Journal of Biopestcides and Environment
ISSN: 2449-1284 (Online); 2449-1292 (Print)
http://www.biopjournal.com

CURRENT CHALLENGES OF MOLECULAR MARKERS ADOPTION IN IMPROVEMENT OF AQUACULTURE SPECIES

Nya, E. J.
Biotechnology & Genetics Research Centre, Akwa Ibom State University, P.M.B.1167. Uyo, Nigeria.
E-mail address: elijahnya@aksu.edu.ng

ABSTRACT

Molecular marker is any assays that enabled the detection of specific sequence differences between two or more individuals among plants or animals. However, its includes such methods as markers assisted selection MAS, restriction fragment length polymorphisms (RFLPs), microsatellite or minisatellite, simple-sequence repeat (SSR) markers, RAPD (random- amplified polymorphic DNA) and AFLP markers (Amplified Fragment Length polymorphism), among others. Various difficulties encountered in the application of molecular markers have been highlighted. Marker's assisted selection and germplasm characterizations appeared most common and are seen as being cost effective, easy to handle and free of any radioisotope requirement, than the advances with AFLP (amplified fragment length polymorphism) and SSR (Simple-sequence repeat) markers. SSR marker is considered as the most suitable and reliable system for DNA characterization. SSR marker detects variation in the number of short repeat sequences; usually two or three base repeats and currently are used to detect multiple alleles. The prohibitive cost of molecular markers development, the inconsistency of results obtained with different marker systems and the inadequate institutional, governmental and public support constitute the challenges of molecular markers adoption in improvement of Aquaculture species It is anticipated that application of markers will remain challenging in aquaculture industry till the allele-specific markers are available and the cost of marker development and analysis is reduced significantly.

***Key words**:* Aquaculture species, restriction fragment length polymorphisms (RFLPs), DNA markers, Marker's assisted selection *MAS*, Simple-sequence repeat SSR, quantitative trait loci *QTLs*, random- amplified polymorphic DNA *RAPD*.

1. INTRODUCTION

Molecular markers refer to any assays that enable the detection of specific sequence differences between two or more individuals among plants or animals. However, its includes such methods as markers assisted selection MAS, isoenzyme and other protein-based markers, restriction fragment length polymorphisms (RFLPs), microsatellite or minisatellite, simple-sequence repeat (SSR) markers, RAPD (random- amplified polymorphic DNA) and AFLP markers (Amplified Fragment Length polymorphism)

One of the earliest types of DNA-based molecular markers, was restriction fragment length polymorphisms (RFLPs), used for the detection of variation in restriction fragment length as detected by Southern hybridization. The types of sequence variation detected by this procedure were known to be caused by single base changes. This led to the creation or removal of a restriction endonuclease recognition site showing a detectable shift in fragment size. But at present this technique has been replaced by microsatellite or simple-sequence repeat (SSR) markers and is hardly used today in screening materials for breeding programs. In spite of this, it remains an important research tool. SSR markers detect variation in the number of short repeat sequences, usually two or three base repeats. The number of such repeat units has been found to change at a high frequency and thus allows the detection of multiple alleles. (Kawabe *et al.* 1997).

While there are several applications of molecular markers in breeding, the most promising for species development is the marker assisted selection MAS. MAS refer to the use of DNA markers that are tightly-linked to target loci as a substitute for or to assist phenotypic screening (Collard & Mackill, 2006). By determining the allele of a DNA marker, an organism that possesses particular genes or quantitative trait

loci (QTLs) may be identified based on their genotype rather than their phenotype.

In each method used in molecular markers, DNA sequence variation is detected. However, each method analyses different aspects of DNA sequence variation and different regions of the genome. For example, RFLPs were detected using cDNA (complementary DNA) clones, mostly variation in the coding sequence, whereas SSR markers have been obtained from non-coding regions although, recently three-base repeats has been used as the source of SSR markers. Other markers such as RAPD and AFLP markers appear to frequently target repetitive regions of the genome. The stability of the sequence differences may also be an issue in some cases. SSRs are seen as being too unstable for some applications since the mutation rate may be high in some cases. The decision about the most appropriate marker system to use varies greatly depending on the plant or animal species, the objective of the marker and available resources.

Current challenges for the adoption of molecular markers in aquaculture are varied and include the following;

1. **The low reliability or the inconsistency of results obtained with different marker systems.**

Accurate information on genetic diversity of animal and indeed Aquaculture germplasm could conveniently be accessed through the use of molecular markers. This is particularly eminent in cases where germplasm to be utilized is closely related or its ancestry or pedigree is obscured or unknown. Interestingly, on the other hand, molecular marker technology has not yet been able to make a widespread acceptance on characterization, management and utilization of germplasm. One of the reasons is the inconsistency of results obtained with different marker systems. As mentioned earlier, RFLPs are the most reliable markers to be used for this purpose as exemplified in sugarcane (Oropeza and Eva, 1996), wheat (Sasanuma *et al.,* 1996), rice (Zheng *et al.,* 1994), alfalfa (Pupilli *et al.,* 2000) and many other crop species. However, they were not found suitable for interspecific comparison (Castagna *et al.,* 1997). RAPD markers are the most commonly used marker system due to its consistency, cost effectiveness and ease of handling. For example, when sorghum germplasm consisting of 42,000 accessions was analyzed with RAPD markers, the clusters developed were not even close to those obtained on the basis of morphological data (Dahlberg *et al.,* 2002). It was thus, concluded that if core collections are to be developed and their validity checked, a single technique would not be adequate. On the other hand, Beebea *et al.,* (2000). analysized 269 land races of common beans with RAPD markers, and found that the grouping corresponded in part to that formed earlier on the basis of morphological and agroecological criteria. Also, Zenglu *et al.,* (2001) found differences in soybean cultivars originated in USA and China and genetic diversity in maize population were accurately determined through RAPD markers. Contrary to this, when RAPD marker obtained in tomato was converted into SCAR (sequence characterized amplified region), it was difficult to differentiate two parental cultivars under a variety of polymerase chain reaction conditions (Yiping and Stommel, 2001). Farooq *et al.* (1998) successfully fingerprinted rice using RAPD markers.

2. **The challenge of cost reduction in molecular markers development**.

Although the application of molecular markers such as markers assisted selection MAS, isoenzyme and other protein-based markers, restriction fragment length polymorphisms (RFLPs), microsatellite or simple-sequence repeat (SSR) markers, RAPD (random-amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) markers in aquaculture improvement have been established as an innovative breakthrough in modern research in many breeding programmes, they are a lots of challenges inherent in their development and widespread use. A basic problem for the exploitation of these molecular markers in fish breeding is their prohibitive cost. It is known that to obtain one data from

molecular marker analysis costs about 0.5 USD, besides the costs associated with the marker development (Ellis *et. al,* 2005). Deploying markers with this cost in breeding programmes presupposes a high commercial value of the crop, or a great willingness for public sector investment. Reports from literatures analyzing the economics of using MAS versus conventional breeding leave much to be desire, even though the cost-effectiveness varies considerably between studies (Dreher *et. al,* 2003). Besides, development and maintenance of molecular marker laboratory requires a considerably large initial cost. A major challenge therefore is to reduce the cost of molecular markers in plant lines breeding, but a major means to reduce cost is to ensure that the molecular markers methods are appropriate to the context in which they are used with technical development.

3. Lack of Institutional, Governmental and Public Support

For the adoption of molecular markers in improvement programmes to succeed, there must be well-structured breeding programmes which is not the case in all third world countries. Often, poorly funded and supported breeding programmes are common place (Ajani *et al*, 2009). The lack of institutional and public support may limit the adoption of molecular breeding activities to mere lips service. In such circumstances, it is unrealistic to anticipate the adoption of innovative biotechnologies.

The challenge remains that institutions and government are not committed to more in food security and agricultural growth such as the scenario found in the Comprehensive Africa Agriculture Development Programme (CAADP). Besides governmental institutions, there is need for increasing participation by foundations, non-governmental organizations (NGO) and the private sector in the country's Food Security. This is critical for the adoption of molecular markers technologies in general (Moose and Mumm, 2008). Notable example is the one shown by international initiatives coordinated by CGIAR (Consultative Group on International Agriculture Research), where several successes have been recorded.

4. Hindrances to genetic progress in aquaculture species.

Despite all the advantages of aquaculture species availed to the geneticist over terrestrial animals, genetic progress has been hindered by:

i. The general lack of knowledge of the biology of many aquatic species. The different spawning behaviours and the miniature size of larvae (young aquatic organisms) make it very difficult for the adoption of molecular marker technology at an early stage of their development. In the past, most phenotypic traits improvement programmes in aquaculture has been through the use of mass selection approach such as shape, colour, scale pattern, growth rate, body conformation and size. Mass selection has been known to result in rapid inbreeding with attendant depression unless assortative mating step are taken to minimize this phenomenon (Tave, 1992).

ii. Tagging technology (TT), is a commonly known feature of salmon and trout improvement programmes worldwide. It has been improved upon, from the use of external tags or freeze branding to the widespread adoption of Passive Integrated Transponder (PIT) tags that are injected intramuscularly or intra-peritoneal into the fish. However, not all hatchery producers can afford a PIT tagging and not all aquaculture species can be stripped individually and their young reared separately for reasons of complex biology. Recent advances in DNA marker development makes it possible to individually identify offspring from known genotyped parents. This enabled the reconstruction of pedigrees retrospectively after a period of rearing, even in large communal populations. With this technology brood stock improvement, replacement and management becomes available. This approach could only open up the possibilities for traditional breeding programmes for greater numbers of fish in commercial environments. Here the adoption of PIT tags and molecular markers are difficult and cannot be practiced.

However, overcoming the main disadvantages of working with aquatic organisms using molecular markers and undertaking bloodstock genetic

improvement and management using a variety of molecular approaches that matches their biology is indeed taxing. The speed of technological development particularly in genomics has been rapid and its uptake in the aquatic sciences is yet to become popular. It is still unclear how much of this will eventually benefit the aquaculture industry. Aquaculture scientists are in a phase of developing these tools and effectively using them to inform stakeholders, farmers, breeders, feed companies and vaccine manufacturers on how they could integrates this knowledge into their existing procedural practice.

5. Inadequate record- keeping & methodology

Keeping records of desirable traits by breeders and methodologies for obtaining data is highly needed for key traits like feed conversion efficiency, weight gain, protein efficient ratio etc, which at present cannot be recorded on family lines or on individual head at an acceptable cost. Principally, genetic improvement of feed efficiency relies on correlated responses through selection for increased growth rate and by changing the composition of the end product e.g. lowering body fat content (Thodesen *et al.* 2001; Ogata *et.al*. 2002; Quillet *et al*. 2007). Presently, near-infrared spectroscopy (NIR) is now used to obtain records of fat content in the live breeding candidates of Atlantic salmon with acceptable precision and accuracy (Folkestad *et al.* (2006)).Methods for carcass quality traits are lacking and decisions are still based on sib information, for this reason, most actual traits are not directly selected due to lack of appropriate recording technologies. The need for new technologies to obtain records on new traits and preferably on live animals is a challenge to molecular breeding.

The availability of genomic information now offers new possibilities for selective breeding for disease resistance. Information on quantitative trait loci (QTL) may be implemented in breeding programs either through gene- assisted selection (GAS) or marker-assisted selection (MAS). By these methods candidate's trait may be selected based on individual markers (QTL), rather than on phenotypic status. The widely accepted assumption that disease resistance in aquaculture species is a complex one, has been challenged by results obtain from resistance to viral diseases in marine shrimp (Cock *et al*., 2009) as well as from infectious pancreas necrosis (IPN) in Atlantic salmon. This has also been supported by a major QTL that explains most of the genetic variance for resistance to IPN as identified in independent studies in Scotland (Houston *et al.,* 2008) and Norway (Moen *et al,*. 2009) among populations of Atlantic salmon.

6. Challenges of Chromosome set manipulation.

Many aquatic species are easily given to artificial phenotypic expression display. This together with their lack of genetic imprinting makes it possible for a range of chromosomal and environmental manipulations to be undertaken in aquatic species. However, these manipulations are not and cannot be seen as genetic modification (GM), as many of the outcomes have been observed naturally in wild fish populations. Another area is the phenomenon of external fertilisation by some aquatic species which presents a unique opportunity for geneticists to manipulate the gametes and generate novel genotypes. Similarly, the most powerful phenomenon is related to parthenogenesis. Parthenogenesis offers the possibility to induce spawning and obtain offspring from maternal or paternal origins. This is in contrast to other farm animals. (Komen and Thorgaard, 2007; Dunham, 2004).

The challenges with these Chromosome set technologies lies in the fact that they are achieved by destroying the nuclear DNA in either the egg or milt, using ionizing radiation (Gamma or UV); the treated gametes is then fused with an untreated milt or egg, to produce a haploid embryo. Eggs treated with UV or gamma radiation, before fused with normal milt produces a haploid embryo that can be made diploid by a disruption of the first mitotic division to produce an androgenetic double haploid (DH) individual that is 100% homozygous. They are on average homozygous

at 50% of their loci because they retain a pair of chromosomes, sister chromatids, which have just undergone recombination.

Moreover, use of electrical shocks that interfere with the second meiotic division can cause the retention of the second polar body especially if the shock is used or administered during the first mitotic division. Then two haploid copies of the maternal chromosomes can be retained to produce a mitotic gynogenetic offspring which is 100 % homozygous. This is because they arise from the duplication of a single chromosome set and are thus described as double haploid (DH) individuals.

Eggs fertilized with normal milt and subjected to shock treatments are used to generate an embryo that has 3 chromosome sets genetically referred to as triploid (3n) embryo. A late shock administered at the first mitotic division will retain two diploid sets and produce an embryo with 4 sets of chromosomes which is referred to as tetraploid (4n) embryo. These types of animals with extra sets of chromosomes are known as polyploids. Polyploidy has been certified to be lethal in higher vertebrates and birds but has been widely applied in plants and aquatic organisms (Chourrout et al 1986). The challenge with polyploidy lies in technical know-how and resource management.

The disruption of the meiotic and mitotic divisions have been achieved by using a variety of shocks, most commonly used shocks are the hot and cold temperature shocks and pressure shocks. In addition anaesthetics and chemicals such as colchicines treatments can also be used to achieve the same purpose. Optimization experiments are needed to determine the timing of the shock, (be it at the 2nd meiotic or 1st mitotic division) and at given ambient water temperature, the strength and duration of the shock to maximize it effect for a given species. The challenge here spelts out time consumption and resources management. Dunham (2004) has produced a list of over 30 different fish and shellfish species and published the optimized shock treatments and outcomes.

However, direct applications of gynogenetic or androgenetic techniques on aquacultured fish are rare and these techniques are usually undertaken in research laboratories. Nevertheless, these techniques can be used to generate unique genotypes for new species. The development of YY male tilapia used in producing all–male XY tilapia can be generated directly by androgenesis as any male offspring of Nile tilapia *Oreochromis niloticus* will have the YY genotype (Myers *et al* 1986). Alternatively, eggs produced by neo-female (males sex-reversed to female phenotype) and crossed to normal males (*XY x XY*) will also generate XX females and XY and YY male offspring (Mair *et al* 1997). However, all the male fish in such crosses have to be tested to identify their genotype. This approach has also been used in the development of all female fish (Pongthana *et al.* 1995). In silver carp (*Puntius goniontus*) females are the preferred sex as the ovaries are a delicacy.

2. CONCLUSION

The above review undoubtedly emphasizes that despite recent advances and successful utilization of molecular markers in aquaculture, one of the current challenges is funding. Funding agencies must absolutely expand their portfolio of projects support by requiring proposals to integrate basic research endeavours with improvement outcomes. Existing examples that deserve emulation include the Harvest- Plus program and U.S. Department of Agriculture - Coordinated Agricultural Projects. The private sector must also continue investments that stimulate integration and provide the appropriate training environment for future scientists entering the molecular breeding workforce. Another challenge is a strong background knowledge of the biology of aquaculture species and the skill in identifying those gene combinations that can lead to significant species improvement among others. This review also suggests the integration of different activities and research methods that form core components of molecular breeding as the most effective approach for the adoption and use of molecular markers.

REFERENCES

Ajani EN, Madukwe MC, Agwu AE, Onwubuya EA (2009). Assessment of technology generating institutions in biotechnology innovation system of South-Eastern Nigeria. *African Journal of Biotechnology*, 8, 2258-2264.

Beebea, S., P.W. Skroch, J. Tohme, M.C. Duque, F. Pedraza and J. Nienhuis, (2000). Structure of genetic diversity among common bean landraces of middle American origin based on correspondence analysis of RAPD. *Crop Science*, 40, 264-273.

Castagna, R., S. Gnocchi, M. Perenzin and M. Heun, (1997). Genetic variability of wild diploid wheat *Tritium errata* by RFLP and RAPD markers. Theor. Appl. Genetic., 94: 429-430.

Chourrout, D., Guyomard, R. and Houdebine, L. (1986). High efficiency gene transfer in rainbow trout (*Salmo gairdneri*. Rich.) by microinjection into egg cytoplasm. *Aquaculture* 51, 143-150.

Cock J., Gitterle T., Salazar M., Morten R. (2009). Breeding for disease resistance of Penaeid shrimps. *Aquaculture* 286 1–11 10.1016/j.aquaculture

Collard, B and Mackill, D (2006). Marker assisted breeding for rice improvement; a review. Philosophical Transactions of the Royal Society of London Series B (2006).

Dahlberg, J.A., X. Zhang, G.E. Hart and J.E. Mullet (2002). Comparative assessment of variation among sorghum germplasm accessions using seed morphology and RAPD marker measurement. *Crop Science*, 42, 291-296.

Dreher K, Khairallah M, Ribaut J, Morris M (2003) Money matters (I): costs of field and laboratory procedures associated with conventional and marker-assisted maize breeding at CIMMYT. *Molecular Breeding* 11, 221-234

Dunham, R.A. (2004), Aquaculture and Fisheries Biotechnology: genetic approaches. CABI . ISBN 0-85199-596-9.

Folkestad A, Wold J P, Rorvik K A, Techudi J, Haugholt K H, Kolstad K (2008).Rapid and non invasive measurements of fat and pigment concentration in live and slaughtered Atlantic Salmon (Salmo salar L) *Aquaculture* 280, 129-135

Farooq, S., N. Iqbal and M. Arif, (1998). Detection of genetic variability in Basmati and non- Basmati rice varieties and their radiation induced mutants through the use of Random Amplified Polymorphic DNAs (RAPDs). In: Application of DNA based Marker Mutations for Improvement of Cereals and other Sexually Reproduced Crop Plants. IAEA-TECDOC-1010. International Atomic Energy Agency, Vienna, Austria.

Ellis, T. H. N; M.R. Knox, M. R and Mackay, I (2005). Molecular Marker Techniques for Crop Improvement. Course Manual. Kirkhouse Trust - John Innes Centre - UAS Bangalore. pp. 7- 8

Houston R. D., Haley C. S., Hamilton A., Guy D. R., Tinch A. E., Taggart J. B (2008). Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). *Genetics* 178, 1109–1115

Kawabe T , Muslin AJ , Korsmeyer S. J (1997). HOX11 interacts with protein phosphatases PP2A and PP1 and disrupts a G2/M cell-cycle checkpoint. *Nature*. 30, 385 (6615):

Komen, H. and Thorgaard G.H. (2007)..Androgenesis, gynogenesis and production of clones in fish: *A review of Aquaculture* 269, 150-173.

Mair, G.C., Abucay, J.S., Skibinski, D.O.F., Abella, T.A. and Beardmore, J.B. (1997). Genetic manipulation of sex ratio for large scale production of all-male tilapia *Oreochromis niloticus*L. Canadian *Journal of Fisheries and Aquatic Sciences* 54, 396-404.

Mair, G.C., Nam, Y.K. and Solar, I.I. (2007). Risk management: Reducing risk through confinement of transgenic fish.pp209-238. In Environmental Risk Assessment of Genetically. Modified Organisms. Vol. 3. Methodologies for Transgenic Fish (eds. A.R Kapuscinski *et al.*). ISBN-13: 9781 84593 2961. CABI.

Meuwissen, T.H.E., Hayes, B. J. and Goddard, M. E. (2001). *Genetics* 157: Pp 1819-1829.

Moen T, Baranski M, Sonesson AK, Kjoglum S: (2009). Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic

salmon (*Salmo salar*): population-level associations between markers and trait. *BMC Genomics* 2009, 10, 368

Myers, J.M., Hershberger, W.K., and Iwamoto, R.N. (1986). The induction of tetraploidy insalmonids. *Journal of the World Aquaculture Society* 17, 1-7.

Moose S P and Mumm R. H (2008). Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiology* 147:969-977.

Ogata, H.Y., Oku, H. and Murai, T., (2002). Growth, Feed Efficiency and Feed intake of Offsring from Selected and Wild Japanese flounder (*Paralichthys olivaceus*).*Aquaculture*, 211, 183-193.

Oropeza, M. and G. Eva, (1996). Use of molecular markers for identifying sugarcane varieties. Phyton., 61: 81-85

Pongthana, N., Penman, D. J., Karnasuta, J. & McAndrew, B. J. (1995). Induced gynogenesis in the silver barb (*Puntius gonionotus* Bleeker) and evidence for femalehomogamety. *Aquaculture* 135, 267-276.

Pupilli, F., P. Labombarda, C. Scotti, S. Arcioni, (2000). RFLP analysis allows for identification of alfalfa ecotypes. *Plant Breeding*. 119, 271-276.

Quillet E, Le Guillou S, Aubin J, Labbé L, Fauconneau B (2007) Response of a lean muscle and a fat muscle rainbow trout (*Oncorhynchus mykiss*) line on growth, nutrient utilization, body composition and carcass traits when fed two different diets. *Aquaculture* 269, 220–231

Sasanuma, T., N.T. Miyashita and K. Tsunewaki, (1996). Wheat phylogeny determined by RFLP analysis of nuclear DNA.3. Intra and inter-specific variations of vie *Aegilops sitopsis* species. *Theoritical Applied Genetics*, 92, 928-934.

Septiningsih EM, Pamplona AM, Sanchez DL, Neeraja CN, Vergara GV, Heuer S, Ismail AM, Mackill D. J. (2009). Development of submergence-tolerant rice cultivars: the Sub1 locus and beyond. *Annual Botany*. 103: 151-160.

Tave, D. (1992). Genetics for fish hatchery managers. Kluwer Academic Publishers. ISBN 0-442-00417-6.

Thodesen J, Gjerde B, Grisdale-Helland B, Storebakken T (2001) Genetic variation in feed intake, growth and feed utilisation in Atlantic salmon (Salmo salar). *Aquaculture* 194, 273–281

Yiping, Z. and J.R. Stommel (2001). Development of SCAR and CAPS markers linked to the beta gene in tomato. *Crop Science*. 41, 1602-1608.

Zheng, K., H. Qian, B. Shen, J. Zhuang, H. Lin and J. Lu, (1994). RFLP based phylogenetic analysis of wide compatibility varieties in *Oryza sativa* L. *Theoretical Applied. Genetics*., 88, 65-69.

Zenglu, L., Q. Lijuan, A.T. Jeffery, M.M. Welsh and L.N. Randall, (2001). Molecular genetic analysis of U.S. and Chinese soybean ancestral lines. *Crop Science*., 41, 1330-1336.

****