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Technology Engineering of Aquaculture Snakeheads [*Channa striatus* (Bloch, 1793)] using Cross Breeding from Different Waters for Determining the Genetic Variation of Superior Seeds

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Abstract

The objective of this research was to investigate the superior seed for snakeheads was result on the cross-breeding parent of snakeheads from different waters in Central Java. The material used snakeheads size 700 g ± 0.05 g (female and male) from different water in Central Java. Methods design completely randomized four treatments and three replications, the study in the first place from February 2015 to November 2015. Seed snakeheads caught from waters Rawa pening is crossbreeding caught from waters Segoro anakan (A), then is crossbreeding parent snakeheads from Gajah Mungkur waters (B), crossbreeding parent snakeheads from Rembang rivers (C) and crossbreeding from the parent snakeheads from Ujung Pangkah waters (D), superior seed treatment results from the production was given by pellet 5% / biomass / day. Study to produce snakeheads the size of consumption with the result of rapid growth, moderate and slow proceed anyway analysis of genetic variation genetic code, heterozygote, polymorphism snakeheads consumption size super. The results showed that of the maintenance of improved seed of cross-breeding waters of the Swamp Dizziness cork mated with parent fish caught from Ujung Pangkah (D), which is the highest result (165 337 mg) and lowest from waters Segoro anakan A (141.5 mg). Next polimorpisame analysis results using a micro-satellite to fish yielding seeds snakeheads on various treatment caught from waters Rawa pening be mated with parent from the waters Segoro anakan of snakeheads (A, band 1,2), the parent snakeheads Gajah Mungkur (B, band 3.4).

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Keywords: : Albumine; DNA; mikrosatelite analysis; snakeheads [*Channa striatus* (Bloch, 1793)]

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1. Introduction

Nowadays, the extinction of the snakehead fish is caused by the overfishing, habitat disruption (Balkhis et al., 2011), and pollution (pesticides, soap sand organic matter, gradual repair of damaged muscles necrosis, macrophage infiltration, fibrosis and mycotic granulomas (Uthayakumar *et al*., 2014, Rao *et al*., 2015), also that Qiufen *et al*. (2013) reported, that causes death in *Channa striatus* to solve the problem is prevent the extinction through basic research to discover the theories and methods of fish farming to improve of snakeheads fish (Istiyanto, 2011) and environmentally friendly so as to increase the production of *C. striatus* (Hariyanti, 2013), so as to increase the production of *C. striatus* (Bijaksana, 2012).

Another problem is the using of mikrosatelite variability of fish growth for both broodstock *C. striatus* and the superior broodstock seed marriages, with differences in geographical location (in Rawa Pening, reservoirs Gajah Mungkur, Solo River, Segara Anakan waters, Ujung Pangkah waters broodstock of snakeheads with the difference in geographical location and type of waters in Central Java was obtained by the difference in quality snakesheads fish broodstock and improved seed are: baseline differences incomposition and molecular weight of the DNA, heterogenetas, genotype and allele frequencies, this basic data helpful. Highly for the development of fish farming snakeheads in Central Java , especially in choosing fish seed parent and a good quality snakesheads. Targeted research was the basis of data obtained by different types of parent snakeheads with different growth rates (highestt, medium and low) and based on the analysis of genetic variation mikrosatelite as one type of fish germ plasmah nutfah of snakeheads in Central Java which was currently unclear, and has not been found by the genetic engineering approach mikrosatelite methods. So it needs to know that the potential genetic (Bijaksana, 2003; Bijaksana, 2006) and freshwater aquaculture efforts, through interbreeding future with out - breeding techniques. The importance of this study is to increase the production of *C. striatus* which contains albumin and useful in acceleration of healing and post-surgical blood clots, as well as assist in the protection / conservation of snakeheads fish from extinction plasmah nutfah Central Java Province. Based on information obtained basic data such as genetic purification, then used as the basis for *C. striatus* and assist in the selection of qualified of broodstock snakedsheads fish farming technology developed with cross-breeding technique is out-breeding Sakhare (2015) reported fecundity of air breathing fish *C. striatus* that also observation made on the fecundity of the air-breathing fish from waterbodies in Beed district Maharashtra India are reported. The ovarian ggs were found to be of different sizes (Bijaksana 2006). The number of ova $\cdot$ g^{-1} mature ovary ranged from 477 to 695 and the number of of ova $\cdot$ g^{-1} body weight, from 36 to 68, the average being 49. The gonad weight and fecundity showed an increase with the increase in size of fish. The objective of this research was to investigate the superior seed for snakeheads was result on the cross-breeding parent of snakeheads from different waters in Central Java.

1. Material and methods

1.1. Preparation of animal test

The research activities will be conducted in the month (February 2015) in the laboratory of Prof. Dr. Gatot Laboratory Coastal Eco Development, University of Diponegoro and Laboratory Faculty of Mathematics, University of Diponegoro in Semarang, Indonesia.

2.2. Animal testing

Animal test used is the snakeheads was crossbreeding are taken from public waters of the area (geographic) which can represent a different public waters in Central Java, Indonesia. Methods of seed snakeheads using basic design completely randomized with four treatments and three replications, namely the implementation of the study year I carried out from February 2015 to November 2015 which examines the character of genetically superior seeds snakeheads most good growth, namely snakeheads caught from waters Rawa pening (A) will be mated with a parent snakeheads caught from waters Segoro Anakan, then mated with parent snakeheads derived Gajah Mungkur (B), and is mated with a parent who came from Rembang waters (C) and mated from the parent fish the of the

snakeheads from Pangkah of waters public (D), followed by giving the type of feed given pellet 5 % / biomass / day (C).

2.3. Implementation research

Implementation of research growth, namely snakeheads caught from waters superior seed snakeheads with the best growth is the parent (type male and female) snakeheads caught from waters Rawa pening is crossbreeding parent snakeheads caught from waters Segoro anakan (treatment A), then is crossbreeding parent snakeheads from Gajah Mungkur waters (B), and is crossbreeding parent snakeheads from Rembang rivers (C) and crossbreeding parent snakeheads from the parent snakeheads from public waters Ujung Pangkah (D), superior seed treatment results from the production was given by pellet 5 % / biomass / day. Study to produce snakeheads the size of consumption with the result of rapid growth, moderate and slow proceed anyway analysis of genetic variation genetic code, heterozygote, polymorphism snakeheads consumption size super.

2.4.Ingredients for mixture

Materials used snakeheads development of fish farming seeds cross-breeding of in Central Java waters.

2.5.Equipment

The equipment used in the development of aquaculture (seed and measure consumption) snakeheads by using, among others, are the surgical tools (tweezers, knives, scissors, watch glass, a petri dish, glass objects, a pipette, hotplate, tissue paper, a microscope (magnification 1 000×, 1 500×).

2.6.Research method

The methods used in this study was parent of snakeheads size 700 g ± 0.05 g (female and male) from different water in central Java. Methods used Completely Randomized Design four treatments and three replications, namely the implementation of the study in the first place from February 2015 to November 2015 resulted in superior seed snakeheads with the best growth is the parent (type male and female) snakeheads caught from waters Rawa pening is crossbreeding parent snakeheads caught from waters Segoro anakan (treatment A), then is crossbreeding parent snakeheads from Gajah Mungkur waters (B), and is crossbreeding parent snakeheads from Rembang rivers (C) and crossbreeding parent snakeheads from the parent snakeheads from public waters Ujung Pangkah (D), superior seed treatment results from the production was given by pellet 5% / biomass / day. Study to produce snakeheads the size of consumption with the result of rapid growth, moderate and slow proceed anyway analysis of genetic variation genetic code, heterozygote, polymorphism snakeheads consumption size super.

2.7. Ingredients for mixture

The materials used for the development of fish farming snakeheads broadstock mikrosatelite analysis sample extract fish snakeheads, reagents such as PCR Kit: 10 x PCR buffer, 2.5 mM dNTP mix, primer IS-GB1F 5-ATT TGT CCC TCA TTT CTC CA-3 and of Islam GB 1 R 5-ACC ATC AAC ACT GCA TCT CT-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE), and primary -GB2 IS F oligo BASE TYPE 5-AGA AGA AGA AGA AGC CGA GT-3 and IS -GB2 R 5-AAA GAA AGG AGC CAG AAC AC-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE) and primary, as well as, 5 uL. Tag Polymerase, akuadest and mt-genome DNA in 0.2 mL PCR Tube, Universal primer OPA 4, 1 % agarose gel in 1× TBE (tris boric acid EDTA) buffer, Lodder 100 bp DNA, ethidium bromide, UV transilliuminotor, enzyme Hind III restriction (A'AGCTT); Bam HI (G'GATCC); EcoR V (GAT'ATC) 17 HAE III (GG'CC), solution 10× buffer, 100× BSA, restriction enzymes and akuadest and mt-DNA template, 1.5 % agarose gel in 1× TBE buffer.

2.8. Equipment

The equipment used in the development of snakeheads fish farming using mikrosatelite analysis including surgical tools (tweezers, knives, scissors, watch glass, petri dish, glass objects, pipette, hotplate, tissue paper, a microscope (magnification 1 000×, 1 500×) and the extraction and purification equipment mt-DNA, mt-genome PCR amplification of DNA, Restriction Fragment Length Polymorphism. equipment such as: eppendorf tube, water bath, heating equipment, centrifuges with size capacity was more than 13 000 rpm (1 rpm = 1/60 Hz), PCR amplification, 0.2 mL PCR tube, UV Transilluminator, cameras gel, electrophoresis.

2.9. Mikrosatelite analysis

Mikrosatelite analysis method was performed as follows:

2.9.1.Method and data collection instruments

Strategy 1 catfish holding elections on the basis of genetic markers. This research used samples of the parent fish with a length of 31.5 snakeheads to 50.3 cm and weight 262 g to 1 037 g original from Central Java waters (Gajah Mungkur, Rawa Pening, Solo River) Each sample group was 20 head.

2.9.2.Extraction and purification of mt-DNA

Method of extraction and purification of mt-DNA of fish snakeheads based method Jamsari et al. (2011) was conducted by means of genome mikrosatelite obtained through modification of the method of extraction followed Ovendem (2000). Network snakeheads fish (fins, meat fish snakeheads) made an extraction destroyed in 500 mL of 10 % Chelex-100 were included in the eppendorf tube and add 5 mL proteinase kinase (10 mg · mL^{-1}) and heated in a 55 °C water bath for 3 h to 4 h. Furthermore, the solution was heated again at a temperature of 89 °C for 8 min and cooled at room temperature to cool before adding 55 mL of TE (Tris-EDTA) buffer pH 8.0. Mt-DNA genome could be obtained by centrifugation for 5 min at 13 000 rpm. Solution in the upper layer and a clear colorless genomic DNA was transferred into a new eppendorf tube and stored at -20 °C for further analysis.

2.9.3.Genomic DNA PCR 3 amplifikasi

PCR Amplification of Genomic DNA by PCR amplification of the genomic DNA samples of snakeheads each treatment began with mixing multiple PCR reagent kit (Qiagen) consisting of 10× PCR buffer, 2.5 mM dNTP mix, primer-primer IS GB1F 5- ATT TGT CCC TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC ATC AAC ACT GCA TCT CT-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE), and primary -GB2 IS F oligo BASE TYPE 5-AGA AGA AGA AGA AGC CGA GT-3 and IS -GB2 R 5-CAG GAA AAA AGC AGG AAC AC-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE), 0.5 uL Taq polymerase, akuadest and genomic DNA in 0.2 mL PCR tube and incubated in a PCR machine with 38 cycles. In this amplification used an initial denaturation temperature of 94 °C for 2 min and a final temperature of 94 °C denaturation for 40 s. For the 60 °C annealing temperature used for 1 min and followed by the initial extension temperature 72 °C for 5 min and final extension temperature of 72 °C for 5 min. Universal primers used in the amplification of DNA was determined based snakeheads species, to determine the banding pattern resulting from the use of DNA amplification poly acrylamide and 1 % agarose gel in 1× TBE (tris boric acid EDTA) electrophoresis buffer with 25 min to 30 min long. As molecular markers used for the analysis of micro-satellite by using primer BP6-2 4 % (w/v) agarose gel metaphore (CAMBREX, USA), a 100 bp DNA ladder, used for ethidium bromide staining by soaking for 10 min and wash with water for 10 min. The results observed under UV Transilluminator and documented by gel camera.

2.9.4.Microsatellite polymorphism

Microsatellite polymorphism determined by using restriction enzymes on the DNA template PCR amplification product was cut with the restriction enzyme Hind III (A'AGCTT); Bam HI (G'GATCC); EcoR V (GAT'ATC) and HAE III (GG'CC). Cutting the DNA template starts with preparing a solution of 10× buffer, 100× BSA, and akuadest restriction enzymes and DNA template PCR amplification products with a certain concentration.

Subsequently incubated in a water bath with a temperature of 37 °C for 2.5 h to 3 h. The use of 1.5 % agarose gel in 1× TBE buffer and electrophoresis process for 30 min to 35 min and staining with ethidium bromide for 10 min, then obtained pieces of fragments of each DNA template. Used as a molecular marker 100 bp DNA ladder, whereas for the control of DNA that do not use templates to experience cuts. The results observed under UV-Transilluminator at 320 nm and documented by gel camera. Confirmation of genotype frequency analysis performed by the GEN-POP Program. Parameters measured include total length and weight of snakeheads, composition and molecular weight of the DNA, heterogeneity, genotype and allele frequencies.

2.10. Statiscal analysis

The data include the growth of the total fish length and weight of snakheads were analyzed by Anova (Hadi, 2004), and knowing the length weight relationship using soft ware Minitap 11, while data on the composition of the molecular weight of the DNA, genotypic heterogeneity, the calculation of the diameter and color eggs, gonadal development (Istiyanto and Sardiyatmo, 2007) is done by analysis ekostat and descriptive.

3.Result and discussions

3.1. Absolute Growth of the Rearing Snakeheads Parent Fish

The observation of the growth of snakeheads on various treatments showed that snakeheads growed normally and good for maintenance in aquaculture pond using pellet by 5% per biomas snakeheads per day. The results showed the highest growth in snakeheads in fish caught in the lake Rawa Pening (Tabel.1).

Tabel.1.Absolute growth of the rearing snakeheads parent fish from crossbreeding of snakeheads (*C. striatus*) were caught from different waters with artificial fed with 35 % protein content

	Treatment (mg)			
	A	B	C	D
1	142.77	148.28	161.25	163.28
2	143.95	157.25	159.29	165.25
3	137.78	155.27	156.28	167.48
Total	424.5	460.8	476.82	496.01
means	141.5^{b}	153.6^{b}	158.94^{a}	165.337^{c}

Note: The snakeheads caught from waters Rawa pening is crossbreeding parent snakeheads caught from waters Segoro anakan (treatment A), then is crossbreeding parent snakeheads from Gajah Mungkur waters (B), and is crossbreeding parent snakeheads from Rembang rivers (C) and crossbreeding parent snakeheads from the parent snakeheads from public waters Ujung Pangkah (D). Different superscript letters in the some columnh significant ndifference betwen sample of the levelsw of ($p < 0.01$).

Table.1, the analysis of variance showed a significant influence ($p < 0.01$) in absolute growth, and that the highest rate of absolute growth weigh in the treatment D (The snakeheads caught from waters Rawa pening crossbreeding parent snakeheads from the parent snakeheads from public waters Ujung Pangkah was 165.337 mg. The result showed that snake heads from the maintenance of improved seed of cross-breeding waters Rawa peningis mated with a parent snakeheads caught from waters Segoro Anakan (A), then mated with parent snakeheadsderived Gajah Mungkur (B), and is mated with a parent derived from Rembang (C) and mated from the parent fish from public waters Ujung cork Pangkah (D), which is the highest result is D (165.337 mg) and lowest was A (141.5 mg). Result growth of snakeheads is better rather than Almaniar et al. (2012), an also used as additional feed chicks frog with the amount of feed given 5 % biomass per day, given in the morning, afternoon and evening (Almaniar et al., 2012; Nam, 2011; Prosperous 2006a; Prosperous 2006b). Also added by Almaniar et al. (2012) to provide feed tubifex per biomas sp 5 % per day can increase the growth of snakehead fish (*C. striata*) biomass weight 29.80 g till 63.20 g. Also added by King (2003) and Makmur (2006) snakeheads are often called snake head is classified as predatory fish with food in the form of zooplankton, frogs, crabs, and others. Fish snakeheads has a length of intestine that never exceed the total length of the fish that are carnivorous fish species. According to Director General of fisheries (1990), at the level of the larvae, snakehead is a type of fish feed protozoa and algae, while the snakeheads fish grown food (Kpogue, 2013) is small fish, insects, worms, shrimp and so on. According Marimutu et

al. (2001), Potts and Wootton (1984), Purdom (1993) reported young seed that feed on plankton, microscopic shrimp and leaves of plants. But when fish highest can increase to grow to eat other smaller fish (De Graaf et al., 1990).

3.2. Results of microsatellite analysis of the Snakeheads fish

The results of the analysis on a variety of broodstock of snakeheads fish Mikrosatelite are different waters in Central Java (Figure 1)

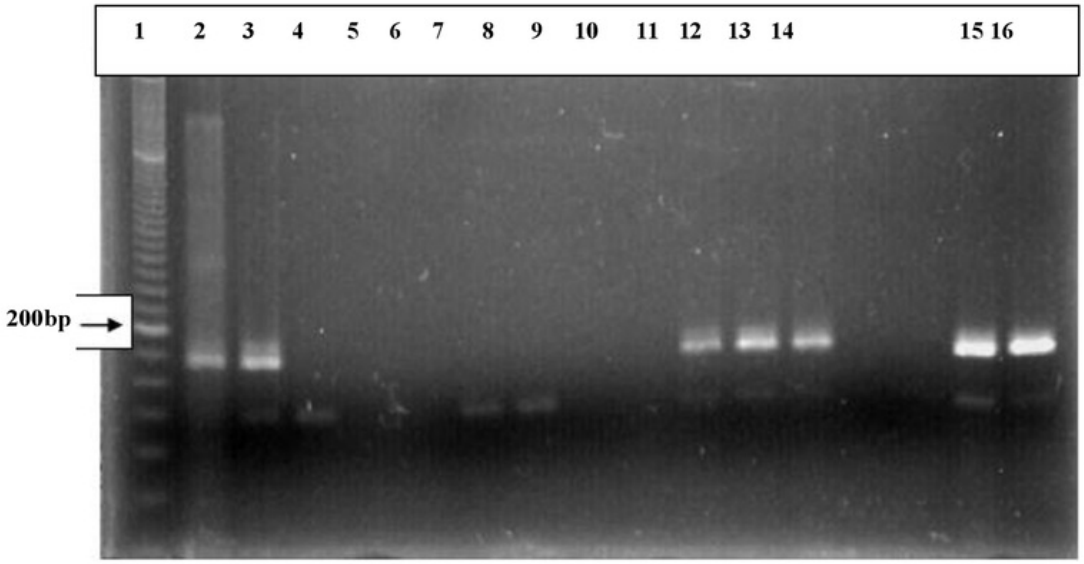

Fig.1. The results of the analysis using micro-satellite Polimorpisme against Fish snakeheads on various treatment The snakeheads caught from waters Rawa pening is crossbreeding parent snakeheads caught from waters Segoro anakan (treatment A), then is crossbreeding parent snakeheads from Gajah Mungkur waters (B), and is crossbreeding parent snakeheads from Rembang rivers C) and crossbreeding parent snakeheads from the parent snakeheads from public waters Ujung Pangkah (D),as the control band 13-16) USING primary IS-GB1F 5-ATT TGT CCC TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC ATC AAC ACT GCA TCT CT-3.

Based on the analysis of micro satellite by using IS-GB1F primer 5-CCC TGT ATT TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC ATC AAC ACT GCA TCT CT-3 (INTEGRATED DNA TECHNOLOGIES SINGAPORE), bright ribbons found in treatment B (bands 4,5,6) of fish caught from waters snakeheads Rowo Pening Ambarawa, Semarang District with ribbon allele 200 bp ladder with 214 bps and 224 bps on the right, and have polimorpism (polymorphism) high. Overall snakeheads caught in waters off central Java has a high heterozygous, so has the quality of parent *C. striatus* products are growing well. Using tape 16, overall show a clear bands. The success of a genetic character snakeheads determined also results from the extraction of DNA, which by using Nanno Photometer ™, UV / Vis (Implen, Munich, Germany) and average concentration for the 12 samples is 0.045µg · mL^{-1}, with good results.

It was good to use of microsatellites in determining the genetic character of snakeheads fish, especially in determation of polymorphic with a single repetition at basepair changes in allele size. This was reinforced by the opinion Prasetiyono et al. (2004) microsatellite is a simple sequence repeated abundant in the genome (Smith et al 2008) of a species. Microsatellite sequences have consecutive repetitions two to four nucleotide sequence motifs as conservative sequences. This marker was very useful as a genetic marker for character codominant, so it can detect allelic diversity at a high level, easy and economical to apply because it uses the PCR process. Repetition of simple forms of DNA (Nam et al., 2011; P[illegible]o et al., 2012) sequences that make repetitive microsatellite markers often called simple sequence repeat (SSR), short tandem repeats (strs) or simple sequence length polymorphisms (SSLPs)

are now becoming one of the most widely used markers for genetic mapping extensively, 5alysis of genetic diversity and evolution studies (Jamsari et al., 2011). Suresh et al. (2015) also reported using of microsatellite DNA analysis of giant freshwater prawn (Macrobrschium rosenbergii) from India genetic diversity of this from five different rivers (Krishna, Mahadi, Hooghly, Narmada and Kalu) of India was investigated using five polymorphicc microsatellite loci. The number of allel across loci varied from four to nine. 2e mean expected and observed heterozygosity at a2oci was 0.8359 and 0.5347. The latest study by Temnykh et al. (2000), Zhang et al. (2012) reported using four polymorphic microsatellite markers were developed for the spotted babylon, *Babylonia areolata*, from a microsatellite enriched library. But using characterizet in 32 individual from one wild population were polymorphic with allele number ranging from 5 to 15 per locus, expected and heterozygosity ranging from 0.60 to 0.92 and from 0.36 to 0.88 respecttively. Hartl and Jone (2000) also reported Temnykh et al. (2000), Nam et al. (2011) these markers appear as markers were very varied and easily repeated, making it is ideal for genome mapping. This microsatellite polymorphism is one type of repetitive, commonly grouped into simple tandem repeat polymorphism (STRP), due to genetic differences between DNA molecules containing a number of copies of short DNA sequences that are repeated several times. STRP which has a repetition two to nine base pairs are often called microsatellites, while the STRP by repetition of 10 to 60 base pairs are often called minisatelit or variable number of tandem repeats (VNTR) (Hartl and Jones, 2000; Yu et al., 2009). Gupta et al. (1996) mentioned microsatellites scattered throughout the genome, whereas most of minisatelit centered near telomeres.

3.3. Genetic variation of Snakeheads

Genetic variation in snakeheads seed hybridization (interbreeding) the parent of the Rawa pening be mated with parent snakeheads caught from waters Segoro Anakan (A), then mated with parent snakeheads derived Gajah Mungkur (B), and is mated to parent derived from Rembang (C) and mated from the parent snakeheads from public waters Ujung Pangkah (D) shows the variation of different types, as well as the number of alleles and heterozigotes different, so i13ll produce superior seeds that have good genetic code. The result of the number of alleles and heterozygotes can be seen in Table 2.

Table. 2. Snakeheads fish genetic variation, the number of alleles, heterozygotes using the primer IS IS GB1 and GB2.

Name primer	Sekuensi primer (5'3')	Motif ulangan (repeat motif)	Temperature anneling	na	ne	Allelic richness	Size range bp	Ho	He
IS-GB1	F:CCC TGT ATT TCA TTT CTC CA-3 R:ACC AAC ACT GCA ATC TCT CT-3	[CTTT]3	57	3	2.6636	3.02	290-305	0.2856	0.6271
IS-GB2	F -AGA AGA AGA AGC CGA GT-3 R. GAA AAA CAG AGC AGG AAC AC-3	[AGAGG]	536	3	2.5445	3.03	213-226	0.6273	0.6097

Information :
Ho: Observed Heterozygosity
He: Expected Heterozygosity
HW8P): Overall Hardy Weinberg Equilibrium P-value
na = observed number of alleles
ne: Effective number of alleles
St. Dev: Standard deviation

In Table 2 shows that the results of the primary analysis using the IS-GB1, F: CCC ATT TGT TCA TTT CTC CA-3 and R: ACC AAC ACT GCA TCT ATC CT-3 and primer IS-F -AGA GB2 is a AGA AGA AGA AGC CGA GT-3 and R. AAA CAG GAA AGG AGC AAC AC-3 Whereas R: ACC AAC ACT GCA TCT ATC CT-3 with the results of microsatellite genetic marker for each sampling location can be seen on. Number of alleles ranged from 2 to. 8. The number of alleles at these snakeheads with ranges of 2 to 8 showed different genetic variations. Likewise heterozigotes and high polymorphic will determine the character of different genetic parent fish that determine the growth and quality of cork different mains different.

According Balkhis et al. (2011) that *C. striatus* or, known as "Haruan", economically important fisheries and aquaculture industry in countries around Asia. DNA sequencing techniques, the snakeheads is very important in determining genetic variation using on the basis of partial segments c cytochrome oxidase subunit 1 (CO1) gene, is used to determine the genetic variations in the sample *C. striatus*. On different geographical, especially on the west coast Peninsular Malaysia has nucleotides, and haplotype diversity of the population with the highest value (= 0.0067, p = 0.835), and the lowest population Tasoh Timah (= 0.0008, p = 0286). As well as geographical differences significant effect on the value FST ($p < 0.05$) in all pairwise comparisons population. So that in genetic mapping in snakeheads (*C. striatus)* provide information about the origin of genetic variation snakeheads are geographically diverse.

Also added by Balkhis et al. (2011) that a genetic analysis using microsatellite on cork fish can distinguish genetic variation in different geographical origins also value genetic heterozygotes and diversity is seen also that of the mutation rate is higher than the base substitution compared with nuclear DNA (Qiongying et al. 2006), that mitochondrial DNA is a genetic penenda, in the study of genetic differentiation. Mitochondrial cytochrome oxidase 1 (CO1) into DNA prime bar-region coding to identify the taxonomy of fish that is snakeheads (Seifert et al,. 2007; Alessandrini et al, 2008, Smith et al, 2008), also added by Yu et al. (2009) that the use of DNA sequencing of mt DNA CO1 can investigate pilogeni group of snakeheads *(C. striatus)* along the west coast of Peninsular Malaysia.

4. Conclusion

The results showed that:

- Superior seed snakeheads highest growth in the seed-yielding seeds snakeheads resulting from the maintenance of improved seed of parent snakeheads from Rawa pening waters crossbreeding the parent snakeheads from public waters Ujung Pangkah (D), which is the highest result is D (165.337 mg) and lowest of waters Segoro Anakan A (141.5 mg).
- The results of the analysis of micro-satellite polimorpisame use of improved seed to snakeheads on various treatment fish, snakeheads caught from waters of the rawa pening mated with parent of snakeheads fish from the waters Segoro anakan (A, bands 1.2), then mated parent fish originating snakeheads Gajah Mungkur (B, bands 3.4), and is mated with a parent who came from Rembang (C, bands 5.6) and mated from the parent fish from public waters Ujung cork Pangkah (D, bands 7 and 8).
- Furthermore, from each of the seeds produced from crossbreeding (hebridisations). Based on the analysis of micro satellite using a primer IS-GB1F 5-CCC TGT ATT TCA TTT CTC CA-3 and IS-GB 1 R 5-ACC AAC ACT GCA ATC TCT CT-3 (Integrated DNA Technologies Singapore), ribbon light found at treatment D (bands 7.8) snakeheads are the results showed that the highest growth of snakeheads on D (parent snakeheads Rawa pening be mated with snakeheads fish parent caught from waters Segoro Anakan waters) with band 200bp allele ladder with 214 bps and 224 bps on the right, as well as having polimorpism (polymorphism) is high.
- The genetic variation in snakeheadsseed hybridization (interbreeding) the parent of the Rawa peningf be mated with snakeheads of parent caught from waters Segoro anakan (A), then mated with parent fish originating snakeheads from Gajah Mungkur waters (B), and mated to a parent who came from Rembang (C) and mated from the parent snakeheads from Ujung Pangkah waters (D) shows the variation of different types, as well as the number of alleles and heterozigotes different, so it will produce seeds that have superior character of genetic good. Overall snakeheads fish seed has a high heterozygous, so has the quality of seed products cork fish that grow well.

Suggestion

Further studies on the results of snakeheads enlargement of the superior seed to reach consumption size by looking at the highest polimorpisme, further mikrosatelite analysis with genetic engineering approach mikrosatelite wear. So it needs to know the potential genetic and freshwater aquaculture efforts, through magnification with artificial feed and natural food or a combination thereof, so as to accelerate growth so as to improve the quality of the fish production of snakeheads. The importance of this research, it is useful to increase the production of snakeheads, snakeheads aquaculture development in Central Java. Snakeheads has the advantage that many contain albumin useful in accelerating healing and post-surgical blood clots, and help the protection/ conservation of snakeheads from extinction plasmah nutfah in the Central of Java Province.

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