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Heritability, Selection Response, Genetic Gain and RAPD Markers of *Anabas testudineus* in Three Generations

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ABSTRACT

The acceleration of fish growth is often accomplished through individual selection programs. Therefore, this study aimed to evaluate growth performance in climbing perch (*Anabas testudineus*) over three generations by analyzing heritability, selection response, as well as genetic gain and distance using the molecular marker RAPD. Climbing perch broodstock was sourced from Musi River in South Sumatra, which produced three individually selected offspring. A total of three treatment types were used, namely first-generation (F1), second-generation (F2), and third-generation (F3). The treatment was replicated four times, and the experiment utilized a randomized design. Furthermore, gene diversity was compared among generations and natural populations using 19 primary RAPD markers. The results showed that low and high heritability was found in F1 (0.18) and F3 (0.40), respectively. The lowest selection response was observed in F1 (3.09g), while F3 (8.04g) demonstrated the highest. F1 also had the smallest genetic gain (5.58%), and F3 produced the largest (13%). Genetic distance (0.28) between F3 and the wild population was not significantly different. The results indicated that the next generation of climbing perch might not experience a significant genetic increase.

Key words: Climbing perch fish, Breeding, Family, Genetic

INTRODUCTION

Climbing perch is an endemic Asian freshwater food commodity with economic value favored by Asians in countries such as India, Malaysia, Cambodia, Thailand, Vietnam and Indonesia (Kottelat et al. 1993; Morioka et al. 2009; Dzerzhinskiy et al. 2019). The population thrives in inland waters, including marshes, rivers, lakes and reservoirs (Perera et al. 2013; Helmizuryani and Muslimin 2016; Helmizuryani et al. 2020). This species is widely distributed in Indonesia across the Sumatra, Kalimantan, and Java Islands (Helmizuryani et al. 2018).

As stated in previous reports, climbing perch has been cultivated in aquaculture for both spawning and rearing purposes (Perera et al. 2013; Helmizuryani and Muslimin 2016; Singh et al 2019). However, a considerable period is required for growth, reaching approximately 10 to 12 months, with a reasonably high feed conversion rate of 3.5-5 (Torang 2013; Helmizuryani and Muslimin 2015). Internal factors, specifically genetic variation, and external factors, including feed quality and quantity, temperature, as well as photoperiod, affect growth (Beveridge and Mangel 2012; Helmizuryani et al. 2020; Muslimin et al. 2023). External factors can be decreased by enhancing genotype characteristics (Ashton et al. 2016; Retnoaji et al. 2023). Individual selection is a selective breeding strategy used in conjunction with genotypic selection to produce specific phenotypic characteristics. Strengthening genotypic characteristics and assessing economic aspects such as growth and carcass make it possible to accomplish effective and efficient climbing perch rearing (Sae-Lim et al. 2015).

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Increased growth performance in fish can be attributed to selection of individuals from several generations (Gjedrem 2012). Genetic engineering is capable of developing breeds with increased growth and environmental tolerance through an individual selection of family members (Liyong et al. 2015). Furthermore, this approach is used to obtain fish with a high degree of genetic variety, affecting growth rate, heritability, selection response, and genetic gain (Lind et al. 2012). Improved genetic quality in the subsequent generation may result in improved gene repair (Lind et al. 2012), as demonstrated in *Oreochromis niloticus* (Bentsen et al. 2012), *Oreochromis aureus* (Robisalmi et al. 2023), Oyster (Dégremont et al. 2015) and white leghorn (Veeramani et al. 2012). RAPD marker is one of the methods for detecting genotypic diversity (Gustiano et al. 2013; Yustiati et al. 2021). Therefore, this study aimed to examine individual selection of climbing perch in three generations, with a focus on evaluating heritability values, selection response, as well as genetic gain and distance.

MATERIALS AND METHODS

Synthetic population

This study was carried out from May 2020 to October 2021 at Mulia, a small-scale fish breeding unit in Plaju District, Palembang City. The test fish were broodstock (*Anabas testudineus*) domesticated in South Sumatra's Lubuk Lampam flooded wetlands. Subsequently, new fish (F0) were generated, among which 10 males and 10 females were mated, resulting in three generations, namely the first (F1), second (F2), and third (F3).

Male parents were selected with an average weight of 33.8±5.56g and an average length of 12.5±0.5cm, while female broodstock values of 32.4±0.96g and 12.6±0.29cm respectively. The broodstocks were mated using semi-natural spawning methods, with a male: female ratio of 1:1. This mating was enhanced by an extra hormone namely GnRH (ovaprim), injected into male and female broodstocks at a rate of 0.5mL/kg (Perera et al. 2013). The fish were then relocated in pairs to a 100x60x40cm^3 aquarium to allow for natural mating.

After 1x24 hours of egg release, the broodstocks were separated and hatching was performed in an aerated tank. Nurseries were conducted in tanks using natural feed in the form of artemia then after 40 days, or the juvenile phase, the fish were used as specimens, and pellets were provided *ad libitum* for a period of 140 days.

A total of three post-juvenile generations of F0 offspring, namely F1, F2, and F3, were used for treatment with three replications. The fish were reared in 1x1x1.25m^3 nets with a stocking density of 30 per fishing net. The rearing lasted three months, while pellets containing 30% protein were supplied *ad libitum* during the day and night.

Selection

Individual selection was used in this study and after the investigation, the fish were measured. Standard weight and length were used to determine phenotypic characteristics. The data were sorted by phenotypic size, and selection limit was 10% of the population with the best size. Heritability value, selection response, diversity coefficient, genetic gain, and phylogenetic tree analysis of RAPD marker gene were all used as parameters.

Heritability (h2) and selection response

Heritability is an estimate of the growth rate and genetic diversity of an organism's phenotype (Tamamdusturi and Basuki 2012). According to Gjedrem and Baranski (2009), heritability is the percentage of phenotypic characteristics inherited by the previous parent due to genotype addition. This genetic trait may manifest as growth and resistance to environmental toleration (Liyong et al. 2015). The following equation represents the relationship between heritability of growth with selection response and differences (Tave 1986).

$$R = S \times h^2$$

Where:
h^2 = heritability
R = selection response, namely the derived population's mean weight (W_{p+1}) – average of broodstocks weight (W_p)
S = *Selection differential*, namely the average weight of selected fish (W_s) – the population's average weight (W_p).

Coefficient of Variance and Differential Selection

Coefficient variance (CV) and differential selection were used to determine the degree of variability required for selection (S) and the equation is as follows (Singh and Chaudhary 1977):

$$CV = \frac{SD}{X} \times 100\%$$

Where:
CV= coefficient of variance
SD = standard deviation
X = weight average
Differential selection was accomplished by applying the formula:

$$S = x' - x$$

Where:
S = selection differential
x' = average of phenotype after selection
x = average phenotype in a population

Genetic gain

Direct selection of fish has a potential effect on the average population growth rate, referred to as genetic gain. In other terms, genetic gain is defined as the average of superior individual offspring relative to the preceding generation using the equation (Gjedrem and Baranski 2009):

$$\Delta G = i.h^2.\delta$$

Where:
ΔG = genetic gain
i = selection intensity, i.e., $S/\delta p$
δ = standard deviation and δp is standard deviation in the population

Random Amplified Polymorphic DNA (RAPD)

The extraction procedure started by isolating 200mg of gills from each specimen preserved in 96 percent ethanol. The gills were obtained by acquiring Animal Ethical Welfare approval from the Research Ethics Committee of Muhammadiyah University of Palembang. DNA extraction was performed at the end of rearing using three specimens

from F1, F2, F3, and wild stock generations. The extraction procedure and PCR product were performed at the fish pest and disease laboratory, Department of Fisheries, Universitas Gadjah Mada, Indonesia using a geneaid kit. Protocols for DNA extraction and isolation were in line with a previous study (Muslimin et al. 2020).

A total of 19 primers were used for climbing perch (Durna 2009) to determine genetic diversity over generations and the nucleotides are reported in Table 1. The material was then transferred to a microtube filled with PBS and pulverized until homogeneous, followed by centrifugation at 300g for 5min, with the supernatant discarded. To begin the lysis, 150µL RBC lysis, 150µL GT, and 200µL GB buffer were added. DNA purification was performed by adding 5µL RNAse and incubating at 60°C for 10min.

Table 1: Nineteen primers listed in this study.

Primer	Sequencing (5'-3')
OPA-1	CAGGCCCTTC
OPA-3	AGTGAGCCAC
OPA-4	AATCGGGCTG
OPA-5	AGGGGTCTTG
OPA-7	GAAACGGGTG
OPA-8	GTGACGTAGG
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCGT
OPA-12	TCGGCGATAG
OPA-13	CAGCACCCAC
OPA-14	TCTGTGCTGG
OPA-15	TTCCGAACCC
OPA-16	AGCCAGCGAA
OPA-19	CAAACGTCGG
OPB-5	TGCGCCCTTC
OPB-8	GTCCACACGG
OPC-2	GTGAGGCGTC
OPC-3	GGGGGTGTTT
OPC-4	CCGCATCTAC

About 200µL absolute ethanol was added to DNA and inverted for 10s. The liquid was transferred to 2mL collection tubes and centrifuged at 14,000rpm for 2min. Subsequently, DNA washing was carried out using 400µL W1 buffer, followed by centrifugation at 14,000rpm for 60s, and discarding GD column contents. At the end of washing, 600µL wash buffer was added and centrifuged 14,000rpm for 3min. DNA elution was performed by adding 100µL of pre-heated elution buffer, allowed to stand for 3min, and then centrifuged at 14,000rpm for 30s.

PCR reaction replicated DNA extraction product and for PCR mix, 5µL NFW, 6µL bioline, 0.5µL primer, and 0.5µL template were required. DNA was amplified in a thermocycler with a 12µL total capacity and 35 cycles at various temperatures. Denaturation was carried out at 94°C for 3.50min, annealing at 50°C for 3s, and extension at 72°C for 6.5min. PCR quality of the product was determined using PCR band generated on an agarose gel, comprising 30mL Tris base, acetic acid and EDTA (TAE), 0.3g agarose, and 0.75µL flourosafe.

Data analysis

The diversity coefficients, heritability values, selection responses, and genetic gains were analyzed using Microsoft Excel 365 and IBM SPSS version 23.0. Meanwhile, the phylogenetic trees, genetic distances and FST values were calculated using MEGA X and POPGene 1.31 software packages.

RESULTS

Coefficient of Variance (CV)

CV percentages (standard-length character and weight) of climbing perch for F1, F2, and F3 generations are summarized in Table 2. Based on the results, F3 had the highest CV score (9.27%), while F2 had the lowest (7.61%). Furthermore, CV value for the weight character was highest in F3 (25.71%) and lowest in F2 (23.31%). The weight character denotes a moderate CV, which serves as a criterion for the following individual selection process.

Table 2: Standard-length and weight character diversity coefficients for the F1, F2, and F3 generations.

Generation	Coefficient of Variance	
	Standard length (%)	Weight (%)
F1	8.64	24.00
F2	7.61	23.31
F3	9.27	25.71

Heritability value and selection response

The fish were selected based on a 10% average weight character of the entire population. Table 3 shows the average results for individual selection, response, heritability, and genetic gain. Individual selection cutoffs began at >50.11g (F1), >51.79g (F2), and >57.82g (F3). Based on the results, F3 had the highest average weight (64.02±6.71g) while F2 had the lowest (53.82±6.71g).

F3 had the highest selection response (8.34 g) and heritability values (0.40), while F1 had the lowest scores of (3.09) and (0.18), respectively. The largest and lowest genetic gain value was found in F3 (8.34 %) and F1 (3.08 %), respectively.

The weight distribution of climbing perch generations F1, F2, and F3 are presented in Fig. 1. Fish were selected based on 10% of the population with the highest weight. F1 (Fig. 1A) weighed between 24-62.8g, with individuals selected from>50g. Selected individuals in F2 (Fig. 1B) started at >48.9g, while those in F3 (Fig. 1C) started at >53.2g.

Phylogenetic trees and genetic distance

The results of DNA amplification from the electrophoresis process are presented in Fig. 2. Although a total of 19 primers were used in the amplification process, electrophoretic bands indicated that only seven primers produced good results. These included OPA1, OPA3, OPA4, OPA10, OPA11, OPA15, and OPA16, with the length of DNA bases ranging from 100 to 1,000. The seven primers were used to detect DNA fragments in F1, F2, and F3 and natural generations as a comparison.

Fig. 3 shows the phylogenetic trees based on the frequency of alleles in the three generations of climbing perch. Clustering used the Unweighted Pair Group Method with Arithmetic (UPGMA), consisting of wild (cluster I), as well as F1 and F2 (cluster II), and F3 (cluster III).

Table 4 shows genetic distance and p-value (< 0.05) of the three generations compared to wild stock. F1 and F2 had a significantly different lowest genetic distance (0.03). The greatest genetic distance (0.28) was found between F3 and wild stock, but it was not significantly different.

Table 3: Cut off, the average weight of individual, the average weight of selected individuals, selection response, and heritability of climbing perch from F1, F2, and F3

Generation	Cut off	Average individual weight±SD (g)	Average±SD weight (g) of selected individuals	Selection response (g)	Heritability (h^2)	Genetic Gain (%)
F1	50.11	38.21±9.15	55.29±4.29	3.09	0.18	5.58
F2	51.79	38.64±9.01	53.82±6.41	3.52	0.23	6.54
F3	57.82	43.46±11.17*	64.02±6.71*	8.34	0.40	13

Note: *Significant values are denoted by superscripts.

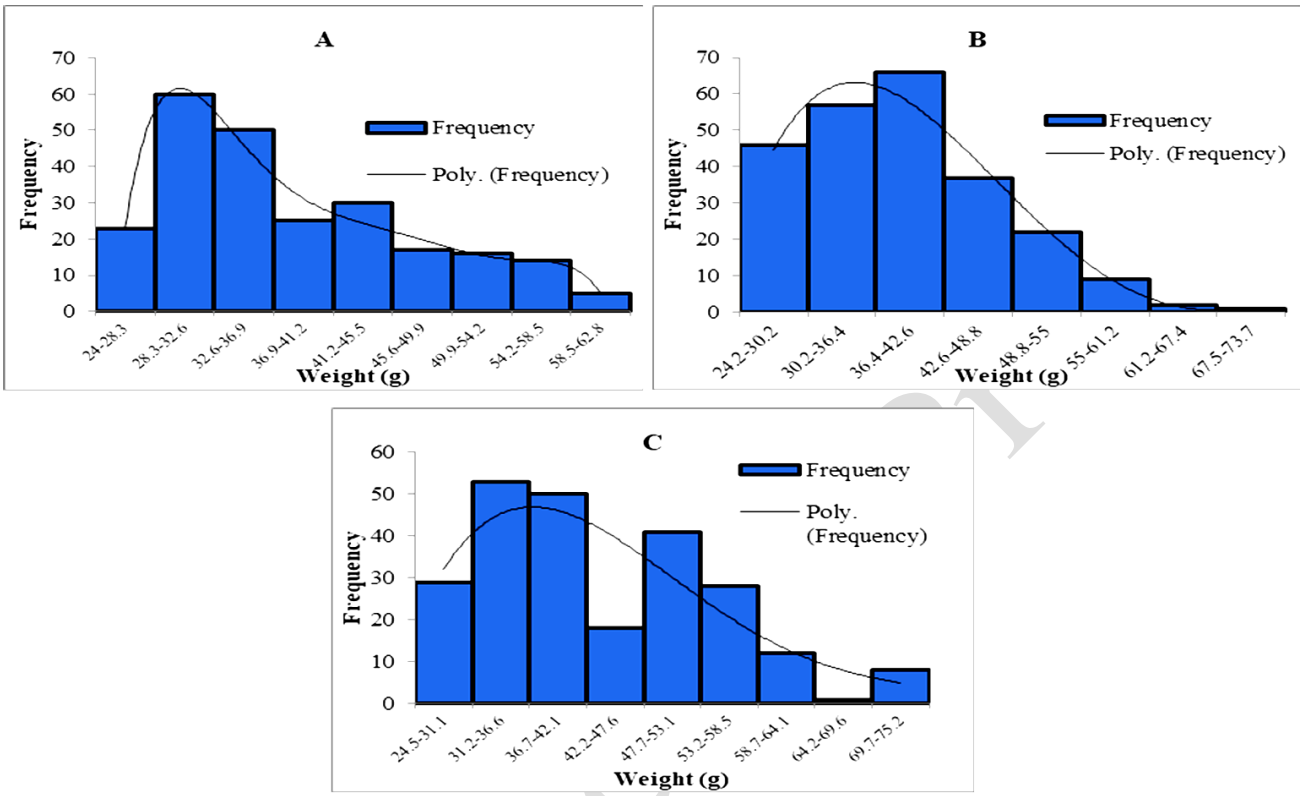

Fig. 1: Data on the weight distribution of climbing perch frequency in three generations, namely F1 (1A), F2 (1B), and F3 (1C).

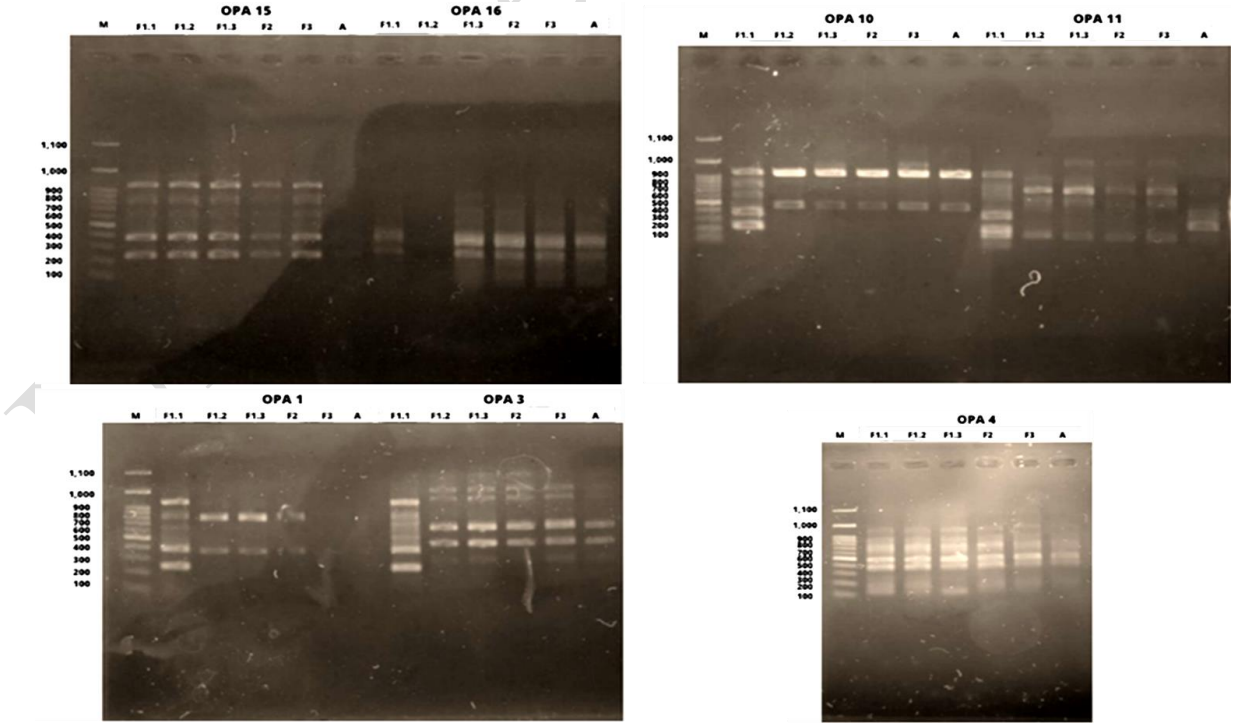

Fig. 2: Seven primers were used to amplify DNA: OPA 1, OPA 3, OPA 4, OPA 10, OPA 11, OPA 15, and OPA 16. Information of M (a DNA size marker indicator), F1 (first generation), F2 (second generation), F3 (third generation), and A (the wild stock).

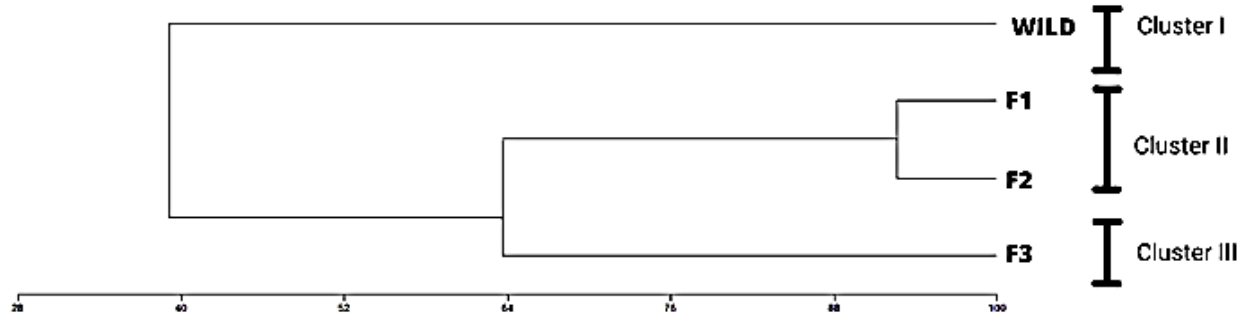

Fig. 3: Dendrograms of climbing perch of three generations (F1, F2, and F3) and Wild types as a comparison.

Table 4: Genetic distance and at the level of significance (P<0.05)

Population	F3	F2	F1	Wild
F3	0	0.6	0.55	1.04
F2	0.21	0	0.05*	0.05*
F1	0.2	0.03*	0	0.45
Wild	0.28	0.18*	0.19	0

Note: *Significant genetic distances.

DISCUSSION

The coefficient of diversity is divided into three categories, namely low (0-20%), medium (20-50%), and high (>50%) (Gunadi et al. 2016). This study showed that CV was low on the standard-length character (7.61-9.27%). In contrast, the percentage of heavy character diversity in the medium category (23.31-25.71%) was not significantly different between generations. Moderate CV suggested that the character of growth in a population had a fair level of diversity or heterogeneity (Radona et al. 2016; Husmaini et al. 2023). Weight character had a higher CV value than standard-length in blue tilapia (*Oreochromis aureus*) (Gunadi et al. 2016). Due to the wider range of expression than the standard-length character, the weight character was used as a phenotype indicator in climbing perch.

CV values in the weight of each species vary, for example, *Scophthalmus maximus* L. and *Pangasius djambal* had a low CV (6-16% and 6.97-19.53%) (Liyong et al. 2015; Tahapari et al 2020), while blue tilapia had a high value (19.62-53.47%) (Gunadi et al. 2016). Each fish produced different results due to the heavy growth and length response. A diversity coefficient of >0.25 is associated with a high heritability value (Gustiano et al. 2013), and CV in this study was 0.40. CV percentage correlated with heritability, selection response, and genetic gain, serving as a preliminary indicator of selection success.

Selection response value ranged from 3.09 to 8.04g, implying an increase in the weight of each generation. This result was higher compared to the red tilapia strain nilasa (7.95g), otherwise lower than GIFT tilapia (31.1g) and *Pangasius djambal* (24.29g) (Gustiano et al. 2013; Tahapari et al. 2020).

Heritability values are divided into four groups, namely low (0.05-0.0.15), moderate (0.20-0.40), high (0.45-0.60), and very high (>0.65) (Liyong et al. 2015). In this study, heritability scores in F1 (0.18) and F2 (0.23) were low, while F3 was in the medium category (0.40). This result was higher than the individual selection program for tilapia (0.39) (Gustiano et al. 2013). According to a previous study, individual fish selection can increase genetic gain between 8.4-9.5% (Da Silva et al. 2019), moreover, more than 10% per generation (Gunadi et al. 2016). The genetic gain value for F3 reached approximately 13%.

Based on the results, genetic gain, selection response, and heritability all increased. Genetic gain, selection response, and heritability are indicators for the interconnected response of genetic advances that determine breeding success (Lucas and Southgate 2014). However, heritability and selection response values indicated that the results were not maximized due to feed factors, water quality, and a wide range of fish sizes (Nugroho and Priyanto 2016). Inbreeding in the same allele in several loci affects heritability values and selection responses (Tamamdusturi and Basuki 2012). The quality of heritability could be improved by continuing selection process for more than five generations, as well as conducting family selection, and crossbreeding (Asih et al. 2012; Gjedrem 2012; Pwipong et al. 2016).

Dendrogram clustering had a coefficient value of >0.75 or>75% due to high genetic diversity (Matta et al. 2015). Based on these results, the wild stock population (40%) and F3 generation had low similarity to UPGMA dendrogram results (64%). A medium genetic distance was found between the wild stock and F3 generation.

Genetic distance values are divided into three, namely low (0.010-0.099), medium (0.1-0.99), and high (1.00-2.00) (Nei 1972). In this study, genetic distance ranged from low (0.03) to moderate (0.18-0.28). The low genetic distance was the same as in tilapia fish (Gustiano et al. 2013). In this context, the effects of heterosis inherited from previous broodstock caused a medium genetic distance. Heterosis potentially leads to low genetic variation due to genetic drift (Nugroho et al. 2014). Individual selection programs of more than five generations could achieve heterozygosity (Gjedrem 2012). However, reduced and increased gene variations might be found in the next generations, such as in red tilapia (Nugroho et al. 2014). This underscores the need for assessing genetic diversity for each generation using family selection methods when individual selection results are not ideal.

Conclusion

In conclusion, heritability, selection response, and genetic distance value of climbing perch (*Anabas testudineus*) in F3 population had a moderate genetic increase compared to previous generations. However, evaluation of selection breeding (family or crossbreeding) for the next generation was required.

Author's contribution

The authors have been engaged in various duties. The research management and draft paper writing was performed by Helmizuryani. Laboratory analyses and experimental research were carried out by Boby Muslimin, Rosmiah, Danang Yonarta, and Dewi Apriyanti. Data analyses and finalization of the original paper conducted by Meika Puspitasari and Khusnul Khotimah.

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