Original Research Article

Genetic Analysis Of Tilapia (*Oreochromis niloticus*) Nirwana Strain That Is Cultivated In Aceh Besar And Wanayasa By Using Random Amplified Polymorphic DNA Method

ABSTRACT

This research held in June to November 2017. This research aims to analyze genetic relationship of tilapia (*Oreochromis niloticus*) nirwana strain which is cultivated in Aceh Besar and in Wanayasa with RAPD-PCR technique. 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. The amplification is done with 2,0 µl primer, 2,0 µl DNA template, 12,5 µl GoTaq® green master mix dan 8,5 µl NFW. Then put into thermocycler and got amplified with 45 cycles. Data analysis is done by using Microsoft Excel, *NTEdit* dan *NTSYS* programs. The stage of genetic level of tilapia nirwana which is cultivated in Aceh Besar and Wanayasa is 67%. The genetic relationship level of tilapia nirwana strain fish cultivated in Aceh Besar with those cultivated in Wanayasa is 67%. The existence of environmental differences results in genetic diversity between Aceh Besar and Wanayasa tilapia fish. Factors that causing grouping movement are external factors such as: geographical isolation and habitat fragmentation as well as internal factors such as mutation, natural selection, genetic drift, and gene flow.

Keywords : Aceh Besar, DNA, tilapia , nirwana, amplification, Wanayasa

1. INTRODUCTION

Tilapia is a type of fish with high economic value, where the demand for seeds and fish consumption from year to year tends to continue to increase along with the expansion of aquaculture and market demand for tilapia continues to increase from year to year (Suria et al 2006). Efforts to improve the quality of tilapia are needed to increase production and profits of tilapia farmers. Mains and seeds that have high quality are absolutely necessary in cultivation activities because of the superior parent it is expected to get quality seeds as well. Quality seeds can be seen from the rapid growth rate, low FCR, resistance to disease, so that later can reduce production costs and increase the benefits of farmers.

The activity of crossing between species of fish that is still in one species results in genetic traits passed down from the parent to the offspring experiencing degradation or decline in genetic quality. To prevent the decline of dominant genes where the dominant genes will be reduced, it is necessary to do hybridization efforts that are able to produce new individuals that have similarities with the parent, in terms of the body shape of the tilapia (Oreochromis niloticus) nirwana strain relatively wider with a shorter head length, terms this makes it has a thicker meat structure compared to other tilapia. The

purpose of this study was to analyze the genetic relationship of tilapia (Oreochromis niloticus) nirwana strain cultivated in Aceh Besar and compared with tilapia (Oreochromis niloticus) nirwana strain cultivated in Wanayasa using the RAPD-PCR technique.

2. 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.

2.1 PROCEDURES

Research procedures include:

2.1.1 DNA extraction

Use the Genomic DNA Purification Kit (Promega). Fish fin samples were taken as much as 0.1 gr. Nuclei Lysis Solution was added as much as 300 µl, then homogenized using vortex for 10 seconds, then incubated in a water bath at 650 C for 30 minutes. Then RNAse Solution was added as much as 1.5 µl, flipped 2-5 times, then in a water bath at 370 C for 30 minutes, then cooled at room temperature for 5 minutes. Adding Protein Precipitation Solution as much as 100 µl was homogeneous using vortex for 10 seconds , then cooled in cured ice for 5 minutes and centrifuged for 4 minutes at a speed of 13,000 rpm centrifuge. The formed upernatant was transferred into a new tube which was filled with isopropanol as much as 300 µl, then homogenized and centrifuged at 13,000 for 1 minute. Furthermore, the supernatant was removed and 70% ethanol was added as much as 300 µl and centrifuged again at a speed of 13,000 rpm for one minute. The unneeded ethanol was removed and dried by pellets by aerating for 15 minutes. Next Rehydration Solution is added as much as 50 µl to the tube that contains pellets and then incubated in a water bath at 65 oC for 1 hour

2.1.2 Electrophoresis Process

Prepare agarose gel with a concentration of 1%. Agarose gel 1% is made by weighing agarose powder as much as 0.4 g for the amplified sample and 0.56 grams for the isolation result, then it is put into the erlenmeyer and 40 mL TBE solution is added for the amplified sample and 56 mL for the isolation result. Then cooled. Then prepare the agarose gel mold complete with a comb to print wells on agarose gel, then agarose solution is poured into agarose molds. Agarose gel is allowed to stand for 10 minutes or until frozen. Put frozen agarose gel in a TBE running buffer submerge solution. The results of DNA amplification were filled in each agarose gel well with a composition of 4 µl PCR DNA and 2 µl loading dye, the other wells were filled with 2 µl Ladder DNA 1 kb plus 2 µl loading dye as a marker. Furthermore, electrophoresis was 70 minutes at a voltage of 75. Whereas for electrophoresis the results of isolation were carried out for 40 minutes. Then soak the agarose gel in a mixture of 100 ml of Aquadest solution and EtBr as much as 5 µl for 30 minutes, then soak again in aquadest as much as 100 µl for 7 minutes. Furthermore, agarose gel is added and put into the UV Transilluminator machine. Documented electrophoresis results using a digital camera for later analysis.

2.1.3 Amplification Process

The amplification process was carried out by combining 2 DNAl DNA templates (DNA sample tests), 1.25 primerl primers,

12.5 masterl master mix, 8.5 nuclel nucleus free water. Then homogenized. Then amplified using a thermal cycler machine.

2.2 DATA ANALYSYS

The results of the data obtained in the form of ribbons that arise in agarose are analyzed descriptively through observational studies with supporting data and related literature. Data analysis was then performed using the NTSYS program on a computer to obtain a genetic relationship tree (phylogeny) to determine whether genetic differences occurred in samples cultivated in Aceh Besar and in Wanayasa.

3. RESULTS AND DISCUSSION

3.1 DNA Isolation

DNA isolation is a technique of separating DNA molecules from other molecules in the cell nucleus. Three samples of tilapia nirwana fish tail originating from Aceh Besar and three samples of tilapia nirwana fish tail originating from Wanayasa were successfully isolated using the Wizard®Genomic Purification Kit (Promega) to isolate the genomic DNA used in accordance with the standard protocol recommended isolation kit Promega Sampling on the tail aims to obtain parts that can present cells in the body of fish samples in good condition and sampling on the tail can be taken without having to kill fish samples (Mulyadi et al 2017).

The isolation genomic DNA can be known for its presence and quality by using electrophoresis to determine the results of qualitative tests conducted by looking at the presence of DNA bands in agarose gels immersed in a solution of Etidium bromide (Etbr) containing fluorescent compounds that will bind to DNA, so that when exposed. UV light DNA will fluoresce in agarose gel.

The results of DNA isolation can be seen in Figure 1:

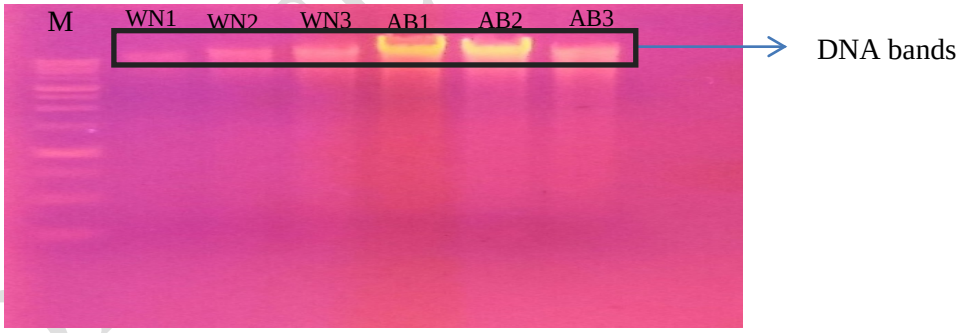

Figure 1. DNA Isolation Results

Note :

M: DNA Ladder Marker 1 Kb
WN1: Wanasaya 1
WN2: Wanayasa 2
WN3: Wanayasa 3
AB1: Aceh Besar 1
AB2: Aceh Besar 2
AB3: Aceh Besar 3

Based on Figure 1, shows the results of the visualization of DNA isolation electrophoresis on agarose gels that still have smears. In DNA isolation, can cause smears in agarose cells signifying the presence of material other than DNA that was also isolated, so that smears appearing at the bottom of the DNA Smear band contained in the band can also be caused by the presence of RNA content that is still present in the sample (Santella 2006) . The way to reduce the presence of smears is by adding RNAse, because RNAse can reduce the smear contained in DNA isolates.

The next step is to determine the quality of the genomic DNA based on the measurement of the concentration value of DNA content and the purity value obtained from the measurement results using a spectrophotometer that has been done based on the ratio ratio of absorbance values A260 nm and A280 nm.

Tabel 1. Purity of samples

No	Sample Name	DNA Purity		Value of DNA Purity
		Abs 260nm	Abs 280nm	
1	Wanayasa 1 (WN1)	0,063	0,041	1,573
2	Wanayasa 2 (WN2)	0,078	0,049	1,592
3	Wanayasa 3 (WN3)	0, 059	0,032	1,844
4	Aceh Besar 1 (AB1)	0,437	0,245	1,784
5	Aceh Besar 2 (AB2)	0,257	0,142	1,810
6	Aceh Besar 3 (AB3)	0,184	0,105	1,752

Note :

WN3: Wanayasa 1 AB1: Aceh Besar 1
WN1: Wanasaya 2 AB2: Aceh Besar 2
WN2: Wanayasa 3 AB3: Aceh Besar 3

Based on the genomic DNA purity values obtained in all samples, the purity values ranged from 1.5-1.8. The purity value of the samples WN1 1.573, WN2 1.592, WN3 1,844, AB1 1,784, AB2 1,810 and AB3 1,752. The results of DNA purity and integrity have a very important influence on the success of the PCR amplification process, especially RAPD-PCR (Triana et al 2010). According to Tenriulo (2001), a value of 1.5-1.8 is still considered a pure isolate criterion because it is close to 1.8. All samples show that the sample is suitable for the amplification process.

3.2 DNA Amplification

PCR amplification can be done after the DNA isolation stage and after knowing the genomic DNA purity value on the DNA isolation results. The results of PCR amplification based on genetic characteristics can be seen using OPA primers (Operon Primary set-A).

DNA amplification uses the RAPD method with primer OPA 2. Other solution components are GoTaq ® green master mix and DNA template. After the DNA amplification process is carried out, it is followed by isolation treatment to see the quality of DNA. The results of amplification of amplified DNA can be seen in Figure 2:

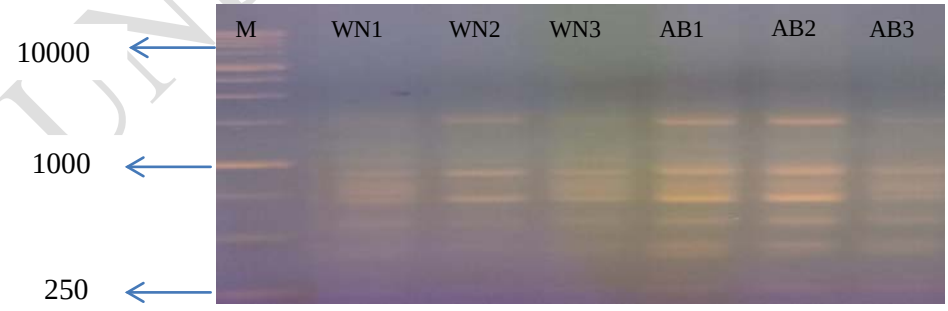

Figure 2: DNA Amplification Results

Note :

M1: Marker DNA Ladder 1 Kb AB1: Aceh Besar Sample 1
WN1: Wanayasa Sample 1 AB2: Aceh Besar Sample 2
WN2: Wanayasa Sample 2 AB3: Aceh Besar Sample 3
WN3: Wanayasa Sample 3

DNA amplification is done by using two primers that can produce bands that are interpreted from the results of amplification of agarose gel electrophoresis which is translated in binary matrix by looking at the presence or absence of the tape. DNA amplification requires a lot of optimization to get the best results (all samples can produce variations of DNA fragments). The primary optimization used in primary selection must be correct. Optimizing the use of primers to analyze genetic genetic relationship of tilapia nirwana stran using OPA-02 and OPA-03 primers.

DNA amplification was carried out using two primers which can produce polymorphic bands and monomorphic bands after primer optimization in each sample of amplified tilapia. The results of genomic DNA amplification using OPA-2 and OPA-5 show different results in each primer. Based on the results of isolation from amplified genomic DNA, it shows that the DNA produced is of good quality, showing that there are bands on OPA 2 (Figure 2) that produce as many as 18 bands, whereas in OPA 5 there are fewer interpreted bands so that no analysis is performed. Furthermore. This shows that OPA 2 primers are sufficiently complementary to be used as primers for part of the test fish network.

Differences in DNA Amplification are due to differences in the number of primary attachment sites and the distance between the two primers attached to the DNA template. According to Wels et al. 1998 in Lia 2006, the number and size of fragments produced generally depend on the order of nucleotides and DNA sources. The bands which were interpreted as the result of amplification of agarose gel electrophoresis by using OPA-2 Primer showed results that were complementary to the DNA of tilapia nirwana in the base pair position (bp) and ended with a complementary sequence on bp which was translated in binary matrix by looking at the presence and the absence of the tape in Table 2.

Table 2. Results of DNA bands using OPA 2

Y (BP position)	WN1	WN2	WN3	AB1	AB2	AB3
16652,55						--*
16223,51						
16065,77		--^				--^
16039,63	--^	--^		--^	--^	--^
15248,59				--^	--^	
14807,41	--^	--^	--^	--^	--^	
14379					--*	
14238,86			--^	--^	--^	--^
14146,08	--^	--^	--^	--^	--^	--^
13872	--^		--^	--^	--^	
13603,24	--^		--^	--^	--^	--^
13382,88		--^	--^	--^	--^	--^
13274,36	--^		--^	--^	--^	--^
12827,18				--*		
12702,46			--^	--^	--^	--^
12599,16	--^	--^	--^	--^	--^	--^
11977,79			--^	--^	--^	--^
11861,34	--^	--^	--^	--^	--^	--^

Note: - ^ = Monomorphic Tape; - * =Polymorphic Tape

WN 1: Wanayasa Sample 1 AB 1: Aceh Besar Sample 1
WN 2: Wanayasa Sample 2 AB 2: Aceh Besar Sample 2
WN 3: Wanayasa Sample 3 AB 3: Aceh Besar Sample 3

Based on the results of the Table. 2, it can be seen a variety of polymorphic and monomorphic bands in three samples of tilapia nirwana that are cultivated in Aceh Besar Besar and three samples of tilapia nirwana that are cultivated in Wanayasa which are implied using OPA-2. Monomorphic bands are bands that are present in some samples so that they do not have variations and polymorphic bands are images of DNA bands that appear at certain sizes, and are not found in other samples (Willian and Ronald 1990). Based on the analyzed bands, then the percentage of monomorphic and polymorphic bands is made. Each primer has a different forging character in each test fish sample, so that it has its own attachment site in each sample.

The percentage of bands can be seen in Table 3.

Table 3 Comparison of Monomorphic and Polymorphic Bands

	Number of bands	Number of polymorphic	Number of monomorphic	Polymorphic (%)	Monomorphic (%)
WN1	8	0	8	0	100
WN2	8	0	8	0	100
WN3	11	0	11	0	100
AB1	14	1	13	6,7	92,3
AB2	14	1	13	6,7	92,3
AB3	12	1	11	8,3	91,7

WN 1: Wanayasa Sample 1 AB 1: Aceh Besar Sample 1
WN 2: Wanayasa Sample 2 AB 2: Aceh Besar Sample 2
WN 3: Wanayasa Sample 3 AB 3: Aceh Besar Sample 3

The primers used in RAPD analysis influence the polymorphism of the fragments produced because each primer has different attachment properties so that fragments generated from genomic DNA from the amplification results using different primers will produce polymorphic or monomorphic bands with the number of fragments and molecular weights different. The results of this DNA band emergence data are based on the use of OPA-2 primers which are complementary to the sample used. Polymorphic and monomorphic bands generated 18 DNA bands in all test samples. Fish samples that gave rise to the most bands were the samples of AB1 and AB2 nirwana strain as many as 14 DNA bands while the fish samples that gave rise to the least bands were WN1 and WN2 nirwana strain samples of 8 DNA bands.

The genetic variation in the amplification results using OPA-2 primers is marked by the appearance of polymorphic bands. In all test samples of tilapia nirwana there are polymorphic bands visualized in fish samples AB1 12827.18 bp showing the results of polymorphism percentage of 6.7, AB2 14379bp showing the results of polymorphism percentage of 8.3%, AB1 12827.18 bp showing the results of polymorphism percentage of 6.7, AB2 14379bp showing the results of polymorphism percentage amounting to 6.7% and AB3 16652.55bp shows the results of the percentage polymorphism of

8.3%, each containing one polymorphic band.

The existence of variations within a population indicates the strength that can maintain variation and affect the adaptation of a population to its environment (Handiwirawan, 2006). According to Kusmini et al. (2012), the high level of polymorphism in the population shows the effectiveness of individuals in the selection process (random mating) and reproduction in their habitat. Conversely, low levels of population polymorphism are thought to be related to gene flow constraints by environmental factors or limited population size that causes interpopulation so as to reduce intrapopulation genetic diversity. Tilapia Nirwana fish that is cultivated in Wanayasa with environmental conditions an average water temperature of 16-28^{o} C, rainfall 1,937 mm / year and an altitude of 600 meters above sea level. These environmental conditions are different from the conditions in Aceh Besar where the water temperature ranges from 26-28^{o} C, rainfall is 1,251-1693 mm / year and a height of 0-800 meters above sea level. Differences in environmental conditions can cause polymorphic bands in fish samples, resulting in genetic differences in tilapia.

In the results of the OPA-2 amplification, it can also produce two monomorphic bands in all samples of Aceh Besar and Wanayasa tilapia fish at the same bp at 11861.34 bp and 12599.16 bp. This shows that the same ribbon size in all test fish samples indicates a close genetic relationship. The emergence of monomorphic bands due to fish cultivated in Aceh Besar was originally the result of offspring from tilapia nirwana II which are the same as those cultivated in Wanayasa. According to Ministerial Decree 23 of 2012 the superiority of tilapia nirwana II is that this fish has a growth rate of 15% faster than tilapia nirwana I, so that it is used as superior seeds to be spread in several regions in Indonesia, one of which is in the Greater Aceh Jantho Fish Seed Center.

3.3 Genetic Relationship Analysis

The existence of DNA band fragments on the results of genomic DNA amplification using OPA-2 can be translated into a binary matrix by observing the presence or absence of DNA band fragments on the results of genomic DNA amplification marked with (1) indicating the band appears and the value (0) indicating the band does not appear . The next stage is to analyze the level of genetic relationship in the test sample. Genetic relationship level analysis using the help of some software namely Microsoft Excel, Corel Draw X7 and for making the genetic relationship tree NTEdit and NTSYS programs are used to show genetic distance in each test sample, the distance contained in the phylogenic tree will provide information on how close the genetic traits are

After the binary matrix is completed, an analysis of the calculation of the coefficient of equation (simple matching) of the data and then made a genetic relationship tree using the UPGMA (Unweighted Pair Group Method With Arithmetics Average) method through the NTSYSPc program and from this genetic relationship tree can be seen the genetic relationship relationship of the test fish samples .

The results of the genetic relationship tree can be seen in Figure 3.

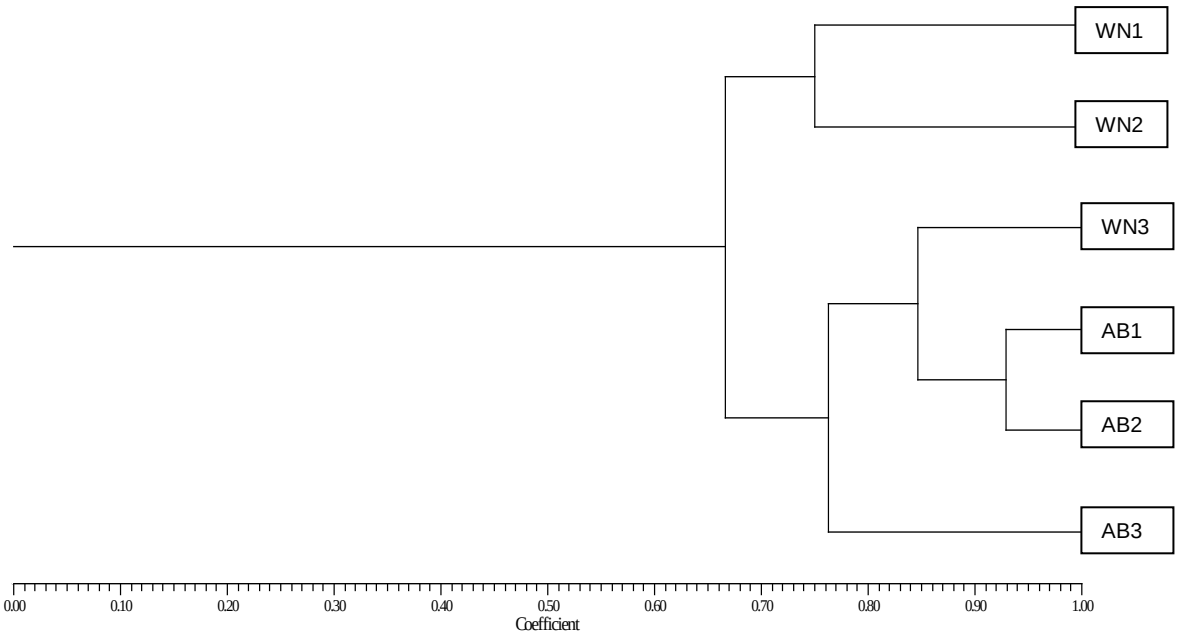

Figure 3. Fendogram of Genetic relationship Using OPA-2

Note :

WN 1: Wanayasa Sample 1	AB 1: Aceh Besar Sample 1
WN 2: Wanayasa Sample 2	AB 2: Aceh Besar Sample 2
WN 3: Wanayasa Sample 3	AB 3: Aceh Besar Sample 3

Based on the UPGMA analysis results in Figure 4, using OPA-2 from three DNA samples of the Greater Aceh tilapia fish genome and three DNA samples from the Wanayasa nirwana tilapia genome, it can be seen that all samples were obtained by two large genetic groups using OPA-2 that is, in the first group consisting of WN1 and WN2 has a coefficient of 0.76 and a similarity in genetic traits of 76%. This shows that there is a close relationship between the two fish samples. This can be explained based on Table 3 which shows that the absence of polymorphic bands that appear in the sample so that the genetic relationship of the sample is quite close.

The second group WN3, AB1, AB2 and AB3 have a coefficient of 0.76 and a genetic similarity of 76%. This shows that there is a close relative of the four fish samples. All sample groups AB1, AB2, AB3, WN1, WN2 and WN3 have a coefficient of 0.67 and have a genetic similarity of 67%. This shows that there is a close relative of the whole fish sample. This shows that AB1 and AB2 samples have a degree of genetic relationship far compared to other groups with a large similarity index value.

This shows that the genetic relatinship between tilapia nirwana cultivated in Aceh Besar and in Wanayasa has a close genetic relationship but does not reach 100%. Based on phylogenic trees there is a genetic difference between samples from Aceh Besar and Wanayasa. The occurrence of grouping differences based on genetic distance due to genetic variations or the emergence of polymorphic bands in the results of amplification among populations of tilapia nirwana fish that indicate the presence of genetic structure as the beginning of the process of starting a new species. This can be seen based on the emergence of monomorphic and polymorphic bands from the results of Table 3 which shows that the appearance of the same monomorphic band at certain base pairs between one sample with another,

resulting in a sample of genetic relationship between one another.

The existence of genetic interaction of tilapia nirwana with its cultural environment and every individual tilapia nirwana cultivated in Aceh Besar does not all have the same level of adaptation, so that the interaction between genetic and the environment can result in genetic differences in fish that are cultivated which causes low genetic relationship between populations tilapia nirwana fish are characterized by differences in the characteristics of these fish.

This can be explained by several assumptions, for example due to external factors such as: geographical isolation and habitat fragmentation as well as internal factors such as mutation, natural selection, genetic drift, and gene flow. According to Slatkin (1987), states that these factors are the cause of variations in genetic structure.

4. CONCLUSION

Based on research that has been done, it can be concluded that the level of genetic relationship of tilapia nirwana cultivated in Aceh Besar with those cultivated in Wanayasa is 67%, the existence of environmental differences results in genetic diversity between Aceh Besar tilapia and Wanayasa, a factor that causes grouping. namely external factors such as: geographical isolation and habitat fragmentation and internal factors such as natural selection, genetic drift, and gene flow.

References

1. Barbas, C.F., D.R. Button, J.K. Scott, and G.J. Silverman. 2001. Quantitation of DNA dan RNA. Adapted from ”General Procedure” appendix 3. Cold Spring Harbor, NY. USA
2. Bardakci, F. 2001. *Random Amplified Polymorphic DNA (RAPD) Markers*. Turkish Journal of Biology. 25:185-196.
3. Erlich, H.A. 1989. PCR technology: Principles and Applications for DNA Amplifications Using a Pseudo-Testcross: Mapping Strategy and RAPD Markers. Genetics 137, 1121-1137. Stockton Press, NY.
4. Falconer, D.S. and T.F.C. Mackay. 1996. Introduction to Quantitative Genetics. Longmann. Malaysia.
5. Iskandariah, Otong ZA, Rudhy G, Irin IK. 2011. *Variasi Genetik Hasil Persilangan Nila Best Dengan Red Nifi Dan Nirwana Menggunakan Penanda RAPD*. Balai Riset Perikanan Air Tawar. Bogor
6. Fergusson, A.J., A.J. Taggart, P.A. Prodohl, O.McMeel, C. Thompson, C. Stone, P. McGinnity and R.A. Haynes, 1995. The application of molecular markers to study and conservation of fish population, with special reference to salmo. Journal of Fish Biology 47 : 103-126.
7. Imron, O.Z., Arifin dan Subagya. 2000. *Karakterisasi Truss Monomorfik Pada Ikan Mas (Cyprinus carpio) Galur Majalaya, Rajadanu, Wildan, dan Sutisna.* Prosiding Seminar Hasil Penelitian Perikanan 1999/2000. Puslitbang Eksplorasi Laut dan Perikanan. Departemen Eksplorasi Laut dan Perikanan. Jakarta.
8. Judantari, S., Khairuman dan Amri. 2008. *Nila Nirwana Prospek Bisnis dan Tekhnik Budidaya Nila Unggul*.Gramedia. Jakarta.
9. Kirpichnikov, V. S. 1981. Genetic Bases of Fish Selection. Springer Veerlag. Berlin Heidelberg. New York. 410p.
10. Lante S, A Tenriulo, dan NN Palinggi. 2012. *Variasi genetik ikan Beronang (Siganus guttatus) asal perairan Barru, Lampung dan Sorong menggunakan penanda RAPD (Random Amplified Polymorphism DNA)*. Jurnal Riset

Akuakultur 7, 195 – 204.
11. Lia, E., 2006. Analisa Keanekaragaman Genetik Ikan Gurame (Osphronemus gouramy Lac) Varietas Bluesafir dengan Menggunakan Metode RAPD. Skripsi S1, Jurusan Pendidikan Biologi. Universitas Pendidikan Indonesia (UPI) Bandung.
12. Linacero, R., J. Rueda & A.M.Vazquez. (1998). Quantification of DNA. Pages 18-21 in Karp, A., P. G. Isaac, and D. S. Ingram (Eds.) Molecular Tools for Screening Biodiversity: Plants and Animals. Chapman and Hall. London, Weinheim, New York, Tokyo, Melbourne, Madras
13. Old, R.W. and Primrose, S.B., 1985. Principles of Gene Manipulation: An Introdcution to Genetic Engineering. Blackwell Scientific Publications, London, Great Britain.
14. Pranawaty, N., Buwono, D., Liviawaty, E. 2012. Aplikasi Polymerase Chain Reaction (PCR) Konvensional dan Real Time PCR untuk Deteksi White Spot Syndrome Virus pada Kepiting. Jurnal Perikanan dan Kelautan. 3 (4):61-74
15. Rustidja, 2005. Penggunaan Sinar Laser Untuk Mempercepat Kematangan Gonad Ikan Nila. Universitas Brawijaya. Malang.
16. Saanin H. 1984. Taksonomi dan Kunci Identifikasi Ikan. Binacipta.Bandung Sambrook J, Fritsch EF, & Maniatis T. (1989). “Molecular cloning: A laboratory manual”. USA: Cold Spring Harbor Lab ress.
17. Satia, Y., P. Octorina Dan Yulfiperius. 2015. Kebiasaan Makanan Ikan Nila (Oreochromis Niloticus) Di Danau Bekas Galian Pasir Gekbrong Cianjur. Universitas Muhammadiyah Sukabumi, Jawa Barat
18. Stickney, R.R., 2005. Aquaculture: An introductory text. CABI Publishing. USA.256p.
19. Suria, D., Junior, Z.M., Sjafei, S.D., Manalu, W., dan Sudrajat, O.A. 2006. *Kajian Performans Reproduksi Perbaikan pada Kualitas Telur dan Larva Ikan Nila (Oreochromis niloticus) yang diberi Vitamin E dan Minyak Ikan Berbeda dalam Pakan*. IPB: Bogor.
20. Tao W., Rosa, Gudrun A. 2009. *Total Phenolic Coumpound, Radical Scavenging and Metal Chelation of Extract from Iceland Seaweeds*. *Food Chem*., 116 : 240-248
21. Yoon, J. M., H. Y Park. 2001. *Genetic Similarity and Variation in the Cultured and Wild Crucian Carp (Carassius carassius) Estimated with Random Amplified Polymorphic DNA.* Departemen *of Marine Biomedical Science College of Ocean Science and Technology*. Kunsan National University.Kunsan.