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AKP and SOD related Quantitative Trait Locus (QTL) detection in common carp

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Abstract: To find out the chromosomal regions, that contribute to variability in alkaline phosphatase (AKP) and Superoxide dismutases (SOD) enzyme, a Quantitative trait locus (QTL) analyses was conducted in common carp. In all tissues, there are some enzymes that catalyze the hydrolysis of inorganic or organic phosphate esters with a broad specificity for ligand, including pyrophosphate. Importantly the status of these enzymes is to be said as disease indicator. Therefore, identifying chromosomal region related with these important enzymes are essential for genetic breeding of such important aquaculture species. A F1 family of common carp consisted on 92 individuals was utilized for QTL analysis. In present investigation a total of ten QTLs were identified, five for each, on eight linkage groups (LGs) i.e LG1, LG2, LG3, LG4, LG7, LG18, LG21 and LG22. LG1 and LG21 were found with the QTLs related with both enzymes, AKP and SOD. Three significant ($P< 0.05$) QTLs (*AKP-1*, *AKP-4* and *AKP-22*) were found related with AKP. While only two significant ($P< 0.05$) QTLs (*SOD-7* and *SOD-21*) were found associated with SOD. A maximum LOD 5.02 was found for *SOD-3* and minimum 3.0 for *SOD-7*. Maximum and minimum phenotype variance was found 37.1% and 18.7% respectively. These findings would lead to produce strain of common carp with strong immunity and will contribute to highest aquaculture production.

Keywords: Common carp, *Cyprinus carpio*, immunity, QTLs, AKP and SOD.

1. INTRODUCTION

Superoxide Dismutases (SOD) and Alkaline phosphate (AKP) are enzymes, various tissues contain such enzymes those plays important role of antioxidant defense in the cells (Lob *et al.,* 2010). Due to increasing human activity (agriculture and industry) heavy metals have been spread in various water bodies, including even Sea and Rives (Falahi-Ardakani, 1984; Taylor and Kesterton, 2002; Lovley and Coates, 1997; Nordberg *et al.,* 2007). Hence, these metals significantly bioconcentrated and bioaccumulated in the tissue of aquatic species. Same time, these pollutants are great threat to survival and growth of aquatic species, due to defective immune. That causes significant mass mortality in aquaculture species. It is considered as great loss of aquaculture production. Therefore, in order to increase aquaculture production and decrease the mass mortality it is essential to develop the fish population with a major genes associated with SOD and AKP.

Genetics has a great impact on animal breeding because of DNA markers, linked to genes, affecting special traits. Such type of markers play important role in plant and animal breeding. These are used tofindout quantitative trait loci (QTL) that controls important traits of interest and to select organism for marker-assisted breeding.

Common carp is one of the important aquaculture species, popular all around the world. At present a genome sequence of *C.carpio* has been completed and a genetic linkage map was constructed with 307 markers, with an average interval of 11 cM.(Zhang *et al.,* 2013). This linkage map was utilized for detection of QTLs associated with SOD and AKP. Present study will contribute to develop the markers for genetic breeding of *C.carpio*.

2. MATERIALS AND METHODS

A F1 family of *C.carpio* was constructed at theAquaculture Experimental Station, Harbin, China. 92 individuals (F1 family) were kept in with water circulating rectangular tanks (120 Liter) and the conditions in the tanks as; water temperature were maintained at 20°C and fishwere fed at 8:00 am, 12:00 noon, 4:00 pm and 8:00 pm. Fish was discouraged from resting to motivate them swimming constantly. The fish were underwent biochemical and genetic analysis after sampling.

A sample of white muscle tissue about 10.0 g from each individual wastaken to assays SOD and AKP enzyme activity.

AKPactivity of the white muscles wasidentified spectrophotometric ally at 520 nm (Biophotometer

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6131, Eppendorf, Germany), with alkaline phosphatase (AKP) detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The amount of enzyme that reacted with the matrix and produced 1 mg phenol in 30 min at 37°C was considered as one unit of AKP.

SOD activities of the white was determined spectrophotometrically at 550 nm (Biophotometer 6131, Eppendorf, Germany). SOD enzyme detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was selected. The amount of tissue extracts that inhibited the rate of xanthine reduction at 25 °C by 50% was considered as one unit of SOD activity, and the specific activity was expressed as SOD units (mg protein−1).

The DNA was isolated by standard phenol–chloroform protocol method, described by Sambrook and Russell (2001) and quality was checked on1% agarose gel. The quantity was measured by usingNanodrop 2000 (Thermo Fisher, USA).A linkage map of *C.carpio* was constructed (Zhang *et al.*,2013) and utilized for QTL detection. The map was resulted on 51 linkage groups, consisted on 109 Simple Sequence Repeats (SSRs), 31Expressed Sequence Tag-SSR (EST-SSR), and 167Single Nucleotide Polymorphism (SNP) markersderived from ESTs.

The QTL analysis was carried out by theMapQTL 4.0 program (Van *et al.*, 2002). A Multiple interval mapping method was applied to detectsignificant associations between enzyme activities and marker loci.

MapQTL 4.0 was utilized to find out LOD scorebypermutation tests (n=10,000).A one-way ANOVA test was conducted tocharacterize the QTL allele substitution effects.

Thegenotypes of the marker locus lying closest to genomicregions were defined as m1f1, m1f2, m2f1, andm2f2, where m1, m2 and f1, f2 denoting thegenotypes of the father and mother, respectively.

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3. RESULTS AND DISCUSSION

The phenotypic values of SOD and AKP traits were measured with an average of 1272.504±295.541 and 3.334±2.052 U in white muscles of the F1 family. A total of ten QTLs were detected, associated with both enzymes, on eight linkage groups (LGs) i.e LG1, LG2, LG3, LG4, LG7, LG18, LG21 and LG22. Five QTLs *AKP-1*, *AKP-2*, *AKP-4*, *AKP-21* and *AKP-22* were found related with AKP on LG-1, LG-2, LG-4, LG21 and LG-22 at nearest marker SNP0989, SNP1003, SNP0674, SNP1282 and SNP 1171 respectively (Fig.01). While five QTLs *SOD-1*, *SOD-3*, *SOD-7*, *SOD-18* and *SOD-21* were found related with SOD at nearest marker HLJ440, SNP0758, HLJ1171, HLJ1499 and SNP1198, respectively (Fig.02). There were three significant ($p<0.05$) QTLs *AKP-1*, *AKP-4* and *AKP-22* associated with AKP. On other hand there were only two significant ($p<0.05$) QTLs (*SOD-7* and *SOD-22*) were found for SOD. LG1 and LG21 were found with the QTLs related with both enzymes, AKP and SOD. While, significant QTLs were detected on same linkage group (LG-21) for both enzymes, at different marker position, with a phenotype variance of 29.8% and 19.5%, respectively (Table 01). The maximum and minimum LOD was found 5.02 and 3.0 respectively. While, maximum phenotype variance was found 37.1% and minimum was 18.7%.

The above results suggest that SNP0989, SNP0674, SNP1171, HLJ1171 and SNP1198 markers are closely related with the traits and having a great importance. Therefore, these markers would be investigated in further and might be useful for gene prediction/discovering associated with SOD and AKP enzymes. Hence, these results would contribute in genetic breeding of *C.carpio* and would lead higher production and reduce mass mortality as well as increase the survival rate.

ACKNOWLEDGEMENT

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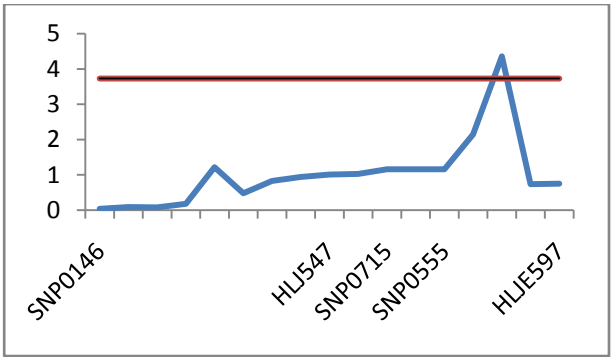

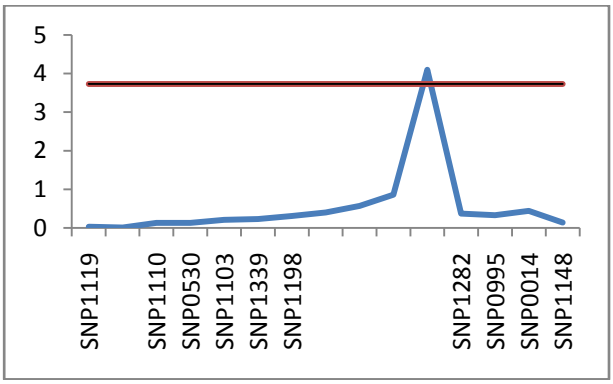

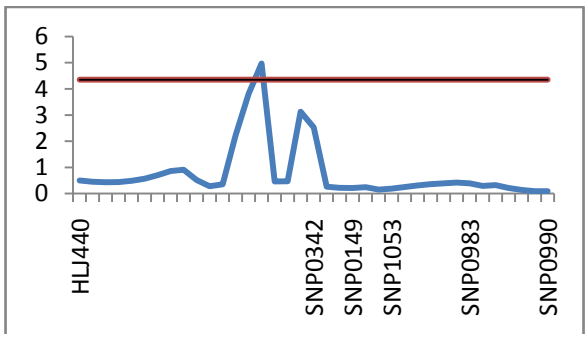
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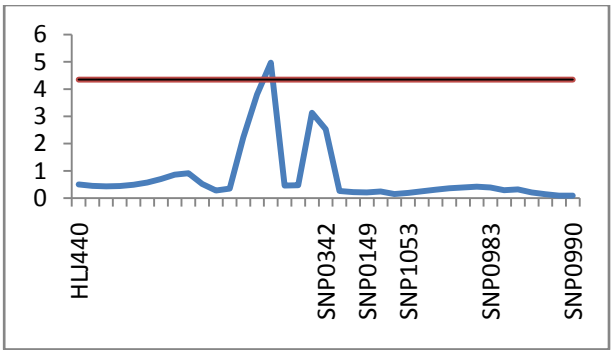
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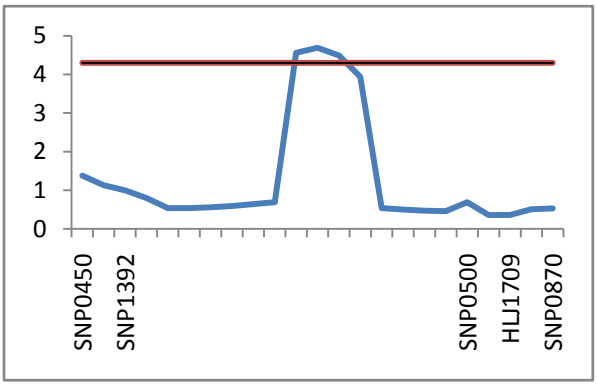
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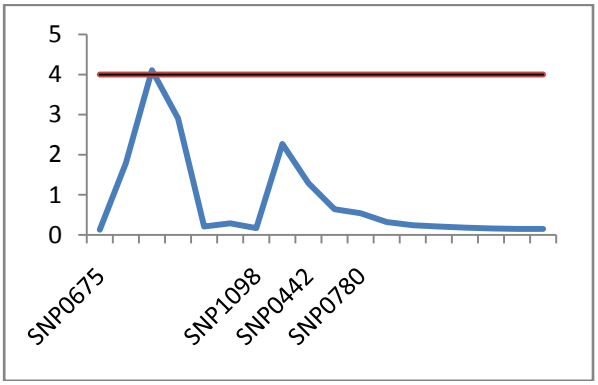
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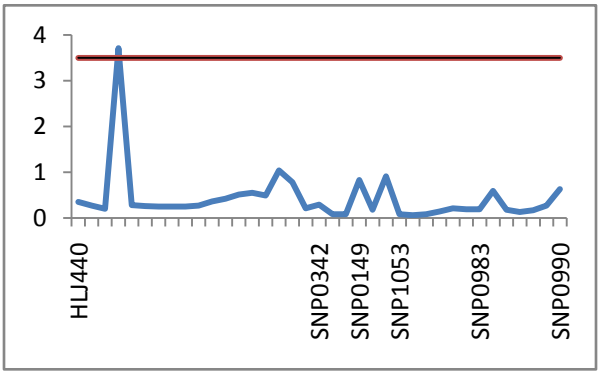
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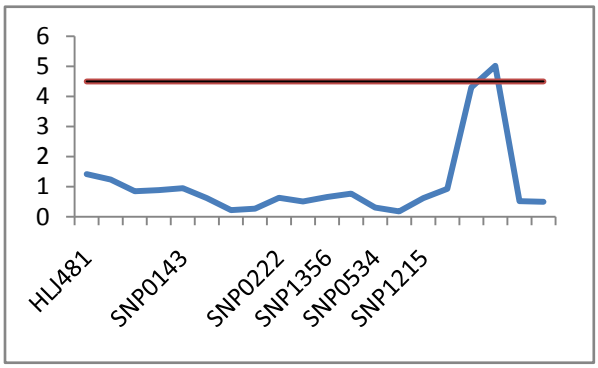
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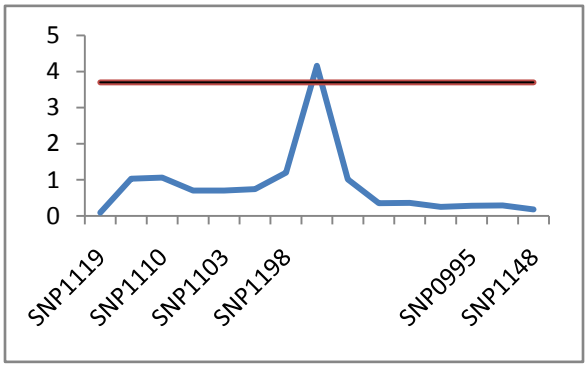
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Table 01. Complete information of detected QTLs associated with AKP and SOD.

Trait			Nearest		LOD	Location		Phenotype mean		Significance
	QTL	LG	Marker	LOD	Threshold	cM	PVE%	m1f1	m1f2	*p*
AKP										
	AKP-1	1	SNP0989	4.97	4.35	63.8	30.9	313.7±2.178	343.0±2.14	0.05
	AKP-2	2	SNP1003	4.36	3.73	43.5	28.1	339.2±1.1642	320.7±2.135	0.07
	AKP-4	4	SNP0674	4.11	4	5.1	37.1	322.6±1.968	366.2±2.355	0.04
	AKP-21	21	SNP1282	4.1	3.73	30.3	29.8	332.1±2.324	341.2±2.134	0.09
	AKP-22	22	SNP1171	3.54	3.35	31	28.9	351.3±2.126	370.2±2.316	0.04
SOD										
	SOD-1	1	HLJ440	3.71	3.5	15	19.4	12.65±1.99	12.99±2.33	0.08
	SOD-3	3	SNP0758	5.02	4.5	58.7	23.2	12.77±2.72	12.74±3.14	0.09
	SOD-7	7	HLJ1171	3.24	3	5	18.7	13.01±2.35	12.80±2.95	0.05
	SOD-18	18	HLJ1499	4.69	4.3	45.4	20.2	12.80±3.03	12.72±2.78	0.07
	SOD-21	21	SNP1198	4.16	3.7	15.3	19.5	12.93±2.87	12.67±2.56	0.05

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