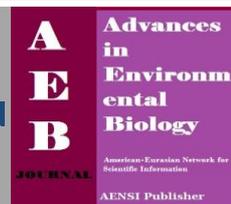




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Uba Gene Application for Parentage Assignment in Iranian Wildcommoncarp (*Cyprinus carpio*)

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ABSTRACT

Reproductive success is very important in aquaculture production especially in species that are candidate for future production. As many investigation on fish sperm competitions show males have biased competition, so some functional gene alleles may be lost during mixed-milt fertilization. Major Histocompatibility Complex genes are one of the most important adaptive immune system genomes which should be conserved during artificial spawning. This issue becomes more critical in case of species like common carp (*Cyprinus carpio*) that are in risk of inbreeding depression. In this study, analysis of MHC class I gene (UBA locus) was used by polyacrylamide gel electrophoresis (PAGE) in 30 brood stocks and 50 offspring (F1) larvae. Results indicated that some genotypes become more frequent and some of them decreased or lost during propagation. It is concluded that these genes can be good candidate for two purposes simultaneously: larvae-parent assignment and genotype-assisted selection.

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INTRODUCTION

Common carp (*cyprinus carpio*) is one of the main aquaculture fish native to East-Western part of Caspian Sea, and there are many ecological concerns about this species. Iran fishery Organizations and many private research centers try to overcome these concerns. During last years, Iranian fishery research centers tried to introduce this specie as a candidate for native aquaculture production. Today, this center has obtained many successes to domesticate it to captive conditions. According to Iran fishery organization data, 500000 larvae of introduce to different farms from Mazandaran to Zanjan in 2012. As there are many trends to diversify species cultured in all over the world, specially this species in Iran, and breeding potential of this species, it is very important to use long-term breeding strategies and programs like selective breeding. For the best selective program based on quantitative trait loci (QTL) selection, the approach of marker-assisted selection (MAS) would be very informative. Progression of genetics methods like sequencing and acquiring the sequence of many protein coding regions and QTLs has revolutionize breeding strategies. One of the most polymorphic nuclear coding genes in vertebrates is immune related genes. These genomes code pathogen driven molecules that present self and non-self-antigens (viruses, bacteria and other pathogens) to T lymphocytes. Until now, many researches have been done on disease resistance polymorphism of these genomes in aquaculture fish species like rainbow trout, Atlantic salmon and brown trout [4,5]. As these genomes have higher Polymorphic Information Content (PIC) and Dn/Ds than neutral molecular markers such as microsatellites, they may be the most realistic response of environmental selections. Investigations on fish sperm competition show males have biased competition and probability lost of some genotypes and another functional important genome alleles. Therefore, this study has been conducted to analysis MHC genes polymorphism by polyacrylamide gel electrophoresis (PAGE) and evaluate potential of this marker for parentage assignment and genotype-assisted selection for disease resistance breeding strategies.

MATERIAL AND METHOD

Sampling: in this study the captive population of common carp (*Cyprinus carpio*) located in Mazandaran province was selected. 30 individual fin clips were picked and fixed in ethanol 96% immediately during propagation of brood stocks and in the time of fish anesthesia. Also the 50 larvae of those parents sampled randomly and fixed in ethanol.

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Molecular analysis: DNA became extracted by DNA Extraction Cinnapure kit. The DNA extracted quality and quantity controlled by 1% agarose gel and Nanodrop 260/280nm analysis, respectively. High quality DNA was selected for PCR amplification. The specific primers were used for PCR amplification of UBA locus (F: 5-GTGTTTTATTGGGTTTCTTTTCTC-3; R: 5-CTCTAAATTA-CTTCTCTCTTAC-3) (Langefors *et al*, 2000). A PCR reaction was performed in a total volume of 12.5 ml containing approximately 10-50 nggDNA, 1.25ul of 10X PCR buffer, 1.5 mM MgCl₂, 200 mMdNTPs, 200 nM of each primer and 1 unit of Taq polymerase. Amplification was performed in a BIOER Xpcycler thermal cycler using the following cycling profile: 10 min at 95°C, 35 cycles of 30s at 94°C, 30s at 64°C and 30s at 72°C, and a final extension step for 5 min at 72°C. Results of the amplifications were visualized in 2% agarose gels and resulted in a single band of 300–320 bp. Single Strand Conformation Polymorphism (SSCP) method was applied for genotyping according to method of Sunnucks *et al* (2000). SSCP pattern was analyzed by Gel Scanner program (Version 6) and genotype data was exported to POPGENE program (ver, 1.32) for statistical analysis of further population.

RESULT AND DISCUSSION

For PCR amplification of UBA locus of Immune systems genes it was found the single and sharp band about 300 bp (figure 1) that are concordant with PCR results of another species like salmon fish (Miller, *et al* 2004). SSCP analysis of these PCR products showed the reproducible and dominant patterns with both parents and progenies fish had the high average heterozygosis (78% and 74% respectively). In parents there were 7 different alleles but 4 alleles were found in their progenies. The Missed allele was belonged to male parents (3 alleles) that indicated some of males didn't anticipate in fertilization or their progenies had low viability.

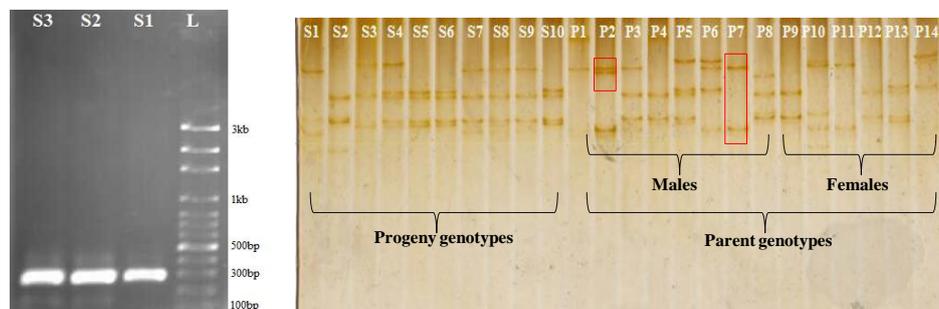


Fig. 1: left: PCR amplification of DAA locus of MHC class II on 1% agarose gel, right: SSCP analysis of PCR products of parent and progeny. Lost alleles indicated by red rectangles.

According to study of Campton [6] males had biased sperm competition occurred during mixed-milt fertilization specially in captive conditions that less competent parents forced to compete with another males. As these fish became domesticated nearly so for breeding this species it is very important to use parentage assignment and marker-assisted selection for most immuno-competent progenies at the next generations.

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