



## Evaluation of Genetic Variation in Hybrid Catfish (Sangkuriang × Thai) Employing PCR-RAPD Markers

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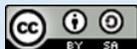


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### ABSTRACT

Hybridization is the process of crossing individuals with different genotypes to produce offspring with superior traits. In this study, Sangkuriang catfish were crossed with introduced Thai catfish to obtain heterosis in hybrid seeds. The objective was to analyze the genetic diversity of these hybrid seeds using the PCR-RAPD method. Specifically, the primers OPA-13, OPB-08, OPC-19, and OPD-02 were used. Crossbreeding involved Sangkuriang female and Thai male broodstock, with four pairs of broodfish used via a semi-natural spawning method. After spawning, hybrid seeds from each pair were reared separately in containers under uniform conditions for 1.5 months. DNA samples were then collected from the fin tissue of each replicate of the crosses, and were grouped according to size categories: large (7-8 cm), medium (5-6 cm), small (3-4 cm), and broodstock, with 30 samples per size category for each replicate. The results revealed a polymorphism percentage of 72.95-93.88%, heterozygosity values of 0.167-0.232, and an average genetic distance among hybrid seeds from the three size categories and broodstock ranging from 0.001-0.018.

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## INTRODUCTION

Catfish (*Clarias gariepinus*) is a leading aquaculture commodity in Indonesia, accounting for 1.17 million tons of national fisheries production in 2024 (KKP, 2024). The Sangkuriang strain is notable for strong endurance, rapid growth, high survival, and low cannibalism. It can withstand high stocking densities, resists disease, and is produced by crossbreeding second-generation (F2) female and sixth generation (F6) male dumbo catfish (Maryeni et al., 2022; Ukat et al., 2023).

Hybridization, the crossbreeding of closely related fish species or strains differing in certain traits, is a method used in fish breeding programs (Hafidah et al., 2021). Sunarma (2016) found that crossing Thai male and Sangkuriang female catfish at BBPBAT Sukabumi, West Java, resulted in higher fertilization (86.05±3.88%) and hatching rates (87.17±8.20%) than the reverse cross (82.20±2.14% and 55.74±3.83%, respectively).

Genetic diversity in individuals guides broodstock selection for hybridization (Hafidah et al., 2021). Diversity and gene pools are key to adaptive capacity, survival, conservation, and management. Advances in molecular markers and statistical methods improve analysis of genetic variation in wild and cultured stocks (Andayani et al., 2001; Tamanna et al., 2012; Hasan & Goswami, 2015).

In fish, as in other taxa, population size and genetic diversity are shaped by genetic drift, gene flow, mutation, and natural selection. Greater genetic diversity increases adaptation and long-term survival and usually correlates with a larger effective population size. Large populations maintain variation because genetic drift is weaker, while small populations, affected by bottlenecks or unequal reproduction, lose diversity and adaptability (Welsh, 2014; Ellegren & Galtier, 2016; Hague & Routman, 2016).

One of the methods considered effective for analyzing genetic diversity is the polymerase chain reaction (PCR) using the random amplified polymorphic DNA (RAPD) approach (Buwono et al., 2018; Hafidah et al., 2021). This technique can detect both monomorphic and polymorphic DNA fragments, and the results can be visualized as a phylogenetic tree (Mulyadi et al., 2017). The PCR-RAPD method has been widely applied to various fish species to assess genetic diversity, including catfish (Kristanto et al., 2017; Omer et al., 2020). The objective of this study was to specifically investigate genetic diversity in offspring from crosses between Sangkuriang and Thai catfish, using the PCR-RAPD method.

The novelty of this study lies in its specific application of

the PCR-RAPD approach to assess the genetic diversity of hybrid offspring from crosses between Sangkuriang and Thai catfish. Although PCR-RAPD has been widely utilized to assess genetic variability in various fish species, including catfish, previous studies have primarily focused on pure strains rather than hybrid progeny from distinct commercial lines. Therefore, this research contributes new insights by characterizing the genetic structure and polymorphism patterns specifically in inter-strain hybrids, which are increasingly important in aquaculture breeding programs.

## MATERIALS AND METHODS

This research was conducted from December 2024 to April 2025. Fish rearing was carried out at the Experimental Pond Facility of the Department of Aquaculture, and the molecular analysis was performed at the Laboratory of Aquatic Organism Reproduction and Genetics, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University.

### Materials

The catfish broodstock used in this study were obtained from BBPBAT Sukabumi, West Java. The female broodstock used were Sangkuriang catfish reared in concrete pond number 1, with an average weight of 0.560±0.09 kg, while the male broodstock were Thai catfish reared in concrete pond number 5, with an average weight of 0.545±0.03 kg. The feed used was the Matahari Sakti brand, containing 39-40% protein for the initial stage (fry) and 31-33% protein for the growth stage. Feeding was carried out twice daily using the restricted method at 3% of the total fish biomass (Lubis et al. 2019).

### Methods

#### Experimental design

This study used an experimental method, performing crossbreeding between female Sangkuriang catfish and male Thai catfish, following the procedures described by Sunarma (2016). The crosses were intentionally designed using ♀ Sangkuriang × ♂ Thailand combinations to maintain consistency in maternal lineage and minimize variation associated with maternal effects. In fish, maternal contributions can strongly influence offspring performance and genetic outcomes. By standardizing the female broodstock origin, we aimed to reduce confounding factors so that the observed genetic variation would primarily reflect nuclear genetic recombination between strains rather than maternal inheritance. Four different pairs of broodstock were used as replicates, each labeled with the codes A through D (Table 1).

**Table 1.** Experimental design of catfish broodstock crossbreeding.

Code	Spawning Crossbreeding
A	♀ Sangkuriang A × ♂ Thailand A
B	♀ Sangkuriang B × ♂ Thailand B
C	♀ Sangkuriang C × ♂ Thailand C
D	♀ Sangkuriang D × ♂ Thailand D

### Spawning broodstock

The spawning containers used were four concrete tanks measuring 3 × 2 × 1 m, each equipped with three aeration points. Mature catfish broodstock were combined in the tanks. Each spawning tank was stocked with one pair of broodstock (♀ Sangkuriang × ♂ Thai). Ovulation and spermiation induction were carried out by injecting Ovaprim at a dose of 0.2 mL/kg for males and 0.5 mL/kg for females. The hormone was diluted in 0.9% NaCl physiological solution at a 2:1 ratio. Injections were administered intramuscularly at a 45° angle. After the injection, the injection site was gently massaged to ensure that the hormone solution was fully absorbed into the fish's body (Ahmed & Talib, 2018).

Each tank was equipped with six spawning mats (*kakaban*) weighed in the center to prevent them from shifting. The *kakaban* served as an egg attachment medium for catfish and was made of fibers, shaped, elongated, and stacked so that eggs could adhere to every side. Hormone-injected broodstock pairs were left overnight to spawn. Egg checking was carried out the following day.

Catfish broodstock were removed in the morning or afternoon, depending on broodstock and egg conditions. Care was taken during removal to avoid disturbing the eggs. Afterward, the broodstock were returned to their original tanks. *Kakaban* were collected one to two days after broodstock removal from the spawning tanks. The *kakaban* were gently shaken to prevent larvae from getting trapped.

### Larvae rearing to fingerling stage

Newly hatched larvae were not fed during the first two days, as they still relied on the yolk sac as a nutrient source (Mukai & Lim, 2011). After that, the larvae were fed tubifex worms ad libitum three times a day at 08:00 AM, 12:00 PM, and 04:00 PM until day 7. From day 8 to day 10, the larvae were given transitional feed consisting of a combination of tubifex worms and PF-0 pellets to facilitate the transition from natural to artificial feed.

Fingerlings were reared to a size of 7–8 cm over about 1.5 months. During rearing, they were fed pellets that matched their mouth gape. Feed types began with PF-0 pellets, followed by PF-100, PF-500, and PF-800. Feeding

was done three times daily at 08:00 AM, 12:00 PM, and 04:00 PM. Feed was provided to satiation (Amin et al., 2020).

### Fingerling grading and sampling

Catfish fingerlings were graded and sampled every two weeks until reaching 7-8 cm, after which tissue samples were taken. Grading involved placing fingerlings in a size-based grading device. Those fitting the size passed through the holes and were collected in water. A portion of these fingerlings, measured by one scoop, was weighed and counted to determine the number of individuals. Sampling was replicated three times. Weighing and counting data were recorded and averaged to determine the average weight per size category.

### Water quality monitoring

Water quality was monitored three times during rearing: at the beginning, middle, and end. Parameters measured included temperature, dissolved oxygen (DO), and pH at three locations in each pond. Each week, 30% of the initial water was replaced.

### DNA extraction

Catfish fingerlings from crossbreeding at the same age were sorted into large (7-8 cm), medium (5-6 cm), and small (3-4 cm) categories. For each size, 30 individuals were sampled per cross replicate. All samples were preserved in 96% ethanol for DNA extraction. Samples included fin tissue from broodstock and fingerlings from each replicate. DNA extraction was performed according to the Geneaid™ DNA Isolation Kit protocol.

### PCR-RAPD

Genetic diversity analysis was conducted using the RAPD method via PCR (Mulyani et al., 2023). This process required DNA samples from all sizes of fish, RAPD primers, and PCR program settings adjusted to the primers used. The RAPD primers employed were OPA-13, OPB-08, OPC-19, and OPD-02 (Table 2). The PCR-RAPD amplification products were separated by electrophoresis on a 1.5% agarose gel in Tris-Boric-EDTA (TBE) buffer at 150 V and 400 mA for 35 minutes. This was followed by visualization and band detection using a Gel-Doc UV system.

Table 2. RAPD primer sequences and annealing temperatures.

Primer	Base Sequence	AT (°C)
OPA-13	5'CAG-CAC-CCA-C'3	37.7
OPB-08	5'GTC-CAC-ACG-G'3	32.3
OPC-19	5'GTT-GCC-AGC-C'3	34.0
OPD-02	5'GGA-CCC-AAC-C'3	36.4

Note: At = annealing temperature.

### Genetic diversity analysis

The visualization and detection of bands from PCR-RAPD amplification for each primer were converted into binary data, with notations of 1 and 0. The value 1 indicates the presence of a detected band, while 0 indicates the absence of a band. From this binary data, genetic diversity was analyzed in terms of heterozygosity, polymorphism percentage, and genetic distance, based on the formulas proposed by Nei (1972). Subsequently, a dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with the Simple Match-

ing (SM) coefficient.

$$D = -\log_e I$$

Notes:

D=genetic diversity

I = normalized gene identity between two populations

### Statistical analysis

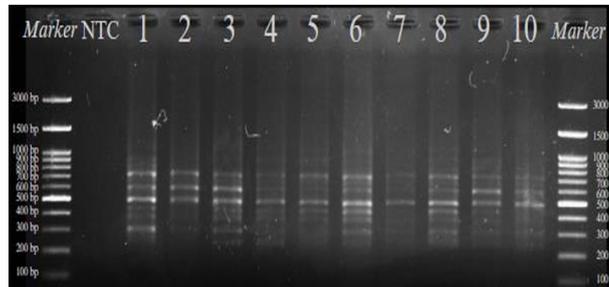
Data analysis was performed using Ms. Excel, GenAIEx 6.51b2, and NTSYS 2.11a software. Genetic diversity and clustering, based on heterozygosity values, polymorphism percentage, and genetic distance, were analyzed in GenAIEx 6.51b2 according to Nei's (1972) formulas, which are preprogrammed in the software. The dendrogram was constructed using NTSYS 2.11a software with the UPGMA method based on the Simple Matching (SM)

coefficient.

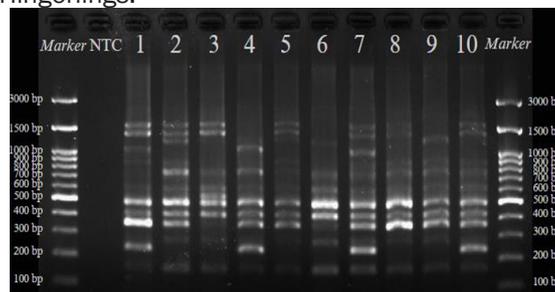
## RESULTS AND DISCUSSION

### Results

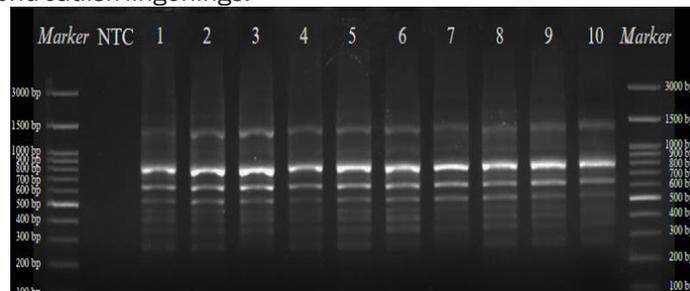
The amplification results using the OPA-13, OPB-08, OPC-19, and OPD-02 primers on 10 individual hybrid catfish fingerling samples, visualized with Gel-Doc UV, yielded a total of 27, 50, 37, and 29 amplified loci, respectively (Figures 1-4).



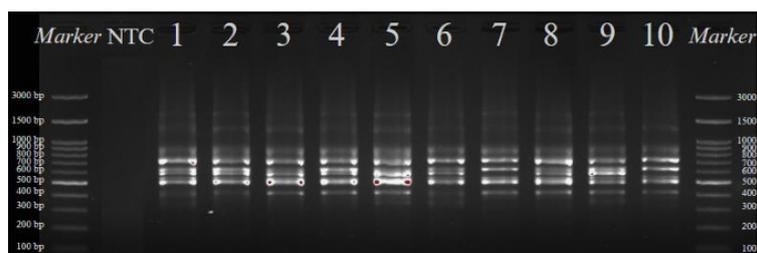
**Figure 1.** Electrophoregram of PCR-RAPD amplification using the OPA-13 primer. Marker = 100 bp DNA ladder from GenedireX, NTC = non-template control (negative control), numbers 1-10 = PCR-RAPD samples from 10 individual small hybrid catfish fingerlings.



**Figure 2.** Electrophoregram of PCR-RAPD amplification using the OPB-08 primer. Marker = 100 bp DNA ladder from GenedireX, NTC = non-template control (negative control), numbers 1-10 = PCR-RAPD samples from 10 individual medium hybrid catfish fingerlings.



**Figure 3.** Electrophoregram of PCR-RAPD amplification using the OPC-19 primer. Marker = 100 bp DNA ladder from GenedireX, NTC = non-template control (negative control), numbers 1-10 = PCR-RAPD samples from 10 individual medium hybrid catfish fingerlings.



**Figure 4.** Electrophoregram of PCR-RAPD amplification using the OPD-02 primer. Marker = 100 bp DNA ladder from GenedireX, NTC = non-template control (negative control), numbers 1-10 = PCR-RAPD samples from 10 individual small hybrid catfish fingerlings.

Genetic diversity in the hybrid catfish fingerlings resulting from the cross between Sangkuriang and Thai catfish, analyzed using four different primers, showed variation based on both size and cross replicate. Heterozygosity values for all samples ranged from 0.167 to 0.232, with

the highest value of 0.232 found in the medium-sized sample from replicate A, and the lowest value of 0.167 observed in the small-sized sample from replicate B (Table 4).

**Table 4.** Heterozygosity values hybrid catfish fingerlings using RAPD primers.

Size of fish sample	Heterozygosity			
	A	B	C	D
Large	0.222	0.198	0.203	0.196
Medium	0.232	0.178	0.210	0.176
Small	0.227	0.167	0.218	0.212

Note: A-D = hybrid fingerlings according to broodstock pair replicate codes.

The polymorphism level for all samples ranged from 72.95% to 93.88%. The highest polymorphism of 93.88% was observed in the small-sized sample from replicate A,

while the lowest value of 72.95% was found in the small-sized sample from replicate B (Table 5).

**Table 5.** Polymorphism levels of hybrid catfish fingerlings using RAPD primers.

Size of fish sample	Polymorphism (%)			
	A	B	C	D
Large	92.86	90.98	75.40	80.28
Medium	88.78	79.51	85.71	80.99
Small	93.88	72.95	89.68	88.73

Note: A-D = hybrid fingerlings according to broodstock pair replicate codes

The heterozygosity and polymorphism values presented in the Table 6 are population-based estimates calculated per cross replicate (ST.A-ST.D). Heterozygosity values for each broodstock pair ranged from 0.094 to 0.142, with polymorphism levels of 22.66%-34.88%. The highest

heterozygosity, 0.142, was obtained from the broodstock pair in cross replicate A, while the lowest value, 0.094, was from the pair in cross replicate D. The highest polymorphism level, 34.88%, was observed in the broodstock pair of cross replicate A, whereas the lowest, 22.66%,

**Table 6.** Heterozygosity and polymorphism levels of broodstock are based on RAPD primers.

Sample Code	Heterozigosity	Polimorphism (%)
ST. A	0.142	34.88
ST. B	0.110	26.56
ST. C	0.123	29.69
ST. D	0.094	22.66

Note: S = female Sangkuriang catfish, T = male Thai catfish, A-D = cross replicate codes for Spawning.

was found in cross replicate D.

ing GenAlEx 6.51b2 software, showed the closest value between samples B and K at 0.001, and the farthest value between samples K and I at 0.018 (Table 7).

The genetic distance values from the binary data of each primer (OPA-13, OPB-08, OPC-19, OPD-02), analyzed us-

**Table 7.** Genetic distances of hybrid fingerlings of different sizes and their broodstock.

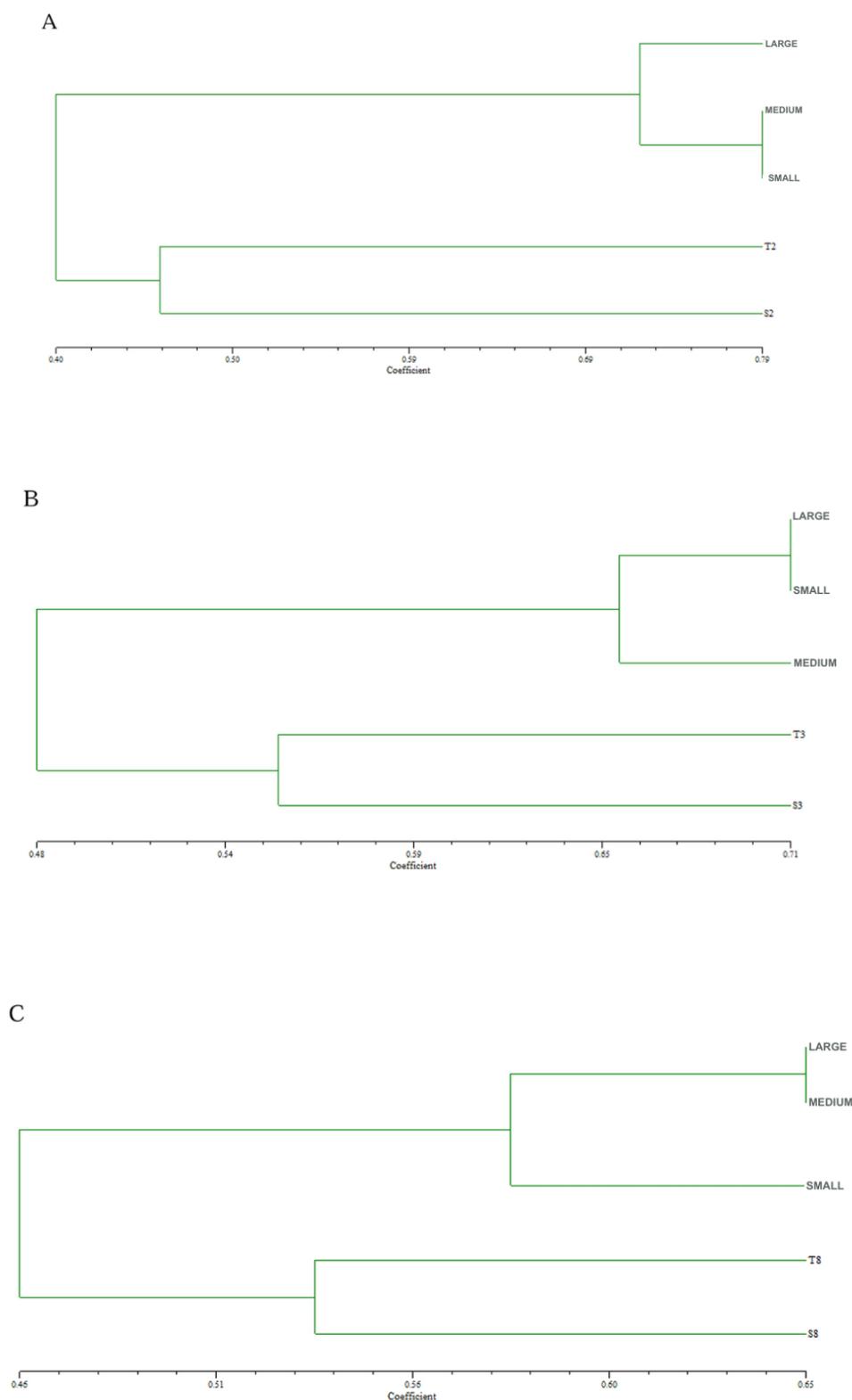
Size of fish samples	L	M	S	B
L	-			
M	0.002	-		
S	0.001	0.002	-	
B	0.016	0.013	0.018	-

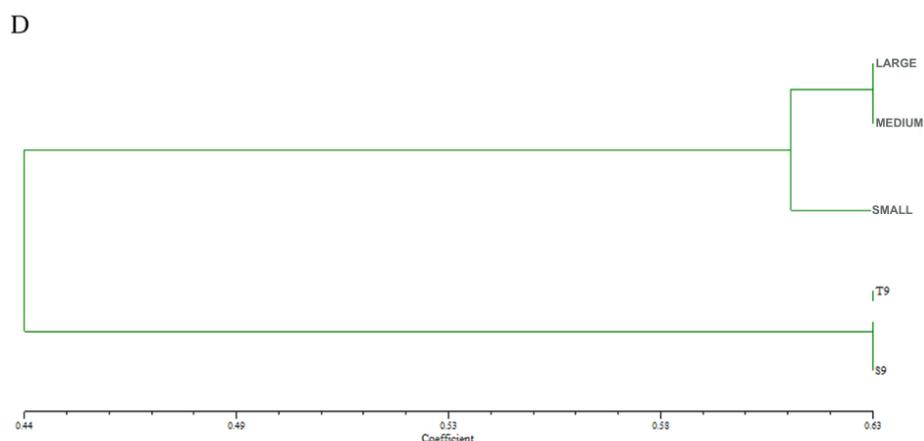
Note: L = large-sized fingerling sample, M = medium-sized fingerling sample, S = small-sized fingerling sample, B = broodstock sample.

The genetic distance values obtained were used to construct a dendrogram illustrating the relationships among fingerlings of three sizes (large, medium, and small) and the two broodstocks (♀ Sangkuriang × ♂ Thai). The dendrogram was constructed using the UPGMA method with the Simple Matching (SM) coefficient in NTSYS 2.11a.

The dendrogram results (Figure 5) for the seedlings of

three different sizes showed coefficient values ranging from 0.63 to 0.79, while the coefficient values for the broodstock (♀ Sangkuriang and ♂ Thai) ranged from 0.40 to 0.48. The dendrogram visualization and these coefficient values indicate that the three groups of seedlings with different sizes result from genetic combinations from both broodstocks.





**Figure 5.** Dendrogram based on genetic distances between fingerlings of three different sizes and their broodstock, conducted using the UPGMA method with the Simple Matching coefficient in NTSYS 2.11a. Large = 7-8 cm seed sample, medium = 5-6 cm seed sample, small = 3-4 cm seed sample, T = male parent of Thai catfish, S = female parent of Sangkuriang catfish, A-D = repeat code in crossbreeding.

Crossbreeding among catfish species has influenced fingerling weight through heterosis, the increased vigor exhibited by hybrids (Xie *et al.* 2024). Results for hybrid catfish fingerling sampling and grading are shown in Table

8. Hybrid fingerling weights were consistent across replicates: 3-4 cm fingerlings ranged from 0.42-0.47 g, 5-6 cm from 2.35-2.54 g, and 7-8 cm from 3.46-3.74 g.

**Table 8.** Length and weight of hybrid catfish fingerlings during rearing.

Crossbreeding		Fish Body Length		
		3-4 cm	5-6 cm	7-8 cm
Weight (g)	A	0.46±0.03	2.35±0.10	3.74±0.00
	B	0.47±0.01	2.36±0.07	3.46±0.12
	C	0.42±0.01	2.54±0.05	3.61±0.00
	D	0.45±0.02	2.40±0.00	3.48±0.23

Note: A-D = hybrid catfish fingerlings according to the broodstock pair replicate codes.

Genetic diversity plays a fundamental role in enhancing population resilience and enabling adaptation to environmental fluctuations. Degraded water quality can exert strong selective pressure, promoting the survival of individuals carrying genotypes suited to stressful conditions. Therefore, alleles associated with lower tolerance may decline in frequency, ultimately reducing overall genetic

variability within the population (Willi *et al.*, 2006; Ellegren & Galtier, 2016; Frankham *et al.*, 2017). Water quality in the rearing ponds for hybrid fingerlings remained within optimal ranges for all parameters. Water temperature in the ponds ranged from 27.9-30.2 °C, dissolved oxygen (DO) ranged from 4.5-6.7 mg/L, and pH ranged from 6.7-7.6 (Table 9).

**Table 9.** Water quality during the rearing period.

Parameter	A	B	C	D	Standard
Temperature (□)	29.5-30.2	29.5-30.1	27.9-28.9	28.8-29.3	25-30*
DO (mg/L)	4.8-6.7	4.5-6.6	5.4-5.9	5.6-6.1	>3 mg/L**
pH	6.7-7.4	6.7-7.6	7.3-7.5	7.3-7.5	6.5-8.6*

Note: \*SNI 01-6484.4-2000

\*\*SNI 6484.3-2014

A-D = hybrid catfish fingerlings according to the broodstock pair replicate codes.

### Discussion

Hybridization is the process of mating two individuals with different genotypes to produce offspring with superior traits (Arifin *et al.*, 2017). Fish breeding and genetic improvement through hybridization are effective methods for obtaining offspring with desired characteristics. Success of hybridization can be achieved when the two broodstocks being crossed share similarities in chromosome number, biological traits, and reproductive traits (Hafidah *et al.*, 2021). The selection of quality broodstock should be based on information about each individual's genetic diversity as a foundation for the hybridization process. Through proper broodstock selection, hybridization can produce offspring that inherit superior traits (Kristanto *et al.*, 2017).

One method for assessing genetic diversity is the random amplified polymorphic DNA (RAPD) technique (Omer *et al.*, 2020). RAPD is chosen because a single specific primer can amplify multiple nucleotide sequences in an individual, producing a high level of polymorphism without requiring prior knowledge of the individual's genome (Nugroho *et al.*, 2016; Kristanto *et al.*, 2017). RAPD primers are not designed to bind to specific genes; instead, they attach to nucleotide sequence regions in the sample that match the primer's base sequence during amplification. Thus, genetic diversity among individuals, species, or populations can be identified through the percentage of polymorphism generated using PCR-RAPD with appropriate primers (Omer *et al.*, 2020; Hayati *et al.*, 2021).

DNA bands observed in PCR-RAPD analysis are classified into two types: polymorphic bands and monomorphic bands. Polymorphic bands generate polymorphisms. Polymorphism is an indicator of genetic diversity, calculated as the percentage of polymorphic loci relative to the total loci detected in a single amplification (Hayati *et al.*, 2021). This polymorphism percentage can be used to assess genetic diversity at the genotypic level in fish (Buwono *et al.*, 2018).

The use of small, medium, and large fish samples in PCR-RAPD analysis is generally intended to evaluate whether differences in genetic diversity are associated with growth performance or phenotypic traits. According to Utomo *et al.* (2020), growth rate is a key trait in selection programs aimed at developing superior fish strains. Selection based on growth performance can be carried out by using individuals from the same cohort and classifying them into size groups, either by body length or weight.

The polymorphism percentage in this study ranged from 72.95% to 93.88%, based on four RAPD primers applied to fingerlings of three different sizes in each replicate. These values are considered high compared to the polymorphism percentages of three catfish strains (Masamo, Sangkuriang, and Paiton), which only reached 40-70% using four RAPD primers (Nugroho & Putera, 2018), and African catfish, which ranged from 76.97% to 83.33% with three RAPD primers (Ikpeme *et al.*, 2015). Differences in polymorphism percentages are due to variations in the sequences and binding sites of RAPD primers, resulting in different values (Nugroho & Putera, 2018). Additionally, the accuracy of the PCR-RAPD method is influenced by the number of primers and samples used; the more primers and samples analyzed, the higher the likelihood of detecting polymorphic DNA bands. These polymorphism values are used to identify superior traits inherited from the

broodstock to their offspring (Hayuningtyas & Kadarini, 2016).

The heterozygosity parameter is used to assess genetic diversity within a population by measuring the frequencies of alleles (alternative forms of a gene) at each locus (location on a chromosome). The extent of variation among individuals is reflected in their heterozygosity values (Iza, 2017). Heterozygosity is considered an accurate indicator of genetic variation within individuals, while genetic diversity among populations can be evaluated by averaging heterozygosity across multiple loci (Safran & Kurnianto, 2017).

The heterozygosity values of hybrid catfish fingerlings in three categories ranged from 0.167 to 0.232. For other freshwater fish, heterozygosity values include 0.236–0.305 for gourami (Nugroho, 2013), 0.203–0.1756 for kalui fish (Nugroho *et al.*, 2016), and 0.075–0.153 for uceng fish (Ath-thar *et al.*, 2018). According to Hayuningtyas *et al.* (2018), higher heterozygosity reflects greater genetic diversity, whereas values near 0 indicate low genetic diversity and those near 1 indicate high diversity (Ajogbasile *et al.*, 2021). Factors such as mutation rate, population size, mating patterns, migration, and broodstock selection influence heterozygosity (Iza, 2017). When sample sizes for small fish are too limited, allele frequencies can be biased. Although Nugroho (2013), Nugroho *et al.* (2016), and Ath-thar *et al.* (2018) did not specify fish sample sizes, each used 5-10 mg fin samples. To reduce bias and increase accuracy, ten fish fin samples are preferable. Genetic diversity can also be evaluated by genetic distance values. These values, calculated with Nei's formula (Pranata *et al.*, 2024), provide numerical measures of genotype differences. By comparing genetic distance, one can assess relatedness: values near 0 indicate closely related individuals, while values approaching or exceeding 1 suggest distant relationships (Saleky *et al.*, 2021).

The average genetic distance values of fingerlings of three different sizes and the broodstock ranged from 0.001 to 0.018. Genetic distance analysis was performed using GenAlEx 6.51b2 and Nei's formula. These low values suggest close genetic relatedness among all size groups and broodstock. This is expected, as all samples originated from the same species and breeding population. Generally, genetic distance within a species is lower than that observed between species (Pranata *et al.*, 2024). High genetic similarity within populations means shared ancestry and gene flow, while greater divergence is typical of interspecific comparisons. High genetic diversity at certain loci is often associated with lower relatedness among individuals (Safran & Kurnianto, 2017). In this case, the minimal genetic distance shows that the different size classes are not genetically distinct groups.

Importantly, interpreting genetic distance values must also consider sample size. Unequal or limited sampling can affect allele frequency estimates and bias calculations. Smaller sample sizes may reduce the likelihood of detecting rare alleles, leading to an underestimation of differentiation. In this study, all groups were analyzed using comparable sampling schemes within the same species. This made comparisons among size classes and across broodstock consistent. Nevertheless, increasing the number of individuals per group would further improve the precision and robustness of the genetic dis-

tance estimates.

Based on genetic distance values (a measure of how genetically different samples is), a dendrogram (a tree-like diagram that shows the relatedness between groups) can be constructed to visualize relationships among samples. In this study, the dendrogram was analyzed with NTSYS 2.11a (a statistical software package for analyzing genetic data). Fingerlings of three sizes were grouped into three populations (large, medium, small) and two broodstocks (Sangkuriang and Thai), showing consistent patterns across replicates. Coefficient values in the dendrogram (numerical values that represent genetic similarity) indicate genetic similarity: higher coefficients mean closer genetic distance (i.e., more genetically similar), while lower coefficients mean greater genetic distance (i.e., less genetically similar) (Banerjee et al., 2016).

Accordingly, the dendrogram results indicate that the fingerlings of three different sizes are a genetic combination of both broodstock, as reflected by the differences in coefficient values between the fingerlings and the broodstock. The coefficient values of the fingerlings ranged from 0.63 to 0.79, forming cluster 1 in the dendrogram, while the coefficient values of the two broodstock ranged from 0.40 to 0.48, forming cluster 2, which is distinct from the fingerling cluster in each replicate.

High genetic diversity in offspring can be achieved through crossbreeding of parents with high levels of genetic variation (Suprpto & Iswanto, 2022). The level of genetic diversity among populations is influenced by factors such as gene mutations, natural selection, and random mating, which can alter a population's genetic composition. Mutations and migration also contribute to increasing genetic diversity within a fish species. Populations with high genetic diversity, as indicated by high levels of polymorphism, heterozygosity, and genetic distance, generally exhibit better adaptability to their environment, thereby affecting survival and growth performance (Nurnaningsih et al., 2022).

Differences in size classes may reflect underlying biological processes such as differential growth rates, selective mortality, or non-random parental contribution. If certain genotypes are associated with faster growth, larger individuals could, in theory, display slightly different allele frequencies than smaller individuals due to selection. However, in the present study, the relatively similar heterozygosity and low genetic distance values among size groups suggest that body size variation is not accompanied by substantial genetic differentiation.

## CONCLUSION AND RECOMMENDATION

### Conclusion

The study results showed that hybrid seedlings of three different sizes, produced from the cross between Sangkuriang catfish and Thai catfish, analyzed using the PCR-RAPD method with four RAPD primers, exhibited a high level of genotype diversity. This was indicated by a polymorphism percentage of 72.95–93.88% and heterozygosity values ranging from 0.167 to 0.232.

### Recommendation

Breeding of Sangkuriang and Thai catfish should be carried out to improve the quality of the broodstock used to produce hybrid seedlings.

## AUTHORS' CONTRIBUTIONS

KAR is supervision and written manuscript. PNS is doing research and written original manuscript. AMD is supervision. DTS is supervision.

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