NORMALA BINTI JALIL MASTER OF SCIENCE 2012

MORPHOLOGICAL CHARACTERISTICS AND GENETIC ANALYSES ON TRIPLOID AFRICAN CATFISH (*Clarias gariepinus*)

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Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirements for the degree of Master of Science

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Triploids are organism with three chromosome sets. They are known to be sterile and easily induced in many species of fish and shellfish. However, suitable and rapid method for triploid identification for large scale production is still limited. This is due to the inability to accurately determine this characterization which currently is determined based on erythrocyte and karyotyping method. The specific objectives of this study were to determine the erythrocyte size, morphometric characteristic and genetic variation in triploid African catfish. Triploid was produced by cold shock method (5°C cold shock for 20 minutes, starting 3 minutes after fertilization). Results showed that 99% triploid erythrocyte was oval in shape whereas 97.5% of the diploid erythrocyte was rounded in shape. There were significant differences of size for cell major axis, cell minor axis, nucleus major axis, nucleus minor axis, cell volume, cell area, nucleus volume, and nucleus area between the two different ploidy individual. Cell major axis had the highest percentage (76%) of non-overlapping in erythrocyte size between both treatments compared to other parameters. It is suggested that the determination of triploid African catfish can be done based on measurement of the cell major axis. Independent T-test analysis showed 12 parameters having significant differences. However there was no specific range or

value for parameter mentioned that can be used to differentiate between both treatments. Principal Component Analysis (PCA) showed high correlation between both treatments. The biplot was not separated into two groups and the individuals from both sets clustered together. The Varimax rotation factor scatter plot also showed no separation between the triploid and diploid individuals. Results from PCA and Varimax rotation factor analyses revealed that triploid and diploid had similar morphological characters among individuals. Genetic analyses were conducted using three RAPD primers (OPB 16, OPC 14, and OPD 12). Genetic similarity ranged from 0.348 to 0.897, 0.378 to 0.913 and 0.533 to 0.968 for OPB 16, OPC 14 and OPD 12, respectively. The UPGMA diagram did not separate into two clusters and showed unclear differentiation between both ploidy individuals. RAPD analyses found 48, 59 and 52 genotypes specifically for OPB 16, OPC 14, and OPD 12, respectively. Out of this, result show 22, 29 and 22 genotypes for OPB16, OPC14 and OPD12, respectively this is more specific to triploid individuals. This genotype can be used to differentiate triploid individual. It can be seen that, out of the three methods erythrocyte measurement, morphological characteristics and genetic variation, erythrocyte measurement was the easiest and most rapid method to differentiate between triploid and diploid African catfish.

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CIRI-CIRI MORFOLOGI DAN ANALISIS GENETIK KE ATAS IKAN KELI AFRIKA (*Clarias gariepinus*) TRIPLOID

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Triploid ialah organism dengan 3 set kromosom. Ia di kenali sebagai mandul dan senang dihasilkan ke atas ikan dan kerangan. Walaubagaimanapun, kaedah yang sesuai dan cepat untuk mengenalpasti ikan triploid untuk penghasilan berskala besar masih terhad. Ini merujuk kepada ketidakupayaan untuk menentukan ciri-ciri dengan tepat yang mana boleh ditentukan berdasarkan kaedah pengukuran eritrosit dan karyotyping. Objektif dalam kajian ini untuk menentukan ciri eritrosit, ciri morphologi, dan variasi genetik antara triploid dan diploid ikan keli Afrika. Triploid dihasilkan menggunakan kaedah kejutan suhu sejuk (5 °C kejutan suhu untuk 20 minit selepas 3 minit persenyawaan). Keputusan menunjukkan 99 % bentuk oval untuk eritrosit ikan triploid di mana 97.5% bentuk bulat dalam ikan diploid. Terdapat perbezaan yang signifikan untuk sel major axis, sel minor axis, nukleus major axis, nukleus minor axis, isipadu sel, dan isipadu nukleus. Sel major axis mempunyai peratus paling tinggi (76%) untuk saiz eritrosit yang tidak bertindih antara triploid dan diploid. Cadangan untuk menentukan triploid keli Afrika boleh dibuat berdasarkan pengukuran sel major axis. Analisis t-test bebas menunjukkan 12 parameter mempunyai perbezaan yang signifikan, walaubagaimanapun, tiada julat atau nilai yang spesifik boleh digunakan untuk membezakan kedua-duanya. Analisis

PCA menunjukkan korelasi yang tinggi antara kedua-duanya. Biplot tidak terasing kepada dua kumpulan tetapi berkumpul bersama antara triploid dan diploid. Faktor pusingan Varimax juga menunjukkan tiada pengasingan kepada dua kumpulan sample ikan. Ini menunjukkan diploid dan triploid mempunyai ciri- ciri yang sama. Analisa genetik dijalankan menggunakan tiga primer RAPD (OPB 16, OPC 14, dan OPD 12). Julat persamaan genetik ikan keli triploid 0.348 ke 0.897, 0.378 ke 0.913 dan 0.533 ke 0.968 untuk OPB 16, OPC 14 dan OPD 12, masing-masing. Diagram UPGMA tidak terasing kepada 2 kluster and menunjukkan perbezaan yang tidak jelas. Analisis RAPD menjumpai 48, 59 dan 52 genotip yang spesifik untuk OPB 16, OPC 14, dan OPD 12, masing-masing. Daripada ini, keputusan menunjukkan 22, 29 dan 22 genotip adalah spesifik untuk individu triploid. Genotip ini boleh digunakan untuk menentukan individu triploid. Ini jelas dilihat, daripada ketiga-tiga kaedah (pengukuran eritrosit, morphologi dan variasi genetik) analisis, pengukuran eritrosit adalah kaedah paling senang dan cepat untuk membezakan triploid keli Afrika.