Using RAPD markers potential to identify heritability for growth in *Fenneropenaeus indicus*

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Abstract

Sampling was done using 90 post larvae which were produced by reproduction of some broodstocks of *Fenneropenaeus indicus* in one day and reared in the same situation for 4 months. Samples were divided into 3 groups: high, medium and low growth (based on weight and length). Genomic DNA was extracted from muscle tissue using the phenol-chloroform method. The polymerase chain reaction (PCR) was carried out using 21 RAPD loci and PCR products were separated on 3% Agarose gel. From 21 loci studied, 12 produced polymorphic bands. The most polymorphic produced bands using OPAQ 9 and the least by OPAQ 7. Search for specific markers in *F. indicus* one specific band was observed in the low growth group using OPAQ4. The highest genetic distance (0.457) was between the low growth group and the medium and the lowest (0.091) between high growth and medium groups, therefore the highest genetic identity (0.912) was between high growth and medium groups and the lowest (0.633) between low growth group and the medium. Neighbor-joining resulted in two groups, the first including high and medium growth groups and the second low growth group. It appears that low growth group depended on separated population. Considering the mean weight of F_1 (mean weight of 90 specimens) (16.25±1.5 g), parental generation mean weight of 15 ±1.2 and mean weight of parent 31.6 g, response to selection (R) and heritability for growth in this species were estimated to be 1.2±0.2 and 0.07±0.01 respectively.

Keywords: *Feneropenaeus indicus*, RAPD marker, Heritability, Growth, Iranian Fisheries

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Introduction

The Indian white shrimp (*Fenneropenaeus indicus*) inhibits in the Indian Ocean coast from north of South Africa to India, South-East Asia, Indonesia, and Northern Australia (Benzie, 2009). In Iran this species has been distributed in the Persian Gulf and Oman Sea. *F. indicus* is non-burrowing, active in both day and night and prefers a sandy mud bottom (Afsharnasab et al., 2005). Its maximum total length is 184 mm (male) and 228 mm (female); its maximum carapace length is 56 mm (FAO, 2009). The shrimp mature and breed mostly in marine habitats and spend the juvenile and sub-adult stages in coastal estuaries from 2 to 90 m brackish waters or lagoons (FAO, 2009). Many natural shrimp stocks are in decline and farmed shrimps provided more than 50% of the world's production for several years (Benzie, 2009). Despite the high potential of aquaculture for increasing production, the sustainability of shrimp farming is treated by low production efficiency (Hetzel et al., 2000) and vulnerability to diseases (Afsharnasab et al., 2005; Gitterle et al., 2005; Lu and Sun, 2005; Olivier and Roel, 2009). The development of genetically improved stocks and domesticated breeds selected for commercial traits is one approach to overcoming these threats (Moss et al., 2007; Hoa, 2009; Pourkazemi et al., 2010), but the high coefficient of variation that comes from the strong environmental influence on the phenotypes make the selection of individuals with genetic advantages for these traits difficult (Falconer, 1998; Hoa, 2009) then the establishment of genetic marker to assist in selecting individuals for breeding would be of great benefit in speeding the domestication of these species (Moore et al., 1999; Donato et al., 2005; Benzie, 2009). The benefits of domestication have been demonstrated in some aquatic species such as Rainbow Trout (Gjedrem, 1992), Atlantic Salmon (Gjedrem and Fimland, 1995), Pacific Oyster (Taris et al., 2007) and Gaint Catfish (Kednapat et al., 2007). But despite the amenity of shrimps to genetic selections and because of their comparatively high fecundity, short generation time and larger genetic gain (Keys et al., 2004), the shrimp farming industry has been slow to adopt selective breeding programs (Goyaed et al., 2008; Cock et al., 2009). It is said by Pullin et al. (1998) that this reluctance has been due in part to the past perceptions of low genetic variability and difficulties in the domestication of shrimps (Benzie, 2009). Hence, it is necessary to challenge these perceptions and to quantify the responses to selection and heritability estimates for commercial traits. Low levels of protein variation and highly unlikely benefits of allozyme variation have been reported by Garcia and Benzie (1995) in shrimps. Although subsequent studies, using allozyme (Benzie et al., 1997; De la Rosa-Velez et al., 1999) have revealed considerable genetic variation within populations, but DNA analysis [RAPD (Garcia and Benzie, 1995); microsatellite (Wolfus et al., 1997); mtDNA and microsatellite (You et al. 2008)] would provide a better source of markers in penaeid prawns. Garcia and Benzie (1995) in a study of RAPD markers of potential use in penaeid prawn (*P. monodon)* breeding programs found that levels of

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variation obtained by RAPD method is similar to those observed in other taxa, and are likely to be adequate for obtaining markers to assist selective breeding programs. RAPD producers were first developed by Welsh and McClelland; Williams et al.(1990) using PCR randomly amplifying anonymous segments of nuclear DNA with an identical pair of primers 8-10 bp in length that have been used as useful markers in breeding programs and gene mapping. The objective of this study was to assess RAPD markers in screening the high growth of wild and cultured brood stock before reaching the final maturation stage.

Material and methods

Sample collection

Sampling was done using muscles of 90 post larvae which were produced by reproduction of some brood stocks of *F. indicus* in one day and reared in the same conditions for 4 months in Bushehr province during Feb.-Aug. 2007. Samples were classified in 3 groups, high growth, medium and low growth. At first all samples were arranged in a row according to their size and divided into three groups, the first ten, low growth group; the second, medium and the third high growth group. Then the groups were weighed, the average weight for the selected low growth prawns was 13.74±1.95 gr, medium growth 15.75±1.5 gr and high growth 19.1±1.4 gr and using SPSS there was a significant difference between them (Table 1). Selection response and heritability were measured for growth. Selection response was measured by the difference between mean body weight of the first and parental generations. Selection differential was calculated by the difference between the mean body weight of the selected parents and the parental generations. Real heritability was calculated by the ratio of selection response to selection differential (Falconer, 1998).

DNA extraction

Genomic DNA was extracted from a $1cm^2$ (50-60 mg) piece of muscle tissue using the phenol-chloroform procedure described by Hillis et al. (1996). The quality and concentration of DNA from samples were assessed by electrophoresis on a 1% agarose gel and Spectrophotometer, and then the samples were stored at -20˚C for further analysis.

PCR amplification and electrophorsis

DNA from each individual was amplified through PCR. A single 10 nucleotides Oligomer of random sequence which existed in a general random amplified polymorphisms Kit (Table 2) were used for each reaction. A total of 12 different primers were tested. Each PCR reaction (final volume 25 µl) was composed of 5 µl of 10X reaction buffer, dNTPs 10 mM, $MgCl_2$ 50 mM, primer 20 pmol, genomic DNA 100ng and 1.5-2 units of Taq polymerase. The temperature profile consisted of a 3-mininitial denaturation at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at the respective annealing temperature, and 1 min at 72°C, ending with 5 mins at 72°C (Master cycler Ependorf model 384). PCR products were separated on 3% agarose gel, and stained with ethidium bromide. RAPD PCR which was carried out using different PCR blocks and slightly varying conditions showed that the RAPD patterns were robust and reliably replicated.

Statistical analyses were carried out using Pop Gene Version (1.31) (Yeh et

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al., 1999) and SPSS Version (10.5). Heterozygosity, genetic distance, identity between three groups and Dendrogram Based on (Nei, 1972) Genetic distance were measured using Pop Gene and selection response and heritability for weight were estimated using SPSS.

Results

From 21 studied loci, 12 produced polymorphic bands. The most produced band using OPAQ10 and the least by OPAQ11. The most polymorphic band produced using OPAQ9 and the least by OPAQ7 (Table 3). The estimated level of polymorphism in this study was 22%. Searching specific markers in *F. indicus* one specific band was observed just in low growth group using OPAQ4 (Fig. 1). The highest genetic distance (0.457) was between low growth and medium groups and the lowest (0.091) between high growth and medium groups, therefore the highest genetic identity (0.912) was between high growth group and medium and the lowest (0.633) between low growth group and medium groups (Table 4). Neighbor-joining tree (Fig. 2) resulted in two groups, the first including high and medium growth groups and the second low growth group, it appears that low growth groups are depended on separated population of the two others. With considering the mean weight of F_1 (16.25±1.5), mean weight of 15 ±1.2 and mean weight of parent 31.6, response to selection (R) and heritability for growth in this species were estimated 1.2±0.2 and 0.07±0.01, respectively.

Table1: Mean ±SD weight for different groups

Factor	High growth	Medium growth	Low growth
Weight	19.1±1.4 c	15.75±1.53 b	13.74±1.95 a

Different superscripts indicate significant differences (p≤0.05)

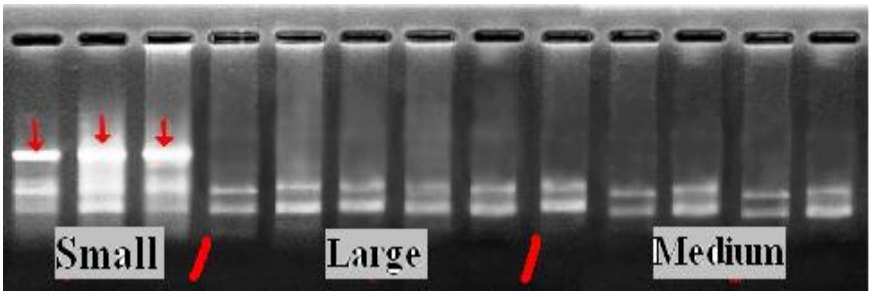

Figure 1: PCR product of *F.indicus* using OPAQ4, 13 samples of 3 groups (Small, Medium and Large), arrows show specific bands in small groups

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Table2: List of the 12 primers used to amplify RDPD markers in *F. indicus*

Primer	Anneling (°C)	
		3'→5'
OPAQ1	35	GGTGGCGGGA
OPAQ2	39	GAGGTCCAGA
OPAQ3	37	GCTGCTGGAG
OPAQ4	37	GCTGTAGTGT
OPAQ5	35	GCGGTTGAGG
OPAQ6	34	CAAGGGAGGT
OPAQ7	35	GGGCACGCGA
OPAQ8	32	ACGGCCGACC
OPAQ9	51	CGGAGAGCGA
OPAQ10	50	TGGGCTCGCT
OPAQ11	53	ACTTGTGCGG
OPAQ12	53	GCGGGAGACC

Table3: Produced bands, Polymorphic bands and Monomorphic bands used primer

Primer's number	Polymorphic bands	Monomorphic bands	Produced bands
OPAQ1	4	3	7
OPAQ2	-	6	6
OPAQ3	3	3	6
OPAQ4	4	2	6
OPAQ5	2	4	6
OPAQ6	2	4	6
OPAQ7	1	5	6
OPAQ8	5	2	7
OPAQ9	8	-	8
OPAQ10	7	2	9
OPAQ11	5	-	5
OPAQ12	3	3	6
Total	43	34	78

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Table 4: Distance matrix, upper rectangle is Nei's identity and lower rectangle is Nei's distance

	Medium growth	High growth	Low growth
Medium growth	***	0.912	0.633
High growth	0.0916	***	0.6469
Low growth	0.4571	0.4355	***

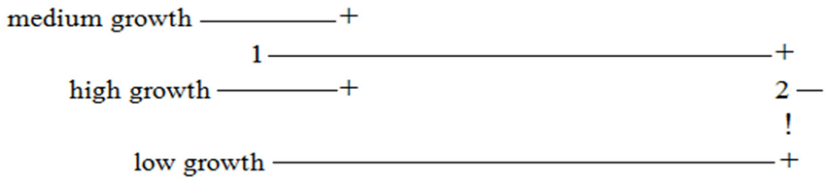

Figure 2: Dendrogram Based Nei's Genetic distance: Method= UPGMA Modified from Neighbor procedure of Phylip Version 3.5

Discussion

Hetzel et al. (2000) did their experiments in different rearing environments of parent and offspring generations as they didn't find any reports in the literature on genetic correlations between growths of prawns in pond vs. tanks. Preston et al. (2004) in the study of growth rate in selected and non-selected *Metapanaeus japonicus* in a controlled environment and open pond found there is no significant differentiation between the two groups when the environment condition is in favorite range for this species. This study was done in open pond in environment condition in favorite range for this species during experiment time. Garcia and Benzie (1995) observed a specific band in one of the studied families, screened all families to find whether the band is truly family-specific marker, but results showed it was probably derived from bacterial or algal epicommensals contaminating the sample. Wolfus et al. (1997) in the study of specific markers in *Litopanaeus vannamei* found 23 specific markers in this species 2 of which were observed in single families from one population. In the current study in searching of specific markers in *F. indicus* one specific band was observed only in the low growth group, and we are continuing tests on sequencing specific bands to develop suite markers for this purpose. On the other hand according to Dendrogram Based Nei's Genetic distance, UPGMA method, it appears that low growth group is dependent on separated population of the two others. Nelson and Hedgecock, (1980) using Alozyme studies suggested that prawn have generally low levels of variation. Garcia and Benzie (1995) using RAPD reported 6-7% polymorphism in *Panaeus monodon* and suggested that randomly amplified polymorphic DNA (RAPD) approaches will be as useful in providing markers for prawn breeding programs as they have been for other species. Wolfus et al. (1997) saw high levels of heterozygosity (45-100%) in *L.*

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vannamei and found the microsatellite technique to be a valuable tool for aquaculturists to use in analyzing the genetic diversity of breeding programs. Microsatellite genetic diversity and nucleotide divergence among haplotypes were demonstrated 0.63-0.74 and 0.2-16.3% respectively, by You et al. (2008). The estimated level of polymorphism in this study (22%) and previous data are promising and indicate RAPD markers are likely to access enough genetic variation for the establishment of a marker-assisted selective breeding program in prawn. The heritability of a metric character is one of its most important properties. It expresses, as we have seen the proportion of the total variance that is attributable to differences of breeding values, and this is what determines the degree of resemblance between relatives (Falconer, 1998) but the most important function of heritability in the genetic study of metric characters is its predictive role, expressing the reliability of the phenotypic value as a guide to the breeding value (Falconer, 1998; Moss et al., 2007). Recently, studies on genetics indicated a very good heritability on productive traits for penaeid shrimp and this could open a good outlook to detect related genes for marker assisted selections in future shrimp selective breeding programs (Hoa, 2009). Estimates of heritability for harvest weight have been reported 0.42±0.15 in *L.vannamei* (Carr et al., 1997); 0.16 to 0.31 in *M. japonicus* (Hetzel et al., 2000); 1.32±0.18 in *L.vannamei* (Perez-Rostro et al., 1999); 0.24±0.05 (full-sib family), 0.17±0.04 (half-sib family) in *L.vannamei* (Gitterle et al., 2005b) and 0.24-0.35 (unvariate animal model), 0.37-0.45 (multivariate animal model) in *L.vannamei* (Castillo-Juarez et al., 2007). Heritability of growth, TSV (Taura Syndrome Virus) resistance and tail percent were estimated 1±0.12, 0.28±0.12 and 0.15±0.12 respectively, in *L. vanna*mei (Argue et al., 2002). Heritability value for body length was demonstrated 0.22 at 119 days of age (Perez-Rostro et al., 2003) and 0.43 at 25 days of age (Campos et al., 2006) in *L. vanna*mei. The heritability for resistance to disease (Gitterle et al., 2005a; Ibarra et al., 2007) and reproductive traits (Arcos et al., 2004; Arcos et al., 2005; Macbeth et al., 2007) in *L. vannamei* have been evaluated. Data in different commercial hatcheries with different abilities confirm the role of genetical control on size and growth in penaeidae (Chow and Sandifer, 1991). The range of heritability (0-1) in the larval stage of *L.vannamei* and *L.stirostris* shows effects of environmental factors on growth (Lester and Lauser, 1990). Argue et al. (2002) studied heritability of sex ratio in *L.vannamei* and were not significantly different from zero. This differs from the results reported in turtles and fish, which exhibit significant heritability estimates for sex ratio (Lester et al., 1989), hence, instead of selective breeding, it may be possible to produce more females by manipulating the androgenic gland or exposing shrimp to exogenous hormones (Sagi and Cohen, 1990; Moss et al. 2002). In this study, heritability of weight for *F. indicus* was estimated 0.07 which is lower than those reported for other prawn that could be explained by lack of genetic care during the domestication period. Estimation of response to selection in *M. japonicus* (Hetzel et al., 2000), *L.stylirostris* (Goyard et al., 2002), and *L.*

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vannamei (Argue et al., 2002) were averagely 4-18%, 10.7% and 25% after one generation. In this study, response to selection was estimated 1.2± 0.2 (8%) in one generation that is lower than those which have been observed in other marine species. Low rates of response observed in our study are presumably because of the relatively low selection intensity applied. Nevertheless, our study has demonstrated that growth will respond to selection in *P. indicus*. It is expected that the response to the selection is valid only in the first generation, but it has been shown in the experiments that response with little change has been maintained during several generations (up to 5, 10 or even more generations). Over the longer term, phenotypic variation as well as heritability may decrease, resulting in lower rates of genetic change. In addition, negative genetic correlation can arise and reduce long term genetic gain (Falconer, 1998). This preliminary investigation of RAPD's in panaeid has provided methods to obtain RAPD's from *F. indicus*, and is likely to be adequate to obtain markers to assist selective breeding programs. We are pursuing our study by sequencing the specific bands, designing primers and consequently examining them on the same age specimens.

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