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Genetic Variability of Wild and Cultured Populations of *Tilapia zilli* by Randomly Amplified Polymorphic DNA (RAPD) Markers

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A B S T R A C T

The analysis of genetic variability between wild and cultured *Tilapia zilli* (*T. zilli*) species collected from New Bussa, Niger State was carried out using randomly amplified polymorphic DNA (RAPD) markers. Four RAPD primers were used for the DNA amplification which generated a total of 55 band loci ranging from 750-7126 bp for both wild and cultured *Tilapia zilli.* The average percentage of polymorphic loci for wild and cultured *Tilapia zilli* was 30.8 and 65.63%, respectively. The percentage of molecular variance within and among species for wild and cultured *Tilapia zilli* was 70 and 30%, respectively, indicating fairly high genetic variation within species and high genetic variation among species. The PhiTP value further affirmed high genetic variation among wild and cultured *Tilapia zilli* having the PhiTP to be 0.013 and data value to 0.281, PhiPT (0.302)>data (0.001), shows significant difference. The wild *T. zilli* shows decrease genetic variability and diversity, therefore, urgent step need to be taken to avert its risk of extinction.

Key words: *Tilapia zilli*, RAPD marker, polymorphism, genetic variability and diversity

INTRODUCTION

Tilapia zilli (Gervais, 1848), is a native of Africa and are widely distributed across the tropical and subtropical region of Africa (Boyd, 2004). Fresh water species and feed on varieties of food such as phytoplankton, periphyton, aquatic plants, invertebrates (Elias *et al*., 2014). *Tilapia zilli* is commercially and ecologically important as food fish, aquarium fish, weed control and recreational fishery in its country of origin (Mehanna, 2004).Genetic variation is said to be variation in the aggregate of alleles, trait or character of alleles, qualities, chromosomes and qualities game plans on the chromosomes that are available inside and among constituent populaces (Çiftci and Okumu, 2002). Determining genetic variation in wild fish populaces or cultured stocks is critical for clarification, understanding and successful managing of these populaces or stocks (Okumu and Çiftci, 2003). Genetic markers are used to recognize different traits in DNA sequence that can be utilized to differentiate between individuals in a population, or to classify

individuals between different varieties within a species, thus, molecular markers give an answer for the evaluation of hereditary variety in species (Warwick Crop Centre, 2011). The RAPD marker uses 10 base pair random primers to locate random segments of sequence DNA to reveal polymorphisms and with the included advantage that primers are financially accessible and no need of past information of the objective DNA arrangement or gene association (Lopes, *et al.*, 2007).The use of genetic makers especially RAPD has gained momentum in aquaculture where genetic analyses of different fish species have been carried out. Garg (2009), reported genetic variation in two populations of *mystusvittatus* (bloch) using RAPD marker with negligible morphological variation. Jongand Hong (2002), reported genetic similarity and differences between wild and cultured crucian carp *carassius carassius* estimated from RAPD marker and that the genetic diversity in wild is 1.5 times higher than that of cultured. Danish *et al*., (2012), reported molecular characterization of two populations of cat fish *Clarias batrachus* using RAPD marker and reveals genetic variation between the two populations and concluded that, it may be as result of different habitat or breeding strategies.

The objective of this work is to reveal the genetic structure based on genetic variability between wild and cultured *Tilapia zilli* by RAPD marker, as to give an insight in better management and conservation of wild and cultured species.

MATERIALS AND METHODS

Sample collection: Ten wild *Tilapia zilli* (juveniles) was collected from aquaculture unit of National Institute for Freshwater Fisheries Research (NIFFR) in New Bussa and ten (10) cultured *Tilapia zilli* (juveniles) was collected from Kainji Lake with the assistance of the local fishermen, for each species that was used for the experiment, the caudal fin was cut, put in micro tube containing 90% absolute ethanol, appropriately label and store at 5-8°C in a refrigerator for further processing.

Extraction, purity and quantification of genomic DNA: The genomic DNA was extracted from the caudal fin using QIAamp mini kit protocol, edited 2012 (www.qiagen.com/handbooks), following the manufacturer guidelines with little modifications. The purity of the genomic DNA extracted was determined by measuring the ratio of absorbance at 260nm to absorbance at 280nm to be 1.7-2.0 using Nano-drop spectrophotometer.

The DNA quantity was determined by electrophoresis in 1% agarose gel buffered with 0.5xTBE (500 mM Tris-HC1, 60 mM boric acid and 80 mM EDTA) at 80 volts for 1.5 h and stained with ethidium bromide (5 µL), then, the gels image was visualized under UV light (thermo scientific, USA), photographed and saves directly into the system. The 200 µL DNA extracted from each samples were diluted with 10 µL of autoclave water and stored at -17°C for 2 days before being used for RAPD amplification.

RAPD amplification and electrophoresis: A series of optimization experiment was conducted following the protocol of Skoric *et al*., (2012), four RAPD primers (OPO-10, OPO-02, OPB-01 and OPT-02 were screened (Operon Technologies, USA) for the amplification. The amplification reactions was performed in final volumes of 10 µL, containing 3 µL of genomic template DNA, 0.4 µL of 50 mM $MgCl_2$, 0.8 µL of DNTPs, 1.0 µL of the primer and 0.1 µL of Taq DNA polymerase (Fermentas Life science). The total volume of the amplified products were evaluated in 2% agarose gels buffered with 0.5x TBE (500 mM Tris-HC1, 60 mM boric acid and 80 mM EDTA) at 80 volts for 1.5 h and stained with 5 µL ethidium bromide, after electrophoresis, the gel picture was visualized under UV light (thermo scientific, USA), photographed and directly save in the system. The gel pictures were scored and used for the analysis of the amplified products.

Data analysis: Amplified fragments were scored as binary data, i.e presence as 1 and absence as 0, for homologous bands. Only data generated from reproducible bands were used for statistical analysis. The number of polymorphic loci, percentage of polymorphic loci (P%), number of different alleles (n_a), effective number of alleles (n_e), Nei's gene

diversity(h), Nei's unbiased gene diversity(uh) and Shannon information index (I) was estimated using the program GENALEX 6.501 (Peakall and Smouse, 2012). Non-parametric analysis of molecular variance (AMOVA) was used as a measure of genetic differentiation to estimate the genetic structure of wild and cultured *Tilapia zilli* within population and among population using the same program.

RESULTS

RAPD amplification products: The amplicons generated using 4 operon primers (OPO-02, OPO-10, OPB-01, OPT-02) to reveal the genetic variability between cultured and wild populations are shown in plate I- IV.

The RAPD amplification revealed a relatively high polymorphism for cultured population and low polymorphism for wild population. A total 55 bands loci were amplified for both wild and cultured *Tilapia zilli* with band size ranging from 750-7126 bp in which cultured *T. zilli* has 34 polymorphic bands and wild *T. zilli* has 20 polymorphic bands and 1 monomorphic band. The number of polymorphic bands for both cultured and wild *T. zilli* ranges from 5-10, respectively as shown in Table 1.

Genetic diversity: High genetic diversity is exhibited by cultured *Tilapia zilli*, having the mean major allele frequency, allele number, gene diversity and PIC to be 0.25, 8.25, 0.85 and 0.8285, respectively as shown Table 2.

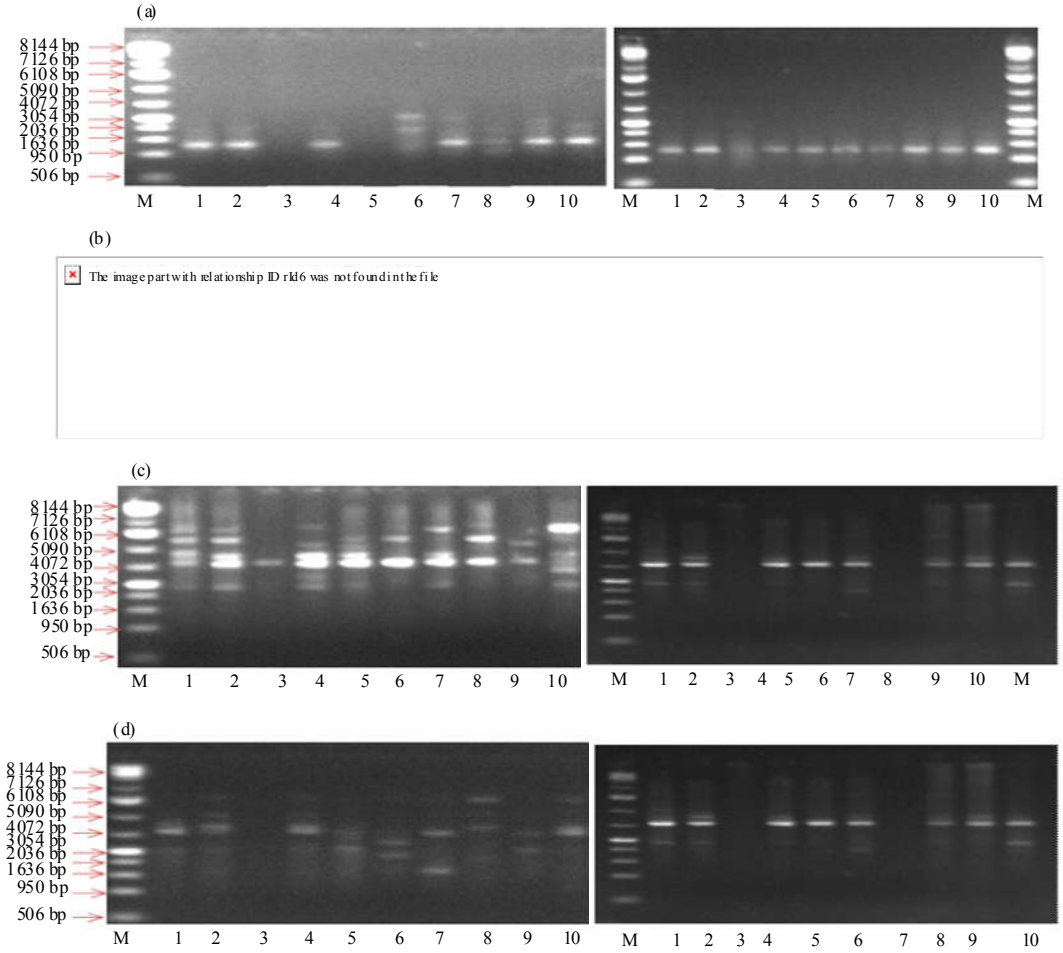

Plate 1-4(a-d): *Tilapia zilli* cultured and *Tilapia zilli* wild (a) Prime OPO-02, (b) OPO-10, (c) OPB-01 and (d) OPT-02

Table 1: Amplification analysis of wild and cultured *Tilapia zilli* using four RAPD primers

			Tilapia zilli			P (%)		
Primers	Ta (°C)	TPC	TPW	TMB	TBL	Cultured	Wild	Band size range (bp)
OPO-02	38	6	0	1	7	85-71	0	5090-950
OPO-10	38	9	9	0	18	50	50	5090-750
OPB-01	38	9	5	0	14	64.29	35.71	7126.2036
OPT-02	38	10	6	0	16	62.5	37.5	6150.1636
Total		34	20	1	55	262.5	123.2	
Average		8.5	5	0.25	13.75	65.63	30.8	

Ta: Annealing temperature, TPC: Total polymorphic band cultured, TPW: Total polymorphic band wild, TMB: Total monomorphic band, TBL: Total bands loci, P (%): Percentage of polymorphism, bp: base pair

Table 2: Genetic diversity within population of cultured *Tilapia zilli*

	Tilapia zilli cultured				
Primer	Sample size	Major allele frequency	Allele No.	Gene diversity	PIC
OPO-02	10	0.30	7	0.82	0.7978
OPO-10	10	0.40	7	0.78	0.7578
OPT-02	10	0.10	10	0.90	0.8910
OPB-01	10	0.20	9	0.88	0.8680
Mean	10	0.25	8.25	0.85	0.8286

Table 3: Genetic diversity within population of wild *Tilapia zilli*

	Tilapia zilli wild				
Primer	Sample size	Major allele frequency	Allele No.	Gene diversity	PIC
OPO-02	10	1.00	1	0.000	0.0000
OPO-10	10	0.20	9	0.880	0.8680
OPT-02	10	0.50	4	0.640	0.5812
OPB-01	10	0.20	8	0.860	0.8442
Mean	10	0.46	5.5	0.595	0.5733

Table 4: Genetic distance above the diagonal and genetic identity below the diagonal for cultured and wild *Tilapia zilli*

Populations	*T. zilli* cultured	*T. zilli* wild
T. zilli cultured	0.000	0.239
T. zilli wild	0.787	0.000

Table 5: Summary of Analysis of molecular variance (AMOVA) for cultured and wild *Tilapia zilli*

									MV (%)	
Populations		N	Na	Ne	I	h	uh	p (%)	Within	Among
T. zilli cultured	Mean	10.000	1.838	1.627	0.519	0.354	0.393	91.890	70.000	30.000
	SE	0.000	0.091	0.056	0.034	0.026	0.029			
T. zilli wild	Mean	10.000	1.108	1.306	0.280	0.185	0.205	54.050		
	SE	0.000	0.163	0.058	0.046	0.032	0.035			

While wild *Tilapia zilli* have the mean major allele frequency, allele number, gene diversity and PIC to be 0.46, 5.5, 0.595 and 0.5733, respectively, indicating a fairly high genetic diversity as shown in Table 3.

Genetic distance and genetic identity: The genetic distance and identity between wild and cultured *Tilapia zilli* was 0.239 and 0.787, respectively, indicating a fairly genetic distance and non-identical as shown in Table 4.

Genetic variability: The percentage of polymorphism based on genetic variability within cultured and wild *Tilapia zilli* were 91.89% and 54.05% respectively and molecular variance within and among population were 70 and 30%, respectively, indicating high variation within cultured *T. zilli* compared to that of the wild and significant variation among populations as shown in Table 5.

Table 6: AMOVA PhiPT value below the diagonal and data value above the diagonal for cultured and wild *Tilapia zilli*

Populations	*T. zilli* cultured	*T. zilli* wild
T. zilli cultured	0.000	0.001
T. zilli wild	0.302	0.000

Significant variation PhiPT>data

The AMOVA PhiPTreveals the total proportion of genetic variation, PhiPTvalues and data value for wild and cultured *T. zilli* were 0.302 and 0.001 respectively which shows significant variation as shown in Table 6.

DISCUSSION

Reduction in genetic variation and diversity of most species (little but quantifiable) is caused by overfishing and over exploitation which leads to loss of evolutionary potential, adaptive abilities and ultimately extinction (Pinsky and Palumbi, 2013; Allendorf *et al*., 2008). Therefore the major concern of fish conservationist and geneticist should be the prevention of overfishing and overexploitation of economically important fish species as well as preserving existing genetic diversity and variability, high level of heterozygosis and allelic abundance. In this study, a total of 55 band loci were generated from four RAPD primers for both wild and cultured *Tilapia zilli* with molecular band size ranging from 750-7126 bp (Table 1).Danish *et al*. (2012), used five primers and obtained a total of 120 band loci, having band size ranging from 200-1360 bp, Popoola *et al*. (2014), also reported a total of 425 band loci using nine primers and size ranging from 250-2700 bp. Yoon and Park (2001), observed a total number of 1084 band loci and band size ranging from 120-4270, using five primers. The variation in band loci and band size as reported by various authorscould result from dependence of band loci and band size on the number of primer used, number of samples, types of species and the source of DNA.

Sultana *et al*. (2010), reported 83.87% polymorphic loci in *Heteropneustes fossilis* (Nebauer, *et al.*, 2000), reported 98.9% polymorphic loci in *Digitalis* species. Danish *et al*. (2012), reported polymorphic loci for wild and cultured *Clarias batrachus* to be 44.61 and 39.83%, respectively, Popoola*et al*. (2014) also reported 89.9 and 74.7% polymorphic loci for wild and cultured species of *Clarias gariepinus*, respectively. The different percentages of polymorphic loci reported by the authors are also dependent on the number of polymorphic band generated and the number of primer used which is expressed in percentage. In contrast, cultured *Tilapia zilli* showed higher percentage of polymorphic loci than the wild *Tilapia zilli* in this study, which was 65.63 and 30.8%, respectively (Table 1). This might be due to sample size effect (Taniguchi and Perez-Enriquez, 2000).

The mean value of genetic diversity obtained for cultured *Tilapia zilli* was 0.85 or 85% (Table 2),which indicates high gene diversity, thus, revealing a fairly good culture management, this is very important for adaptation, long term survival, ability to resist disease, fast growth and high reproduction (Pinsky and Palumbi, 2013). While wild *Tilapia zilli* has 0.59 or 59% (Table 3), indicate fairly low gene diversity which might be due to overfishing. Wild *T. zilli* needs urgent attention to improve its gene diversity, in order to avert all the negative effect of low gene diversity as well as save guarding it from extinction.

The genetic distance and identity between cultured and wild *Tilapia zilli* were 0.239 or 23.9 and 0.787 or 78.7%, respectively (Table 4), indicating a quiet genetic distant and non- identical, this might be due to low polymorphic loci exhibited by wild population of *Tilapia zilli*. According to Thorpe and Sole-cava (1994), the value of genetic identity between species of the same genera should be considered high when it is above 0.85 or 85%.

The genetic variability between cultured and wild population of *Tilapia zilli* were evaluated to reveal the genetic structure of both species using non-parametric analysis of molecular variance (AMOVA). From the AMOVA analysis, the Shannon's information Index (I) and Nei's gene diversity (h), Nei's unbiased gene diversity (uh) and percentage polymorphism (%p) that were obtained for cultured *Tilapia zilli* are 0.5190.034, 0.3540.026, 0.03930.029 and 91.89%, respectively while wild *Tilapia zilli* has 0.2800.046, 0.1850.032, 0.2050.035 and 54.05%, respectively and percentage of Molecular variance (%MV) within and among species was 70 and 30%, respectively (Table 5), indicates high heterozygosis, high genetic diversity and variation in cultured *T. zilli* and fairly low heterozygosis,

fairly low genetic diversity and variation in wild *T. zilli* compared to the cultured species. The low heterozygosis, low genetic diversity and variability in wild *T. zilli* is an indication that the wild *T. zilli* might be going through a trouble time and urgent attention is needed to combat all the factors that lead to low heterozygosis, low genetic diversity and variability, so as to avoid the risk of extinction. Different values for Shannon information Index and gene diversity for different species has been reported by several authors. Suresh *et al*. (2013), observed genetic diversity in four Indian populations of *Mugil cephalus* such as Gujarat, Mahrashtra and hra and Pradesh to be 0.37170.1460, 0.53160.1720, 0.44190.2112 and 0.40120.1310, respectively. Abdel-Kadir*et al*. (2013) reported Shannon's information index and gene diversity for *O. niloticus*, *O. aureus* and *T. zilli* to be 0.318, 0.347, 0.363, respectively and 0.219, 0.238, 0.249, respectively.

AMOVA PhiPT value measures the total proportion of genetic variance among species, PhiPT value and data value for cultured and wild *Tilapia zilli* are 0.302 and 0.001, respectively (Table 6). PhiPT value been greater than data value (PhiPT>Data), further affirmed significant genetic variability among cultured and wild *T. zilli*. Wild *T. zilli* showed a deviation from usual reports by (Jongand Hong, 2002; Alarcon, 2004; Mustafa *et al*., 2009; Pereira *et al*., 2010; Popoola *et al*., 2014) that, wild populations are more diverse, have high polymorphism and high genetic variability than the cultured species, the reason why wild *T. zilli* deviates in this research might be due to sample size effect, the type of marker used, decrease in population as a result of exploitation or overfishing and exposure to toxic chemical that can cause mutation (Taniguchi and Perez-Enriquez, 2000; Pinsky and Palumbi, 2013).

CONCLUSION

The result of this study reveals the genetic structure and genetic variability between wild and cultures *T. zilli* cultured *T. zilli* shows high genetic diversity and variability while wild *T. zilli* shows genetic divergent, undeniable decrease in genetic diversity and variability. Therefore, the wild *T. zilli* need to be further investigated and urgent step needs to be taken to avertitsrisk of extinction.

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