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Research Article
Genetic Variation Between Cultured and Wild Populations of *Oreochromis niloticus* Deduced from Randomly Amplified Polymorphic DNA (RAPD) Markers

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Abstract
Background and Objective: Overexploitation and overfishing is one of the major factors causing depletion or extinction of commercially important fish species. In order to curb this effect, restocking and stock enhancement have been utilized to revive population of numerous species that have gone extinct or threatened in the wild. The reduction in genetic variation within the culture populations has raised worries in the use of culture population for restocking and stocking enhancement. The objective of this study was to reveal the genetic variation between cultured and wild *Oreochromis niloticus* and ascertained whether the cultured species can be used for restocking and stocking enhancement programme. **Materials and Methods:** In this study, Randomly Amplified Polymorphic DNA (RAPD) markers were used to analysis the genetic variation between 20 samples of cultured and wild *Oreochromis niloticus* (*O. niloticus*) species collected from New Bussa, Niger State. Four RAPD primers were used for the DNA amplification which generated a total of 69 band loci ranging from 750-7126 bp for cultured and wild *Oreochromis niloticus*. Non-parametric analysis of molecular variance (AMOVA) was used to estimate the genetic variation of cultured and wild *Oreochromis niloticus* within populations and among populations, using software GENALEX 6.501 and the diversity and genetic distance was determined using the same software. **Results:** The average percentage of polymorphic loci for cultured and wild *Oreochromis niloticus* was 48.87 and 49.34%, respectively. The percentage of molecular variance within and among species for cultured and wild *Oreochromis niloticus* was 99 and 1%, respectively, indicating the high genetic variation within species and very low genetic variation among species. The total proportion of genetic variation (PhiTP value) was 0.013 and data value was 0.281, PhiPT (0.013)<data (0.281), no significant difference. The result from PhiTP value further shows low genetic variation among cultured and wild *Oreochromis niloticus*. **Conclusion:** The high genetic variation within species is very important for species long term survival and low genetic variation among cultured and wild *Oreochromis niloticus* and give the possibility of using cultured species for restocking and stocking enhancement.

Key words: *Orechromis niloticus*, RAPD markers, population genetics, management and conservation, genetic variation, genetic diversity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oreochromis niloticus is an indigenous species of African, found in fresh water and feed on different kinds of food such as aquatic plants, invertebrates, benthic fauna, detritus and bacterial films[1].

Fishery owners, managers and scientists have utilized stocking strategy to enhance fisheries that are frequently practiced throughout the world[2].

Thousands of stocking events that took place involves millions of individual fish annually in managing fisheries[3]. The reasons for these activities are perfectly acceptable, for instance, to make up for environmental loss due to pollution, river engineering or a man-made obstruction to migration such as construction of a dam, or to enhance fish yield[4].

In recent times, concerns have also been raised about the potential risks associated with stocking of fish, particularly with respect to ecological imbalance and change in community structure and loss of genetic integrity of wild populations[2,5].

The main genetic problem of fisheries enhancement programsis the discovery of genetic variation between wild and cultured stocks[6]. The reduction of genetic variation within cultured stocks through inbreeding and genetic drift and the interbreeding between wild and cultured stocks causes reduction in the fitness of the wild stocks[7].

Randomly Amplified Polymorphic DNA (RAPD) marker is a polymorphic assay based on the amplification of random DNA sequences using primers with arbitrary nucleotide sequence. The RAPD marker uses 10 base pair random primers to locate random segments of sequence DNA to show polymorphisms[8]. It has all the advantages of a Polymerase Chain Reaction (PCR) based marker, with the added benefit that primers are commercially available and do not require prior knowledge of the target DNA sequence or gene organization[8].

Several authors have utilized RAPD marker to reveal genetic variation in fish species. Genetic differences between wild and hatchery population of *Diplodus sagus* and *Diplodus vulgaris* inferred from RAPD marker. The percentage of genetic variation within *D. sargus* and *D. vulgaris* reached 82 and 90%, respectively and considerable decrease in genetic variation in hatchery populations especially in *D. sargus* was observed[9].

Genetic variability between cultured and wild *Clarias gariepinus* using RAPD marker. Variability occurs between wild and cultured *C. gariepinus* and that wild populations are more diverse than the cultured population. High genetic variation within species is important for adaptability and long term survival of the species[10].

The discovery of low genetic variability within culture population has limit the use of culture population to augment depleted wild population. Therefore, checking the genetic variation between culture and wild populations forms the baseline for any restocking and stocking enhancement programme. The objective of this study was to reveal the genetic variation between cultured and wild *Oreochromis niloticus* and also gives the possibility of using culture population for restocking and stocking enhancement programme.

MATERIALS AND METHODS

Sample collection: Ten cultured *Oreochromis niloticus* juveniles was collected from aquaculture unit of National Institute for Freshwater Fisheries Research (NIFFR) in New Bussa and 10 wild *Oreochromis niloticus* 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.

Isolation, purification and quantification of genomic DNA: The genomic DNA was isolated from the caudal fin using the QIAamp mini kit protocol, following the manufacturer guidelines with little modifications. The purity of the genomic DNA extracted was determined by measuring the ratio of absorbance at 260 nm to absorbance at 280 nm to be 1.7-2.0 using nano-drop spectrophotometer (thermo-scientific 200, USA). The DNA quantity was determined by electrophoresis in 1% agarose gel buffered with 0.5×TBE (500 mM tris-HC1, 60 mM boric acid and 80 mM EDTA) at 80 V 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 sample were diluted with 10 μL of autoclave water and stored at -17°C for 2 days before being used for RAPD amplification. These were carried out at biotechnology center of International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

RAPD amplification and electrophoresis: A series of optimization experiment was conducted following the protocol[11], four RAPD primers (OPO-10, OPO-02, OPB-01 and OPT-02 were screened (Operon Technologies, USA) for the amplification. The amplification reactions were performed in the 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 was evaluated in 2% agarose gels buffered with 0.5×TBE (500 mM tris-HC1, 60 mM boric acid and 80 mM EDTA) at 80 V 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. These were carried out at bioscience center of International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Data analysis: Amplified fragments or bands 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), a 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 software GENALEX 6.501[12]. Non-parametric analysis of molecular variance (AMOVA) was used as a measure of genetic differentiation to estimate the genetic structure of cultured and wild *Oreochromis niloticus* within populations and among populations (GENALEX 6.501)[12]. The genetic identity and genetic distance was estimated using the same software. The number of alleles, frequency of major alleles, gene diversity and polymorphic information content was estimated using power marker version 3.25[13].

RESULTS

RAPD amplification products: The amplicons generated using four operon primers (OPO-02, OPO-10, OPB-01, OPT-02) to reveal the genetic variation between cultured and wild populations are shown in Fig. 1-4. The M is the molecular band size, lane 1-10 is the samples bands and bp is base pair, which range from 506-8144.

The RAPD amplification revealed a relatively high polymorphism for cultured and wild *Oreochromis niloticus*. A total of 69 bands loci was amplified for cultured and wild *Oreochromis niloticus* with band size ranging from 750-7126 bp in which 34 were polymorphic and 1 monomorphic band for cultured *O. niloticus* and 34 were polymorphic for wild *O. niloticus*, as shown in Table 1.

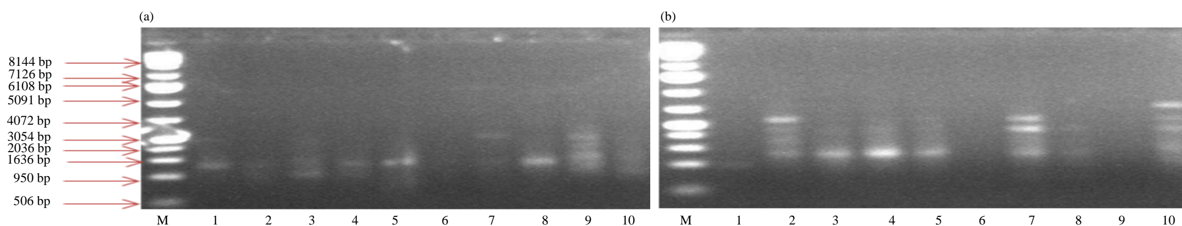

Fig. 1(a-b): Gel images from primer OPO-02 (a)*Oreochromis niloticus* culture and (b) *Oreochromis niloticus* wild

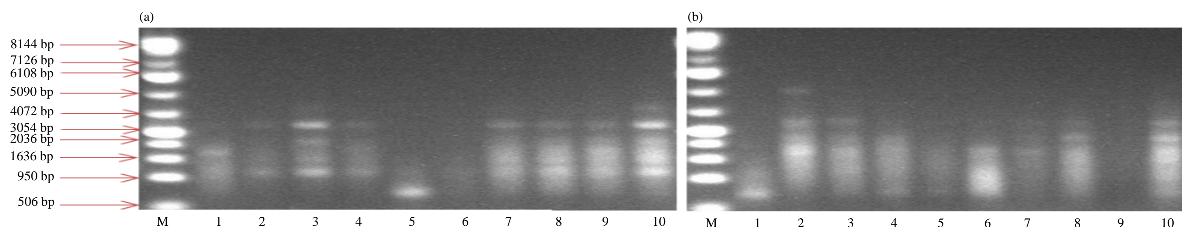

Fig. 2(a-b): Gel images from primer OPO-1 (a) Bp *Oreochromis niloticus* culture and (b) *Oreochromis niloticus* wild

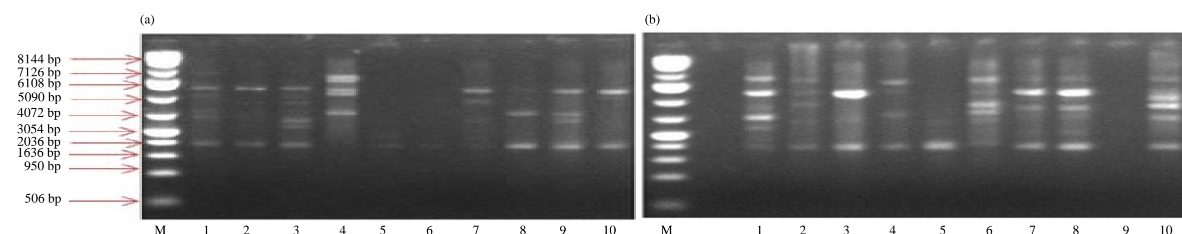

Fig. 3(a-b): Gel images from primer OPB-01 (a) *Oreochromis niloticus* culture and (b) *Oreochromis niloticus* wild

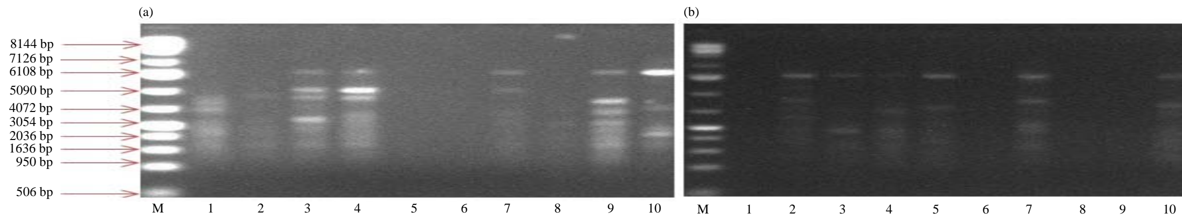

Fig. 4(a-b): Gel images from primer OPT-02 (a) *Oreochromis niloticus* culture and (b) *Oreochromis niloticus* wild

Table 1: RAPD primers amplification analysis for cultured and wild *Oreochromis niloticus* (derived from scoring of bands in Fig. 1-4)

	Oreochromis niloticus					P (%)	
Primers	Ta (°C)	TPC	TPW	TMB	TBL	Culture	Wild
OPO-02	38	7.0	7.0	0.00	14	50.00	50.00
OPO-10	38	6.0	7.0	1.00	14	42.86	50.00
OPO-01	38	10.0	9.0	0.00	19	52.63	47.37
OPO-02	38	11.0	11.0	0.00	22	50.00	50.00
Total		34.0	34.0	1.00	69	195.49	197.4
Average		8.5	8.5	0.25	17	48.87	49.34

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

Table 2: Genetic diversity within population of cultured *Oreochromis niloticus*

	Oreochromis niloticus cultured				
Primers	Sample size	Major allele frequency	No. of allele	Gene diversity	PIC
OPO-02	10	0.5	6.00	0.70	0.6730
OPO-10	10	0.3	8.00	0.84	0.8232
OPT-02	10	0.2	9.00	0.88	0.8680
OPB-01	10	0.2	8.00	0.86	0.8442
Mean	10	0.3	7.75	0.82	0.8021

PIC: Polymorphic information content

Table 3: Genetic diversity within population of wild *Oreochromis niloticus*

	Oreochromis niloticus wild				
Primers	Sample size	Major allele frequency	No. of allele	Gene diversity	PIC
OPO-02	10	0.40	5	0.74	0.7014
OPO-10	10	0.30	7	0.82	0.7978
OPT-02	10	0.40	7	0.78	0.7578
OPB-01	10	0.20	9	0.88	0.8680
Mean	10	0.33	7	0.81	0.7812

PIC: Polymorphic information content

Genetic diversity: The genetic diversity within species is shown in Table 2 and 3. In cultured *Oreochromis niloticus*, the mean major allele frequency, allele number, gene diversity and Polymorphic Information Content (PIC) was 0.3, 7.75, 0.82 and 0.8021, respectively, this indicates a high genetic diversity (Table 2). While wild *Oreochromis niloticus* have the mean major allele frequency, allele number, gene diversity and PIC to be 0.33, 7, 0.81 and 0.7812, respectively, this is also an indication of high genetic diversity (Table 3).

Genetic distance and genetic identity: The genetic distance and identity between cultured and wild *Oreochromis niloticus* was 0.066 and 0.936, respectively, indicating a very close and identical genetic structure.

Genetic variation: Analysis of molecular variance (AMOVA) results revealed 91.89% polymorphism for both cultured and wild *Oreochromis niloticus* and molecular variation within and among population to be 99 and 1%, respectively, indicating high variation within populations and no significant variation among populations as shown in Table 4.

The AMOVA PhiPT value shows the proportion of total genetic variation among populations, the PhiPT value is 0.013 and data value is 0.281, PhiPT<data, indicates no significant variation among the two populations.

Table 4: Summary of analysis of molecular variance (AMOVA) for cultured and wild *Oreochromis niloticus*

									MV (%)	
Populations		N	Na	Ne	I	h	uh	P (%)	Within	Among
Oreochromis niloticus cultured	Mean	10.000	1.865	1.572	0.498	0.334	0.371	91.890	99.000	1.000
	SE	0.000	0.079	0.053	0.033	0.025	0.028	0.000		
Oreochromis niloticus wild	Mean	10.000	1.838	1.595	0.509	0.343	0.381	91.890		
	SE	0.000	0.091	0.053	0.033	0.025	0.028	0.000		

N: No. of sample, Na: No. of different alleles, Ne: No. of effective alleles = $1/(p^2+q^2)$, I: Shannon's information index = $-1\times(p\times Ln(p)+q\times Ln(q))$, h: Nei's gene diversity = $1-(p^2+q^2)$, uh: Nei's unbiased diversity = $(N/(N-1))\times h$, Where for haploid binary data, p: Band frequency and q = 1-p, SE: Standard error, P (%): Percentage of polymorphism, MV (%): Percentage of molecular variance

DISCUSSION

In this study, four RAPD primers were used to reveal the genetic variation between cultured and wild populations *Oreochromis niloticus* and the gel images are shown in Fig. 1-4. A total number of 69 band loci were generated for both cultured and wild *Oreochromis niloticus* with molecular band size ranging from 750-7126 bp. A total of 215 band loci using fifteen primers and band size ranging from 118-2556 bp[14], also, a total number of 142 band loci using five primers and band size ranging from 200-2,000 bp[15]. Different band loci and band size were reported by different authors, this is due to the fact that, the number of band loci and number of band size is dependent on the amount of primer use, number of samples, type of species and the source of DNA.

The average percentage of polymorphic loci obtained in the present study in cultured and wild *Oreochromis niloticus* were 48.87 and 49.34%, respectively (Table1). Approximately 45% average polymorphic loci in four Indian carp[16], 46.5% of polymorphic loci in Indian carp[17] also, 57.69% polymorphic loci in wild and farmed *Labeo calbasu*[1]. The various percentages of polymorphic loci reported by various authorsare also dependent on the number of polymorphic bands generated by number of primer used which is expressed in percentage. Approximately 89.9 and 74.7% polymorphic loci was reported for wild and cultured species of *Clarias gariepinus*, respectively and that the lower percentage polymorphic loci exhibited by the cultured species could be attributed to inbreeding effect[10].

The mean value of genetic diversity obtained for cultured *Orechromis niloticus* and wild *Oreochromis niloticus* are 0.82 or 82% and 0.81 or 81%, respectively (Table 2, 3), which indicates high gene diversity in the both groups, thus, revealing a fairly good culture management, this is very useful for adaptation, long term survival, ability to resist disease, fast growth and high reproduction[18].

The genetic distance and identity between cultured *Oreochromis niloticus* and wild *Oreochromis niloticus* were 0.066 or 6.6% and 0.936 or 93.6%, respectively, indicating a very close genetic distance and highly identical. It agrees with the value of genetic identity standard given by Thorpe and Sole-Cava *et al.*[19], that, the value of genetic identity between species of the same genera should be considered high when it is above 0.85 or 85%. Genetic identity between populations of *Oreochromis niloticus*, *Orochromis aureus* and *Tilapia zilli* to be 0.736, 0.667 and 0.686, respectively[13] while 0.83 genetic identity was obtained between two populations of *Clarias gariepinus*[10]. The higher genetic identity obtained in this study may be attributed to the fact that, National Institute of Fresh Water Fisheries Research (NIFFR) being a research institute understands inbreeding or depression effect, as such, maybe constantly replacing new brood stock from the wild, thereby making the cultured and the wild populations to be genetically highly identical. Therefore, the culture species can be used for restocking and stocking enhancement without the fear of genetic pollution.

The AMOVA reveals high values of Shannon's information index (I), Nei's gene diversity (h), Nei's unbiased gene diversity (uh) and percentage of polymorphism (P) for cultured *Oreochromis niloticus* which ware 0.4980.033, 0.3340.025, 0.3710.028 and 91.89%, respectively and wild *Oreochromis niloticus* were 0.5090.033, 0.3430.025, 0.381 0.026 and 91.89%, respectively and percentage of molecular variance (MV) within and among species was 99 and 1%, respectively (Table 4), indicating high heterozygosis, high genetic variation within species which is very necessary for species adaptability against hash environmental conditions, high reproduction, fast growth and as well as long term survival and very low genetic variation among species shows possibility of using the cultured population for restocking and stocking enhancement but this kind of action should be monitored.

Several authors observed different values for Shannon information Index and gene diversity in different species. 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[13], genetic diversity in four populations of *Mugil cephalus* such as Gujarat, Mahrashtra Andhra and Pradesh to be 0.37170.1460, 0.53160.1720, 0.44190.2112 and 0.40120.1310, respectively[14], gene diversity and Shannon's information index in hatchery

population of *Labeo calbasu* to be 0.1224 and 0.1779, respectively, wild population from Jamuna River to be 0.1726 and 0.2506, respectively and wild population from Padma River to be 0.1238 and 0.1756, respectively[1]. The total proportion of genetic variance among species, AMOVA PhiPT value for cultured and wild *O. niloticus* was 0.013 and data value was 0.281, PhiPT value less than data value (PhiPT<Data), further affirmed no significant genetic variation among cultured and wild *O. niloticus*.

The results of this study are dependent on the sample size, the number and the type of primer use. In this study, one location, small number of samples and primers were utilized due to the financial implications of genetic analysis. Further study should utilize large sample size and different genetic markers that can reveal high polymorphism.

CONCLUSION

In conclusion, the results from this study reveal no significant variation between cultured and wild *O. niloticus*, both have high genetic variation within species and low genetic variation among species, therefore, the cultured species can be used for restocking and stocking enhancement programme but when embarking on such programme the result should be monitored afterward.

SIGNIFICANCE STATEMENTS

- This study revealed low genetic variation between wild and culture *Oreochromis niloticus*, thus, when the wild population is been stretched, the culture population can be utilized for stocking enhancement
- This study also revealed reduction in genetic diversity and variability in culture population as compared to wild population of *Oreochromis niloticus*, thus, good management strategies should be adopted by constantly replacing the brood stock from the wild to avoid effects of low genetic diversity and variability
- The high genetic variation and diversity exhibited in both wild and culture species needs to be sustained for high reproduction, long-term survival and as well as withstanding hash environmental conditions

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