Genetic Relationship of Four Strains of Guppy (Poecilia reticulata Peters, 1859) Using RAPD-PCR Method

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Authors’ contributions

This work was carried out in collaboration among all authors. Author ITN wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Author AY designed the study and wrote the protocol. Authors AY, AAH and IB managed to check and revise the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study aims to determine the genetic relationship between four strains of guppy, albino full platinum (AFP), albino german yellow (AGY), top sword (TS) and guppy yellow cobra (GYC) using the RAPD-PCR method. This study used explorative method without experimental design and analyzed by descriptive qualitative and quantitative. The obtained genetic relationship data could be used as data reference for hybridization between strains of guppy fish that have been researched. The research was conducted in October 2020-April 2021. The three fish samples (AFP, TS and GYC) obtained from fish breeder in Cilengkrang-Bandung and AGY sample obtained from fish breeder in Tanggerang-Banten. Based on the results of amplification using OPA-03 primer (AGTCAGCCAC), four strains of guppy fish showed 30 DNA bands that included polymorphic and monomorphic bands. The AFP strains had 19 monomorphic bands, AGY had 21 DNA bands (20 monomorphic bands and one polymorphic bands), TS had 19 DNA bands (17 monomorphic bands and two polymorphic bands) and GYC had 15 DNA bands (14 monomorphic bands and one polymorphic band).
polymorphic band). Phylogenetic tree analyzed by NTSys program. It is shown between AFP and AGY strains had 95% relationship index, then between TS and GYC strains had 82% relationship index and between AFP-AGY and TS-GYC had 50% relationship index.

Keywords: Genetic relationship; strains; Poecilia reticulate; RAPD-PCR; genetic variation; phylogenetic tree.

1. INTRODUCTION

Guppy is an ornamental fish with small body size. The fins of male guppy are longer, patterned and have a striking color than female guppy. Therefore, male guppies are in high demand because the various patterns and color of their fins and tail. Female guppy had a longer body, which is 7 cm long compared to male guppy fish that only measures 4 cm [1].

In aquaculture activities, guppy fish is one of the potential fish to be developed. That superiority of body pattern and color of guppy will increase its selling value. Improving the quality of body patterns and colors can be done by conducting hybridization activities. Hybridization means a cross between two different individuals aimed to getting better offspring [2]. The crossing between these two individuals can be done on closely related individuals or further related individuals [3]. This hybridization activity regularly carried out by ornamental fish breeders to increase the variety of patterns and body colors. However, the genetic relationship data of the guppy fish is still few researches found. In fact, the genetic relationship in guppy fish needs to be known, so inbreeding behavior can be avoided in crossing. Therefore, genetic relationship research between strains of guppy fish is needed to know the relationship of one individual and another, one of the techniques is by molecular approach using RAPD (Randomly Amplified Polymorphic DNA) PCR method, this method cause the polymorphic of each fish will appear.

With the polymorphic knowledge of each fish, it will facilitate hybridization activities. With the identification of genetic relationship in strains of guppy fish, the cross will get better results because it is based on data. Based on the description above, genetic relationship analysis of four strains of guppy, albino yellow cobra, albino german yellow and top sword needs to be done as an effort to decrease failure degree in hybridization activities, for example is to reduce the error of inbreeding in ornamental fish industrial activities.

2. MATERIALS AND METHODS

Research were conducted in October 2020 to April 2021. The process of preservation sample, DNA isolation, DNA amplification, and electrophoresis were performed in the biotechnology laboratory of Building 3 Fisheries and Marine Sciences Faculty, and the process of calculating the purity of DNA were performed in the Central Laboratory of Padjadjaran University.

The research used explorative method without using experimental design and analyzed by descriptive qualitative and quantitative. The fish used is guppy with four different strains. The obtained data from this study was collected through the RAPD-PCR method in the form of monomorphic and polymorphic bands, then used the NTSys program (Numerical Taxonomy and Multivariate Analysis System) and produced a phylogenetic tree.

2.1 Procedures

Research procedures include:

2.1.1 DNA isolation

DNA isolation aims to separate chromosomal DNA from other components of cells. Part of the sample taken as a DNA carrier is the fin part of the fish caudal. The sample was isolated with the Wizard Genomic DNA Purification Kit (Promega). 10 mg caudal fins are inserted into a 1.5 ml eppendorf tube, smoothed with sterile chopsticks. Added 300 μl nuclei lysis solution and homogenized with vortex for 10 seconds, then incubated in waterbath (temperature 65°C for 30 minutes). Added 1.5 μl RNAse Solution and homogenize, then incubated in waterbath (37°C for 30 minutes), and let stand 5 minutes on ice gel. Added 100 μl Protein Precipitation Solution and vortex for 10 seconds, chill on ice gel for 5 minutes then centrifuge for 4 minutes (13,000 rpm). Then the supernatant is transferred to a new 1.5 ml eppendorf that has been filled with isopropanol as much as 300 μl then centrifugation for 1 minute (13,000 rpm). Then the supernatant was thrown out and added 300 μl ethanol 70%, then
centrifugation for 1 minute (13,000 rpm). Ethanol is discarded and pale dried. Added 50 µl rehydration solution, then incubation in waterbath (temperature 65°C for 60 minutes).

2.1.2 DNA purity calculation

DNA isolation results were checked qualitatively and quantitatively to see the purity value and concentration of DNA. Qualitatively tested with electrophoresis on agarose gel, while quantitatively by measuring the value of DNA absorption using a spectrophotometer (Abs 260 nm and Abs 280 nm). Spectrophotometer turned on, 2 µl DNA sample and 2 µl nuclease free water inserted in blanks. After that the absorbance results were recorded and calculated the purity of the DNA.

2.1.3 DNA amplification

DNA amplification used RAPD-PCR method with 2 primers (OPA-02 and OPA-03). For the next step, only one primer that showed the most bands will be used to see the relationship index. The amplification process used by mixing 12.5 µl GoTaq Green Master Mix (Promega), 2 µl DNA template, 1.3 µl RAPD primer and 9.2 µl nuclease free water. Then vortex for 10 seconds and put into thermal cycler PCR. PCR program settings used were: Pre-denaturation (94°C, 2 minutes, 1 cycle), denaturation (94°C, 1 minute, 45 cycle), annealing (36°C, 1 minute, 45 cycle), extension (72°C, 2 minutes, 45 cycle), final extension (72°C, 10 minutes, 1 cycle), hold or temperature lowering process (4°C, 3 minutes, 1 cycle).

2.1.4 Electrophoresis

DNA samples that passed through the process of isolation and amplification was visualized by performing an electrophoresis process used agarose gel. Agarose powder measured as much 0.8 grams and put in erlenmeyer, added 80 ml TAE solution. Then the erlenmeyer heated in the microwave for 3 minutes. Once its warm, added 0.8 µl gel red and homogenized. Then pour the solution into the agarose mold that was equipped with a comb and let it for 25-30 minutes until it is solid, after that the comb is lifted. Then, pour the TAE running buffer until the gel is all submerged. DNA amplification results were filled in each gel agarose with a composition of 4 µl DNA results and 2 µl loading dye, the most left filled with 2 µl DNA Ladder 1kb and 2 µl loading dye as a marker. Then, the electrification tank is streamed by electricity and make sure the side that contained the amplification result is negative. The electrophoresis process lasts for 45 minutes (for DNA isolation results) and 90 minutes (for DNA amplification results) at 75 voltage.

2.2 Data Analysis

Data analysis was conducted with descriptively, quantitatively, and qualitatively method. Quantitative data was obtained from spectrophotometer results in DNA isolation process for calculate DNA purity. While qualitative data obtained from DNA amplification results using the method RAPD-PCR that visualized. The amplified DNA bands will be presented through the bands that appear on agarose. The visualized band is assigned as one-numeric sign (1) and unvisualized band is assigned as zero numeric sign (0). The bands converted in a binner matrix, then inserted into the NTSys program on the computer. The obtained result is a relationship tree (phylogenetic tree) of the guppy fish tested. The emerging genetic similarity index shows relationship between test samples so the value of genetic distance will be known.

3. RESULTS AND DISCUSSION

3.1 DNA Isolation

The samples tested were albino full platinum (AFP), albino german yellow (AGY), guppy yellow cobra (GYC), and top sword (TS). The test used caudal fin of male guppy. It was because fins are easily destroyed tissues and also easy to obtain the DNA samples [4]. DNA isolation of four samples of guppy used the Wizard Genomic Purification Kit (Promega) isolation kit. This activity aims to separate DNA from other cell components. DNA isolation method using Promega kit is a good isolation method issued by Promega Corporation company, where the results have been tested both in quantity and quality of DNA produced [5]. The DNA isolation results of the four samples can be seen based on qualitative and quantitative tests. Qualitative tests were conducted by looked at the results of DNA isolation electrophoresis, while quantitative tested was done by calculated DNA purity.

Qualitative test, which was tested the quality of DNA by looking at the appearance of band on gel
agrose were showed in Fig. 1. In this test, electrophoresis was performed with 1% agarose gel concentration, voltage of 75 volts, and lasted for 45 minutes. This process of electrophoresis is a technique to separate DNA mixtures based on molecular size.

The results of DNA isolation electrophoresis of the four strains of guppy fish as shown in Fig. 1, show that the four strains are able to bring up DNA bands on agarose gel. AFP, AGY, GYC and TS produce thick DNA bands, although there were smears that appear at the top and bottom of the DNA bands. The appearance of smears can occur because there is still remained a solution during the isolation process, or it can also be DNA degraded in the isolation process [5]. In addition, the appearance of smears can also be caused by the presence of contamination by protein [6]. Even so, the appearance of DNA band on agarose has indicated that the isolated samples already carry out the DNA from each strain of fish and can be used for a quantitative test.

Quantitative test used the Spectrophotometer Multimode Reader Infinite 200 PRO NanoQuant tool, which aims to know the purity value of DNA by see the value of Abs260 nm and Abs280 nm. The results of the four strains (Table 1) show in different purity value ratios. Pure DNA has a purity value between 1.8-2.0. If DNA shows results below 1.8 it may indicate contamination of proteins or phenols. If the value more than 2.0, it caused by contamination in DNA by RNA [7]. The following (Table 1) is the purity value of the DNA isolates of the four strains of guppy fish.

**Fig 1. Electrophoresis of DNA Isolation**

*Description: M = Marker, AFP=Albino Full Platinum, AGY= Albino German Yellow, TS= Top Sword, GYC= Guppy Yellow Cobra*

**Table 1. DNA isolate purity value of four strains of guppy fish**

| No | Samples                        | Abs260 | Abs280 | Purity |
|----|--------------------------------|--------|--------|--------|
| 1. | Albino Full Platinum (AFP)     | 0.18   | 0.08   | 2.12   |
| 2. | Albino German Yellow (AGY)     | 0.19   | 0.10   | 1.86   |
| 3. | Top Sword (TS)                 | 0.24   | 0.13   | 1.88   |
| 4. | Guppy Yellow Cobra (GYC)       | 0.38   | 0.19   | 1.94   |
AFP, AGY, TS and GYC shown a DNA purity value of 2.12; 1.86; 1.88 and 1.94. Of the four purity values, AGY, TS and GYC guppy strains already had the criteria of pure DNA because it has a purity value between 1.8-2.0. While the AFP has a purity value of 2.12. The value exceeds the maximum range, this may indicate DNA contamination by RNA in the isolation sample [7]. Maximum value of DNA can be absorbed at Abs260 nm, while the maximum protein (contaminant) value is absorbed at Abs280 nm [6]. The results obtained by the results of Abs260 nm and Abs280 nm will have an effect on the value of DNA purity. However, because the DNA purity value of the AFP strain guppy is close to the maximum value (2.0) and the emergence of DNA bands in qualitative testing, the isolation result is still feasible to be used as a sample in the next process. The purity of DNA is important because it effects the success of the PCR amplification process, especially by using the PCR RAPD method [8].

3.2 DNA amplification

One of many ways to know genetic variations of DNA amplification results was using OPA (Operon Primer setA). Primer is a piece of DNA strand with a short size, a single thread, and its length ranges from 10-40 bases [9]. The primers used are OPA-02 and OPA-03. The results of this part is OPA-03 show the most DNA bands than OPA-02. Therefore, the next step was used OPA-03 for analyzed the DNA amplification. Documentation of DNA amplification is shown in Fig. 2.

DNA amplification result in Fig. 2 show there are DNA bands that appear in each strain. Strains of AFP, AGY, TS and GYC are capable of generating DNA bands, both monomorphic and polymorphic bands. This suggests that the use of OPA-03 primer with an alkaline sequence of 'AGTCAGCCAC' matches with the guppy strains of AFP, AGY, TS and GYC. The following (Table 2) showed the basepair (bp) size of the DNA bands.

The amplified DNA bands appeared in various basepair sizes, starting from 4850.45 bp - 216.70 bp. All of DNA bands appears polymorphic and monomorphic bands as many as 30 DNA bands. Polymorphic bands describe DNA bands that appear only one sample in a certain size and are not present in other samples of that size [10]. While monomorphic band is a DNA band that appears on some samples [11]. The number of DNA bands from each strain show the AFP strain contained 19 monomorphic bands. AGY appears 21 DNA bands (20 monomorphic bands and one polymorphic band). TS has 19 DNA bands (17 monomorphic bands and two polymorphic bands). GYC with 15 DNA bands (14 monomorphic bands and one polymorphic band).

![Fig. 2. Electrophoresis of DNA amplification](image)

Description: M = Marker, AFP=Albino Full Platinum, AGY= Albino German Yellow, TS= Top Sword, GYC= Guppy Yellow Cobra
Table 2. DNA bands amplified By OPA-03 primer

| Fragment DNA | AFP | AGY  | TS   | GYC  | bp Size |
|--------------|-----|------|------|------|---------|
| 359.44       |     |      | -    | -    | 4850.45 |
| 354.62       |     |      | -    | -    | 3841.67 |
| 352.58       |     |      | -    | -    | 3480.81 |
| 350.17       |     |      | -    | -    | 3097.92 |
| 347.05       |     |      | -    | -    | 2663.97 |
| 344.37       |     |      | -    | -    | 2341.08 |
| 342.86       |     |      | -    | -    | 2175.40 |
| 341.08       |     |      | -    | -    | 1996.38 |
| 339.97       |     |      | -    | -    | 1891.87 |
| 338.09       |     |      | -    | -    | 1727.64 |
| 337.03       |     |      | -    | -    | 1638.94 |
| 335.89       |     |      | -    | -    | 1553.14 |
| 334.77       |     |      | -    | -    | 1471.76 |
| 332.06       |     |      | -    | -    | 1290.44 |
| 330.88       |     |      | -    | -    | 1219.23 |
| 328.92       |     |      | -    | -    | 1108.45 |
| 327.55       |     |      | -    | -    | 1037.45 |
| 325.87       |     |      | -    | -    | 957.01  |
| 322.91       |     |      | -    | -    | 829.11  |
| 321.24       |     |      | -    | -    | 764.82  |
| 319.77       |     |      | -    | -    | 712.31  |
| 318.27       |     |      | -    | -    | 662.60  |
| 315.68       |     |      | -    | -    | 584.44  |
| 312.53       |     |      | -    | -    | 501.79  |
| 308.63       |     |      | -    | -    | 415.67  |
| 306.59       |     |      | -    | -    | 376.63  |
| 304.29       |     |      | -    | -    | 337.00  |
| 302.28       |     |      | -    | -    | 305.83  |
| 300.68       |     |      | -    | -    | 283.00  |
| 295.16       |     |      | -    | -    | 216.70  |

*Description: (--*) = monomorphic band; (--*) = polymorphic band

The number of polymorphic bands amplified from the four strains of guppy fish is as much as four bands. One band on AGY, two bands on TS and one polymorphic band appear on GYC strains. While the AFP strain of guppy fish does not give rise to polymorphic band. The polymorphic bands that appear on each strain are amplified at different sizes. Polymorphic band is a DNA band that is amplified at a certain size and appears only on one sample. The appearance of polymorphic band in each sample indicates a genetic variation of the sample [8]. The crosses individuals who have genetic variations, it will increase genetic diversity [12]. This is reinforced by [13] that the presence of genetic variations or the appearance of polymorphic bands could give rise to new genetic structures in subsequent individuals.

AGY brings up a variation of polymorphic band at 1108.45 bp. This polymorphic band presumed expressed the diversity of genetic variations of the AGY strain. By phenotype, this strain has a characteristic body that has metallic yellow color. TS has two variations of DNA bands at 1553.14 and 1290.44 bp. These two DNA bands variations certainly show the genetic variations that TS strains have. As the name, TS had a top sword caudal fin type whose shape tapered upwards like a sword. In addition, TS strains have snakeskin patterns on their bodies, snakeskin patterns that TS strains have is a horizontal direction.

GYC presents a variation of the DNA band, located at 4850.45 bp. One variation of the DNA band or polymorphic band indicates the presence of genetic variations of GYC strains. Typical GYC strains have snakeskin patterns on the body and caudal fins, which in GYC strains snakeskin pattern has a vertical direction on the penducle. This is the difference between GYC strains and others, that it can show a single variation of DNA band.
The appearance of polymorphic and monomorphic bands of the four guppy strains on the agarose gel indicated that the OPA-03 primer matched with the DNA of four guppy strains tested. This result is different from a similar study conducted by [14] in the study, primers that matched with the DNA of guppies were OPH-15 and OPJ-04 primers. The different primers used in the same species does not rule out DNA amplification. This can occur because both OPA, OPH, and OPJ primers are RAPD primers (10 base sequences) with random sequences that are not specific to a particular gene [15].

3.3 Relationship Analysis

The DNA bands produced from the DNA amplification process using the OPA-03 primer is then altered in the form of a matrix binner. It is based on the emergence or absence of DNA band amplification results. The DNA band that appears will be numbered (1) and the band that does not appear will be numbered (0). Furthermore, the binary matrix is translated into NTSys program and generates phylogenetic tree. The dendogram or phylogenetic tree is the result of a combined analysis of the primer that groups populations based on the degree of genetic similarity [16].

Based on phylogenetic tree of the analysis using NTSys showed in Fig. 3, used OPA-03 primer for albino full platinum (AFP), albino german yellow (AGY), top sword (TS) and guppy yellow cobra (GYC) was obtained by two large groups. The first group are AFP and AGY strains with 95% similarity index. The second group is TS and GYC strains with 82% similarity index. Then these two large groups have an association with the similarity index of 50%.

The first group consisted of AFP and AGY with 95% similarity index. Phenotypes of these two strains have a lot in common. AFP and AGY are strains of albino guppy. Albino strains are known as Real Red Eye Albino (RREA) or guppy strains that have red eyeballs [17]. This corresponds to the color of the eyeballs possessed by the AFP and AGY strains, both of which have red eyeball colors. Because it is albino, these two fish are also unable to produce black pigment on their body [18]. In addition, both strains have the same type of caudal fin, that is delta tail type [1].

The phenotype difference between AFP and AGY is the body color. AFP is metallic white, while AGY is metallic yellow. When reviewed by the origin of the cultivated place, these two fish come from different places. AFP comes from ornamental fish breeders in Cilengkrang-Bandung area, while AGY strains come from breeders in Tangerang-Banten area. The difference between these two places of origin has an effect on the genetic differences of fish. The differences in environmental conditions in a fish population will cause variations in phenotypes and fish genotypes. This is because the fish needs to adapt to environment [19].

**Fig. 3. Phylogenetic Tree of Four Guppy Strains**

*Description: The phylogenetic tree is the result of OPA-03 primer and analyzed by NTSys program.*
The magnitude of the genetic relationship value between the AFP and AGY strains will also increase the success rate when these two strains are crossed. This is because the magnitude of the value of genetic relationship can indicate the existence of similar numbers, shapes or sizes of chromosomes. This is reinforced by the opinion of [20] that the success of interspecific hybridization is influenced by two things, the suitability of cariotypes (number, size or shape of chromosomes) and the suitability of reproductive biology of the two individuals to be crossed. So if crossing two individuals with the same number of chromosomes, the success rate will be greater.

The second group, TS and GYC, had 82% genetic relationship index. Phenotypes, these two strains have similarities to their body patterns. TS and GYC strains both have snakeskin body patterns. Snakeskin patterns on TS have a horizontal pattern with yellow colour, while GYC had vertical pattern snakeskin with black colour in the penducle. The direction of the snakeskin pattern is vertical on the pendule of the fish, according to [21] better known as cobra. Both snakeskin and cobra are actually the same type of body pattern, which resembles snake skin. In addition to the pattern of the body, the similarity of these two strains guppy is both cultivated in the same place (Cilengkrang-Bandung). Phenotype differences between TS and GYC strains, among others, are found in caudal fins. Strain TS, as the name implies has the form of fins caudal type top sword or also known as sword tail, is guppy fish with the upper edge of the tail fin has an elongated shape like a sword [22-23]. While GYC strains had delta tail type of caudal fins.

Between the two groups (AFP and AGY with TS and GYC) had 50% similarity index. According to [24], a similarity index showing results greater than or equal to 50% indicates that the samples are the same species. The statement corresponds to the results of the percentage of genetic relationship between the four strains of fish used in this study, because the four strains are one species of fish that is Poecilia reticulata. The split of the four strains into two different groups occurred because each group is able to express their own characteristics. The first group consisting of AFP and AGY strains is expressed through the albino gene, while the second group of TS and GYC is expressed based on the same body pattern of snakeskin.

4. CONCLUSIONS

The results of the phylogenetic tree between the four strains of fish were divided into two major groups. The first group, namely the full platinum albino (AFP) and albino german yellow (AGY) strains, had a genetic relationship value of 95%. The second group is between the top sword (TS) strain and the guppy yellow cobra (GYC) which has a genetic relationship percentage of 82%. Then these two large groups have a relationship with bringing up a genetic relationship value of 50%. The highest genetic variation was owned by the TS strain which was able to produce two polymorphic bands using OPA-03 primers. Meanwhile, the AGY and GYC strains each produced as much as one polymorphic band.

5. SUGGESTIONS

For a high success rate of hybridization, cross between albino full platinum and albino german yellow strains. Meanwhile, to bring up new genetic variations in the offspring, cross between the top sword, guppy yellow cobra, or albino german yellow, where all three produce a polymorphic band.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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