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GENETIC ANALYSIS OF TILAPIA NIRWANA STRAIN (*Oreochromis niloticus*) CULTURED IN LUMAJANG EAST JAVA AND WANAYASA WEST JAVA BY USING RANDOM AMPLIFIED POLYMORPHIC DNA

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Abstract

This research was aimed to analyze genetic relationship of Tilapia Nirwana strain (*Oreochromis niloticus*) cultured in Lumajang and Wanayasa using RAPD-PCR method. This research was conducted from July 2017- October 2018. Sample that was used in this research was Tilapia Nirwana strain which has been cultured in Lumajang and Wanayasa. The method used in this research was explorative with RAPD-PCR technique using primary OPA-03 and OPA-05 and analyzed by descriptive qualitative with NTSYS-PC. The result showed that using OPA-03 could show many DNA bands in samples of fish which cultured in Lumajang and Wanayasa compared to OPA-05. This showed that primary OPA-03 is more efficient than OPA-05 to analyze genetic trait of Tilapia Nirwana strain. The results of analysis with fenogram UPGMAM using primary OPA-03 indicated that the relationship is relatively high with value of 71%.

Keywords: *Tilapia Nirwana strain, Primer OPA-03, RAPD-PCR, Lumajang, Wanayasa, Genetic Relationship.*

INTRODUCTION

Tilapia (Oreochromis niloticus) is one of the leading cultivation commodities that is expected to meet the government's target of increasing aquaculture production by 353% in 2015 (2014 KKP). Tilapia consists of various types of strains, one of which is Tilapia Nirwana strain , which is spread throughout most regions in Indonesia, one of them is Lumajang.

Nirvana Tilapia (O. *niloticus*) is a hybrid fish product among 18 families of GIFT tilapia (*Genetic Improvement of Farm Tilapia)* and 20 families of GET tilapia (*Genetic Enchanted Tilapia*) developed at the BPPINM in Wanayasa Purwakarta West Java and collaborated with the Faculty of Fisheries, Bogor Agricultural Institute (IPB) for three years (Khaeruman 2007). Then Tilapia Nirwana strain is spread to almost all regions in Indonesia, one of which is in the Lumajang area of East Java.

The waters condition in Wanayasa is different from the waters in the Lumajang area where the BPPINM in Wanayasa has environmental conditions with an average water temperature of 18-23°C, pH 6.5-7, DO 3-5 mg/L, annual rainfall is 3,093 mm/year and altitude of 600 mdpl, while the BBI in Lumajang has environmental conditions with an average water temperature of 20-26°C, pH 4.5-7.5, annual rainfall 4,176 mm / year and altitude of 500- 700 mdpl.

With this condition the possibility of Tilapia Nirwana strain will undergo genetic changes due to adjustments to the new environment where the fish is now cultivated, so research on the genetic Tilapia Nirwana strain is cultivated in Lumajang with Tilapia Nirwana strain cultivated in Wanayasa.

Hybrid fish can be seen from the phenotypic characteristics (morphology and color patterns) of the fish, but genetic evidence can clearly be seen from the fish polymorphism by conducting molecular tests at the DNA level. Polymorphism is defined as the presence of different genetic traits in several individuals living together in a population and having a frequency that does not change due to a genetic mutation (Nursida 2011). One alternative to determining this genetic variation can be done molecularly with various methods, including the *Random Amplified Polymorphism* DNA (RAPD).

The RAPD technique is a fast and inexpensive DNA analysis technique in obtaining genetic molecular data (Beaumont and Hoare 2003). Polymorphic DNA fragments detected using the RAPD method can interpret hybrid relationships with male and female parents. Therefore, polymorphic analysis of tilapia is cultivated in Lumajang East Java and Wanayasa West Java.

MATERIALS AND METHODS

The research was conducted in June 2017-October 2018. The DNA isolation activity of Tilapia Nirwana strain was carried out in building 4 of the Biotechnology Laboratory of FPIK UNPAD Jatinangor campus and the PCR process was carried out in building 3 of the Biotechnology Laboratory of FPIK UNPAD Jatinangor.

This research uses descriptive exploratory methods, where research is exploratively examined and analyzed

descriptively qualitatively. Research was carried out by taking tail fin from Tilapia Nirwana strain which was cultivated in Lumajang and Wanayasa areas as much as 5 grams. The fish sample was then tested for DNA in the laboratory. DNA testing was carried out using the RAPD-PCR method (*Random Amplified Polymorphic DNA - Polymerase Chain Reaction*). The DNA test results are then analyzed using the NTSYS - PC program.

DNA Extraction

Isolation process using the Wizard Genomic DNA purification Kit (Promega). 10 mg of caudal fins were inserted into a 1.5 mL tube and mashed until smooth, added 300 µl of Nuclei Lysis Solution, homogenized by vortex for 10 seconds, incubated at 65 ^{o}C for 30 minutes, added 1.5 µl of RNAse Solution, reversed 2-5 times, incubated at 37° C for 30 minutes, cooled at room temperature for 5 minutes, added 100 µl of Protein Precipitation Solution, homogenized by vortex for 10 seconds, cooled in ice cured for 5 minutes, centrifuged at 13,000 rpm for 4 minutes, Supernatan transferred to the new tube, added 300 µl of isopropanol, homogenized and centrifuged at 13,000 rpm for 1 minute, Supernatan was discarded, added 300 µl of ethanol 70%, centrifuged at 13,000 rpm for 1 minute, Ethanol was discarded, pellets dried for 15 minutes, added 50 µl of Rehydration Solution, incubated at 65 ^{o}C for 1 hour, the tube was stored at -20 ^{o}C.

Calculation of DNA Concentration

Measurement of DNA quantity was carried out by spectrophotometric method using spectrophotometer at wavelength (λ) 260 and 280 nm. Switched on the spectrophotometer, select the DNA test program, prepare 250 µl of standard solution (aquadest) and put it in a special cuvet for aquadest. The Cuvet is inserted into a spectrophotometer and select measure blank and taken 245 µl of aquadest and 5 µl of template DNA and put into a special cuvet for the test sample.

DNA Amplification

The amplification process was carried out by mixing 2 ql of DNA template (test DNA sample), 1.25 ql of primer, 12.5 ql of master mix, 9.25 ql of nucleus free water as filler or balancer. Then flashed for a few seconds on the centrifugator to homogenize.

The solution mixture was put in a thermalcycler machine (PCR) with program: Pre Denaturation (94^{o}C, 2 minutes, 1 cycle), Denaturation (94^{o}C, 1 minute, 34 cycles), Annealing (36^{o}C, 1 minute, 34 cycles), Extension (72^{o}C, 2 minutes, 34 cycles), Final Extension (72^{o}C, 7 minutes, 34 cycles), Hold (4^{o}C, 3 minutes, 1 cycle).

Electrophoresis

Weighed 0.56 grams of agarose and added 40 mL TBE, heated in a microwave for one minute until boiling and homogeneous. Cooled the solution, then poured into agarose gel mold. After harden, the gel is placed on an electrophoresis container which has been added with a TBE buffer solution.

Each test sample piped 4 ql and 2,5 ql of marker, placed on paper film, then added 2 ql of staining solutions (loading

dye). Mixed well using a micropipette, then the mixture of the solution is injected into the agarose gel well. Add the TBE Buffer solution to cover agarose gel. Electrophoresis was carried out with 75 Volt for 80 minutes. Whereas the electrophoresis process of the isolation result was carried out with 75 Volt for 45 minutes and 1% agarose gel concentration.

DNA visualization

Soaking agarose gel in a mixture of 100mL aquadest and 6 µl EtBr for 20 minutes, then rinsed by soaking the agarose gel in 100mL of aquadest for 7 minutes. Furthermore, the agarose gel was placed on top of the UV-Transluminator and documented with the camera for analysis.

Data Analysis

The length of the base sequence of the sample DNA can be known by comparing the distance of the standard DNA displacement (DNA marker) with Corel Draw X6 and Microsoft Excel program. Calculation of similarity index is done using the NTSYS-PC program. The results obtained in the form of Relationship tree diagrams (phylogeny).

The genetic distance matrix can be obtained from the results of genetic relationship analysis (Lee 1998) with the formula:

$$S = 1 - GS$$

Note :
S = genetic distance
GS = Genetic Similarity

The Genetic Similarity level is estimated from the data on the number of alleles using the Jaccard coefficient (Rohlf 2000) with the formula:

$$GS = \frac{m}{(n + u)}$$

Note :
m = Number of DNA band with same position
n = Number of DNA Band
u = Number of DNA band with different position

RESULT AND DISCUSSION

Genomic DNA Isolation

DNA isolation is a technique of separating DNA molecules from other molecules found in the cell nucleus. The method of DNA isolation in this study uses the *Wizard Genomic DNA Purification Kit* (Promega).

The test samples used were caudal fins as samples in the process of isolation because the caudal fin is a soft tissue that is easily destroyed so it is easy to get samples and will not interfere with fish activity.

In Figure 1, based on isolated DNA electrophoresis, several samples of DNA isolates visualized in the agarose gel still have many smears. The appearance of smears signifies the existence of material other than DNA which is isolated, for example RNA or protein. To reduce the presence of smears, you can add RNAse Solution and Isopropanol.

The next step is to determine the quality of genomic DNA produced by the DNA isolation process using a spectrophotometer based on the ratio of the ratio of absorbance values of A_{260} nm and A_{280} nm. The results of genomic DNA isolation that have good purity levels are

ranging from 1.8 to 2.0 (Sambrok and Russel 2001).

The absorbance ratio at wavelength (A_{260}/A_{280}) which is above the range of pure DNA values shows that there are RNA contaminants, whereas for values below 1.8, there are protein contaminants (Santella 2006). In Table 1, the results of samples of Nirvana tilapia cultivated in Lumajang, East Java and Wanayasa, West Java, showed purity values ranging from 1,742 - 1,893 indicating that the samples were suitable for the amplification process.

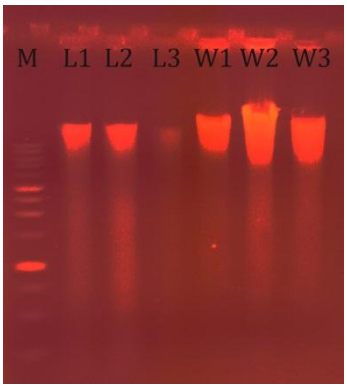

Figure 1. Electrophoresis Results of Genome DNA Isolation in Lumajang East Java and Wanayasa West Java

Note:

M : Marker 1 kb

L1 : Lumajang East Java Tilapia Nirwana strain Fin Samples 1
L2 : Lumajang East Java Tilapia Nirwana strain Fin Samples 2
L3 : Lumajang East Java Tilapia Nirwana strain Fin Samples 3
W1 : Wanayasa West Java Tilapia Nirwana strain Fin Samples 1
W2 : Wanayasa West Java Tilapia Nirwana strain Fin Samples 2
W3 : Wanayasa West Java Tilapia Nirwana strain Fin Samples 3

Table 1. Purity of DNA Isolation Results

No	Sample	DNA Purity		Value of DNA Purity
		Abs 260nm	Abs 280nm	
1	Lumajang (L-1)	0,155	0,089	1,742
2	Lumajang (L-2)	0,199	0,114	1,746
3	Lumajang (L-3)	0,053	0,028	1,893
4	Wanayasa (W-1)	0,437	0,245	1,784
5	Wanayasa (W-2)	0,330	0,182	1,813
6	Wanayasa (W-3)	0,255	0,143	1,783

DNA Amplification RAPD Method and Polymorphism Analysis

The results of PCR amplification based on genetic properties can be seen using the OPA primer (*Operon Primer set-A).* Test results from primary use (OPA-03 and OPA-05) showed different results from each primer used, for the primary OPA-03 produced 16 bands while for the primary OPA-05 produced 12 bands. According to Wels *et al.* 1998 *in* Lia 2006, generally the number and size of fragments produced depends on the nucleotide sequence and DNA source.

Each primer has different fragment attaching characters because each primer has its own attachment system so that fragments from amplified DNA using different primers will produce polymorphic with different fragments and molecular weights (Roslim 2001).

In Figure 2, the results of DNA amplification using OPA-03 and OPA-05 primers can give rise to many polymorphic and monomorphic bands which vary in all samples showing good results. This shows that OPA-03 and OPA-05 primers have a primary base sequence suitable for use with genomic DNA samples of Tilapia Nirwana strain cultivated in Lumajang East Java and Wanayasa West Java.

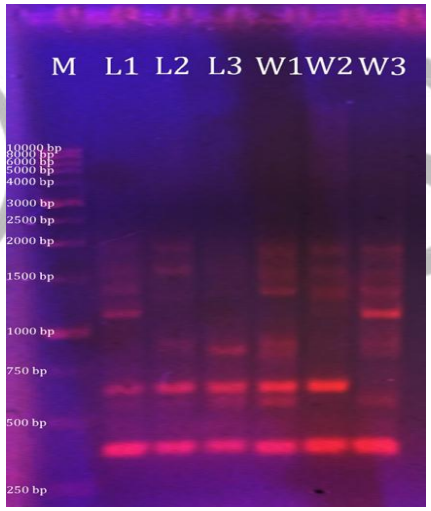

Figure 2. DNA Amplification Results Using OPA-03

Note:

M : Marker 1 kb

L1 : Lumajang East Java Tilapia Nirwana strain Fin Samples 1
L2 : Lumajang East Java Tilapia Nirwana strain Fin Samples 2
L3 : Lumajang East Java Tilapia Nirwana strain Fin Samples 3

W1 : Wanayasa West Java Tilapia Nirwana strain Fin Samples 1
W2 : Wanayasa West Java Tilapia Nirwana strain Fin Samples 2
W3 : Wanayasa West Java Tilapia Nirwana strain Fin Samples 3

Table 2. Polymorphic Band and Monomorphic Band From OPA-03

L1	L2	L3	W1	W2	W3	M (Fragment Distance)	Y
	..		..	..	..	127,722	2191
..	..	..	..	..	..	130,367	2108
..	..		..	..	..	132,536	2043
..	..	..	..	..	..	134,006	2000
..	..	..	..	..	..	146,209	1675
..		..	..	..	..	159,438	1383
	..*					165,259	1271
..					..	172,138	1150
	..		..		..	187,749	917
	..	..	..		..	193,305	846
	..	..	..	..		212,884	637
..	..	..	..	..		215,001	618
		..	..		..	220,557	570
..	..	..	..		..	223,732	544
		..		..	..	246,222	393
..	..	..	..	..	..	248,074	382

Note : ..* Polymorphic Band| .. Monomorphic band

Based on the results of Table 2 it can be seen that by using OPA-03 various polymorphic and monomorphic bands that appear in each sample both from Lumajang East Java and Wanayasa West Java.

The appearance of polymorphic bands indicates genetic variation in Nirwana Lumajang and Wanayasa tilapia samples, while the number of emerging monomorphic bands indicates that Nirwana Lumajang and Wanayasa tilapia have close relationship.

Table 3. Results of DNA Amplification Products OPA-03

Sample	Amplified Fragments	Polymorphic Bands	Monomorphic Bands	(%) Polymorphic	(%) Monomorphic
L1	9	0	9	0%	100%
L2	12	1	11	8,33%	91,67%
L3	11	0	11	0%	100%
W1	13	0	13	0%	100%
W2	10	0	10	0%	100%
W3	13	0	13	0%	100%

From the results of amplification using the OPA-03 primer showing the number of different bands in each test sample obtained from Lumajang East Java and Wanayasa West Java. This is due to differences in environmental conditions in each sample. Tilapia Nirwana strain from West Java can not produce polymorphic bands. The appearance of polymorphic bands defines the variation of genetic traits

in Tilapia Nirwana Lumajang and Wanayasa samples. In Table 3, a single Nirwana Lumajang tilapia sample (L1) contained a polymorphic band which showed a percentage of polymorphism of 8.33%.

In the phenogram (Figure 3) the results of Unweighted Pair Group Method Arithmatic Mean (UPGMAM) analysis using OPA-03 primers from all samples from Lumajang East Java and Wanayasa West Java obtained three large groups of six test samples. The first group consisted of samples of Tilapia Nirwana strain Lumajang two (L2), Wanayasa one (W1), and Wanayasa three (W3), with a similarity index value of 0.78, this indicates that there is a very close level of relationship from the three samples both from Lumajang East Java, and samples from Wanayasa West Java, and can be interpreted by the similarity of the genetic characteristics of 78%. This is caused by the number of DNA bands in each test sample that appear at the same distance from the fragment.

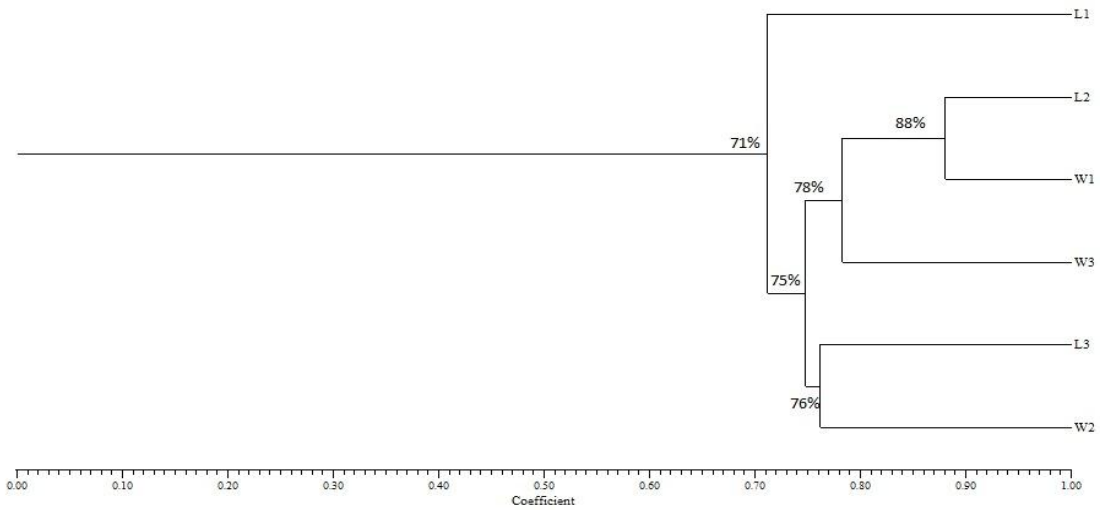

Figure 3. Phenogram of OPA-03 Tilapia Nirwana strain Lumajang and Wanayasa

The second group consisted of samples of Tilapia Nirwana strain Lumajang three (L3) and Wanayasa two (W2) with a similarity index value of 0.76 which means it has a very close similarity in genetic traits of 76%, indicating a very close level of relationship from the two the sample. This is probably due to the Nirwana Lumajang tilapia breeders that still originate from Wanayasa, and the Wanayasa and Lumajang regions have environmental conditions that do not differ greatly so that genetic changes do not occur.

The third group is between samples of Nirwana Lumajang Tilapia 2 (L2), Wanayasa one (W1), and Wanayasa three (W3), Lumajang three (L3) and Wanayasa two (W2) with similarity index values of 0.75, this indicates that the level of kinship seen from the genetic traits is quite close from the two samples and can be interpreted by the similarity index value of 75%.

In all samples of genetic relationship of Tilapia Nirwana strain cultured in Lumajang and Wanayasa the similarity index is 71%. This shows that Nirwana

tilapia in Lumajang and Wanayasa has a high level of relationship even though it does not reach 100%. This is because the ribbon fragments that appear in the sample have different sizes and not all are in the same size of the fragment.

CONCLUSION

The genetic similarity between Tilapia Nirwana strain cultivated in Lumajang East Java and Wanayasa West Java, is 71%. Only one polymorphic band appeared in the Lumajang East Java sample. This shows that Nirvana tilapia has not experienced genetic changes and can adapt well.

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