2002)**. This, therefore, might have possibility to make each hatchery as a genetically isolated self-sustaining closed unit which would lead to accumulate the genetic deterioration over generations due to negative selection, inbreeding and genetic drift **(Eknath and Doyle, 1990; Smith, 2009; Maqsood and Ahmad, 2017)**. However, inbreeding as well as genetic deterioration can be avoided by the proper knowledge of genetic structure, diversity and variability of the species **(Bert *et al.*, 2007)**. In this regard, polymerase chain reaction (PCR) based random amplified polymorphic DNA (RAPD) could be an efficient and sensitive molecular tool which has been used for evaluating genetic variation and genetic relationships in several aquatic species including *Anguilla* **(Takagi and Taniguchi, 1995)**, *Penaeus monodon* **(Tassanakajon *et al.*, 1997)**, *Salmo salar* **(Elo *et al.*, 1997)**, *Symphysodon sp*. **(Koh *et al.*, 1999)**, *Ictaluras punctatus* and *Ictaluras furcatus* **(Liu *et al.*, 1998)**, *Micropterus salmoides* **(Williams *et al.*, 1998)**, *Oncorhynchus mykiss* **(Afzali *et al.*, 2013)**.

The tool has also been used in several occasions to infer population structure and genetic variability of different tilapia populations, both from hatchery and wild sources **(Bardakci and Skibinski, 1994; Dinesh *et al.*, 1996; El-Alfy *et al.*, 2009)**. In Bangladesh, RAPD technique has been successfully used to detect genetic variation for rohu, *Labeo rohita* **(Barman *et al.*, 2003; Islam and Alam, 2004**) and catla, *Catla catla* **(Rahman *et al.*, 2009)**, the two main economically important aquaculture species; kalibasu, *Labeo calbasu* **(Mostafa *et al.*, 2009)**, sing, *Heteropneustes fossilis* **(Islam *et al.*, 2011)** etc. Hitherto, the genetic characterization of tilapia stock has yet been studied in Bangladesh though genetic deterioration or genetic diversity assessment of the hatchery reared populations are very much essential to gain insight for future development of the brood stocks as well as seed production. Therefore, this study was

carried out for genetic monitoring of the species by evaluating intra- and inter-population genetic variability of five different private hatcheries using RAPD tool.

MATERIALS AND METHODS

A total of 25 fish samples of monosex tilapia (average weight 91.3±5.7 gm) were collected from five different research ponds in Jessore district of Bangladesh during July 2012. Each of the ponds was assigned in production related research separately stocked with tilapia fry of five different hatcheries **(Table 1)**.

Table 1. Tilapia populations used in the study.

Populations	District	Sample code	No of Sample
1. Matshay-kanon hatchery	Noapara, Jessore	MK*1	5
2. Khan hatchery	Boshundia, Jessore	KA*1	5
3. Nancy hatchery	Sherpur	NC*2	5
4. City hatchery	Mymenshing	CT*2	5
5. Brac hatchery**	Magura	BR*1	5

**1: Closely located of about 10-30 km distances to each other; *2: Closely located of about 25-30 km distances to each other; Distance between *1&*2 was about 300-350 km.*

***A fisheries part of BRAC, an international development organization based in Bangladesh.*

Genomic DNA extraction and primer selection

Genomic DNA was extracted from the muscle tissue of the sample using Invitrogen PureLink™ Genomic DNA extraction kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol. Absorbance at 260 nm and the ratio of absorbance at 260 nm and 280 nm were used to check the concentration and purity of DNA, respectively using a UV-visible Spectrophotometers (Hitachi U-2910, Tokyo, Japan). Randomly sequenced of ten oligonucleotide decamer primers (Bioneer Corporation, Korea) were used for analyzing genetic variations among the five hatchery populations **(Table 2)**.

Table 2. Profile of the selected primer used in the RAPD-PCR.

Primer	Sequences (5'-3')	MW	TM	Primer	Sequences (5'-3')	MW	TM
OPA1	AGTCTCCTGG	2989	34°C	OPA10	GTGATCGCAG	3068	32°C
OPA2	CAGGCCCTTC	2963	34°C	OPA18	AGGTGACCGT	3068	32°C
OPA3	AGTCAGCCAC	2097	32°C	OPB2	TGATCCCTGG	3019	32°C
OPA4	AATCGGGCTG	3068	32°C	OPB12	CCTTGACGCA	2987	32°C
OPA9	GGGTAACGCC	3053	34°C	OPC3	GGGGGTCTTT	3090	32°C

MW= Molecular weight and TM= Melting temperature.

RAPD-PCR amplification and visualization

The PCR reactions were performed for each DNA samples in a 25 µl reaction mixture containing 2 µl of genomic DNA (20 ng/µl), 1 µl of Taq DNA polymerase (2 unit), 2 µl of 10 pm/µl each primer, 2 µl of 10 mM dNTPs mixture, 5 µl of 5X reaction buffer and 13 µl sterile deionized nuclease free distilled water. All the reagents used in the mixture were procured from Bioneer Corporation, Korea. Amplifications were carried out in a C1000^{TM} thermal cycler (Bio-Rad Laboratories, USA). The cycling conditions were empirically determined firstly which were finally carried out following an initial 3 minutes at 94ºC for the first round followed by 35 cycles of denaturation for 60 sec at 94°C, 60 sec of annealing at 32-34°C, and 60 sec of extension at 72°C. After the amplifications, the products were loaded into 2% agarose gel using a 100 bp DNA marker (Bioneer, Korea) and electrophoresis was done at 120 V for 45-50 minutes. Afterward, the gel was carefully exposed to UV trans-illuminator for observing and taking photographs in a desktop computer.

Data analysis

Intra- and inter-population comparison of individual genotype was carried out based on a single data matrix constructed by the manual scoring and counting of fragments (bands) visualized on ethidium bromide staining agarose gel produced by RAPD amplification. The scoring was formed in binary number excluding week bands where '1' was designated for presence of fragments and '0' for absence of fragments. The data matrix was used to calculate polymorphic loci profile, gene flow (N_m), genetic differentiation (F_{ST}), genetic distance (D) and pair wise genetic similarities (SI) among the populations with the aid of POPGENE software package (1.31 version) **(Yeh *et al.*, 1999)** in a computer. The pair-wise genetic similarities were estimated according to the formula, $SI=2N_{ab}/(N_a+N_b)$ where, N_{ab} is the number of bands common in individuals 'a' and 'b', while N_a and N_b are total number of bands possessed by the individuals 'a' and 'b', respectively **(Nei and Li, 1979; Lynch, 1990).** Moreover, an unweighted pair group method of arithmetic mean (UPGMA) dendrogram was constructed with the POPGENE software for analyzing cluster wise genetic distance.

RESULTS AND DISCUSSION

This study was an attempt in determining genetic variation of five hatchery produced tilapia populations in Bangladesh using RAPD markers. The PCR products (bands) generated through RAPD technique were used to evaluate polymorphisms as well as genetic variability within and between five private tilapia hatchery populations. On an average, each of the selected primers produced 18.1 (total 181) number of scorable RAPD bands ranged in molecular size from approximately 100bp to 2000bp in which

11.6 per primer (total 116) ranging from 4 (OPA10) to 23 (OPA2) bands were found to be polymorphic in replicate amplifications **(Table 3)**. The number of fragments generated per primer in this study was relatively higher than the study observed by **Bardakci and Skibinski (1994)**, who found between 6 and 17 bands per primer in identification of tilapia species applying RAPD marker. The fragment generation between the two studies was varied probably due to the nucleotide sequence difference of primers and amplification profile of different loci within the genomes, the strains or stocks differences, the source of the template DNA and the influence of environment **(Vithanage and Winks, 1992; Bardakci, 2001; Islam *et al.*, 2011; Maqsood and Ahmad, 2017)**.

Table 3. Primer-wise amplified/scorable and polymorphic bands, their size range and proportion documented by RAPD-PCR of tilapia populations.

Primer codes	No of bands scored/amplified	No of polymorphic bands	Proportion of polymorphism (%)	Size range (bp)
OPA1	12	07	58.33	400-1600
OPA2	28	23	82.14	200-2000
OPA3	17	17	100	200-2000
OPA4	06	06	100	500-1600
OPA9	13	13	100	300-1600
OPA10	04	04	100	300-1000
OPA18	18	13	72.22	100-1600
OPB2	07	07	100	300-1600
OPB12	44	14	31.82	200-2000
OPC3	32	12	37.50	100-1200
Total	**181**	**116**	**64.1**	**-**
Average/primer	***18.1***	***11.6***	**-**	**-**

The primer-wise maximum and minimum polymorphisms were recorded as 100% and 31.82% with an average of 64.1% **(Table 3)** which was higher than the polymorphisms obtained by **Basavaraju *et al.* (2007)** and **Fadly *et al.* (2016)**, but was more or less similar to **Mahboob *et al.* (2019)** by RAPD analysis in tilapia. Variable polymorphic patterns found in the present study revealed the suitability of the markers for effectively assessing genetic variability among the studied populations. Accordingly, the ten primers yielded a total of 77 distinct bands across the population of which 64 or 83.1% were polymorphic. The brac population had the highest genetic variability attaining 0.51 proportions of overall polymorphic loci **(Table 4)** and lower variability co-efficient were observed among the rest of the populations. Among other factors, maintenance of large random mating populations with an infinite number of effective individuals (Ne) might be a cause of the highest genetic variation in the brac hatchery.

The genetic variation within populations is a key measure of species adaptation to environmental changes and, hence, of species successful survival and growth **(Sofia *et al.*, 2006)**. When a population loses genetic plasticity by narrowing genetic pool, it becomes increasingly vulnerable to environmental changes and, hence, lowers the overall fitness and tends to decline the productivity **(Guttman and Berg, 1998; Berg *et al.*, 2002)**.

Table 4. Estimates of genetic variation across the hatchery population.

Description	Populations				
	MK	**KA**	**NC**	**CT**	**BR**
Overall number of scorable bands	26	34	46	23	52
Number of polymorphic bands (%)	13 (50)	21 (62)	33 (72)	10 (43)	39 (75)
Proportion of polymorphic bands (%)	0.16 (16)	0.27 (27)	0.43 (43)	0.12 (12)	0.51 (51)

Number of overall polymorphic bands across populations was 64; Proportion of overall polymorphic loci across populations was 0.83 (83%); Population code "MK" denotes Matshay-Kanon; "KA" denotes Khan; "NC" denotes Nancy; "CT" denotes City; "BR" denotes Brac.

The lower genetic variability in the rest of the four hatcheries might be resulted from the repeated propagation of a limited number of individuals over a long period in domestication. Similar to the finding, numerous other studies using genetic markers have shown that domestication process in fish species is associated with a decrease in genetic variability **(Koh *et al.*, 1999; Reilly *et al.*, 1999; Islam and Alam, 2004)**. This phenomenon has been proposed to be associated with several facts, such as use of few hatchery brood stocks **(Allendorf *et al.*, 1987)**, practice of wrong breeding techniques **(Gile and Ferguson, 1990)**, and even genetic drift and inbreeding **(Tave, 1986; Ferguson *et al.*, 1998)**. A gradual loss of genetic variation due to inbreeding appears to be most common aspect in cultured fish stocks after several generations **(Koh *et al.*, 1999; Hansen *et al.*, 2000)**. Despite the low genetic variations within four of the tilapia populations, the overall population-wise polymorphic loci appeared to be more or less similar to the observation of catfish **(Saad *et al.*, 2009; Sultana *et al.*, 2010; Islam *et al.*, 2011)**, however, the polymorphism was much higher than carps **(Kholmann and Kersten, 1999; Das *et al.*, 2005)** using microsatellite and RAPD markers. This variations were probably caused by taxonomic difference **(Welsh and McClelland, 1990; Charlesworth and Charlesworth, 2016)**, life- history characteristics **(Frankham *et al.*, 2014**), habitat and ecosystem differentiation **(DeWoody and Avise, 2000; Martinez *et al.*, 2018)**.

The Higher range of inter-population similarity was estimated among four of the five hatchery populations (75-82%), except with brac (55-62%) **(Table 5)**, though the inter-population similarity co-efficients were comparatively higher than the similarity index found among three tilapiine species **(El-Alfy *et al.*, 2009)**. The higher similarities

among four hatchery stocks, especially between khan & city hatchery tilapia populations indicated that comparatively more RAPD fragments were shared among the populations originated from similar sources. Moreover, this implied that individuals of the four hatcheries were genetically more similar to each other than the individuals of brac hatchery because closely related individual share fewer patterns in common with high similarity indices and vice-versa **(Bardakci and Skibinski, 1994; Dinesh *et al.*, 1996)**. This high level of inter-population band sharing-based similarity indices was indicative of frequent sharing of brood stocks among the hatchery and not maintaining genetically diversified brood stocks. The indication can be supported by a survey-based finding of **Mekkawy *et al.*, (2017)** who characterized the mono-sex tilapia hatcheries in Bangladesh and reported a very similar management system across the country where brood stocks were frequently replaced in the hatcheries every two years from the current stocks.

Table 5. Inter population genetic index (SI±SD) of the hatchery populations.

Population	Similarity Index (SI)	Percentage (%)	Population	Similarity Index (SI)	Percentage (%)
MK-KA	0.78±0.18	78	KA-CT	0.82±0.13	82
MK-NC	0.61±0.25	61	KA-BR	0.61±0.20	61
MK-CT	0.64±0.22	64	NC-CT	0.75±0.14	75
MK-BR	0.55±0.24	55	NC-BR	0.62±0.17	62
KA-NC	0.76±0.14	76	CT-BR	0.60±0.21	60

Population code "MK" denotes Matshay-Kanon; "KA" denotes Khan; "NC" denotes Nancy; "CT" denotes City; "BR" denotes Brac.

The F_{ST} is the standardizing measure of degree of genetic differentiation among populations, value of which ranges from 0 (no differentiation) to 1 (no alleles shared). The values ranging from 0.05 to 0.15 and 0.15 to 0.25 are indicative of moderate and high genetic structuring, respectively **(Wright, 1978)**. In the present study, the F_{ST} values between brac and rest of the hatchery populations were significantly higher at 95% confidence interval, averagely ranged 0.071-0.048, with moderate genetic differentiation **(Table 6)**. One of the possible reasons for the significant higher F_{ST} values in the brac hatchery was that the individuals of brac population were highly isolated one originated from improved strains of unique genetic characteristics. A similar possibility has been reported by **Mireku *et al.* (2017)** and **Kajungiro *et al.* (2019)** in the assessments of genetic structure of different tilapia strains who found that improved tilapia strain had significantly higher genetic differentiation than others. In contrast, low with insignificant genetic differentiations among matshay-kanon, khan, nancy and city hatcheries **(Table 6)** indicated that those were genetically relevant to each other with some common genetic characters which might be the result of inter-mixing of genetic materials among the populations.

Table 6. Population differentiation (F_{ST}) based on square root transformation of frequencies of the null allele (recessive) genotype followed in parentheses by the 95% confidence interval (CI), gene flow (Nm) and genetic distance (D) values between populations.

Population Pairs	F_{ST}±SD (95% CI)	Range (F_{ST})	Gene Flow (N_m)	Genetic Distances (D)
MK vs KA	0.015±0.009	0.006-0.024	33.12	0.002
MK vs NC	0.019±0.012	0.007-0.038	28.39	0.003
MK vs CT	0.021±0.011	0.010-0.035	25.87	0.004
MK vs BR	0.071* ± 0.043	0.036-0.117	8.91	0.012
KA vs NC	0.017 ± 0.010	0.00-0.026	30.34	0.002
KA vs CT	0.032 ± 0.024	0.022-0.053	19.21	0.006
KA vs BR	0.063* ± 0.038	0.039-0.113	11.73	0.011
NC vs CT	0.020 ± 0.010	0.004-0.029	29.07	0.004
NC vs BR	0.054* ± 0.032	0.032-0.109	13.31	0.010
CT vs BR	0.048* ± 0.031	0.021-0.102	13.89	0.008
Across all the experimental groups/populations			*33.12*	-

**denoted to significantly population genetic differentiation (F_{ST}).*
Population code "MK" denotes Matshay-Kanon; "KA" denotes Khan; "NC" denotes Nancy; "CT" denotes City; "BR" denotes Brac.

Gene flow (N_m) is one of the key indicators of genetic structure of populations, as it determines to what degree every local population of a species act as an independent evolutionary unit **(Slatkin, 1987)**. If there is strong gene flow among closely located populations, they evolve together; in contrast if the gene flow is low, each population evolves independently. Theoretical value of $N_m > 1$ is necessary to avoid random differentiation due to genetic drift **(Slatkin, 1987)**. In general, the N_m values reported in this study ranged from 8.91 to 33.12, suggests that the genetic differentiation observed between them are due to the level of gamete exchange, rather than genetic drift and most likely they originated from a genetically similar source. In our study, the least gene flow was observed in the brac hatchery and high levels were found in among the rest of the hatcheries **(Table 6)**. The high gene flow levels among the rest of the hatchery suggested that those populations were evolving toward homogeneity **(Smith, 2009)**. This could be happened for the intentional or unintentional use of multiple strains which might lead to the movement of gametes, genes or individuals within and between populations. In contrast, the brac hatchery owner might prevent introducing broods of multiple strains and thereby diminish gene flow which resulted in less homogenous unit with high genetic differentiation.

Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on genetic distance analysis supported the previous findings of the distinctiveness (high genetic variation, low similarity, high genetic difference and low gene flow) of brac hatchery populations **(Table 6** and **Fig. 1)**. The dendogram aligned five populations into

two clusters- the brac alone belonged to one cluster and rest of the populations has grouped into another cluster. It should be noted that khan & matshay-kanon hatchery were geographically closest to each other **(Table 1)**, suggesting an intense genetic mix up might be taken place between the populations because of exchanging breeders between hatcheries. The close relationship with small genetic distances among populations due to geographical proximity has been reported by many author**s (Islam and Alam, 2004; Islam *et al.*, 2011).** The brac hatchery located at geographically isolated area as compared to other four hatcheries was likely to prevent interchanging of breeders from others. Not only the geographical barrier, the brac hatchery owners/technicians might be accustomed to operating improved hatchery management and breeding program, resulting in low levels of gene flow and inter population similarities with genetically distant from other populations **(Bert *et al.*, 2007)**.

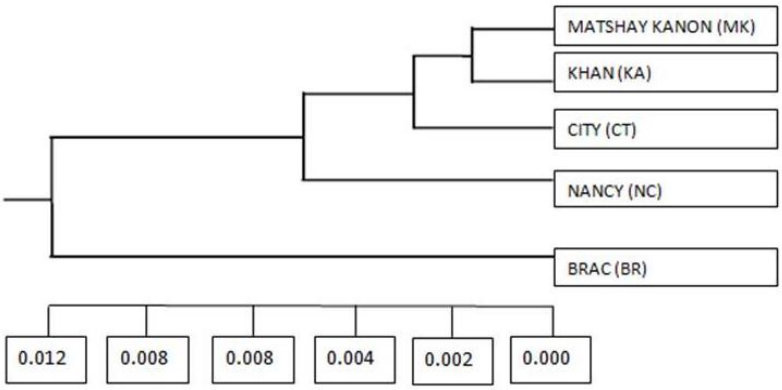

Fig. 1. Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on genetic distance, summarizing data on differentiation between tilapia populations according to RAPD analysis.

In the present study, a distinctive genetic structure was observed in the brac hatchery population as compared to other four populations. With contemporary rapid expansion of mono-sex tilapia aquaculture in Bangladesh, farmers associated the culture are mostly dependent on private hatcheries for seed supply **(Mekkawy*et al.*, 2017)**. However, the quality of hatchery seeds is of great concern, as in our study only a single hatchery (brac) depicted they had good quality brood stock with having moderate genetic variability; and rest are having poor quality brood stock with low genetic variability, and therefore, these hatcheries might produce genetically inferior quality seed. The overall low genetic variability observed in the studied hatcheries, therefore, suggests an urgent tilapia brood stock improvements are needed through increasing genetic diversity. For which following strategies can be followed - for instance, setting up marker assisted breeding program or back crossing of wild fish population with farmed stocks. The cautious selection of potential breeders, based on genetic marker, is a determinant of the

success and sustainability of hatchery programs **(Crozier, 1994)**. The implementation of suggested approaches for tilapia fish breeding in hatchery could ensure production of good quality seed by decreasing the inbreeding rate in each cultivated generations.

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