Original Research Article

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

UNDER PEER REVIEW

ABSTRACT

This research aims to determine the kinship of nirvana tilapia (*Oreochromis niloticus*) cultivated in Wanayasa with Galunggung, Lumajang, Bali, and Minahasa by Random Amplified Polymorphic DNA method. All tilapia cultivated in these four places originated from Wanayasa. This research was conducted in the Biotechnology Laboratory, Laboratory of Microbiology and Molecular Biotechnology, FPIK UNPAD. The research method was an explorative experiment with the RAPD-PCR technique using primary OPA-3 and OPA-5. However, the results of DNA amplification with OPA-3 were better than with OPA-5. Therefore, further analysis was carried out using data from OPA-3. Kinship was analyzed based on the similarity index which was 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 range 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 costs, rapidly growing, has high disease resistance, and has broad tolerance to environmental changes. So, the development of tilapia cultivation is quite widespread in several regions in Indonesia (Iskandariah et al., 2011). At present, the production of tilapia is the second largest aquaculture commodity after the fish commodity from the 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: are 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.

Nirvana Tilapia was developing and spreading to several regions in Indonesia including Minahasa, Bali, Lumajang, and 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 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 et al., 2004).

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

2. MATERIAL AND METHODS

The research was conducted from 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 A260/A280 ratio of 1.8–2.0 (Sambrook and Russel, 2001). The Formula for calculating double-stranded DNA concentrations according to Barbas et al (2001):

$$\text{Concentration } (\mu g/ml) = A_{260} \text{ reading} - A_{320} \text{ reading}) \times \text{dilution factor} \times 50 \mu g/ml$$

$$\text{Dilution factor} = 50 \text{ times}$$

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 1,810. while in the sample from Galunggung (G) was 1,792. In the sample from Lumajang (L) was 1,893. Then in the sample from Bali (B) was 1,724 and the sample from Minahasa was 1,819.

UNDER PEER REVIEW

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.

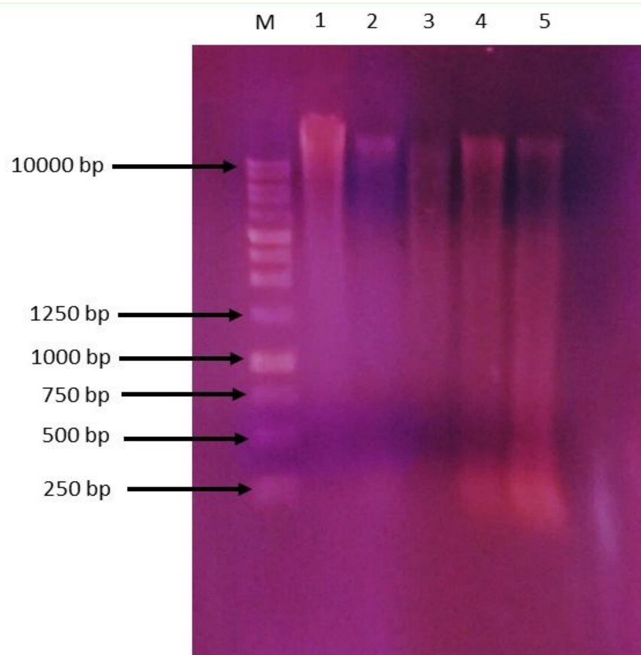


Figure 1. Agarose gel electrophoresis of Tilapia's DNA

M :Marker 1 Kb DNA ladder
1 = WanayasaNirvana tilapia
2 = GalunggungNirvana tilapia
3 = LumajangNirvana tilapia
4 = Bali Nirvana tilapia
5 = MinahasaNirvana 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	25.00
Minahasa (MH)	0.081	0.019	1.819	20.25

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,

UNDER PEER REVIEW

the results of the amplification of Genomic DNA with OPA-5 did not show any bands for each sample. So, the further analysis only uses data from OPA-3.

Based on the results of the amplification interpretation (Table 2), 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 (Table 2).

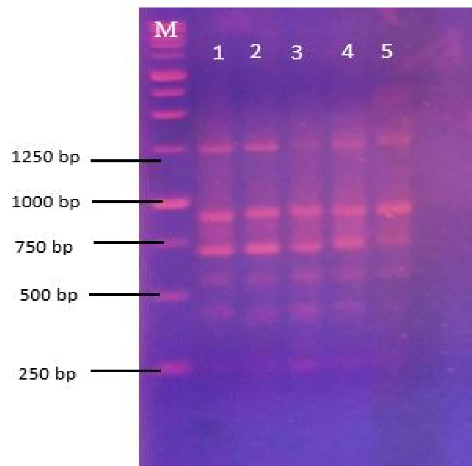
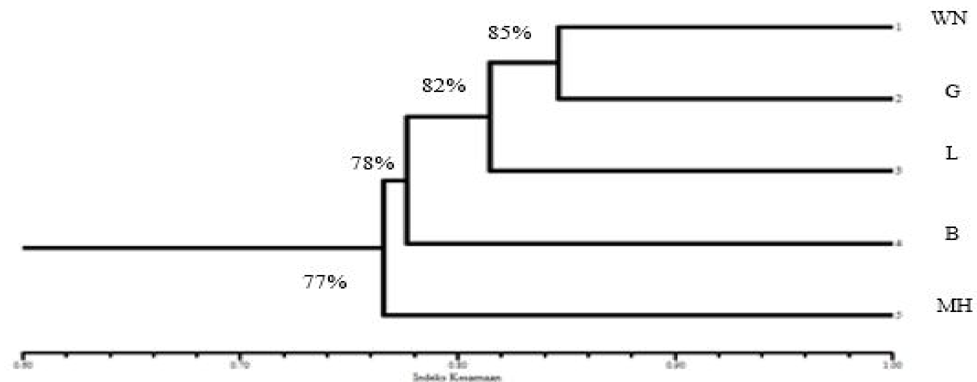


Figure 2. Electrophoresis of DNA amplification of Nirvana tilapia with OPA-3

M : Marker 1 Kb DNA ladder
1 = WanayasaNirvana tilapia
2 = GalunggungNirvana tilapia
3 = LumajangNirvana tilapia
4 = Bali Nirvana tilapia
5 = MinahasaNirvana tilapia



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

Figure 3. Phenogram of Nirvana tilapia

UNDER PEER REVIEW

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

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	--^			--^	

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

Genetic changes that occur were presumably due to differences in temperature, pH, rainfall, altitude, and others. These environmental factors from each place allow the occurrence of genetic mutations in fish. Nugroho et al (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 et al (2000), genetic diversity can affect the organism's ability to respond to natural and artificial selection. High

UNDER PEER REVIEW

diversity populations can also indicate a high capacity to adapt to stressful environments, productivity, and population persistence than low diversity populations (Markert et al., 2010; Madduppa et.al, 2018).

According to Kusmini et al (2012), the high level of polymorphism in the population shows the effectiveness of individuals in the process of selection and reproduction in their habits. 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, 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 shown 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 are Wanayasa Nirvana tilapia (WN) and Galunggung Nirvana tilapia obtaining (G) similarity index of 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 obtained a similarity index of 0.78 or 78%. The fourth group, which is between Bali Nirvana tilapia (WN) and Minahasa Nirvana tilapia (MH) obtained a 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 being 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) and with temperature in Galunggung (22-30ºC) is not much different. The altitude is not too much different. The Environmental factors that were not too different caused low genetic changes in tilapia that were cultivated in Galunggung. So, 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 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. These 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 is 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%.

UNDER PEER REVIEW

REFERENCES

Asagbra, M.C., Adebayo, A.S., Ugwumba, A.A.A.,&Anumudu, C.I. (2014). Genetic characterization of fin fish species from the Warri River at Ubeji, Niger Delta, Nigeria. *African Journal of Biotechnology*,13 (27). http://dx.doi.org/10.5897/AJB2013.11982

Badan Karantina Ikan, PengendalianMutu, dan Keamanan Hasil Perikanan. 2017. Risk Analysis of Tilapia Lake Virus Disease in *Oreochromis niloticus*.Retrieved http://www.bkipm.kkp.go.id

Barbas, C.F., Button,D.R., Scoot,J.K., Silverman, G.J. (2001). Quantitation of DNA and RNA Adapted from "General Procedure", Appendix 3. NY, USA, Cold Spring Harbor.

Chawla, H.S. (2000). Introduction to Plant Biotechnology. USA, Science Publishers. Inc.

Edward, D. D., Dearherage, D. E., and Ernsting, B. R. (2004). Random Amplified Polymorphic DNA Analysis of Kinship Within Host-Associated Populations of The Symbiotic Water Mite *Unionicolafolli* (Acari: Uniocolidae). *Experimental & Applied Acarology* 34 (1-2), 67. DOI: https://doi.org/10.1023/B:APPA.0000044440.80795.48

Fatchiyah, A.E.L., Widyarti, &Rahayu, S. (2011). Molecular Biology. Basic Principle of Analysis. Jakarta. Erlangga.

Gustiano, R. (2009). BEST Tilapia, New Superior Commodity, Quality Expectations. TrobosNovember, 116-117.

Hadrys, H., Balick, M., andSchierwater, B. (1992). Applications of Random Amplified Polymorphic DNA (RAPD) in Molecular Ecology**.***Molecular Ecology*, 1(1), pp. 55-63. https://doi.org/10.1111/j.1365-294X.1992.tb00155.x

Imron, Arifin, O. Z, dan Subagya. (2000). Characterization of Monomorphic Truss in Carp (Cyprinus carpio) Majalaya, Rajadanu, Wildan, and Sutisna Strains. *Proceedings of the Conference on Fisheries Research Results.* Jakarta, Center for Marine Exploration and Fisheries. Department of Marine Exploration and Fisheries.

UNDER PEER REVIEW

Iskandariah, Arifin, O. Z., dan Gustiano, R. (2011). Analysis of Genetic Diversity of Three Red Tilapia Strains (Oreochromis sp) with Anova RAPD. *Journal of Natural Sciences, University of Nusa Bangsa.*1 (1), 18 – 21

Kusmini, I. I., Gustiano R, dan Mulyasari. (2012). Genetic Characterization of Fish (Osteochiluskelabau) from Various Locations in West Kalimantan Using RAPD Analysis. *Biology News*, 10 (4), 449-454. https://10.14203/beritabiologi.v10i4.762

Madduppa, H.H., Timm, J., and Kochzius, M. (2018). Reduced Genetic Diversity in the Clown Anemonefish *Amphiprionocellaris* in Exploited Reefs of Spermonde Archipelago, Indonesia. *Frontier in Marine Sc*ience. 5:80. https://doi.org/10.3389/fmars.2018.00080

Markert, A., Champlin, D. M., Gutiahr-Gobell, R., Grear, J. S., Kuhn, A., McGreevy, T. J., Roth, A., bagley, M. J., and Nacci, D. E. (2010). Population Genetic Diversity and Fitness in Multiple Environtment. *BMC Evolutionary Biology*. 10, 205. https://doi.org/10.1186/1471-2148-10-205

Nugroho, E., Subagja, J., Asih, S., Kurniasih, T. (2006). Evaluation of Genetic Diversity of Tor Soroby Using the Mt. DNA D-Loop and RAPD. *Indonesian Fisheries Research Journal*, 7 (1), 211-217. DOI: http://dx.doi.org/10.15578/jra.1.2.2006.211-217

Pacheco, A. B. F.,Guth,B. E. C., Almeida, D. F. D., and L. C. S. Ferreira. (1996). Characterization of Enterotoxigenic*Escherichia coli*by Random Amplification of Polymorphic DNA. *Research in Microbiology*, 147, 175–182. https://doi.org/10.1016/0923-2508(96)80217-8

Sambrook, J and Russel. (2001). MoleculerCloning : A Laboratory Manual. New York, USA, Cold Spring Harbour Laboratory Press.

Santella, R. M. (2006). Approaches to DNA/RNA Extraction and Whole Genome Amplification. *Cancer Epidemiologi Biomarker*, 15 (9), 185-187. https://doi.org/10.1158/1055-9965.EPI-06-0631

Winarti M, Sulmartiwi L, Lutfiyah L. The effect of salinity on the expression of heat shock protein 70 (hsp70) in the gills and kidneys of srikandi tilapia (Oreochromis niloticus).

UNDER PEER REVIEW

InIOP Conference Series: Earth and Environmental Science 2022 Jul 1 (Vol. 1036, No. 1, p. 012092). IOP Publishing.

Yustiati A, Rahmadewi Z, Suryadi IB, Lili W. Genetic Analysis of Tilapia Nirwana Strain (Oreochromis niloticus) Cultured InLumajang East Java and Wanayasa West Java By Using Random Amplified Polymorphic DNA. GSJ. 2019 Nov;7(11).

UNDER PEER REVIEW