GENETIC VARIABILITY OF GIANT GOURAMY STRAINS REVEALED BY RANDOM AMPLIFIED POLYMORPHISM DNA

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ABSTRACT

Genetic variability of giant gouramy strains has been observed using RAPD Marker. Genomic DNA was extracted from giant gouramy fin clip, and amplified using primers OPA 1-20. There was no significant difference among three strains of giant gouramy analyzed. Based on two primers of RAPD (OPA 4 and 7), the highest variability was observed in Bluesafir with heterogeneity value of 0.3050 and followed by Paris (0.2832) and Soang (0.2360) respectively. The average of Nei's genetic distance is 0.118, and the smallest on is between Paris and Bluesafir.

KEYWORDS: genetic variabilit, giant gouramy, RAPD

INTRODUCTION

Giant gouramy is an economically important fresh water fish in Indonesia, especially in West and Central Java Province. Some strains of giant gouramy have been widely used in aquaculture such as *Soang, Jepang, Paris, Porselen,* and *Bastar* (Sudarto, 1989). Nowadays, farmers in different locations in Indonesia use different names for the sam giant gouramy because there is not clearly identification for this species.

Identification of giant gouramy strain using morphometric and biochemical analysis has been carried out by previous researchers (Soewardi *et al.*, 1995; Soewardi, 1995; Kusmini *et al.*, 2000; Suseno *et al.*, 2000). Analysis of three strains of giant gouramy using new trussmorphometric was conducted by Setijaningsih *et al.*, 2007). However this information has not yet applied in giant gouramy culture.

Nugroho *et al.* (1993) found differences in morphology and growth rate of some strains of giant gouramy. Furthermore Nugroho & Kusmini (2007) revealed that analysis genetic variation of strain *Bastar, Blue,* and *Bluesafir* using allozyme showed there was no significant difference among three strains. Genetic variability knowledge is important feature of its population both for short terms fitness individuals and the long term conservation of the population (Ferguson *et al.*, 1995). The gentic variation can be observed in the DNA Polymorphism (by allelic diversity and heterozygosity). One of several approaches to detect the polymorphism DNA fish is using molecular genetic marker (Carvalho & Pitcher, 1995).

Analysis of genetic variation using DNA markers is needed to determine the genetic potential that may be utilized in the production of superior breeds of giant gouramy. By knowing the genetic variation in each strain giant gouramy will help to determine the appropiate breeding program in the next stage. This study aims to evaluate the genetic variability of three strains of giant gouramy, i.e. Soang, Paris, and Bluesafir using RAPN markers.

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MATERIALS AND METHODS

Samples

Thirty samples of giant gouramy strains were collected from Parung District, Bogor, West Java Province. The strains of giant gouramy used were *Soang* (A), *Paris* (B), and *Bluesafir* (C) (Figure 1).

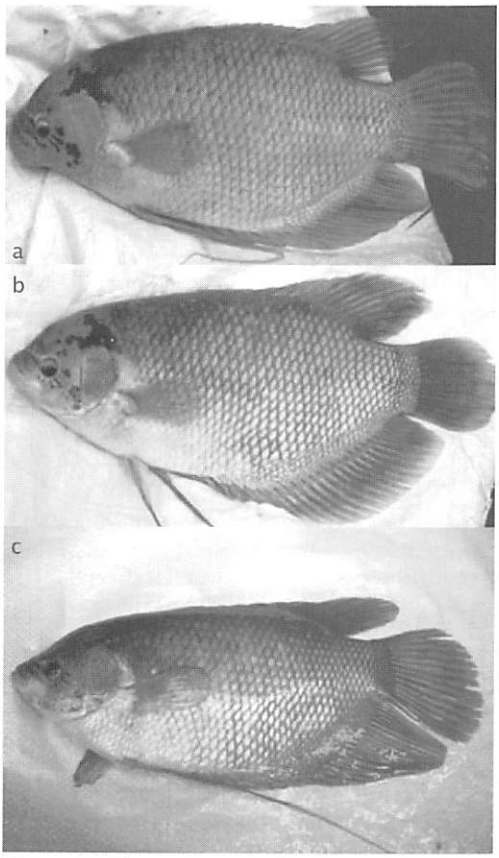

Figure 1. Three strains of giant gouramy

Genomic DNA Extraction

Genomic DNA was extracted from a piece of fish fin using phenol-chloroform method (Harris *et al.*, 1991). Approximately 5-10 mg pieces of fin were mixed with 500 uL TNES Urea in 1.5 mL tube and lysed by addition of 10 ug/mL Proteinase K. Samples were incubated for 12 hours at 37°C. The lysate was extracted ywice with an aqual volume of phenol-chloroform (25:24) and twice with Chloroform-isoamylalcohol (24:1). The nucleic acid were precipated with two volumes of ethanol and 1/10 volume 3M Ammonium acetate and rinsed with 70% alcohol. The pellet DNA was air dried and resuspended in 50-100 uL Tris-EDTA (TE) buffer and stored in 4°C. Before use in the next step, purification of DNA was conducted using Rnase (Lia, 2006).

Random Amplified Polymorphism DNA (RAPD)

RAPD loci were amplified by the Polymerase Chain Reaction (PCR) using 20 primers (OPA 1-20) to assess the level of polymorphism. Screening of primers, conducetd for primary amplification product has to suit giant gouramy DNA.

Polymerase Chain Reaction was conducted in volumes of 25 uL, each consisting of: 10 ug of template DNA, 2 uM of each primer, a "pure taq DNA" (Promega) and H2O. Amplification cycles consisted of one cycle of denaturation at 94°C for 2 minutes, 45 cycles consisting of 94 C for 1 min., 36 C for 1 min and 72 C for 2.5 min. The last cycle was at 72 C for 10 min. Three uL of Brome Phenol Blue were added to each reaction vessel. Results of amplification were separated by electrophoresis using 2-3% agarose gel ing Tris-Boric-EDTA (TBE) buffer and observed with illuminator (UV) and printed with the polaroid camera.

Data Analysis

DNA variation among strains of giant gouramy was evaluated using analysis of Molecular Variance (AMOVA) and Fst tes in TFPGA program (Miller, 1997). While genetic relationship was analyzed using Genetic Distance of Nei (1972).

RESULTS ADN DISCUSSIONS

A preliminary screening of primers on the several individuals from three strains revealed that three primers i.e. OPA 4, OPA 6, and OPA 7 were score readly. However only two primers were used in this study (OPA 4 and 7) due to inconsistency result of primer OPA 6. This was similar with the result obtained by Lia (2006) that found those primers are useful for giant gouramy DNA. The total number of observed bands from two promers was 28 pieces with alength from 300 to 2,000 bp. An example patterns of fragment DNA, resulted by primer OPA 4 was shown in Figure 2.

Level of genetic variation of giant gouramy is influenced by type of strain. In general, giant gouramy has a high level of genetic variation

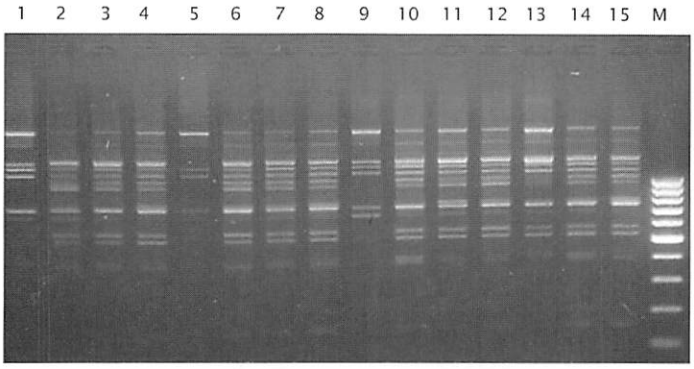

Figure 2. Amplification of RAPD using primer OPA 4. M=Markerline 1-5=Soang, 6-10=Paris, 11-15=Bluesafir

Table 1. Genetic variation of giant gouramy based on the RAPD fragment with primer OPA 4 and 7

	Soang	Paris	Bluesafir
N-Samples	6	10	8
Heterozygosity	0,2360	0,2832	0,3050
Polymorphic loci (%)	70,37	74,07	74,07

that indicated by an average heterozygosity value 0.2747. the highest value of genetic variation is observed in Bluesafir (0.3050), followed by Paris (0.2832), and Soang (0.2360) (Table 1). This value is equivalent to the result of Lia (2006) who obtained an average heterozygosity of Bluesafir strain of 0.310 and heterozygosity giant gouramy tested was higher than that of other freshwater fish. Generaly, genetic variation of freshwater fish is quite low as a result of natural migration limitations, such as carp-kancra (Nugroho *et al.*, 2006).

This phenomenon is possible because of giant gouramy commodities still persist locally in its cultivication stage (widely undeveloped) so that the reduced levels of genetic diversity due to "inbreeding depression" which common in freshwater fish are still relatively, and lower than the commodities that are grown widely and long story of culture, where possibility of "genetic introgression" becomes greater. Relatively high genetic variation also suggest that commodity is used as fish farming potential.

Moreover, the level of heterozygosity of Soang strain was lower than those observed in two other strains that can be understood. It may caused by the farmers who prefer use the strain Soang in giant gouramy culture therefore the possibility of inbreeding matting is higher than two others. Based on field observation, almost all of giant gouramy culture activities use a strain Soang, although with different farming system in each region.

Result of AMOVA based on bands of two primers showed that there was no significant different among strains genetically (P>0.05) (Table 2). Similar result was also obtained using isozyme for Soang, Paris, and Bluesafir (Nugroho & Kusmini, 2007). It was suggested that the possibility of all three strains came from the same ancestor population. The differences in phenotypes i.e. the form of color and size scales may be caused by environmental influences. However, this phenomenon still needs further study.

This condition will be seen clearly in their genetic distance. Genetic distances calculated according to Nei (1972) is listed in Table 3. The avarege genetic distance among giant gouramy strains is approximately 0.118. Genetic distance value of giant gouramy is relatively equal to genetic distance of fish from the same population as carp-kancra fish (Nugroho *et al.*, 2006).

Table 2. Results of pairwiae Fst test

	Soang	Paris	Bluesafir
N-Samples	xxxxxxxxx	0.4929ns	0.4902ns
Heterozygosity		xxxxxxxxx	1.0ns
Polymorphic loci (%)			xxxxxxxxx

Table 3. Nei's genetic distance (1972)

	Soang	Paris	Bluesafir
N-Samples	xxxxxxxxx	0,1587	0,1657
Heterozygosity		xxxxxxxxx	0,0282
Polymorphic loci (%)			xxxxxxxxx

Dendogram formed showed that the genetic distance of strain Bluesafir and Paris is closer than Soang (Figure 3). Based on the observation in the field, Bluesafir and Paris srain have a similar scale type, and the differentiation is only on the color scale. Bluesafir has blue color, while Paris has over gray scale color. This suggested that the possibility of hybridization between Soang x Bluesafir or Soang with Paris and throught hybridization is expected to find better performance in the next generation.

CONLCLUSION

There is no significant differences genetically among giant gouramy strains Soang, Paris, and Bluesafir. The higest variability is in strain of Bluesafir (Heterozygosity of 0.3050), followed by strain of Paris (0.2832) and strain of Soang (0.2360).

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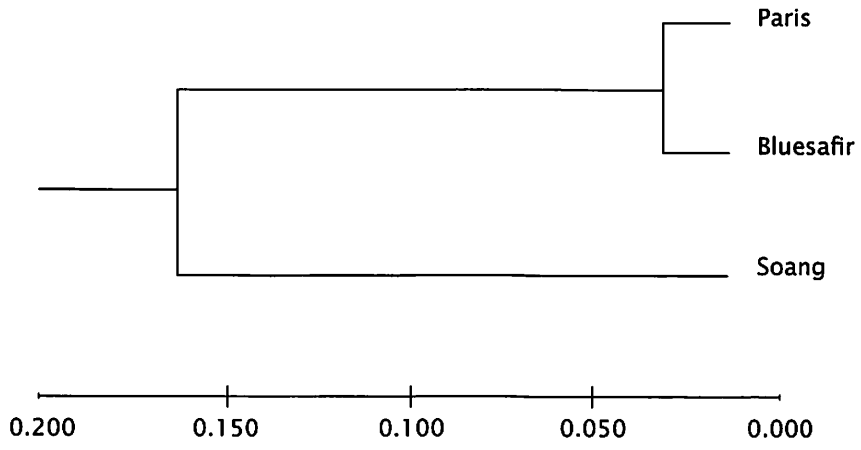

Figure 3. Dendogram of the Nei's genetic Distance for giant gouramy, Soang, Paris and Bluesafir based on RAPD using primer OPA 4 and 7.

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