Review Article

MARKER-ASSISTED SELECTION IN FISH: A REVIEW

ABSTRACT

The important economical traits like body growth, resistance to diseases, meat quality, etc. highly influence the profitability of **the** food animals including fishes. The main target of every selective breeding programme is to produce improved offsprings for these traits. However, improvement of performance traits through traditional phenotype-based selection needs several generations to optimise these characters. Marker-Assisted Selection (MAS) is a type of indirect method of selection of better performing breeding individuals. MAS is beneficial when the traits are difficult, **and** expensive to measure and has both low heritability and recessive traits. MAS facilitates the exploitation of existing genetic diversity in breeding populations and can be used to improve desirable traits in livestock. MAS depends on identifying the link between a genetic marker and Quantitative Ttraits Lloci (QTL). The distance between marker and target traits determines the association of the marker with the QTL. After identifying the markers linked to QTL, they can be used in the selective breeding programme to select the brooders having better genetic potential for the targeted trait. Improvement of performance traits through MAS is fast and more accurate and allows us to understand the genetic mechanism affecting performance traits.

Keywords: Marker-Assisted Selection, Quantitative traits loci, genetic diversity, trait

1.0 INTRODUCTION

Marker-Assisted Selection (MAS) is a type of biotechnology that uses molecular genetic markers as a criteriona for selecting **a** desired traits (Ashraf, 2012). Marker Assisted Selection (MAS) is an indirect selection process where a trait of interest is selected not based on the trait itself but on a marker linked to it (Ribaut and Ragot 2007).

MAS is considered a "revolutionary" approach to traditional tree breeding as it allows breeders to select individuals based on their genotypes, rather than being restricted to phenotypic characteristics (Boopathi *et al.*, 2013).

Sax (1923) was the first to show how genetic factors influencing quantitative traits can be identified using markers.

Recently MAS becaome a very popular method of indirect selection for production of the genetically improved offspring's in aquaculture breeding programme. MAs most of the performance traits such asincluding growth or disease resistance are controlled by multiple genes and are therefore inherited as quantitative traits, analysis of their associated quantitative trait loci (QTL) is an essential part of aquaculture genomics (Liu and Cordes, 2004). QTLs are largely unknown genes that affect performance traits (such as growth rate and disease resistance) and these are important to breeders.

MAS in a breeding context involves scoring indirectly for the presence or absence of a desired phenotype or phenotypic component based on the sequences or banding patterns of molecular markers located in or near the genes controlling the phenotype. The sequence polymorphism or banding pattern of the molecular marker is indicative of the presence or absence of a specific gene or chromosomal segment that is known to carry a desired allele (Brumlop and Finckh, 2011).

Marker-assisted selection method (MAS) or genome-wide marker-assisted selection method (G-MAS) was not widely used in aquaculture, but nowadays its use is increasing due to its ease of use and quicker than traditional phenotype-based selection. Now it becomes a fertile field of research for the aquaculture researchers to discover novel genetic marker that can be used to link with the QTLs in selective breeding programmes (Hauser *et al.*, 2011; Dichmont *et al.*, 2012; Abdul-Muneer, 2014).

In order to manage individual species effectively, identification of different species from a mixed catch becomes important. DNA markers are widely being accepted not only to obtain information about gene flow and allele frequencies in aquaculture practices but also to identify hybrids. The majority of the markers, which are used in inter- and intra-specific disparity, include RAPD (write in full at first) for species and sub-species identification done in tilapia (Bardakci and Skibinski, 1994), and iso-enzyme used in intraspecific variations in Sparidae species (Alarcón and Alvarez 1999). Similarly, Nijman *et al.*, (2003) reported the use of mtDNA markers as an important tool in rapid detection of hybridization between species and subspecies of livestock.

Markers tend not to have any biological effect, but rather can be thought of as notable and constant points of reference within the genome (Guimaraes, Ruane, Scherf, Sonnino, and Dargie, 2007 et al will be appropriate here). Markers can be found within the desired gene or, more commonly, linked to a gene determining a trait of interest (Brumlop and Finckh, 2011; Guimaraes *et al.,* 2007). Unlike genetic engineering, MAS does not alter the original DNA (Vogel and Van Aken, 2009); instead it uses genetic marker to identify naturally-occurring genetic variations among individuals, with the intent of selecting those with the best potential to meet desired criteria and objectives.

Marker Assisted Selection (MAS) provides several other benefits to breeders, in that it can select for genes that demonstrate low heritability, have recessive alleles, and are difficult, expensive, or time exhaustive to determine phenotypically (Boopathi, 2013a; Brumlop and Finckh, 2011; Xu and Crouch, 2008). MAS also allows for gene pyramiding or combining multiple genes within the same breeding line, while having fewer unintentional losses and fewer selection cycles (Boopathi, 2013a; Xu and Crouch, 2008).

Furthermore, MAS may be viewed by the public with more support than genetic engineering as breeders are not manually manipulating the genes, and thus all offspring inheritance occurs naturally (Vogel and Van Aken, 2009). It is also believed that genetic markers may be important in the assessment, conservation and use of diversity in germplasm and varieties (Brumlop and Finckh, 2011).

Molecular marker maps have been constructed for a number of aquaculture species, e.g. tilapia, *Clarias*, giant tiger prawn, kuruma prawn, Japanese flounder and Atlantic salmon, although their density is generally low (Nichols *et al.,* 2003). As many preferred traits are not observed until maturity, MAS eliminates this waiting period by allowing for the early selection of desired genotypes at the seedling stage (Yanchuk *et al.,* 2002).

The desirable phenotypic variations in the performance traits of fishes are used to increase the aquacultural yield, improve incomes of farmers and enhances food security through selective breeding by choosing better-performed individuals. However, phenotype-based selection needed considerable time to optimise the traits, so researchers are now moving from phenotype based selection to genotype-based selection. The **lacking** absence of a molecular marker is the main limiting factor for the realization of genotype based selection potentials in fishes. However, with the advent of DNA-based genetic markers in the late 1970s and now the ease of the marker discovery through the next generation sequencing allowed **the** researchers to identify large numbers of markers spreads throughout the genome

of any species of interest. The markers are used to detect linkage with the traits of interest, thus allowing MAS finally to become a reality (Peterson *et al.,* 1990). This paper aims to provide information regarding the technical aspect of MAS and the current application in fisheries and Aquaculture in other to increase high quality production within a period of time.

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2.0 Marker Assisted Selection

Incorporation of marker information into breeding programs in aiding identification and selection of superior individuals has been widely studied (Bernardo, 1994; Han *et al.,* 1997; Xie and Xu, 1998; Romagosa *et al.,* 1999; Ayoub *et al.,* 2003; Jordan *et al.,* 2003).

Molecular markers in aquaculture and fisheries have been used for over 50 years (Ryman and Utter, 1987; Liu and Cordes, 2004) and their use has steadily increased over the last two decades (Park and Moran, 1994; Chauhan and Rajiv, 2010; Dichmont *et al.,* 2012; Abdul-Muneer, 2014).

An important factor in MAS is the accuracy of estimating the genetic effects related to the trait of interest. In contrast to genetic engineering (GE), MAS does not alter the original DNA. Rather, it identifies whether the desired trait(s) are being expressed, so that individuals with the best potential can be selected (Andersson, 2001).

Molecular marker analysis allows the identification of genome segments, so called Quantitative Trait Loci (QTL), contributing to the genetic variance of a quantitative trait and thus to select superior genotypes as these loci (Cannai *et al.,* 2003). Allelic variation in genetic markers can be linked to the variation in traits of economic interest, and thus the marker provides DNA level information on the inheritance of the traits.

The practical use of markers in selection can be roughly divided into three classes:

1) Removing genetic disorders,

2) Marker breeding value-selection, and

3) Genomic selection.

2.4 MAS versus Phenotypic Selection

Marker-Assisted Selection (MAS) will probably never replace Phenotypic Selection (PS) entirely. There is no general pattern by which it can be predicted whether MAS or PS will be more useful. Empirical comparisons of MAS and PS for increasing gain from selection have been made in several studies. The outcomes of these studies are conflicting. In some studies MAS is reported to be more effective/efficient than PS (**e.g.** Yousef and Juvik 2001; Abalo *et al.,* 2009) while other studies considered the two methods equal (**e.g.** Van Berloo and Stam 1999; Willcox *et al.,* 2002; Hoeck *et al.,* 2003; Moreau *et al.,* 2004). In a

third group of studies PS proved to be more effective/efficient than MAS (**e.g.** Davies *et al.,* 2006; Wilde *et al.,* 2007) and in other comparisons the effectiveness/efficiency of MAS and PS varied within the same study, depending on the populations or on the trait selected for (**e.g.** FlintGarcia *et al.,* 2003b; Robbins and Staub 2009).

2.5 Limitations of MAS

• Cost

• Requirement of technical skill

• Automated techniques for maximum benefit

2.6 Advantages of MAS

In addition to the cost and time savings described above, for a number of breeding scenarios, MAS methods are likely to offer significant advantages compared with conventional selection methods. These scenarios assume the availability of markers for multiple traits and take into consideration the advantages of MAS under optimum situations (Dreher *et al.,* 2002; Dudley, 1993).

1. Gene stacking for a single trait: MAS offers potential savings compared with conventional selection when it allows breeders to identify the presence of multiple genes/alleles related to a single trait, and the alleles do not exert individually detectable effects on the expression of the trait.
2. Early detection*:* MAS offers potential savings compared with conventional selection when it allows alleles for desirable traits to be detected early, well before the trait is expressed and can be detected phenotypically. This benefit can be particularly important in species that grow slowly.
3. Heritability of traits*:* Up to a point, gains from MAS increase with decreasing heritability. However, due to the difficulties encountered in QTL detection, the gains are likely to decline beyond a certain threshold heritability estimate.

2.7 Disadvantages of MAS

Perhaps the greatest disadvantage of MAS is the time and financial investment required to develop markers that are widely applicable for traits of agronomic importance. Often a marker developed in one or a few related genotypes will not work for other genotypes in a breeding scheme due to allelic effects. Furthermore, development of markers, particularly for QTL, is complicated by epistatic interactions and the critical need for good quality phenotypic data.

2.8 Quantitative Trait Loci

In fish, several QTL studies have been published; in salmonids (Jackson *et al.,* 1998; Johansen 1999; Robinson *et al.,* 1999; Sakomoto *et al.,* 1999; Marfyniuk 2001, Ozaki *et al.,* 2001 Somorger 2001. Tao and Bailding 2003), in catfish (Liu *et al.,* 2003), in tilapia (Cnaani *et al.,* 2003) and in silver barb (Hussain *et al.,* 2002).

Marker Assisted Selection (MAS) is followed by two steps, detection of molecular markers associated with quantitative trait locus (QTL) and application of those markers.

The position of the chromosome that controls the economical important trait is termed as QTL.

The concepts for detecting QTL were developed more than 90 years ago (Sax, 1923). In aquaculture species, much effort has been applied for QTL mapping. QTLs are mapped by linkage disequilibrium with molecular markers exhibiting Mendelian segregation. Economically important traits are controlled by the single or group of gene.

The basic concept of QTL studies is to know the number and location of loci associated with phenotypic traits (Mackay, 2001; Mauric io, 2001; Burt and Hocking, 2002; Erickson *et al.,* 2004). Thus, candidate gene or molecular markers, resulted by QTL mapping, could be used in MAS (Groenen *et al.,* 2000). QTL detection is an ongoing effort in aquaculture species. More than 37 important traits have been located in about 20 aquaculture species.

QTL mapping is the practical application of marker-assisted selection in aquaculture (Al-Samarai, 2015). With rapid advancement of molecular technology, it is now possible to use molecular marker information to map major quantitative trait loci (QTLs) on chromosomes *(e . g .* ,Paterson *et al.,* 1988,1991;Hilbert *et al.,* 1991;Jacob *et al.,* 1991; Stuber *et al.,* 1992). Once QTL for a trait are identified, individuals can be selected for breeding on the basis of marker alleles that segregate with favourable phenotypes (Lande and Thompson, 1990). This strategy, known as marker-assisted selection (MAS), is particularly useful for traits that cannot be measured on selection candidates directly, notably disease resistance or meat quality traits (Sonesson, 2007a).

2.8.1 QTL Detection for in Fish

A number of genetic maps have been developed specifically to locate QTL in several fish species. The first of such map was produced in Zebrafish insert scientific name (Postleth wairt *et al.,* 1994; Shimoda *et al.,* 1999), which is a non-aquacultural species. Among cultivable fish groups low-density maps have been developed for salmonids (Sakamoto *et al.,*

2000; Ghabi 2001) for catfish (Liu *et al.,* 2003; Poompuang and Na-Nakorn 2004) for tilapia (Kocher *et al.,* 1998; Cnaani *et al.,* 2003), for Japanese flounder (Sanchez *et al.,* 2003), for red sea beam (Sakamoto *et al.,* 2003), for Oyster (Yu and Geso 2003), and for shrimp (Http://shrimpmap.tag.csiro.au).

2.8.3 QTL Mapping in Fish

Although in fish several studies have confirmed the existence of significant genetic variation for quantitative traits at commercial importance (Kause *et al.,* 2003) and have recognized the potential of MAS for their genetic improvement (Flint and Mott, 2001). Thus far, very few QTL for production traits have been identified in fish (Sonesson, 2003). Much effort is devoted to QTL mapping for growth, feed conversion efficiencies, disease resistance, fecundity, and spawning time (Dunham *et al.,* 2001).

Several QTL studies have been published in rainbow trait for temperature tolerance (Jackson *et al.,* 1998). Danzmann *et al.,* 199, perry 2001), spawning time (Sakamoto *et al.,* 1999; fish back et al 2000, O' Malley 2001); growth (Martynicik 2001), disease resistance (Ozaki *et al.,* 2001), azand fitness traits (Somorjai 2001). Other notable QTL studies published in aquacultural fish species include: in tilapia for temperature and salinity tolerance (Streadman and Kocher 2002; Cnaan *et al.,* 2003), in catfish for feed conversion efficiency and bacterial septicenmia resistance (Liu 2003), in guppy for growth (Nakajima and Taniguchi 2002), in at fautic salmon for infectious anemia resistance (Moen *et al.,* 2003 and in Arctia Charr for growth rates and fitness traits (Johansen 1999, Somorjai 2001).

In salmonids, QTL have been found related to body weight and size (Martyniuk *et al.,* 2003; O'Malley *et al.,* 2003; Reid *et al.,* 2005), for colouration pattern (Streelman, Albertson and Kocher, 2003) and for one form of albinism (Nakamura *et al.,* 2001). Zimmerman *et al.,* (2005) found QTL for pyloric caeca number, a trait related to feed conversion efficiency.

Table 1: QTL studies in selected aquaculture species

Species	Traits	Reference
Arctic charr	Body weight and sexual maturation; Salinity tolerance	Küttner *et al.,* 2011
Asian seabass	Resistance against viral nervous necrosis disease Growth-related trait Omega-3 fatty acids	Wang *et al.,* 2006 Xia *et al.,* 2014

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Atlantic salmo	Growth traits and flesh colour	Baranski *et al.,* 2010;
	Resistance against IPN	Tsai *et al.,* 2014;
	Late sexual maturation	Moen *et al.,* 2009 ;
		Houston *et al.,* 2008 ; 2010
		Gutierrez *et al.,* 2014
Catfish	Columnaris disease resistance	Geng *et al.,* 2015
	ESC disease resistance	Wang *et al.,* 2013; Zhou *et*
	Hypoxia tolerance	*al.,* 2017
	Heat stress	Wang *et al.,* 2016;
	Head size	Jin *et al.,* 2016
		Geng *et al.,* 2016
Common carp	Common carp	Zhang *et al.,* 2011
	Morphometric traits	Boulton *et al.,* 2011
	Swimming ability	Laghari *et al.,* 2014
Eastern oyster	Disease resistance	Yu and Guo, 2006
European seabass	Growth, body weight	Louro *et al.,* 2016
	Morphometric traits and stress	Massault *et al.,* 2010
	Response	
Pacific white shrimp	Growth parameters	Andriantahina *et al.,* 2013
Giant tiger prawn	Disease resistance and sex determination	Robinson *et al.,* 2014
Japanese flounder	Vibrio anguillarum resistance	Wang *et al.,* 2014
Pacific oyster	Growth	Guo *et al.,* 2012
	Resistance against summer mortality	Sauvage *et al.,* 2010
	Viability	Plough and Hedgecock, 2011
		Plough *et al.,* 2016
Gilthead seabream	Skeletal deformities	Negrín-Báez *et al.,* 2015
	Sex determination and body growth	Loukovitis *et al.,* 2011
		Massault *et al.,* 2011
Rainbow trout	Growth related traits	Kocmarek *et al.,* 2015;
		Wringe at al., 2010; Leder *et*
		al., 2006.

Tilapia	Growth traits Sex	Liu *et al.*, 2014; Wang *et al.*, 2015 Palaiokostas *et al.*, 2015

2.8.4 QTL analysis

1. QTL for growth traits

Growth is one of the most important economic traits of all aquaculture species. Up to 2012, QTL analyses have been conducted in more than 20 aquatic species (Yue *et al.*, 2014), and growth **is** was the most popular trait studied. Wang *et al.*, (**Wang *et al.*,** 2006) used 380 F1 Asian seabass to identify five major QTLs and 27 potential QTLs. Of them, three major QTLs for body weight, length, and body length were located at a similar linkage group 2 (LG2) position with the nearby Lca287 microsatellite and accounted for 28.8%, 58.9%, and 59.7% of the phenotypic variations. The other two major QTLs for body weight were located at another LG2 position. These five major QTLs have been confirmed in two other Asian seabass populations (Wang *et al.*, 2008). Further QTL fine mapping of the Asian seabass growth trait identified three candidate "growth genes" (CATHEPSIN D, KCTD15, and CSMD2) affecting body weight, body length, and total length (Wang *et al.*, 2011). The function of the *cathepsin* D gene in humans involves cell proliferation and cell growth; therefore, *cathepsin* D may also be a major "growth gene" in Asian seabass. O'Malley *et al.*, (O'Maller *et al.*, 2008) identified QTLs for body weight in rainbow trout on 10 different LGs.

Wringe *et al.*, (2010) used additional backcrossed families and SSR markers to confirm the O'Malley *et al.*,'s results and found several major candidate growth genes (e.g., *GH2* and *Pax7*). Reid *et al.*, 2004 identified a QTL for body weight in two LGs (AS8 and 11) of Atlantic salmon, and reported that it was homologous to the growth QTL in rainbow trout. Houston *et al.*, (2009) identified QTLs for body weight in LG1 and LG5 of Atlantic salmon. Gutierrez *et al.*, (2012) further used a 6.5 K SNP chip to identify QTLs in six LGs at the genomic level. Cnaani *et al.*, (2004) identified a QTL for tilapia growth on LG23, which is the linkage group with the genetic sex-determining region. Song *et al.*, (**Song *et al.*,** 2012) used 1487 SSRs to produce a high-density genetic linkage map and successfully identified a QTL affecting body weight in LG14 of Japanese flounder.

Some reports have used a candidate gene approach to identify growth-related genes and molecular markers in fish. Tao and Boulding (2003) found polymorphisms in the growth hormone gene (*GH)* that were significantly associated with growth rate of Arctic charr

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(*Salvelinus alpinus*). Li *et al.,* (2009) reported an SNP in the insulin-like growth factor-(IGF)1
gene 5′ untranslated region (UTR) of largemouth bass (*Micropterus salmoides*). Sun *et al.,* (2012) reported that two SNPs in exon 3 of the myostatin (*MSTN*) gene were significantly related to body weight and Fulton's factor in common carp. Liu *et al.,* (2012) also found that a SNP in the *MSTN* 3′ UTR was very significantly associated with total length, body length, and body weight of bighead carp.

2. QTL for feed conversion rate

FCR is one of the most important economic traits in fish, as fish with a better FCR increase profits.

Liu (2005) used AFLP markers to construct a catfish genetic map and found a QTL associated with FCR. Zimmerman *et al.,* (2005) revealed three QTLs for the number of pyloric caeca in three LGs of rainbow trout, and this is an important index associated with FCR.

FCR studies have also been reported in common carp from the Heilongjiang Fisheries Research Institute of the Chinese Academy of Fishery Sciences (Wang, 2012).

3. QTL for sex determination

Sex phenotype and sex determination in fish have specific evolutionary status and diversity. Males and females of some species have significant differences in growth rate or commercial value; therefore, monosex fish culture is a promising strategy. The sex-determining (SD) loci and QTLs have been studied in a limited number of fish, such as tilapia (Lee *et al.,* 2004) rainbow trout (Alfaqih *et al.,* 2009) and salmonids (Davidson *et al.,* 2009). Previous studies have demonstrated that sex QTLs are located on LG1, 2, 3, 6, and 23 of tilapia (Cnaani *et al.,* 2004; Lee *et al.,* 2004; Cnaani *et al.,* 2008) Eshel *et al.,* (2011) reported a major candidate sex QTL that is considered the sex determining region in tilapia. Fifty-one genes in this region have been annotated, and 10 have been confirmed.

The anti-Müllerian hormone gene is the most differentially expressed gene in male and female tilapia. Sun *et al.,* (2014) recently published several sex-specific markers, and one is tightly linked with the sex-determining region discovered by Eshel *et al.,* The sex-determining locus in rainbow trout is located on the LG of RT10, and this locus also significantly affects thermo-resistance and body length. The sex-determining regions in Artic charr (Moghadam *et al.,* 2007) brown trout (Gharbi *et al.,* 2006) and Atlantic salmon (Gilbey *et al.,* 2004) are located on the LGs of AC4, BT28, and AS1, respectively.

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Woram *et al.,* (2003) compared LGs of sex-determining loci in four salmonids and found that although the nucleotide sequences flanking the sex-determining loci were well-conserved, the SD LGs were diverse, suggesting that the regions underwent different recombination events.

Loukovitis *et al.,* (2011) located growth and sex-determining QTLs in gilthead sea bream and showed that these two traits have similar genetic control in LG21. Martínez *et al.,* (2009) located a sex QTL on LG5 of turbot and proposed a ZZ/ZW sex-determining mechanism. Viñas *et al.,* (2012) also found a major sex QTL on turbot LG5. These findings suggest that the sex-determining genes may occur on turbot LG5. Song *et al.,* (2012) used high-density genetic maps to locate seven sex QTLs on the half-smooth tongue sole LG1f, LG14f, and LG1m.

Additional study by Chen *et al.,* (2014) provided insight into ZW sex chromosome evolution and identified sex-determining genes, such as *dmrt1* and *neurl3*.

2.8.5 Factors affecting QTL analyses

The power of mapping QTL can be influenced by a number of factors, such as genetic properties of QTL, experimental design, environmental effects, marker density and informativeness, genotyping errors and precision of trait measurement. Details about how these factors influence the power of QTL mapping can be found in some very good reviews (**e.g.** Crosses 2001; Flint and Mott 2001; Doerge 2002).

2.8.6 Methods of Detecting QTL

Basically, three methods are frequently used for mapping QTL and estimating their effects, namely Single-Marker Association Analysis (SMAA), Simple Interval Mapping (SIM) and Composite Interval Mapping (CIM) (Crosses 2001; Flint and Mott 2001; Doerge 2002).

2.9 Current Status of Applications of MAS in Fish

Molecular marker maps have been constructed for a number of aquaculture species, e.g. tilapia, catfish, giant tiger prawn, kuruma prawn, Japanese flounder and Atlantic salmon, although their density is generally low. Density is high for the rainbow trout, where the map published in 2003 has over 1 300 markers spread throughout the genome – the vast majority are AFLPs but it also includes over 200 microsatellite markers (Nichols *et al.,* 2003). Some QTLs of interest have been detected (e.g. for cold and salinity tolerance in tilapia and for specific diseases in rainbow trout and salmon).

In a recent review of MAS in fish breeding schemes, Sonesson (2003) suggested that MAS would be especially valuable for traits that are impossible to record on the candidates for selection such as disease resistance, fillet quality, feed efficiency and sexual maturation, and concluded that MAS is not used in fish breeding schemes today and that the lack of dense molecular maps is the limiting factor. Marker Assisted Selection (MAS) has become a valuable tool in selecting organisms for desirable traits. MAS is expected to increase genetic gain compared to traditional breeding programs and reduce the cost of progeny testing by early selection. The application of MAS in breeding programmes depends on the knowledge of breeders about variable marker information.

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