Original Research Article

Genetic Analysis of (Oreochromis niloticus) Nirvana Tilapia Cultivated in Wanayasa and Galunggung, Lumajang, Bali, Minahasa Using Random Amplified Polymorphic DNA method

ABSTRACT

This research aims to determine kinship of nirvana tilapia (*Oreochromis niloticus*) cultivated in Wanayasa with Galunggung, Lumajang, Bali, Minahasa by Random Amplified Polymorphic DNA method. All tilapia cultivated in these four places originated from Wanayasa. This research was conducted in Biotechnology Laboratory, Laboratory of Microbiology and Molecular Biotechnology, FPIK UNPAD. The research method was an explorative experiment with RAPD-PCR technique using primary OPA-3 and OPA-5. However, the results of DNA amplification with OPA-3 were better than OPA-5. Therefore, further analysis was carried out using data from OPA-3. Kinship was analyzed based on the similarity index which calculated by *Numerical Taxonomy and Multivariate Analysis System* (NTSYS) program. The results showed that the kinship between Nirvana tilapia cultivated in Wanayasa with Galunggung, Lumajang, Bali, and Minahasa, according to the similarity index was high, about 77-85%. The highest similarity index (85%) was obtained between nirvana tilapia cultivated in Wanayasa with Galunggung, and the lowest similarity index (77%) between tilapia cultivated in Bali with Minahasa. Genetic changes that occur because of differences in cultivation environments ranging from 15-23%.

Keywords: Tilapia, RAPD-PCR, Nirvana, Oreochromis niloticus, Wanayasa, Minahasa, Bali, Lumajang, Galunggung

1. INTRODUCTION

Tilapia (*Oreochromis niloticus*) is an important commodity in freshwater aquaculture (Gustiano, 2009). The fish has inexpensive cultivation cost, rapidly growing, high resistance to disease, and broad tolerance to environmental changes. So that, the development of tilapia cultivation quite widely in several regions in Indonesia (Iskandariah, Arifin, & Gustiano, 2011). At present, the production of tilapia is the second largest as an aquaculture commodity after the fish commodity from family Cyprinidae, with a production of more than 2 million metric tons or about 5% of global production of aquaculture. The main producers of world tilapia, among others: China, Indonesia, Egypt, Philippines, Mexico, Thailand, Taiwan and Brazil (BKIPM, 2017).

Tilapia has several varieties, one of which is Nirvana tilapia (Tilapia Ras Wanayasa). Nirvana tilapia was a result of genetic improvement done by the Development Center for Tilapia Stock and goldfish Wanayasa (BPPSINM), Purwakarta, West Java. Nirvana tilapia has been around for several years, which was released in 2006 (Reference).

Nirvana Tilapia was developing and spreading to several regions in Indonesia including the Minahasa, Bali, Lumajang, Galunggung and still exist in several other places in Indonesia. Environmental changes will cause genetic differences in the nirvana tilapia cultivated in Wanayasa and some of these places. Many factors that influence genetic differences include temperature, pH, rainfall, altitude, etc.

The environmental conditions in Wanayasa have a temperature of around 23.4-30ºC, pH value of about 6.5-8 with an altitude of around 668 masl, and rainfall of 3,093 mm/year. The Galunggung area has temperatures ranging from 22-30ºC, pH 6, rainfall of 2,687 mm/year and an altitude of 613 masl. In the Lumajang area, the average temperature ranges from 20,-6 -21,-7 ºC with 3,763 mm/year of rainfall. In Bali, the temperature ranges from 23-30.4ºC, rainfall is 1,230 mm/year and the height is 100-150 masl. Whereas in Minahasa has a temperature of 25.5ºC, the pH value of the water is around 6.8-8.2 and has an altitude of 600 meters above sea level.

Environmental differences in the four places might cause mutations or genetic changes. The method that can be used to determine genetic changes and kinship was the Random Amplified Polymorphic DNA or commonly referred to as RAPD-PCR (Edward, Dearherage, & Ernsting, 2004).

The RAPD technique was useful and sensitive in differentiating various fish genera and species (Asagbra, Adebayo, Ugwumba, & Anumudu, 2014). This technique was widely used in identifying diversity at the intraspecies (Pacheco, Guth, Almeida, & Ferreira, 1996) and interspecies levels (Hadrys, Balick, & Schierwater, 1992). Therefore, this research aims to determine kinship of tilapia in the four cultivation sites using the RAPD (Random Amplified Polymorphic DNA) method.

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2. MATERIAL AND METHODS

The research was conducted in November 2017 until February 2018. The research activities were carried out at the Biotechnology Laboratory of FPIK UNPAD. The test samples were caudal fin of nirvana tilapia cultivated in Wanayasa, Galunggung, Lumajang, Bali, and Minahasa. All nirvana tilapia cultivated in Galunggung, Lumajang, Bali, and Minahasa originally came from Wanayasa.

DNA Isolation

DNA isolation was carried out to separate chromosomal DNA or genomic DNA from other cell components. Five mg caudal fins were isolated using the Wizard Genomic DNA Purification Kit (Promega).

DNA Quantifications

DNA quantification was carried out by using spectrophotometry. Double band DNA can absorb UV light at λ 260 nm, while protein or phenol contaminants will absorb light at λ 280 nm. DNA purity can be measured by calculating the absorbance value of λ 260 nm divided by the absorbance value of λ 280 nm (A260 / A280). Good-quality DNA for molecular analysis will have an A_{260}/A_{280} ratio of 1.8–2.0 (Sambrook and Russel, 2001). The Formula for calculating double stranded DNA concentrations according to Barbas, Button, Scoot, and Silverman (2001):

Concentration (µg/ml) = A_{260} reading – A_{320} reading) × dilution factor × 50µg/ml
Dilution factor = 50 times

DNA Amplification

The DNA was then amplified using the OPA-3 Primer (5'AGsTCAGCCAC-3 ') and OPA-5 primer (5'AGGGGTCTTG-3'). Amplification was carried out using the PCR method with the composition of the ingredients: GoTaq® green master mix as much as 12.5 µl, OPA-3 primer as much as 1.25 µl, Template DNA 2 µl, and Nucleus Free Water 9.25 µl. Furthermore, it is included in a thermocycler with a cycle of 34 cycles, namely one cycle of initial denaturation at 94ºC for 2 minutes, 34 subsequent cycles consisting of denaturation at 94ºC for 1 minute, 36ºC annealing for 1 minute and elongation of 72ºC for 2 minutes. Final elongation at 72ºC for 7 minutes. The PCR results were seen through electrophoresis.

Kinship Analysis

Kinship can be known by calculating the similarity index based on amplified numeric data bands. Kinship was analyzed based on the similarity index calculated through the Numerical Taxonomy and Multivariate Analysis System (NTSYS) program.

3. RESULTS AND DISCUSSION

DNA Isolation

Isolation of genomic DNA is the process of separating DNA molecules from other molecules in the cell nucleus. DNA isolation is a process to obtain pure DNA that can be used for examination and diagnosis purposes in an organism (Chawla, 2000). The isolated genomic DNA can be known for its existence and quality using electrophoresis. The Electrophoresis process carried out by using a potential power of 75 volts for 45 minutes with an agarose gel concentration of 1%. The agarose gel from electrophoresis was then immersed in Ethidium bromide (Etbr) and visualized with UV light.

The results of electrophoresis in Figure 1 showed that the DNA samples were more than 10.000 bp. Smeared bands were seen on the agarose gel. These were due to the presence of contaminants of protein and RNA in DNA isolates. These contaminants can inhibit the amplification process (PCR) (Fatchiyah, Widyarti, & Rahayu, 2011). Therefore, DNA samples were purified by adding RNAse. Then, DNA purity and concentration were measured by using spectrophotometer.

The DNA concentration results in Table 1 showed that the DNA purity in the sample from Wanayasa (WN) was 1,7810. while in the sample from Galunggung (G) was 1,7792. In the sample from Lumajang (L) was 1,7893. Then in the sample from Bali (B) was 1,7724 and the sample from Minahasa was 1,7819.

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Sambrook and Russell (2001) stated that the results of DNA isolation can be said to be pure if the value of the ratio is λ 260 nm and λ 280 nm between 1,8 to 2,0. The absorbance ratio at a wavelength (A260 / A280) which is above the range of pure DNA values indicates that there are RNA contaminants. Whereas values below 1,8 indicate protein contaminants (Santella, 2006). The results of DNA purity from several samples ranged from 1,7 to 1,8. These indicate that the results of the DNA isolation can be used for the amplification process.

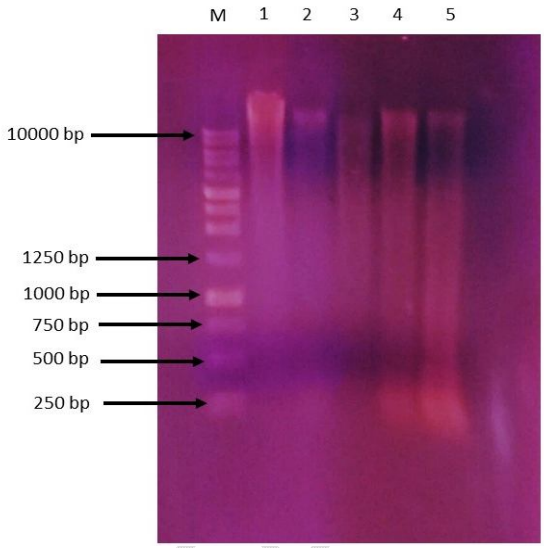

Figure 1. Agarose gel electrophoresis of Tilapia's DNA

M : Marker 1 Kb DNA ladder
1 = Wanayasa Nirvana tilapia
2 = Galunggung Nirvana tilapia
3 = Lumajang Nirvana tilapia
4 = Bali Nirvana tilapia
5 = Minahasa Nirvana tilapia

Table 1. Result of DNA Concentration of Nirvana tilapia

Sampel DNA	Abs 260 nm	Abs 280 nm	Result	Concentration (ng/µl)
Wanayasa (WN)	0,257	0,142	1,810	64,25
Galunggung (G)	0,086	0,048	1,792	21,50
Lumajang (L)	0,053	0,028	1,893	13,25
Bali (B)	0,100	0,058	1,724	25
Minahasa (MH)	0,081	0,019	1,819	20,25

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RAPD-PCR Analysis

The amplification results showed that OPA-3 produces more DNA fragments than OPA-5. Amplification with OPA-3 showed DNA band fragments for each sample. Whereas, the results of the amplification of Genomic DNA with OPA-5 did not show any bands for each sample. So that, further analysis only uses data from OPA-3.

Based on the results of the amplification interpretation (Table 2), ~~Electrophoresis~~ electrophoresis result (Figure 2), and phenogram (Figure 3), an estimation of the genetic changes between the above species was 15-23%. The genetic changes were characterized by the presence of polymorphic bands in each sample. In the Wanayasa (WN) sample, there is one polymorphic band at 1380.25 bp. Whereas in the Galunggung (G) sample, there are two polymorphic bands at 1223.12 bp, and 1478.45 bp. In the sample Lumajang (L) at 581.51 bp and 843.39. Then, in the Bali sample (B), there are two polymorphic bands at 764.83 bp and 1059.45 bp. In the Minahasa sample (MH), there are two polymorphic bands at 1268.95 bp and 1412.98 bp.

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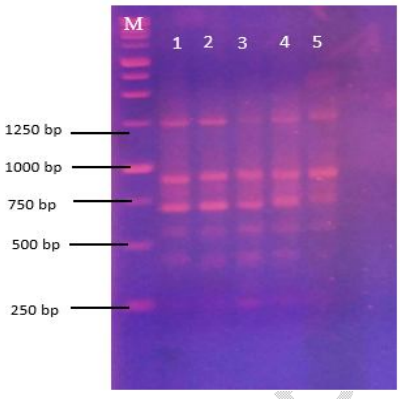
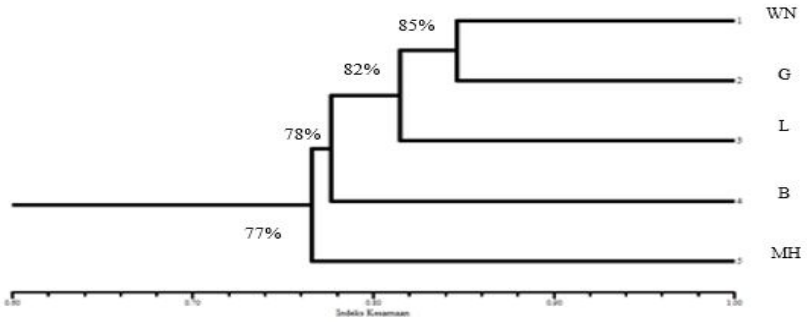


Figure 2. Electrophoresis of DNA amplification of Nirvana tilapia with OPA-3

M : Marker 1 Kb DNA ladder
1 = Wanayasa Nirvana tilapia
2 = Galunggung Nirvana tilapia
3 = Lumajang Nirvana tilapia
4 = Bali Nirvana tilapia
5 = Minahasa Nirvana tilapia



WN = Wanayasa B = Bali
G = Galunggung MH = Minahasa
L = Lumajang

Figure 3. Phenogram of Nirvana tilapia

Table 2. Interpretation of DNA amplification of Nirvana tilapia with OPA-3

---^ = Monomorphic band ---* = Polimorphic band

M (Fragment distance)	WN	G	L	B	MH
1674,86	--^	--^	--^	--^	--^
1629,03	--^	--^	--^	--^	--^
1602,85	--^	--^	--^	--^	--^
1550,47				--^	--^
1478,45		--*			
1412,98					--*
1380,25	--*				
1334,42			--^		--^
1268,95					--*
1223,12		--*			
1196,93	--^	--^	--^	--^	--^
1170,74	--^	--^	--^	--^	--^
1151,13	--^	--^	--^	--^	--^
1124,92	--^	--^	--^	--^	--^
1059,45				--*	
1000,52			--^		--^
961,24	--^	--^	--^	--^	--^
928,59	--^	--^	--^	--^	--^
902,32	--^	--^	--^	--^	--^
843,39			--*		
764,83				--*	
692,81	--^	--^	--^	--^	--^
646,98			--^	--^	
581,51			--*		
516,04	--^	--^	--^	--^	--^
509,59	--^			--^	

Genetic changes that occur were presumably due to differences in temperature, pH, rainfall, altitude, and others. These environtmental factors from each place allow the occurrence of genetic mutations in fish. Nugroho, Subagja, Asih, and Kurniasih (2006) also found that genetic diversity of Tor soro collected from kuningan (Pesawahan, Gandasoli, Ragawacana), and Sumedang was significant different among collection.

In Galunggung (G), Lumajang (L), Bali (B), and Minahasa (MH) samples, there are two polymorphic bands in each region. Whereas in the Wanayasa sample (WN), there is only one polymorphic band. This indicates that Nirvana tilapia in four regions have higher genetic diversity than Nirvana tilapia in Wanayasa. According to Imron, Arifin, and Subagya (2000) genetic diversity can affect the organism ability to respond to the natural and artificial selection. High diversity populations can also indicate a high capacity to adapt to stressful environments, productivity and

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population persistence than low diversity populations (Markert, Champlin, Gutiahr-Gobell, Grear, Kuhn, McGreevy, Roth, Bagley, & Nacci, 2010; Madduppa, Timm, & Kochzius, 2018).

Acording to Kusmini, Gustiano, and Mulyasari (2012) the high level of polymorphism in the population shows the effectiveness of individuals in the process of selection and reproduction in their habit. Conversely, the low level of population polymorphism is thought to be related to barriers to gene flow by environmental factors. The higher the number of polymorphic alleles contained in fish DNA, the easier it is for selection purposes. So that, It can be said that the Nirvana tilapia cultivated in Galunggung, Minahasa, Lumajang, and Bali is superior to Nirvana tilapia in Wanayasa.

From the results of the phenogram showed in Figure 3, it can be seen that the nirvana tilapia cultivated in Wanayasa with Galunggung, Lumajang, Bali and Minahasa using OPA-3 were grouped into 4 groups. In the first group is Wanayasa Nirvana tilapia (WN) and Galunggung Nirvana tilapia obtaining (G) similarity index about 0.85 or if it is set at 85%. The second group is Wanayasa Nirvana tilapia (WN) with Galunggung (G), and Lumajang obtaining a similarity index of 0.82 or 82%. The third group, between Lumajang Nirvana tilapia (L) and Bali Nirvana tilapia obtaining a similarity index of 0.78 or 78%. In the fourth group, which is between Bali Nirvana tilapia (WN) and Minahasa Nirvana tilapia (MH) obtaining the similarity index of 77%.

The highest similarity index (85%) was obtained between Wanayasa Nirvana tilapia with Galunggung Nirvana tilapia. This might be due to the distance between Wanayasa and Galunggung was quite close and within one province. Organisms cultivated in adjacent places will have higher kinship values than organisms in far-off regions.

A high similarity index can also be caused by similar environmental factors. The temperature in Wanayasa (23.4-30ºC) with temperature in Galunggung (22-30ºC) is not much different. The altitude is not to much different. The Environmental factors that were not too different caused low genetic changes in tilapia that were cultivated in Galunggung. So that, the index of similarity with Wanayasa Nirvana tilapia was high

The lowest similarity index (77%) between tilapia cultivated in Bali with Minahasa. This can happen because the two places are on different islands so the environmental conditions are also very different. However, overall, the relationship between Nirvana tilapia cultivated in Wanayasa with Galunggung, Lumajang, Bali and Minahasa, according to the similarity index is high, which ranges from 77-85%. This indicates that between the nirvana tilapia cultivated in Wanayasa with four other regions, Galunggung, Lumajang, Bali, and Minahasa have a close kinship.

Conclusion

Based on the results of the study it can be concluded that:

1. Nirvana tilapia which was originally cultivated in Wanayasa undergo genetic changes when cultivated in Galunggung, Lumajang, Bali, and Minahasa about 15-23%.
2. This genetic changes were characterized by the presence of 2 polymorphic bands in tilapia that are cultivated in these four places, while only 1 polymorphic band found in tilapia that is cultivated in Wanayasa. This signifies the emergence of excellence in response to changes in the environment.
3. The relationship between Nirvana tilapia cultivated in Wanayasa and Galunggung, Lumajang, Bali, and Minahasa, according to the similarity index is high, ranging from 77-85%.

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