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Full Length Research Paper

Identification of different selected genotypes of salinity resistance of each of full-sib Nile tilapia, Blue tilapia and their diallel interspecific hybridization using random amplified polymorphic DNA fingerprinting

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Random amplified polymorphic DNA (RAPD) analysis was applied to identify the three selected genotypes which were defined as; low, medium and high salinity resistance of each of full-sib Nile tilapia (*Oreochromis niloticus*), Blue tilapia (*Oreochromis aureus*) and their diallel interspecific hybridization. Six random primers were used to assay polymorphisms among the different selected genotypes within each species or hybrid. The results reveal that high genetic polymorphic percentages were detected among low, medium and high salinity resistance of different selected genotypes within each species or hybrid using different random primers. The results suggest also that RAPD technique can be successfully used as a rapid and easy way for identification of the different selected genotypes of salinity resistance fish, which considers a great potential for the development and implementation of genetic improvement programs.

Key words: RAPD fingerprinting, salinity resistance, Nile tilapia, Blue tilapia, interspecific hybridization.

INTRODUCTION

Tilapia are important species, especially for tropical aquaculture, and euryhaline fish that can live and thrive in a wide range of salinity from fresh water to full sea water even though some species tolerate a wider range of salinity than others (Philippart and Ruwet, 1982; Guner et al., 2005; Kamal and Mair, 2005). Tilapia is the second most important group of food fishes in the world, next to the carps. Nile tilapia, *Oreochromis niloticus* accounted for a harvest of nearly 2.54 million tones in 2009 (FAO, 2011).

Increasing demands on the use of freshwater for agricultural, industrial and domestic purposes progressively limit freshwater-based aquaculture. The efficient uses of marine and brackish environments for aquaculture become a vital alternative (Suresh and Lin, 1992; El-Sayed, 2006). It has been widely suggested that the euryhaline tilapias could be cultured in higher salinity of brackish water and marine systems, thereby enabling their exploitation in arid lands and coastal areas (Watanabe et al., 1985 a). Many species of tilapia are euryhaline, but the tolerance limits of species vary considerably (Suresh and Lin, 1992). Euryhaline tilapias have the genes for salinity tolerance and could be adapted, grown, and even bred in seawater (Likongwe, 2002).

The evaluation of the resistance to salinity is one of the fundamental key issues for ranking the parental strains and hybrids. Currently, the existing tests of salinity resistance are based on the change in mortality over time for fish transferred directly from freshwater to water of varying salinities (Watanabe et al. 1985 a, b; Villegas, 1990).

The technique of random amplified polymorphic DNA (RAPD) technique (Welsh and McCelland, 1990; Williams

Table 1. The sequences, GC % and the annealing temperatures of the primers used.

Primer	Sequence 5`- 3`	GC (%)	Annealing Tm°C/s
1	CCA GCC GAA C	70	28/30
2	ACC GCC GAA G	70	28/30
3	AAA GCT GCG G	60	28/30
4	AGG GGT CTT G	60	28/30
5	ATG CCC CTG T	60	28/30
6	AGG CCC CTG T	70	28/30

et al., 1990) has been successfully exploited for stock identification and population analysis in fish and aquatic animals (Partis and Wells, 1996; Dong and Zhou, 1998; Bartfai et al., 2003; Ahmed et al., 2004; El-Zaeem et al., 2006; El-Zaeem and Ahmed, 2006; Liu et al., 2007; Chen et al., 2009; Klinbunga et al., 2010; El-Zaeem et al., 2011; El-Zaeem, 2011a, b; Zhou et al., 2011).

Therefore, the aim of this work was to employ the RAPD technique to study and identify the molecular differences among the different selected genotypes of salinity resistance of each of full-sib Nile tilapia, *Oreochromis niloticus*, Blue tilapia, *Oreochromis aureus* and their diallel interspecific hybridization.

MATERIALS AND METHODS

The experimental work was undertaken at two areas: (1) The laboratory of Breeding and Production of Fish, Animal and Fish Production Department, Faculty of Agriculture (Saba-Bacha), Alexandria University and; (2) Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City for Scientific Research and Technology Applications, Alexandria, Egypt.

Fish origin

Nile tilapia, *O. niloticus* and Blue tilapia, *O. aureus* used in this study descended from a randomly mating population at the Middle East Fish Farm, Tolombat Halk El-Gamal, El-Behera Governorate, Egypt.

Fry production

Purebred of each Nile tilapia, *O. niloticus* and Blue tilapia, *O. aureus* spawners (1 female and 1 male) were stocked separately in a rectangle fiberglass tank (350 L) for full-sib of each of Nile tilapia and Blue tilapia fry production. The same previous spawners of Nile tilapia, *O. niloticus* and Blue tilapia, *O. aureus* were used for full-sib of each of diallel interspecific hybridization fry production of ♀*O. aureus* x ♂ *O. niloticus* and ♀*O. niloticus* x ♂*O. aureus*. Water temperature was thermostatiscally regulated and fixed at 28°C. Readiness of females to spawn was ascertained by testing the degree of swelling of the urogenital papilla (Hussain et al., 1991). Males were examined by stripping sperm (Wester and Foote, 1972).

Fry were collected after 10 days from the mouth of females and maintained separately in glass aquaria (100x34x50 cm). Each aquarium was supplied with fresh water and adequate continuous aeration systems and water temperatures were thermoregulated at 28°C. Glass aquaria were cleaned by siphoning, then water was partially changed once daily.

Saline water acclimation

Nine days post-hatching fry (Watanabe et al., 1985b), were obtained from each Nile tilapia, *O. niloticus*, Blue tilapia, *O. aureus*, ♀*O. aureus* x ♂ *O. niloticus* and ♀ *O. niloticus* x ♂ *O. aureus* and gradually acclimated to the salinity of 36 ppt, by raising the salinity at the rate of 4 ppt daily (Watanabe and Kuo, 1985). Salinity was prepared by mixing fresh water with crude natural salt (Likongwe, 2002) obtained from El-Nasr Company for salt, Borg El-Arab, Alexandria, Egypt. Water in each glass aquaria was partially changed once daily and totally every three days. Fry were fed three times daily pellets containing 38% protein, to satiation for 18 days.

Selection procedure

Two genotypes of salinity resistance fish were selected within the acclimation period by collecting the dead individuals of fish as soon as they succumbed to salinity stress. The third genotype was selected from the survival fish at the ninth day after the end of acclimation period. The first genotype (G1) was defined as low salinity resistance; the fish cannot survive at the salinity more than 12 ppt. The second genotype (G2) was defined as medium salinity resistance; the fish can survive only within a range of salinity 12 to 24 ppt. The third genotype (G3) was defined as high salinity resistance; the fish can survive and tolerant the salinity of 36 ppt.

Random amplified polymorphic DNA (RAPD) technique

DNA was extracted from tissue of base generation (F0) of purebred, and their diallel interspecific hybridization following the method described by Bardakci and Skibinski (1994). In this work, 10 base long oligonucleotide primers (Table 1) were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplification. The polymerase chain reaction amplifications were performed following the procedure of Williams et al. (1990, 1993). The reaction (25 µl) was carried out in a reaction that consist 0.8 U Taq DNA polymerase (Fanzyme), 25 pmol dNTPs, and 25 pmol of random primer, 2.5 µl. 10X Taq DNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (ependorff). The PCR programme included an initial denaturation step at 94°C for 2 min followed by 45 cycles with 94°C for 30 s for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 s and final extension at 72°C for 10 min. The samples were cooled at 4°C. The amplified DNA fragments were separated on 1.5% agarose gel and stained with ethidium bromide. ΦX174 DNA ladder marker (bp 1353, 1078, 872,…,72) was used in this study. The amplified pattern was

Table 2. The percentage of polymorphic (PB%) of low (G1) versus medium (G2), low (G1) versus high (G3), and medium (G2) versus high (G3) of salinity resistance Nile tilapia, *O. niloticus*.

Primer	G1 vs. G2			G1 vs. G3			G2 vs. G3		
	NTB	NPB	PB (%)	NTB	NPB	PB (%)	NTB	NPB	PB (%)
1	8	6	75.00	8	6	75.00	6	2	33.33
2	7	5	71.43	7	1	14.28	6	4	66.67
3	4	2	50.00	4	2	50.00	6	2	33.33
4	5	3	60.00	4	4	100.00	3	3	100.00
5	6	2	33.33	6	0	0.00	6	2	33.33
6	7	3	42.86	6	0	0.00	7	3	42.86
Average			55.44			39.88			51.59

NTB, Number of total bands; NPB, number of polymorphic bands.

Table 3. The percentage of polymorphic (PB%) of low (G1) versus medium (G2), low (G1) versus high (G3), and medium (G2) versus high (G3) of salinity resistance Blue tilapia, *O. aureus*.

Primer	G1 vs. G2			G1 vs. G3			G2 vs. G3		
	NTB	NPB	PB (%)	NTB	NPB	PB (%)	NTB	NPB	PB (%)
1	4	4	100.00	2	2	100.00	4	2	50.00
2	7	1	14.28	7	1	14.28	8	0	0.00
3	2	2	100.00	5	3	60.00	5	3	60.00
4	9	1	11.11	5	5	100.00	4	4	100.00
5	3	3	100.00	5	5	100.00	2	2	100.00
6	2	0	0.00	6	4	66.67	6	4	66.67
Average			54.23			73.49			62.78

NTB, Number of total bands; NPB, number of polymorphic bands.

visualized on an UV transilluminator and photographed by Gel Documentation system.

Scoring and analysis of RAPDs

RAPD patterns were analyzed and scored from photographs. For the analysis and comparison of the patterns, a set of distinct, well-separated bands were selected. The genotypes were determined by recording the presence (1) or absence (0) in the RAPD profiles.
Genetic similarity (GS) of the three selected genotypes of salinity resistance from each of full-sib Nile tilapia, *O. niloticus*, Blue tilapia, *O. aureus*, ♀*O. aureus* x ♂ *O. niloticus* and ♀*O. niloticus* x ♂ *O. aureus* based on RAPD fingerprinting were analyzed by the index of similarity using the formula given by Nei and Li (1979):

$B_{ij}=2\ N_{ij}/(N_i + N_j)$,
Where, N_{ij} is the number of common bands observed in individuals i and j, and N_i and N_j are the total number of bands scored in individuals i and j respectively, with regard to all assay units. Thus, GS reflects the proportion of bands shared between two individuals and ranges from zero (no common bands) to one (all bands identical). Genetic dissimilarity (GD) was calculated as: GD = 1- GS (Bartfai et al., 2003).

RESULTS AND DISCUSSION

Identification of the three selected genotypes of salinity resistance of each of full-sib Nile tilapia, Blue tilapia and their diallel interspecific hybridization was made using RAPD technique. Sex random primers (Table 1) were tested for their ability to produce DNA polymorphism in genomic DNA of each genotype selected. All the six random primers examined produced different RAPD bands patterns. The number of amplified fragments detected varied depending on primers, genotype and species. Moreover to ensure that the amplified DNA fragments originated from genomic DNA, not from primer artifacts, negative control was carried out for each primer/ genotype combination. No amplification was detected in the control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions (Tables 2, 3, 4 and 5; Figure 1).Data of genetic diversity among the three selected genotypes of Nile tilapia showed that highest genetic polymorphic percentage (55.44%) was obtained between low and medium salinity resistance fish while the lowest percentage (39.88 %), was recorded between low and high salinity resistance fish (Table 2 and Figure 1). The results of genetic polymorphic of the three selected genotypes of Blue tilapia revealed that the highest percentage (73.49%) was found between low and

Table 4. The percentage of polymorphic (PB%) of low (G1) versus medium (G2), low (G1) versus high (G3), and medium (G2) versus high (G3) of salinity resistance (♀*O. aureus* x ♂ *O. niloticus*).

Primer	G1 vs. G2			G1 vs. G3			G2 vs. G3		
	NTB	NPB	PB (%)	NTB	NPB	PB (%)	NTB	NPB	PB (%)
1	3	3	100.00	6	2	33.33	3	3	100
2	5	5	100.00	10	4	40.00	5	5	100
3	3	3	100.00	6	2	33.33	3	3	100
4	3	3	100.00	4	2	50.00	1	1	100
5	3	3	100.00	6	0	0.00	3	3	100
6	2	2	100.00	2	2	100.00	0	0	-
Average			100.00			42.78			100.00

NTB, Number of total bands; NPB, number of polymorphic bands.

Table 5. The percentage of polymorphic (PB%) of low (G1) versus medium (G2), low (G1) versus high (G3), and medium (G2) versus high (G3) of salinity resistance (♀*O. niloticus* x ♂ *O. aureus*).

Primer	G1 vs. G2			G1 vs. G3			G2 vs. G3		
	NTB	NPB	PB (%)	NTB	NPB	PB (%)	NTB	NPB	PB (%)
1	6	0	0.00	6	4	66.67	6	4	66.67
2	8	2	25.00	7	3	42.86	7	3	42.86
3	4	0	0.00	5	1	20.00	5	1	20.00
4	5	1	20.00	5	5	100.00	4	4	100.00
5	5	1	20.00	5	1	20.00	6	0	0.00
6	3	3	100.00	7	1	14.28	4	4	100.00
Average			27.50			43.97			54.92

NTB, Number of total bands; NPB, number of polymorphic bands.

high salinity resistance fish while the lowest percentage (54.23%), was obtained between low and medium salinity resistance fish (Table 3 and Figure 1).

The highest genetic polymorphic percentage (100%) of ♀*O. aureus* x ♂ *O. niloticus* was found between low and medium and between medium and high salinity resistance genotypes with the same value while the lowest percentage (42.78 %) was recorded between low and high salinity resistance genotypes (Table 4 and Figure 1). Genetic diversity among the three selected genotypes of the hybrid ♀ *O. niloticus* x ♂ *O. aureus* revealed that the highest genetic polymorphic percentage (54.92%) was found between medium and high salinity resistance fish while the lowest percentage (27.50%), was obtained between low and medium salinity resistance fish (Table 5 and Figure 1).

The results of this work show that the genetic diversity of the different selected genotypes of salinity resistance tilapia can be detected using RAPD fingerprinting.

The main advantages of RAPD markers in aquaculture are the possibility of working with anonymous DNA and the relatively low expense, and it is also fast and simple to produce RAPD marker (Hadrys et al., 1992; Elo et al., 1997; Ali et al., 2004; El-Zaeem et al., 2011; El-Zaeem 2011a, b). Moreover, RAPD analysis might be useful for systematic investigation at the level of species and subspecies (Bardakci and Skibinski, 1994), and more sensitive and technically easier to perform and produced results with low statistical error, whereas DNA fingerprinting detected greater genetic differentiation between Nile tilapia strains than other molecular techniques such as multilocus minisatellite marker (Naish et al., 1995). El-Zaeem and Ahmed (2006) reported that RAPD fingerprinting can be used to detect the genetic diversity between sex reversal and normal full-sib Nile tilapia, *O. niloticus*, which considers a great potential for detection of the commercial deceit and protection of human health and also, RAPD marker are successfully used to evaluate and determine the genetic differentiation of different populations of jumbo squid, *Dosidicus gigas* (Sandoval-Castellanos et al., 2007). Chen et al. (2009) reported that RAPD marker can be used to distinguish gender in Yellow River carps (*Cyprinus carpio*). Klinbunga et al. (2010) showed that RAPD marker was successfully used for species identification of the blue swimming crab (*Portunus pelagicus*) in Thailand waters.

In addition, Zhou et al. (2011) demonstrated the potential of RAPD assay for application as a powerful tool for detecting DNA damage induced by nitrofurazone drugs in marine ciliate, *Euplotes vannus* (Protozoa, Ciliophora).

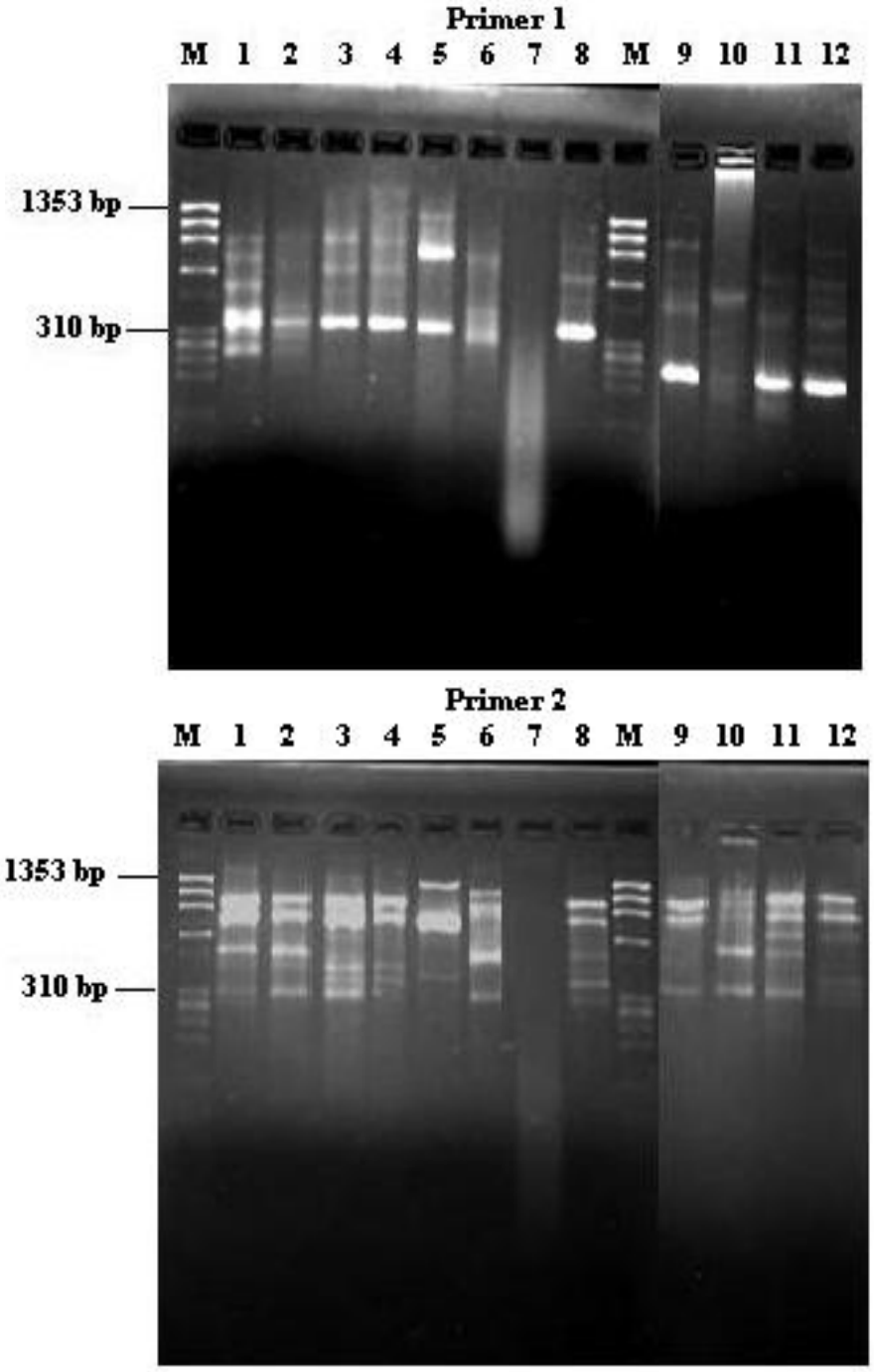

Figure 1. Patterns in different salinity resistance genotypes of each of full-sib Nile tilapia (lanes 1, 5 and 9), Blue tilapia (lanes 2, 6 and 10), ♀Blue tilapia x ♂ Nile tilapia (lanes 3, 7 and 11) and ♀Nile tilapia x ♂ Blue tilapia (lanes 4, 8 and 12) of each primer; lane M, ΦX174 DNA marker, the lanes 1 to 4 of each primer are low salinity resistance of fish; the lanes 5 to 8 of each primer are; medium salinity resistance fish, the lanes 9 to 12 of each primer are high salinity resistance fish.

The results of this study are consistent with these findings.This work represents a first step towards the generation of DNA markers for proposes, such as species diagnosis, detection of molecular markers linked to economic traits, assessment of genetic diversity and studies on molecular systematic. The results suggest also that RAPD technique can be successfully used as a rapid and easy way for identification of the different

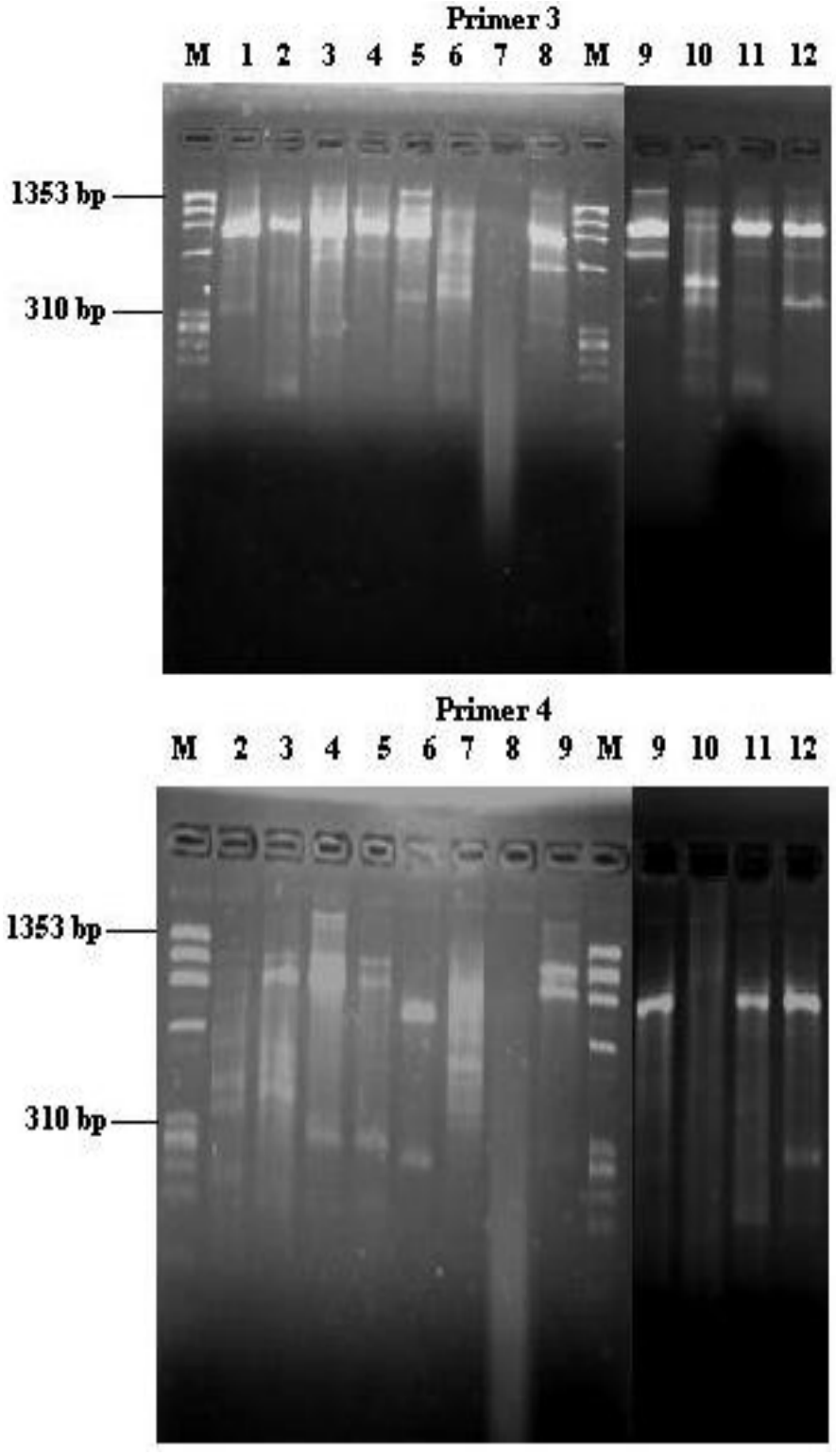

Figure 1. Contd.

selected genotypes of salinity resistance fish, which considers a great potential for the development and implementation of genetic improvement programs.

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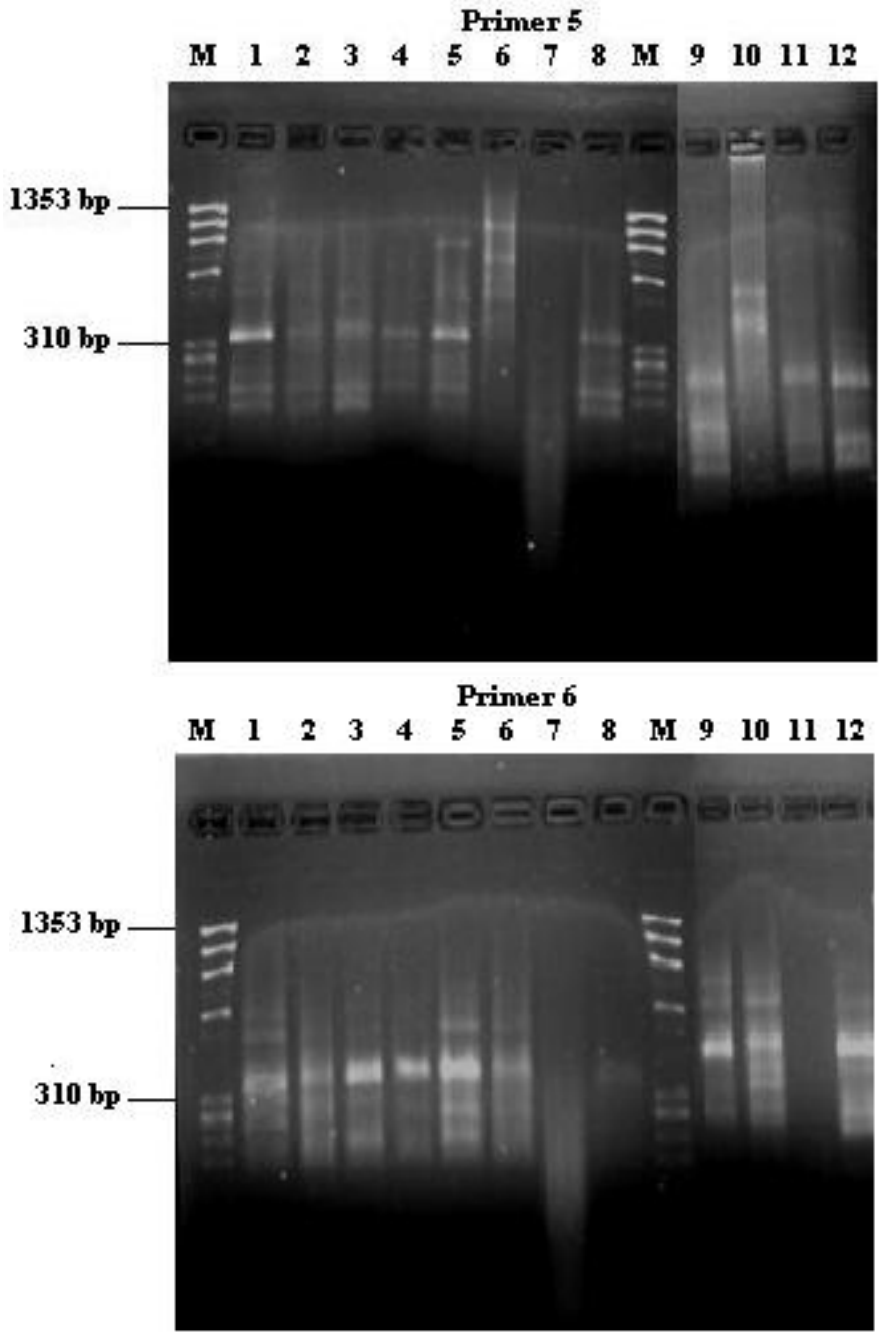

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