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Genetic Variation among F1 and F2 Parents Stocks from Cultured Populations of *Lates calcarifer* in Tok Bali, Kelantan using DNA Polymorphism Markers

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Abstract Seabass belongs to the species of *Lates calcarifer* which is one of the economically marine food fish species in tropical and subtropical regions of Asia Pacific. It has been produced widely in large scale for domestic and export market. Malaysia is one of the major producers of cultured seabass in Asia. In this study, DNA polymorphism markers are used to determine the genetic divergences of the species among the F1 and F2 parents stocks in DIMAN SDN BHD at Tok Bali, Kelantan. After screening, seven primers (OPA 02, OPA 03, OPA 05, OPA 09, OPA 10, OPA13, and OPA 18) were selected and used to analyze the polymorphisms of seabass. The RAPD analyses showed that the genetic distance among three cultured populations (F1/1, F2/1 and F2/2) of *L. calcarifer* was 0.202-0.252 indicating that all populations in the hatchery are genetically closely related. The value of genetic distance among individuals of *L. calcarifer* showed high genetic distance between F1/1(3), male individual and F2/2(1), female individual (0.429). The result obtained through this study can serve as a reference to DIMAN SDN BHD when applying selective breeding program and act as a supportive research in reducing inbreeding depression.

Keywords *Lates calcarifer*, seabass, genetic variation, DNA polymorphism, genetic marker

1. Introduction

Seabass belongs to the Entropomidae family and Lates genus and is a promising species for aquaculture food production. The seabass is a euryhaline and catadromous species that have unique life cycle. Sexually mature fish can be found in the river, lakes or lagoons, meanwhile newly-hatched larvae distributed along the coastline of brackish water estuaries. Under natural condition, seabass grows in freshwater and migrates to more saline water for spawning. However, the major restriction to rapid growth of sea bass culture has been the inconsistent supply of fry collected from the wild. It varies widely from year to year and making forward planning for production difficult (Kungvankij et al, 1985). There is a need for artificial propagation to provide sufficient seeds for aquaculture and stock enhancement for this species. Genetic improvement program for greatest selective breeding for broodstock is essential through analysis of genetic variability in cultured population.

Assessment on the gene pool of the species in a commercial hatchery is crucial to ensure the effectiveness of the breeding program. Large gene pool will provide diversity thus is more stable as compared to small gene pool. Since polymorphism is a common phenomenon that exists in most natural populations (Ayala, 1982), variation among the individuals in a population can be assessed based on polymorphism exhibited by molecular markers. Although modern techniques for studying genetic polymorphisms are useful for genetic interpretation, these techniques have limitation. As an example, RFLP (Restriction fragment length polymorphism) analysis requires fairly large amounts of genomic DNA while the cloned probes may be specific to an organism. Targeted polymerase chain reaction assays, although requiring much less genomic DNA, depends on DNA sequence knowledge

of the organism. One of the technique for detecting DNA polymorphisms is random amplified polymorphic DNA (RAPD) which requires only small amounts of DNA, the laboratory are simple to handle and perform, no prior knowledge of genome is necessary and no radioactive reagents are utilized in the assay. It is a simple, cheap and rapid technique used for population genetic studies to determine genetic diversity and to identify useful genetic markers at different taxonomic levels of various marine organisms. The aim of this study is to establish DNA fingerprinting amongst the F1 and F2 brood stocks populations of seabass (*L. calcarifer*) reared in DIMAN SDN BHD, Tok Bali, Kelantan and to determine the degree of their genetic divergence.

2. Materials and methods

2.1 Fish Samples

The Asian Sea bass, *L. calcarifer*, samples were collected from the hatchery at DIMAN SDN BHD, Tok Bali, Kelantan. Scope net were used to captured a total numbers of 18 samples of Asian Sea bass from three cultured brood stocks tanks in hatchery of DIMAN SDN BHD. After the collection, the Asian Sea bass fin clips were collected and kept in lysis buffer to lyses the fish tissues. The 18 samples of Asian Seabass fin clips were properly labeled according to the three cultured tanks. The samples were stored at 5^0C until use.

2.2 Morphological Identification

The captured Asian Sea bass morphology were observed, weighed, and the sex has been identified based on the scale mentioned by Lai, (2008) and Kungvankij et al, (1985). The *L. calcarifer* samples have been taken on 17th May 2012 where 18 Asian sea bass were randomly picked from the three cultured tanks.

2.3 DNA Preparation and RAPD procedures

Genomic DNA from the fin clips were extracted using Phenol-Chloroform method, modified Cetyltrimethylammonium Bromide (CTAB) method and DNeasy Tissue Kit method. The method that gives better DNA quality was used for further analysis on RAPD. Twenty primers from Operon Technology Kit A (OPA01-20) with 60-70% GC content, were screened. Based on the clear and reproducible bands, seven primers (OPA 02, OPA 03, OPA 05, OPA 09, OPA 10, OPA 13, and OPA 18) were selected for further study. The amplification reaction was consisted of 12.5 µL of 2X MyTaq Mix, primer (0.4 µM), template DNA 50 ng and distilled water in a final volume of 25 µL. The amplification of DNA was performed using a programmable Thermal Cycler (BIO-RAD MJ Mini), programmed at 35 cycles for denaturation (95°C, 15 seconds), annealing (36°C, 30 seconds), extension at 72°C (10 seconds) and final extension (72°C, 5 minutes). The amplification product was separated by gel electrophoresis on 1.2% agarose gel and stained with RedSafe Nucleic Acid Staining Solution. The bands were viewed under the UV-transluminator and photographed using camera (DSLR Canon EOS 100D).

3. Data analysis

The bands were scored as either presence (1) or absence (0) at a particular position of a given amplification product of each individuals. The data matrix of 1's and 0's from the scorable bands were used to calculate the genetic distance values and to construct the Unweighted Pair-Group Method of Aritmethic (UPGMA) dendrogram using RAPDistance and NTSYS Version 2.0.

4. Results and Discussion

The genomic DNA of *L. calcarifer* was successfully extracted using Phenol-Chloroform technique and produced better DNA quality as compared modified CTAB method and DNeasy Tissue Kit method. The DNA purity and quantity *L. calcarifer* obtained from this technique was 1.023±0.007 and 1784±121.12 $\mu g.\mu L^{-1}$ respectively. Roshani (2006) reported that the quantification of DNA is a very important in many molecular research procedures and it is necessary to know the purity and quantity of DNA that is present when performing genetic analysis such as RAPDs.

Among the 20 primers (OPA01-20) screened, seven primers (OPA 02, OPA 03, OPA 05, OPA 09, OPA 10, OPA 13, and OPA 18) were selected for further analysis based on the clarity and sharpness of fragment profiles. This study showed that good fragments of amplified DNA were obtained from the primers containing 60% or slightly more than 60% of GC bases in their sequence in comparison to GC content. Fritsch et al., (1993) also reported that primer with a high GC content (more than 60%) produced better RAPD profile than other primers. All of the primers examined produced different RAPD fragment pattern (Figure 1). Bardakci (2001) noted that some primers do not perfectly match the priming site. On the other hand, non specific amplification with increased band production is also a common occurrence in RAPD reactions. Differences between DNA concentrations could lead to differences in number of bands.

A total of 105 fragments (loci) in size of 150 – 2000 bp and 105 polymorphic fragments were generated by the seven RAPD primers (Table 1). The number of genotype detected within a *L. calcarifer* population was varied from two to six. A similar case was shown in Rajasekar et al., (2012) reported that the totals of 589 bands were scored using 10 random primers in three *L. calcarifer* populations and 93.12% of them were polymorphic. Although there was some physical variation observed between individuals within the population but most of the bands were less variable among individuals in a population. Barman et al., (2003) noted that 45% of polymorphic loci in four Indian major carp population using RAPD primers. Islam and Alam (2004) observed 46.5% of polymorphic loci in four different *Labeo rohita* populations.

Figure 2 shows the dendrogram of *L. calcarifer* in five clusters. The first cluster which consisted of thirteen individuals (F1/1(1), F2/2(4), F1/1(2), F2/1(5), F2/1(1), F2/1(4), F2/2(3), F1/1(6), F2/2(2), F2/2(5), F2/2(6), F2/1(6), and F2/2(1)) shows the dominant individuals from F2/2 tank. The second cluster which consisted of two individuals (F1/1(4) and F1/1(5)) shows the individuals from F1/1 tank. The third cluster to fifth cluster consisted of one individual in each cluster which are F2/1(2), F2/1(3) and F1/1(3) respectively. The result also shows middle variation among individuals (0.07-0.33) and no specific clustering of individuals from specific tank (individual present in different UPGMA cluster from various tanks) was observed. The low variation among individuals indicates inbreeding occurs among individuals of each population or tank. In Figure 3, the genetic distance among populations of *L. calcarifer* of this study shows low values of coefficient dissimilarity (0.24-

0.25). The results of the seabass population study suggested that the breeding program should be improvised in order to increase genetic variation in *L. calcarifer* from different tanks in DIMAN SDN BHD, Tok Bali, Kelantan. As reported by Koh et al., (1999) proper management and breeding programs have to be implemented to preserve genetic variability and prevent inbreeding depression that will probably results from the present state of unplanned breeding. One of the improvement methods to be implemented is to select broadstocks from different region which includes the wild type. Meanwhile, Barbanera et al., (2005) reported that molecular analysis techniques allow the estimation of genetic variability and divergence between species and populations, and can be used for phylogenetic studies, breeding and conservation programs.

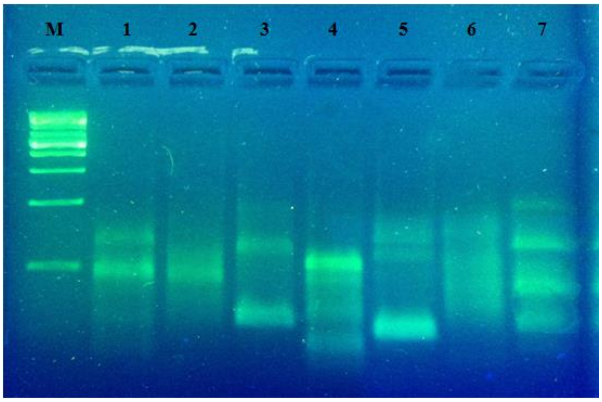

Figure 1: The PCR profile of *L. calcarifer* from FI population generate by RAPD primers. Lane M- VC 1kb DNA Ladder; Lane 1- Primer OPA 02; Lane 2- Primer OPA 03; Lane 3- Primer OPA 05; Lane 4- Primer OPA 09; Lane 5- Primer OPA 10; Lane 6- Primer OPA 13 and Lane 7- Primer OPA 18. Only clear and sharp bands were scored.

Table 1: Total number of fragments, polymorphic fragments, number of genotypes and size range of fragments of *L. calcarifer* between tanks (F1/1, F2/1 and F2/2) at hatchery of DIMAN SDN BHD in Tok Bali, Kelantan.

Populations/Tanks	Total no. of fragments	No. of polymorphic fragments	Number of genotypes	Size range of fragments (bp)
F1/1	37	37	4-6	150-2000
F2/1	33	33	2-5	150-2000
F2/2	35	35	2-5	250-2000
Total	105	105		

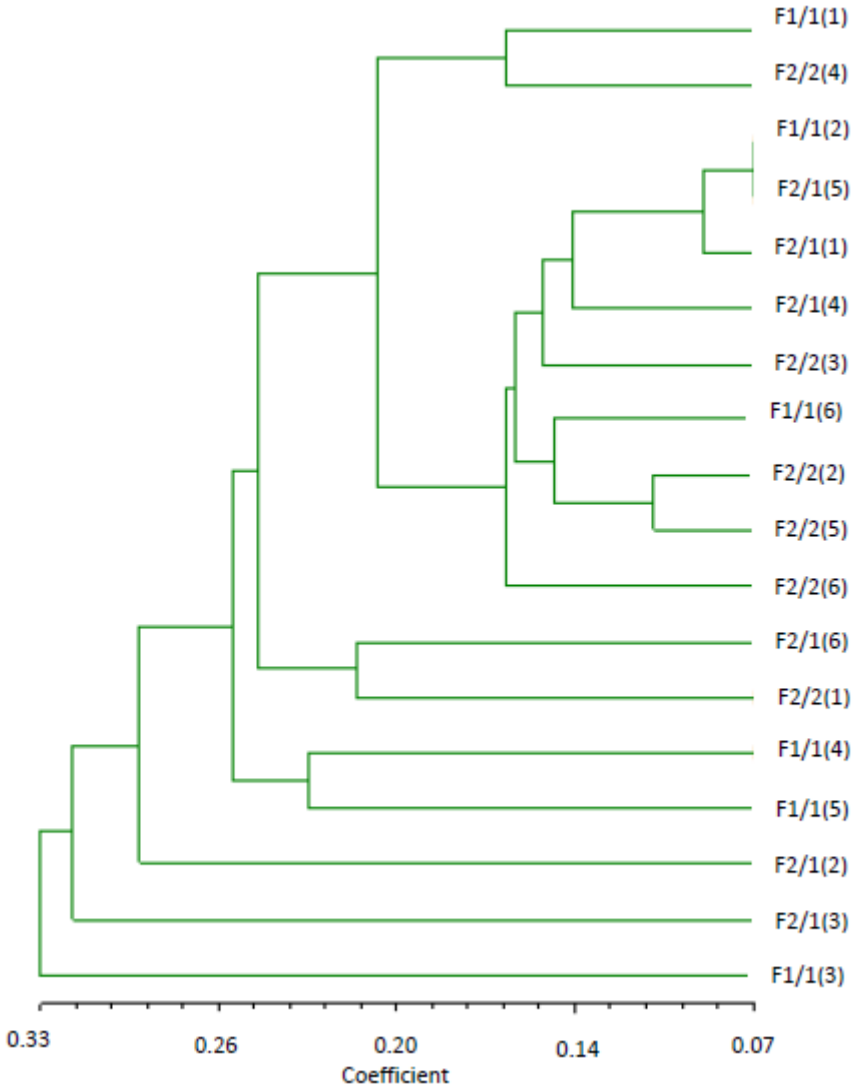

Figure 2: UPGMA cluster analysis of all the individuals based on the genetic distance generated using Nei and Li's indices.

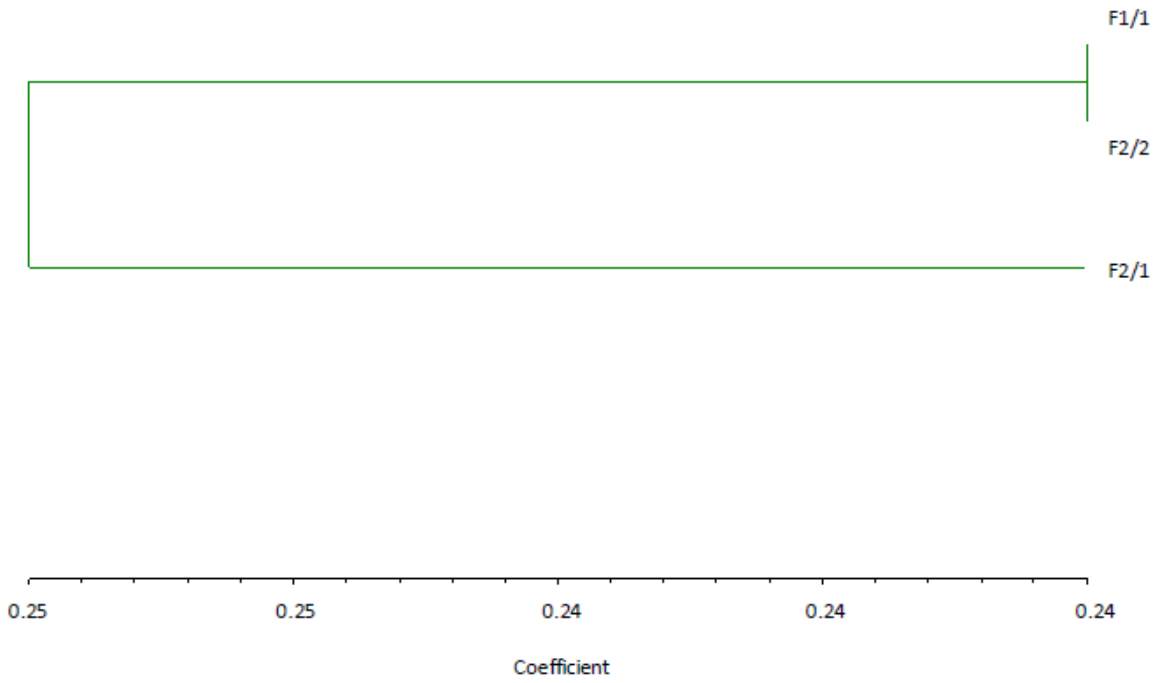

Figure 3: UPGMA cluster analysis of the three populations F1/1, F2/1 and F2/2 based on the genetic distance generated using Nei and Li's indices.

5. Conclusion

In conclusion, the genetic variation among tank populations (F1/1, F2/1 and F2/2) of seabass (*L. calcarifer*) in DIMAN SDN BHD, Tok Bali, Kelantan have narrow genetic distance (0.24-0.25). When comparing all individuals, the furthest genetic distance obtained is between F1/1(3) male and F2/2(1) female, at value of 0.429. These two individuals is suggested to be selected by DIMAN SDN BHD for breeding. The finding of this study is important and can be implemented in breeding programme to produce better quality for market demand and as well as for the conservation of this species. However, the breeder or researcher should enhance the genetic variability of the hatchery species through crossing the wild types from different region and can avoid inbreeding depression.

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