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GENETIC VARIATIONS AMONG FAMILIES OF SELECTIVELY BRED *MACROBRACHIUM ROSENBERGII* (DE MAN) BY RAPD-PCR ANALYSIS

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Giant freshwater prawn, *Macrobrachium rosenbergii* is an important freshwater crustacean widely cultured in several countries including India. Of late, its production has come down due to slow growth rate and disease occurrences. The ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar in collaboration with the WorldFish, Malaysia has initiated a selective breeding programme for growth improvement of this species. In the present study, two groups of families (I. six numbers of families for growth and II. six numbers of families for disease resistance) were selected for experimentation from the families produced in the fourth generation of selection programme. Each group consisted of two extreme sub-groups of three families in each with higher and lower growth (based on weight) under group I and, susceptible and resistant families (based on larval survival following challenge with *Vibrio harveyi*) under group II. RAPD-PCR was used to evaluate the genetic variations between and within groups separately. Twelve selected decamer primers were used to amplify DNA fragments of three individuals of each family and data were analyzed by POPGENE version 1.31 software. In group I, a total of 102 bands were scored by the primers out of which 41 bands (40.19%) found to be polymorphic. Genetic diversity within the group varied from 0.0272 ± 0.0965 to 0.0463 ± 0.1316. UPGMA dendrogram of this group based on Nei's genetic distance showed that families 5 (low growth family 2) and 6 (low growth family 3) are distantly related to high growth families. In the second group of disease resistance, 35 bands (36.46%) were found to be polymorphic out of 96 bands scored. Genetic diversity varied between 0.0301 ± 0.0957 to 0.0438 ± 0.1381 within this group. UPGMA dendrogram showed that families 1 (susceptible group 1) and 2 (susceptible group 2) are distantly related to three resistant families. Thus, the present results showed the existence of genetic variations in both growth and disease resistance traits that could be utilized in the selective breeding programme in *M. rosenbergii*.

INTRODUCTION

The genetic structure of a population is changeable. The degree of change depends on intensities of interventions. Wild populations are less prone to changes in their gene pool than hatchery populations as interventions in wild populations are negligible or very less. Therefore, wide genetic variations are found among the wild populations. Whereas, reduction in genetic variation through inbreeding, negative selection and genetic drift are very common in a hatchery

population (Alam and Islam, 2005). Loss of genetic variation is considered to be the loss of genetic potential for stock improvement and adaptation to environmental changes. It is therefore, essential to maintain the genetic variations in the hatchery populations through a systematic selective breeding programme to avoid inbreeding. The effect of any selection programme will be to change allele frequencies at loci influencing targeted phenotypes because certain alleles will be favoured and less favourable alleles will be reduced in frequency or eliminated (Gjedrem and Thodesen, 2005). Thus the long-term success of any breeding program will depend to a significant extent on the amount of genetic variations available in the parental population (Falconer and Mackay, 1996). Several studies have proved that breeding programs on culture stocks with low amount of genetic variation in the parental population are unsuccessful (Moav and Wohlfarth, 1976; Hulata *et al.,* 1986; Huang and Liao, 1990). Therefore, quantifying the levels of genetic diversity for every generation among produced families is important for the target traits in any selective breeding programme.

Molecular markers offer the realistic method to assess the genetic status of a population, and are powerful tools to detect genetic uniqueness of individuals, populations or species (Chauhan and Rajiv, 2010). Application of molecular markers has allowed rapid progress in investigations of genetic variability and inbreeding, parentage assignments, species and strain identification, and in the construction of high-resolution genetic linkage maps for aquaculture species (Liu and Cordes, 2004). Several molecular markers like mitochondrial DNA (mtDNA) random fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites and single nucleotide polymorphism (SNP) have been used widely to assess the genetic variations among populations. These markers in general have been categorized into different classes by various authors. Danzmann and Gharbi (2001) classified the genetic markers largely into two groups, *i.e*., sequence specific (e.g. microsatellite simple sequence repeat SSR) and sequence-independent markers (e.g. AFLP and RAPD).

RAPD, a multi-locus dominant marker system, is quite popular amongst researchers as it provides multiple markers without any prior knowledge of the DNA sequences. These oligonucleotides serve as both forward and reverse primers and usually are able to amplify fragments from 3 to 10 genomic sites simultaneously. The variable lengths of DNA are inherited as classical Mendelian traits (Williams *et al.,* 1990) and thus can be used for genetic analysis (Horn *et al.,* 1996). RAPDs have gained considerable attention particularly in population genetics, species and subspecies identification, phylogenetic study, linkage group identification, chromosome and genome mapping, analysis of interspecific gene flow and hybrid speciation and as a potential source for single-locus genetic fingerprints (Brown and Epifanio, 2003). These markers have been used for species identification or interspecies genetic relationship in fishes (Naish *et al.,* 1995; Partis and Wells, 1996; Dahle *et al.,* 1997; Das *et al.,* 2005; Lakra *et al.,*

2007), molluscs (Klinbunga *et al.,* 2000), *Argulus* parasites (Sahoo *et al.,* 2013) and shrimp (Shi *et al.,* 1999; Song *et al.,* 1999; Zhuang *et al.,* 2001), and analysis of population structure in shrimp (Tassanakajon *et al.,* 1997; 1998; Klinbunga *et al.,* 2001; Mishra *et al.,* 2009), catfish (Liu *et al.,* 1999) and scampi (See *et al.,* 2008; Islam *et al.,* 2014; Mohanty *et al.,* 2014). Sun *et al.* (2000) applied the techniques of RAPD and AFLP to analyze the relationships among four species of *Artemia* species and strains, and reported that RAPD markers successfully detected diversity and genetic differentiation among them. This technique has also been employed to study the genetic variations among the wild populations in selective breeding programme for establishment of base population of *Penaeus monodon* (Garcia and Benzie, 1995) and *Macrobrachium rosenbergii* (Mohanty *et al.,* 2011), and to identify heritability for growth in *Fenneropenaeus indicus* (Rezvani Gilkolaei *et al.,* 2011). RAPD markers having genetically linked to a trait of interest could be used for individual and pedigree identification, and trait improvement in genetics and breeding programme (Yoon and Kim, 2001; Shikano and Taniguchi, 2002). Mohanty *et al.* (2011) evaluated the genetic variation among the three Indian state (Odisha, Kerala and Gujarat) populations of *M. rosenbergii* by RAPD and reported substantial genetic variation within and between the three populations.

M. rosenbergii is one of the most important cultured species in India and many other Asian countries. The farmed production of *M. rosenbergii* in India has shown phenomenal increase from less than 178 tonnes in 1996 to 42,780 tonnes in 2005 (FAO, 2008). However, the production has been declining steadily since 2006. Poor quality seed and low survival have been found to be main reasons of decrease in production. Besides, the scampi culture industry in India relies mostly on wild or undomesticated lines, which are not pathogen free and often provide inconsistent quality compared with genetically improved lines. Implementation of stock improvement programs for scampi in India was necessary to allow this industry to develop in a sustainable way as it has been demonstrated in other aquatic species, namely Atlantic salmon (Thodesen and Gjedrem, 2006), common carp (Bakos *et al.,* 2006), GIFT tilapia (Eknath *et al.,* 2007) and rohu carp (Mahapatra *et al.,* 2006). Therefore, ICAR-Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar in collaboration with the WorldFish, Malaysia initiated a systematic selective breeding programme for improving growth rate of *M. rosenbergii* in 2007 (Pillai *et al.,* 2011, 2015). In the present study an attempt was made to assess the genetic variations among the produced families of fourth generation (between high growth and low growth groups) and between disease resistant and susceptible groups) through RAPD-PCR method.

MATERIALS AND METHODS

Sample collection

The samples were collected from the ongoing selective breeding programme for *M. rosenbergii* at the Institute. From the families produced in the fourth generation of selection, three higher (HG1-3) and three lower (LG1-3) growth families were identified based on the

weight at final harvest out of 45 families generated (Pillai *et al.,* 2013). In addition, the natural resistance of larvae of generation 4 (G4) to *Vibrio harveyi* was assessed by immersion challenge experiment and a wide variation in survival (0-93.3%) was noticed among the families after bacterial challenge (Patra *et al.,* 2014). The families were grouped into susceptible or resistant based on the challenge test. Three extreme susceptible (<20% survival) (S1-3) and three most resistant (R1-3) families (>80% survival) were selected for present experiments. Pleopods from prawns (weight 30-40 g) belonging to above twelve selected families were collected for DNA extraction.

Isolation of genomic DNA

The genomic DNA was isolated from pleopods of prawns by phenol-chloroform extraction method of Sambrook and Russel (2001) with minor modifications. DNA from three individuals of each family of each group (total 36 individuals of 12 families) was utilized in the current study. The pleopod sample was homogenized using a sterile mortar and pestle in the presence of 700 µl TEN buffer (50 mM Tris-HCL, pH 8.0, 10 mM EDTA, 100 mM NaCl) and transferred to a 2.0 ml eppendorf tube. Proteinase K and SDS were added at a final concentration of 500 µg ml^{-1} and 1%, respectively. The mixture was mixed thoroughly and incubated overnight at 37 ^{0}C. DNA was extracted from the aqueous phase (after centrifugation at 10,000 rpm for 10 min at 4 ^{0}C) of phenol, phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1) and was precipitated by adding 0.3 M sodium acetate and absolute alcohol. DNA pellet was obtained by centrifugation at 10,000 rpm for 10 min at 4 ^{0}C. Further, DNA pellet was washed with 70% ethanol. The pellet was air dried and dissolved in TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0). Extracted DNA was checked for its purity and quantity using NanoDrop (ND1000, Thermo Scientific, Wilmington, DE, USA) and diluted with distilled water for a working concentration of 50 µg ml^{-1}.

RAPD-PCR

The PCR reaction was carried out using 25 ng of genomic DNA as template in a total volume of 25 µl reaction mixture. The reaction conditions optimized for amplification of random fragments were 0.75 U *Taq* polymerase (Genei, India), 1x *Taq* buffer A, 100 mM dNTPs and 20 picomole RAPD primer per reaction. The amplification was carried out in a thermal cycler (MJ research, Waltham, MA, USA) as per the following programme: initial denaturation at 94 ºC for 5 min, 40 cycles of 94 ºC for 1 min, 36 ºC for 1 min and 72 ºC for 2 min followed by final extension at 72 ºC for 7 min. Twelve random decamer primers {(OPA02, OPA04, OPA06, OPA07 OPA08, OPA09, OPA10, OPA11, OPA12, OPA14, OPA15 and OPA17 (IDT Milpitas, CA, USA)} were selected depending upon repeatability and reproducibility of amplified fragment patterns and were used in experiments.

Agarose gel electrophoresis

Amplified products were separated by electrophoresis on 1.5% agarose gel containing ethidium bromide in 1x TBE buffer at 100 v for 2 h. To determine the molecular size, 100 bp DNA ladder was run alongside RAPD products. The gels were visualized in a UV gel documentation system (ALPHA INNOTECH, San Leandro, CA, USA).

Analysis of Data

The bands observed in each lane were compared with all other lanes on the same gel. The reproducible bands were scored visually as either presence (1) or absence (0). Scores with respect to all the primers were used for constructing a single data matrix for growth and disease resistance separately. High growth families (HG1-3) followed by low growth families (LG1-3) were sequentially considered as population 1-6. Similarly, disease susceptible families (S1-3) followed by resistant families (R1-3) were considered sequentially as population 7-12. The data were analyzed using Pop-Gene version 1.31 software (Yeh *et al.,* 1999) for estimation of polymorphic loci, genetic diversity within populations, interpopulation genetic diversity and for construction of dendrogram among populations based on genetic distances (Nei, 1972).

RESULTS

The representative RAPD profiles of 18 individuals of *M. rosenbergii* from growth group generated by primers OPA2 and OPA4 are depicted in Fig.1. RAPD profiles of *M. rosenbergii* obtained by twelve random primers are summarized in Table 1. From all the twelve random primers 102 bands were scored, out of which 41 bands (40.19%) were polymorphic. Number of the scored fragments varied from 2 to 13 with a size range of 125 to 2042 bp. Gene diversity within populations varied from 0.0272±0.0965 to 0.0463±0.1316 (Table 2). Population 3 (HG-3) was found to have maximum gene diversity with 11.76% polymorphic loci and population 1 (HG-1) having the minimum with 7.84% polymorphic loci (Table 2). Genetic similarity among families ranged from 0.8176 to 0.9320. The highest genetic distance was 0.2013 between populations 3 (HG-3) and 6 (LG-3) while the lowest was 0.0705 between populations 3 (HG-3) and 4 (LG-1) (Table 3). UPGMA dendrogram based on Nei's genetic distance for 6 populations of *M. rosenbergii* (Fig. 2) showed that populations 5 (LG-2) and 6 (LG-3) form a different clade and are distantly related to other families.

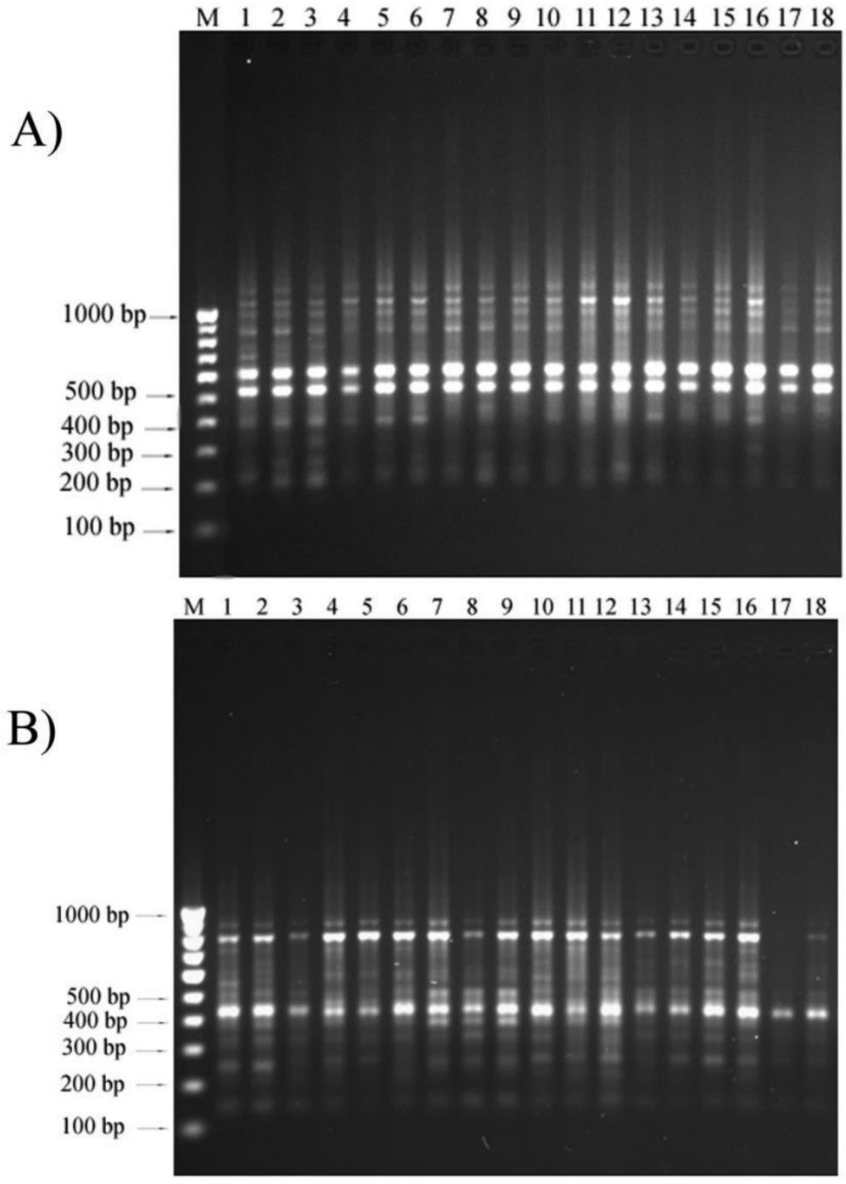

Fig. 1. RAPD profile of 18 individuals of *M. rosenbergii* of growth group with OPA 2 (A) and OPA4 (B) primers. Lane M: 100 bp DNA ladder; lanes 1-18: three samples from each family (lanes -1-3: LG1; 4-6: LG2; 7-9: LG3; 10-12: HG1; 13-15: HG2; 16-18: HG3).

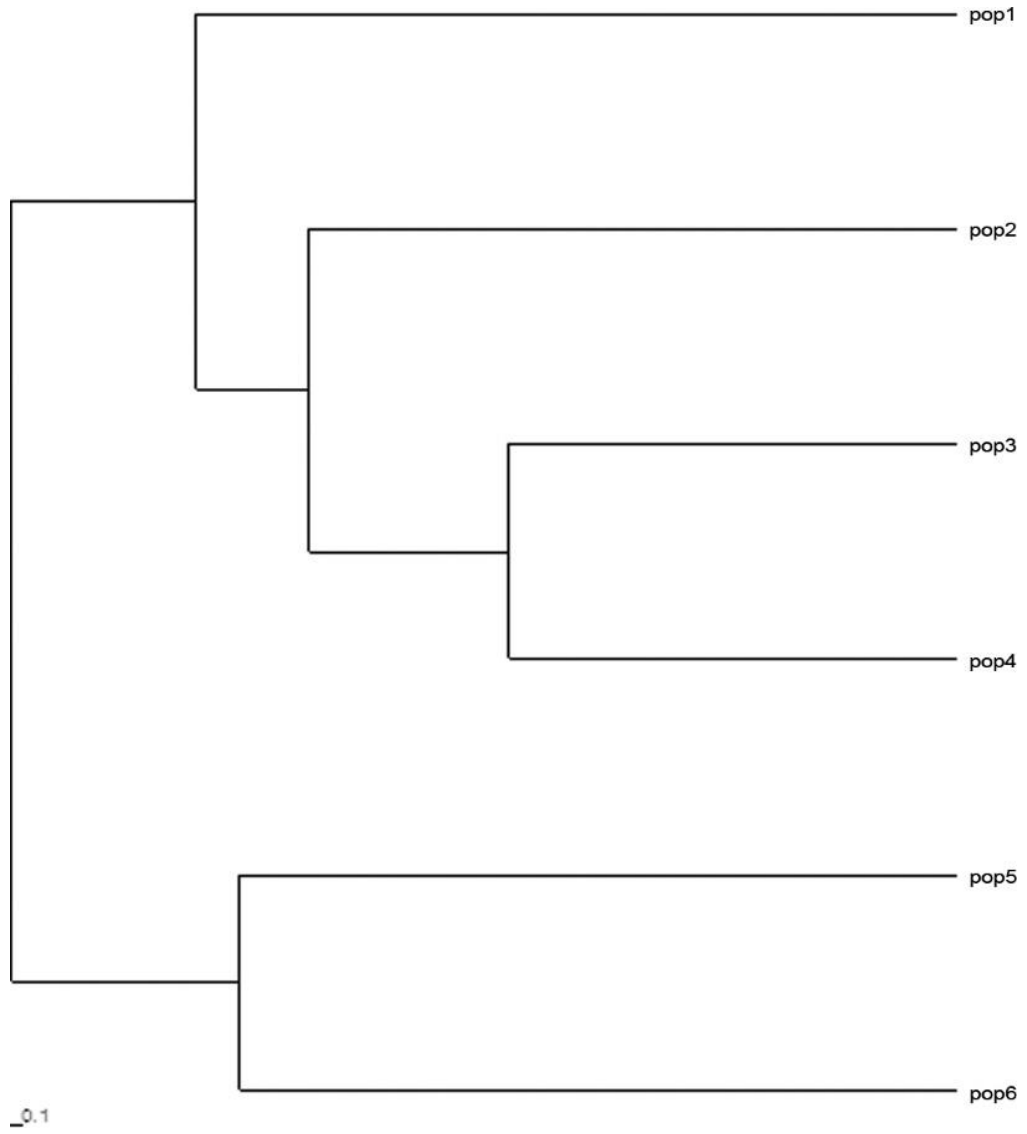

Fig. 2. UPGMA dendrogram of growth group for six populations (families) of *M. rosenbergii* based on Nei's genetic distance (Pop1- HG1; Pop2- HG2; Pop3- HG3; Pop4- LG1; Pop5- LG2; Pop6- LG3)

Table 1 : RAPD profiles of *M. rosenbergii* families from growth group obtained by twelve random primers.

Primer	Sequence (5' to 3')	No. of bands scored	Size of fragments (bp)	Total no. of bands	No. of polymorphic bands	Polymorphic bands (%)
OPA2	TGCCGAGCTG	6-9	207-1504	10	4	40
OPA4	AATCGGGCTG	7-10	160-1018	10	3	30
OPA6	GGTCCCTGAC	10-13	198-1969	13	3	23.07
OPA7	GAAACGGGTG	4-7	233-1625	7	3	42.85
OPA8	GTGACGTAGG	3-7	143-1204	8	6	75
OPA09	GGGTAACGCC	5-7	125-961	7	2	28.57
OPA10	GTGATCGCAG	5-7	179-1200	9	5	44.44
OPA11	CAATCGCCGT	8-11	137-2042	11	3	27.27
OPA12	TCGGCGATAG	3-6	181-1051	7	5	57.14
OPA14	TCTGTGCTGG	7-9	161-1056	9	3	22.22
OPA15	TTCCGAACCC	5-7	378-1563	7	2	28.57
OPA17	GACCGCTTGT	2-4	295-730	4	2	50
Total				**102**	**41**	**40.19**

Table 2 : Number and percentage of polymorphic loci and gene diversity values within *M. rosenbergii* families from growth group.

Population	Polymorphic loci		Gene diversity (Mean ± SD)
	No.	%	
Population 1 (HG1)	8	7.84	0.0272 ± 0.0965
Population 2 (HG2)	11	10.78	0.0416 ± 0.1240
Population 3 (HG3)	12	11.76	0.0463 ± 0.1316
Population 4 (LG1)	10	9.80	0.0331 ±0.1036
Population 5 (LG2)	11	10.78	0.0397 ± 0.1186
Population 6 (LG3)	11	10.78	0.0416 ± 0.1240

HG- Higher Growth, LG- Lower Growth

Table 3 : Nei's unbiased genetic identity (above diagonal) and genetic distance (below diagonal) values between *M. rosenbergii* families from growth group.

Population	1 (HG1)	2 (HG2)	3 (HG3)	4 (LG1)	5 (LG2)	6 (LG3)
1 (HG1)	****	0.8924	0.8681	0.9011	0.8630	0.8265
2 (HG2)	0.1138	****	0.8871	0.9195	0.8553	0.8606
3 (HG3)	0.1414	0.1198	****	0.9320	0.8986	0.8176
4 (LG1)	0.1042	0.0839	0.0705	****	0.8998	0.8762
5 (LG2)	0.1473	0.1564	0.1069	0.1056	****	0.8933
6 (LG3)	0.1906	0.1502	0.2013	0.1322	0.1129	****

The representative RAPD profiles of 18 individuals of *M. rosenbergii* samples from disease resistance group generated by primers OPA2 and OPA4 are depicted in Fig. 3 and RAPD profiles of *M. rosenbergii* obtained by twelve random primers are summarized in Table 4. From all the twelve random primers 96 bands were scored, out of which 35 bands (36.46%) were polymorphic. Number of the scored fragments varied from 2 to 13 with size ranges of 125 to 2042 bp. Gene diversity within populations varied from 0.0301±0.0957 to 0.0438±0.1381 (Table 5). Population 7 was found to have maximum genetic diversity with 11.46% polymorphic loci and population 11 having the minimum with 9.38% polymorphic loci (Table 5). Genetic similarity among families ranged from 0.8706 to 0.9371. The highest genetic distance was 0.1386 between populations 7 (S1) and 9 (S3) while the lowest was 0.0650 between populations 7 (S1) and 8 (S2) (Table 6). UPGMA dendrogram based on Nei's genetic distance for 6 families of *M. rosenbergii* (Fig. 4) showed that families 1 (S1) and 2 (S2) form a different clade, and are distantly related to other families.

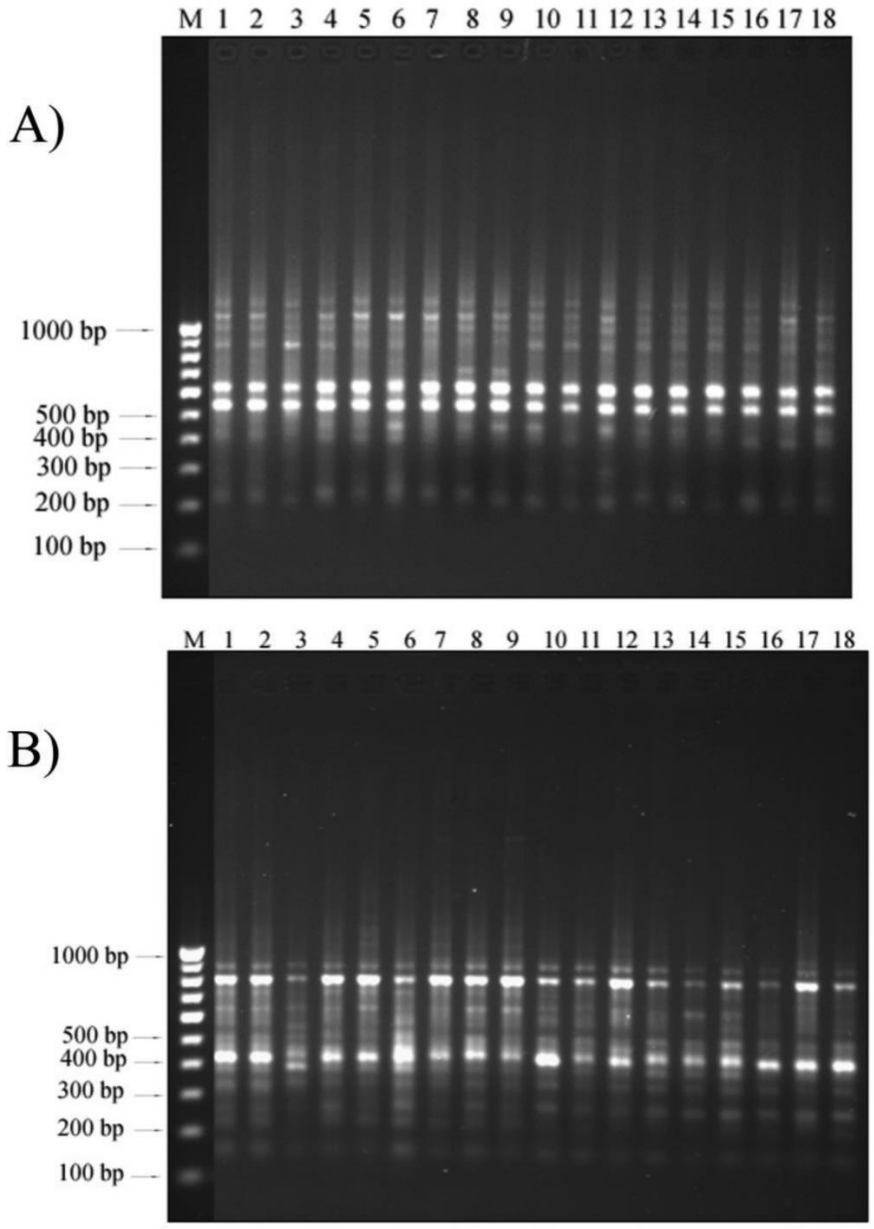

Fig. 3. RAPD profile of 18 individuals of *M. rosenbergii* from disease resistance group with OPA2 **(**A) and OPA 4 (B) primers. Lane M: 100 bp DNA ladder; lanes 1-18: three samples from each family (lanes 1-3: S1; 4-6: S2; 7-9: S3; 10-12: R1; 13-15: R2; 16-18: R3).

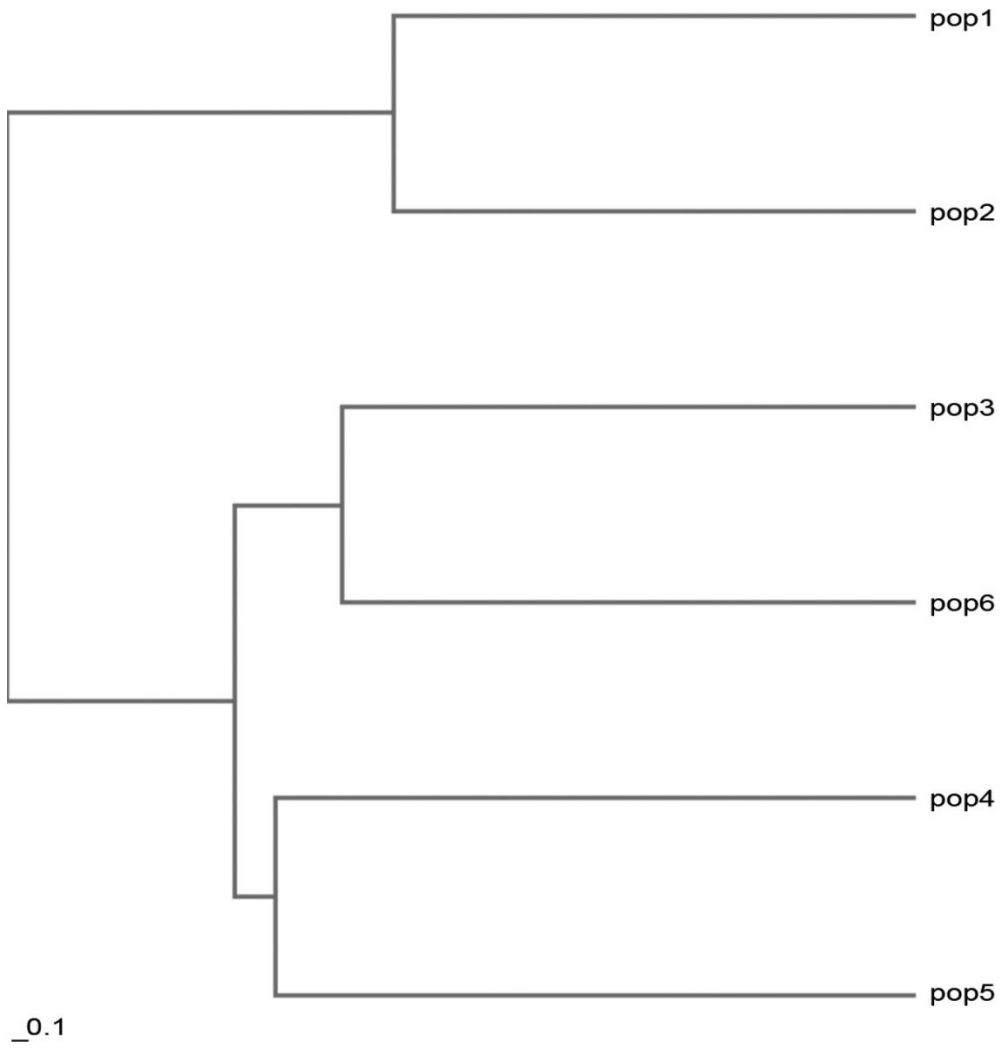

Fig. 4. UPGMA dendrogram of disease resistance group for six populations (families) of *M. rosenbergii* based on Nei's genetic distance (Pop1- S1; Pop2- S2; Pop3- S3; Pop4- R1; Pop5- R2; Pop6- R3).

Table 4 : RAPD profiles of *M. rosenbergii* families from disease resistance group obtained by twelve random primers.

Primer	Sequence (5' to 3')	No. of bands scored	Size of fragments (bp)	Total no. of bands	No. of polymorphic bands	Polymorphic bands (%)
OPA2	TGCCGAGCTG	6-9	207-1504	10	3	30.0
OPA4	AATCGGGCTG	5-10	160-1018	10	5	50.0
OPA6	GGTCCCTGAC	10-13	198-1969	13	4	23.07
OPA7	GAAACGGGTG	4-6	233-1625	6	3	33.33
OPA8	GTGACGTAGG	3-4	143-1204	6	3	50.0
OPA09	GGGTAACGCC	5-7	125-961	7	2	28.57
OPA10	GTGATCGCAG	5-8	235-1200	8	3	37.5
OPA11	CAATCGCCGT	8-10	137-2042	10	2	20.0
OPA12	TCGGCGATAG	3-5	234-616	6	3	50.0
OPA14	TCTGTGCTGG	5-8	161-1197	9	3	33.33
OPA15	TTCCGAACCC	4-6	280-1563	7	2	28.57
OPA17	GACCGCTTGT	2-4	295-730	4	2	50.0
Total				**96**	**35**	**36.46**

Table 5 : Number and percentage of polymorphic loci and gene diversity values within families of *M. rosenbergii* from disease resistance group.

Population	Polymorphic loci		Gene diversity (Mean ± SD)
	No.	%	
Population 7 (S1)	11	11.46	0.0402 ± 0.1160
Population 8 (S2)	9	9.38	0.0438 ± 0.1381
Population 9 (S3)	9	9.38	0.0320 ± 0.1029
Population 10 (R1)	9	9.38	0.0399 ± 0.1276
Population 11 (R2)	9	9.38	0.0301 ± 0.0957
Population 12 (R3)	10	10.42	0.0410 ± 0.1247

S-Susceptible, R-Resistant

Table 6 : Nei's unbiased genetic identity (above diagonal) and genetic distance (below diagonal) values between families of *M. rosenbergii* from disease resistance group.

Population	7 (S1)	8 (S2)	9 (S3)	10 (R1)	11 (R2)	12 (R3)
7 (S1)	****	0.9371	0.8706	0.8934	0.9017	0.8873
8 (S2)	0.0650	****	0.8977	0.9011	0.8890	0.9021
9 (S3)	0.1386	0.1080	****	0.9288	0.9221	0.9308
10 (R1)	0.1127	0.1041	0.0738	****	0.9233	0.8993
11 (R2)	0.1035	0.1177	0.0811	0.0798	****	0.9239
12 (R3)	0.1196	0.1030	0.0717	0.1061	0.0792	****

DISCUSSION

The present study was conducted to assess the genetic variability within and between families of selectively bred *M. rosenbergii* by RAPD analysis. RAPD has long been used in studying genetic variability in various species owing to its capabilities to run without known genetic sequence and to generate high polymorphic loci (Vaseeharan *et al.*, 2013). High number of polymorphic bands in RAPD reflects a high level of polymorphism in the populations. In our study, RAPD generated a moderate level of polymorphism both in growth (40.19%) and disease resistance (36.46%) groups. Mohanty *et al.* (2011) reported a higher level (~76%) of polymorphic bands with eight RAPD primers, while comparing 3 populations (Odisha, Kerala and Gujarat) from India which were used as base populations for the selective breeding program leading to development of populations used in present study. Islam *et al.* (2014) observed a similar level of polymorphism (41%) through RAPD with five primers while studying post larvae of *M. rosenbergii* broods stocked under different male: female ratio in Bangladesh. The reduced level of polymorphism noticed in this study (36.46 and 40.19%) as compared to base population (~76%) might be obvious and due to selection pressure or genetic interventions as the population under study are from fourth generation of selection.

However, the genetic diversity was low at the population level in both growth (percentage of polymorphic loci varying from 7.84 to 11.76; Nei's gene diversity varying from 0.0272 to 0.0463) and disease resistance (percentage of polymorphic loci varying from 9.38 to 11.46; Nei's gene diversity varying from 0.0301 to 0.0438) groups. These observed intra-population diversity in selectively bred populations were lower, when compared with the studies conducted earlier on its base populations (Mohanty *et al.*, 2011). It was reported that percentage polymorphic loci to be 45 to 52.5% and gene diversity to be 0.1330 to 0.1921. See *et al.* (2008) however, detected a very high level of polymorphism (94.3 to 100%) with all five primers while comparing 11 populations of *M. rosenbergii* in Malaysia. Hence, selection of base populations play crucial role in a selection programme.

The result of the present study showed presence of genetic variations among the produced families of the selection programme. The genetic distance between populations varied from 0.0705 to 0.2013 for growth group and 0.0650 to 0.1386 for disease resistance group. A comparison with the earlier study in their base populations (genetic distance varied from 0.1161 to 0.2076) (Mohanty *et al.*, 2011) indicated a reduced genetic distance between some populations. However, Mohanty *et al.* (2014) reported the genetic distance varying from 0.175 to 0.856 while studying genetic diversity of 5 Indian populations of *M. rosenbergii* by microsatellite markers. In UPGMA dendrogram, out of three families of lower growth groups two families were genetically distant and one family closely related from higher growth families. Similarly, out of three susceptible families, two were genetically distant from resistant group whereas one susceptible family genetically close with the resistant families. The results thus indicate that the genetic differentiation between families has not stabilized as per breeding traits after four generations of selection and the populations may require further directional breeding activities to reach the genetic stability. However, data generated from more number of families of successive generations would confirm the robustness of these findings.

Hence, it may be concluded that the genetic diversity within populations (families) has reduced, whereas, the genetic difference between families are still maintained. Hence, further selective breeding program may help to harness the full potential of traits. Though genetic diversity studies on selectively bred populations have not been conducted in freshwater prawn species, there are some reports on marine shrimps and fish. Cruz *et al.* (2004) found selected strains through breeding programs tended to lose genetic diversity compared with wild populations in Pacific white shrimp (*Litopenaeus vannamei*). A similar study was conducted by Li *et al.* (2006) using amplified fragment length polymorphism (AFLP) markers to investigate the genetic structure of a wild base population and three generations of marine shrimp, *Fenneropenaeus chinensis*, selected for fast growth (F5–F7). As time under selection increased, the genetic diversity tended to reduce, the differentiation between generations became less, and the variation of genetic structure of the populations became smaller. Luo *et al.* (2015) studied the genetic diversity and structure of 5 consecutive selected populations of golden mandarin fish (*Siniperca scherzeri* Steindachner) with microsatellite markers and observed reduced genetic diversity over generations and there was increased genetic distance between adjacent generations. They opined that the generation populations of breeding had not fully adopted to the existing selection pressure and environment, and thus the population genetic structure had not yet stabilized. Additionally, the populations may require further breeding activities to reach a stable genetic structure in order to ensure the genetic stability of breeding traits.

The reported polymorphism level found in the present investigation indicates that RAPD markers could be useful to assess genetic variations in selectively bred populations in freshwater prawn. Further, the results of genetic diversity among different families of *M. rosenbergii* based

on RAPD markers can contribute significantly to the development and implementation of further genetic improvement programs. However, using more powerful markers with large sample size may reveal better results which can help to establish genetic relationships among the families in a particular generation of any selective breeding programme.

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REFERENCES

Alam Md. S. and Md. S. Islam, 2005. Population structure of *Catla catla* (Hamilton) revealed by microsatellite DNA markers. *Aquaculture*, **246:** 151-160.

Bakos, J., L. Varadi, S. Gorda, and Z. Jeney, 2006. Lessons from the breeding program on common carp in Hungary. *In*: Development of aquatic animal genetic improvement and dissemination programs: current status and action plans. (Eds. R. W. Ponzoni, B. O. Acosta and A. G. Ponniah) WorldFish Center Conferences Proceedings **73**: 27-33.

Brown, B. and J. Epifanio, 2003. Nuclear DNA. *In*: Population Genetics: Principles and Applications for Fisheries Scientist, (Ed. Hallerman, E. M.) American Fisheries Society, Bethesda, pp. 458-472.

Chauhan, T. and K. Rajiv, 2010. Molecular markers and their applications in fisheries and aquaculture. *Adv. Biosci. Biotechnol.*, **1**: 281-291.

Cruz, P., A. M. Ibarra, H. Mejia-Ruiz, P. M. Gaffney, and R. Perez-Enriquez, 2004. Genetic variability assessed by microsatellites in a breeding program of Pacific white shrimp (*Litopenaeus vannamei*). *Mar. Biotechnol.*, **6**: 157–164.

Dahle G., M. Rahman and A.G. Eriksen, 1997. RAPD fingerprinting used for discriminating among three populations of Hilsa shad (*Tenualosa ilisha*). *Fish. Res.*, **32:** 263-269.

Danzmann, R. G. and K. Ghabri, 2001. Gene mapping in fishes: a means to an end. *Genetica*, **111**: 3-23.

Das, P., H. Prasad, P. K. Meher, A. Barat and R. K. Jana, 2005. Evaluation of genetic relationship among six *Labeo* species using randomly amplified polymorphic DNA (RAPD). *Aquacult. Res.*, **36:** 564-569.

Eknath, A. E., H. B. Bentsen, R. W. Ponzoni, M. Rye, N. H. Nguyen, J. Thodesen and B. Gjerde, 2007. Genetic improvement of farmed tilapias: composition and genetic parameters of a synthetic base population of *Oreochromis niloticus* for selective breeding. *Aquaculture*, **273:** 1-14.

Falconer, D. S. and T. F. C. Mackay, 1996. Introduction to quantitative genetics. Pearson, Harlow, 464 pp.

FAO, 2008. FAO yearbook 2006. Fisheries and Aquaculture Statistics. FAO, Rome, Italy, 57 pp.

Garcia, D. K. and J. A. H. Benzie, 1995. RAPD markers of potential use in penaeid prawn (*Penaeus monodon*) breeding programs. *Aquaculture*, **130:** 137–144.

Gjedrem, T. and J. Thodesen, 2005. Selection. *In*: Selection and Breeding Programs in Aquaculture. (Ed. T. Gjedrem) Springer, the Netherlands, pp. 89-111.

Horn, P. L., J. A. Rafalski and P. J. Whitehead, 1996. Molecular genetics (RAPD) analysis of breeding mapping genes. *The Auk*, **113**: 552-557.

Huang, C. M. and I. C. Liao, 1990. Response to mass selection for growth rate in *Oreochromis niloticus*. *Aquaculture*, **85**: 199-205.

Hulata, G., G. W. Wohlfarth and A. Halevy, 1986. Mass selection for growth rate in the Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **57**: 177-184.

Islam S. S., Md. S. Shah and Md. L. Rahi, 2014. Assessment of genetic variability of prawn (*Macrobrachium rosenbergii*) post larvae (PL) from brood stocks under different sex ratios. *Int. J. Aquacult.*, **4**: 55-63**.**

Klinbunga, S., D. Siludjai, W. Wudthijinda, A. Tassanakajon, P. Jarayabhand and P. Menasveta, 2001. Genetic heterogeneity of the giant tiger shrimp (*Penaeus monodon*) in Thailand revealed by RAPD and mitochondrial DNA RFLP analyses. *Mar. Biotechnol*., **3**: 428-438.

Klinbunga, S., P. Ampayup, A. Tassanakajon, P. Jarayabhand and W. Yoosukh, 2000. Development of species-specific markers of the tropical oyster (*Crassostrea belcheri*) in Thailand. *Mar. Biotechnol*., **2:** 476-484.

Lakra, W. S., M. Goswami, V. Mohindra, K. K. Lal and P. Punia, 2007. Molecular identification of five Indian sciaenids (Pisces: Perciformes, Sciaenidae) using RAPD markers. *Hydrobiologia*, **583**: 359-363.

Li, Z., J. Li, Q. Wang, Y. He and P. Liu, 2006. The effects of selective breeding on the genetic structure of shrimp *Fenneropenaeus chinensis* populations. *Aquaculture*, **258**: 278-282.

Liu, Z. J. and J. F. Cordes, 2004. DNA marker technologies and their applications in aquaculture genetics. *Aquaculture*, **238**: 1-37.

Liu, Z. J., P. Li, B. J. Argue and R. A. Dunham, 1999. Random amplified polymorphic DNA markers usefulness for gene mapping and analysis of genetic variation of catfish. *Aquaculture*, **174**: 59-68.

Luo, X. N., M. Yang, X. F. Liang, K. Jin, L. Y. Lv, C. X. Tian, Y. C. Yuan and J. Sun, 2015. Genetic diversity and genetic structure of consecutive breeding generations of golden mandarin fish (*Siniperca scherzeri* Steindachner) using microsatellite markers. *Genet. Mol. Res.*, **14**: 11348-11355.

Mahapatra, K. D., R. K. Jana, J. N. Saha, B. Gjerde, and N. Sarangi, 2006. Lessons from the breeding program of Rohu. *In*: Development of aquatic animal genetic improvement and dissemination programs: current status and action plans (Eds. R. W. Ponzoni, B. O. Acosta and A. G. Ponniah). WorldFish Center Conferences Proceedings, **73**: 34-40.

Mishra P. S., A. Chaudhuri, G. Krishna, D. Kumar and W. S. Lakra, 2009. Genetic diversity in *Metapenaeus donovani* using RAPD. *Biochem. Genet.*, **47**: 421-426.

Moav, R. and G. Wohlfarth, 1976. Two-way selection for growth rate in the common carp (*Cyprinus carpio* L.). *Genetics*, **82**: 83-101.

Mohanty, J., L. Sahoo, S. Sahu, B. R. Pillai and K. Dasmahapatra, P. K. Sahoo and P. L. Lalrinsanga, 2011. Evaluation of genetic variation in *Macrobrachium rosenbergii* by RAPD-PCR. *In*: Book of Abstracts, Asian Pacific Aquaculture, Kochi, 105 pp.

Mohanty, P., L. Sahoo, B. R. Pillai, P. Jayasankar and P. Das, 2014. Genetic divergence in Indian populations of *M. rosenbergii* using microsatellite markers. *Aquacult. Res.*, **47**: 472-481.

Naish, K. A., M. Warren, F. Bardakci, D. O. F. Skibinski, G. R. Carvalho and G. C. Mair, 1995. Multilocus DNA fingerprinting and RAPD reveal similar genetic relationships between strains of *Oreochromis niloticus* (Pisces: Cichlidae). *Mol. Ecol.*, **4**: 271-274.

Nei, M., 1972. Genetic distance between populations. *Am. Nat.*, **106**: 283-292.

Partis, L. and R. J. Wells, 1996. Identification of fish species using random amplified polymorphic DNA (RAPD). *Mol. Cell. Probes*, **10**: 435-441.

Patra, G., B. R. Pillai, P. K. Sahoo, P. L. Lalrinsanga, A. Das, S. Mohanty, S. Sahu and N. Naik, 2014. Immune responses and susceptibility patterns in different families of selectively bred *Macrobrachium rosenbergii* (de Man) in response to *Vibrio harveyi* infection. *In*: *Book of Abstracts*, 10th Indian Fisheries and Aquaculture Forum, held at National Bureau of Fish Genetic Resources, Lucknow, 12-15 November 2014, AAH- 48, pp: 372.

Pillai, B. R., K. D. Mahapatra, R. W. Ponzoni, L. Sahoo, P. L. Lalrinsanga, W. Mekkawy, H. L. Khaw, N. H. Nguyen, S. Mohanty, S. Sahu and G. Patra, 2015. Survival, male morphotypes, female and male proportion, female reproductive status and tag loss in crosses among three populations of freshwater prawn *Macrobrachium rosenbergii* (de Man) in India. *Aquacult. Res.*, **46**: 2644-2655.

Pillai, B. R., K. D. Mahapatra, R. W. Ponzoni, P. L. Lalrinsanga, H. L. Khaw, S. Mohanty, S. Sahu, G. Patra and N. Naik, 2013. Genetic improvement of *Macrobrachium rosenbergii* (de man) in India through selective breeding, *In*: Souvenir; Final workshop of the collaborative project between Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar and WorldFish Centre (WFC), Malaysia on 'Genetic Improvement of Freshwater Prawn, *Macrobrachium rosenbergii*, (de Man) in India-Phase-II' 20-21 December, 2013.

Pillai, B. R., K. D. Mahapatra, R.W. Ponzoni, L. Sahoo, P. L. Lalrinsanga, N. H. Nguyen, S. Mohanty, S. Sahu, K. Vijay, S. Sahu, H. L. Khaw, G. Patra, S. Patnaik and S. C. Rath, 2011. Genetic evaluation of a complete diallel cross involving three populations of freshwater prawn (*Macrobrachium rosenbergii*) from different geographical regions of India. *Aquaculture*, **319**: 347-354.

Rezvani Gilkolaei, S., R. Safari, F. Laloei, J. Taqavi and A. Matinfar, 2011. Using RAPD markers potential to identify heritability for growth in *Fenneropenaeus indicus*. *Iran. J. Fish. Sci.*, **10**: 123-134.

Sahoo, P. K., J. Mohanty, S. K. Garnayak, B. R. Mohanty, B. Kar, J. K. Jena and H. Prasanth, 2013. Genetic diversity and species identification of *Argulus* parasites collected from major aquaculture regions of India using RAPD-PCR. *Aquacult. Res.*, **44**: 220-230.

Sambrook J. and D. W. Russel, 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, NY, USA.

See, L. M., R. Hassan, S. G. Tan and S. Bhassu, 2008. Genetic characterization of wild stocks of prawns, *M. rosenbergii* using random amplified polymorphic DNA markers. *Biotechnology*, **7:** 338-42.

Shi, T., J. Kong, P. Liu, L. L. Hart, Z. M. Zhuang and J. Y. Deng, 1999. RAPD analysis of genetic diversities in *Penaeus chinensis*: in the western coast of Korean Peninsula. *Oceanol. Limnol. Sin.*, **30:** 609-614.

Shikano, T. and N. Taniguchi, 2002. Using microsatellite and RAPD markers to estimate the amount of heterosis in various strain combinations in the guppy, *Poecilia reticulate* as a fish model. *Aquaculture*, **204**: 271-281.

Song L. S., J. H. Xiang, C. X. Li, B. Z. Liu and R. Y. Liu, 1999. Analysis of RAPD markers of the genetic structures in the natural population and hatchery stock of *Penaeus japonicus*. *Oceanol. Limnol. Sin.*, **30**: 261-264.

Sun, Y., W. Q. Song, Y. C. Zhong, R. S. Zhang and R.Y. Chen, 2000. Phylogenetic study of *Artemia* from China using RAPD and AFLP markers. *Yi Chuan Xue Bao*, **27**: 210-218.

Tassanakajon, A., S. Pongsomboon, V. Rimphanitchayakit, P. Jarayabhand and V. Boonsaeng, 1997. Random amplified polymorphic DNA (RAPD) markers for determination of genetic variation in wild populations of the black tiger prawn (*Penaeus monodon*) in Thailand. *J. Mar. Biotechnol.*, **6**:110-115.

Tassanakajon, A., S. Pongsomboon, V. Rimphanitchayakit, P. Jarayabhand and V. Boonsaeng, 1998. Genetic structure in wild populations of black tiger shrimp (*Penaeus monodon*) using randomly amplified polymorphic DNA analysis. *J. Mar. Biotechnol.*, **6**: 249-254.

Thodesen, J. and T. Gjedrem, 2006. Breeding programs on Atlantic salmon in Norway-lessons learned. *In*: Development of aquatic animal genetic improvement and dissemination programs: current status and action plans (Eds. R. W. Ponzoni, B. O. Acosta and A. G. Ponniah). WorldFish Center Conferences Proceedings, **73:** 22-26.

Vaseeharan, B., P. Rajakamaran, D. Jayaseelan and A. Y. Vincent, 2013. Molecular markers and their application in genetic diversity of penaeid shrimp. *Aquacult. Int.*, **21**: 219-241.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., **18**: 6531-6535.

Yeh F. C., R. C. Yang and T. Boyle, 1999. Popgene 32-version 1.31: Population Genetics Software. University of Alberta, Edmonton, AB Canada T6G 2H1.

Yoon, J. M. and G. W. Kim, 2001. Randomly amplified polymorphic DNA-polymerase chain reaction analysis of two different populations of cultured Korean catfish *Silurus asotus*. *J. Biosci.*, **26**: 641-647.

Zhuang, Z., T. Shi, J. Kong, P. Liu, Z. Liu, X. Meng and J. Deng, 2001. Genetic diversity in *Penaeus chinensis* shrimp as revealed by RAPD technique. *Prog. Nat. Sci.*, **11**: 332-338.