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Application of marker assisted selection for livestock improvement in Bangladesh

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Abstract

Molecular markers usually do not have any biological effect. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted from one generation to the next. Marker assisted selection (MAS) is a novel technique that can complement traditional breeding methods for rapid genetic gains. Genetic gain through selective breeding is the objective of a breeder to achieve long term improvement in animal and plant genomes; however the pace of improvement is inversely proportional to the Generation Interval. Genetic improvement in livestock, particularly those with long generation intervals, requires decades for tangible results. Successful MAS breeding programmes require gene mapping, marker genotyping, quantitative trait loci (QTL) detection, genetic evaluation and finally MAS. Genomic selection is a form of marker-assisted selection. Using markers covering the whole genome could mean potentially that all the genetic variance is explained; and the markers are assumed to be in linkage disequilibrium with the QTL so that the number of effects per QTL to be estimated is small. MAS drastically reduces generation interval and increases selection accuracy. Therefore, a breeding strategy based upon markers making the best use of the two approaches can facilitate rapid genetic gain though selection of markers related to economic traits such as milk and meat production. This review is designed to elaborate the technique of MAS and its application in developing countries. (*Bangl. vet*. 2014. Vol. 31, No. 1, 1 – 11)

Introduction

Since the 1970s, the identification and genotyping of large numbers of genetic markers, the use of this technology to identify genomic regions that control variation in quantitative traits and to show how the resulting quantitative trait loci (QTL) could be used to enhance selection, have raised high expectations for the application of marker-assisted selection (MAS) in livestock. Most of the traits considered in animal and plant genetic improvement programmes are quantitative traits (influenced by many genes and environmental factors) e.g. milk yield and growth rate in animals. In classical and conventional genetics, selection is based on phenotypes, without knowing which genes are being selected. The development of molecular markers was, therefore, a major breakthrough.

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However, despite the considerable resources that have been invested, MAS has not yet delivered its expected benefits in commercial breeding programmes.

This review aims to provide information regarding the technical aspects of MAS, the current application in livestock and applications in developing countries.

Molecular markers

Only a small fraction of the DNA sequence typically makes up genes, while the major share of the DNA represents non-coding sequences, the role of which is not clearly understood. Molecular markers usually do not have any biological effect. Instead, they can be thought of as landmarks in the genome. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted from one generation to the next. Their identification relies on a DNA assay, in contrast to morphological markers that are based on visible traits, and biochemical markers based on proteins produced by genes. Different kinds of molecular markers exist. They may differ in a variety of ways – such as the amount of genetic variation at each marker. The information provided to the breeder by the markers varies depending on the type of marker system used.

Successful MAS breeding programmes require advances in five areas

- ***Gene mapping*:** Identification and mapping of genes and genetic polymorphisms.
- ***Marker genotyping*:** Genotyping of large numbers of individuals for large numbers of markers at a reasonable cost for QTL detection and routine application for MAS.
- ***QTL detection*:** Detection and estimation of associations of identified genes and genetic markers with economic traits.
- ***Genetic evaluation*:** Integration of phenotypic and genotypic data in statistical methods to estimate breeding values of individuals in a breeding population.
- ***MAS*:** Development of breeding strategies and programmes for the use of molecular genetic information in selection and mating programmes.

Steps involved in MAS

1. Validation of molecular markers: Extract the DNA from test individuals and find out whether there is one-to-one relationship with marker and the trait.
2. Extract the DNA of breeding population at the early stage and apply MAS. Select the individuals on the basis of presence of desired molecular markers for the concerned trait.

From markers to MAS

The molecular marker systems described above allow high-density DNA marker maps (*i.e.* with many markers of known location, interspersed at relatively short

intervals throughout the genome) to be constructed for a range of economically important farm animal species, thus providing the framework needed for eventual application of MAS. The next step is that putative genes affecting traits of interest can be detected by testing for associations between marker variants and any trait of interest. These traits might be genetically simple – for example, many disease resistance traits in plants are controlled by one or a few genes (Ruane and Colleau, 1996; Rao, Lakshminarasu and Jena, 2002). Alternatively, they could be genetically complex quantitative traits, involving many genes (*i.e*. so-called quantitative trait loci (QTLs)) and environmental effects. Most economically important agronomic traits tend to fall into the second category. Yue *et al*. (2005) using 280 molecular markers (comprising 134 RFLPs, 131 AFLPs and 15 microsatellites) detected a number of putative QTLs for drought resistance in rice. Having identified markers physically located beside (or within) genes of interest, it is now possible to select identifiable marker variants (alleles) in order to select for non-identifiable favourable variants of the genes of interest.

For example, consider a hypothetical situation where a molecular marker M (with two alleles M1 and M2), that we can identify using a DNA assay, is known to be located on a chromosome close to a gene of interest Q (with a variant Q1 that increases yield and a variant Q2 that decreases yield), that is, as yet, unknown. If an individual has the alleles M1 and Q1 on one chromosome and M2 and Q2 on the other any of its progeny receiving the M1 allele will have a high probability of also carrying the favorable Q1 allele, and would be preferred for selection. With conventional selection, relying on phenotypic values, it is not possible to use this kind of information. The success of MAS is influenced by the relationship between the markers and the genes of interest. Dekkers (2004) distinguished three kinds of relationship:

1) The molecular marker is located within the gene of interest (*i.e*. within the gene Q, using the example above). In this situation, we can refer to gene-assisted selection (GAS). This is the most favourable situation. On the other hand, it is most difficult to find these markers.

2) The marker is in linkage disequilibrium (LD) with Q throughout the whole population. LD is the tendency of certain combinations of alleles (*e.g*. M1 and Q1) to be inherited together. Population-wide LD can be found when markers and genes of interest are physically very close to each other and/or when lines or breeds have been crossed in recent generations. Selection using these markers can be called LD-MAS.

3) The marker is in linkage equilibrium (LE) with Q throughout the whole population. Selection using these markers can be called LE-MAS. This is the most difficult situation for applying MAS.

MAS can, in theory, be applied to any agriculturally important species, and active research programmes have been devoted to building molecular marker maps and to detecting QTLs for potential use in MAS programmes in a range of plant and animal

species. In addition, MAS can be applied to support existing conventional breeding programmes. These programmes use strategies such as: recurrent selection (*i.e.* within-breed or within-line selection, important in livestock); development of crossbreds or hybrids (by crossing several improved lines or breeds) and introgression (where a target gene is introduced from a low-productive line or breed (donor) into a productive line (recipient) that lacks the target gene (a strategy especially important in plants). See Dekkers and Hospital (2002) for more details. MAS can be incorporated into any of these strategies (*e.g.* for marker assisted introgression, by using markers to accelerate introduction of the target gene).

Limitations of MAS

- Cost
- Requirement of technical skill
- Automated techniques for maximum benefit
- DNA markers are not affected by environment but traits may be affected by the environment and show G x E interactions. Therefore, while developing markers, phenotyping should be carried out in multiple environments, and implications of G x E should be understood and markers should be used judiciously.
- DNA marker has to be validated for each the breeding population. Assumptions regarding the validity of markers may be disastrous.

Current application of MAS in livestock

The first reported map in livestock was for the chicken in 1992, which was quickly followed by publication of maps for cattle, pigs and sheep. Since then, the search for useful markers has continued and further species have been targeted, including the goat, horse, rabbit and turkey (see http://www.thearkdb.org). Microsatellite markers have been of major importance. Markers have been identified for almost all farm animal species, including against milk production in dairy cattle (Ansari-Mahyari *et al*., 2008; Lipkin *et al.,* 2008), buffalo (Sarika *et al.,* 2013), growth and carcass traits of beef cattle (Carr *et al*., 2006), chicken (Lipkin *et al*., 2002; Lahav *et al*., 2006), and goat (Shen *et al*., 2004).

There are reports of methodology for MAS (Hayes *et al*., 2007, Kwame AD and Lawrence BS, 2012), genomic selection strategies (Thomasen *et al.,* 2013; Buch LH *et at.,* 2012a), use of molecular technologies for the advancement of animal breeding (Spelman *et al.,* 2013*)*, the efficiency of MAS (Lande and Thompson, 1990) and genomic selection (Florian *et al.,* 2013; Roos *et al.,* 2011), QTLs and epistatic effects (Liu *et al*., 2003), types of selection model (Luo *et al.,* 1997), genome-wide screening for markers (Meuwissen *et al.,* 2007), MAS in dairy breeding (Meuwissen and Van Arendonk, 1992), relationship between MAS and linkage analysis (Ollivier, 1998), relationship between MAS and inbreeding (Pedersen *et al*., 2009) and selection for sex limited characters (Ruane and Colleau, 1996). ISAG–FAO recommended some microsatellite markers for cattle, buffalo, sheep, goat, horse, donkey, camelid, pig,

chicken (FAO, 2011). Holstein Association, USA developed genomic testing technologies and offer a wide array of tests (Holstein Association USA, 2011). Illumina designed cost-effective chips and provides the services to evaluate the genetic merits of cattle (Illumina 2011a; Illumina 2011b; Illumina 2011c). An Animal QTL database (Animal QTLdb) strives to collect all publicly available trait mapping data, *e.g.* QTL (phenotype/expression, eQTL), candidate gene and association data (GWAS). Copy number variations (CNV) mapped to livestock animal genomes was constructed recently, to facilitate locating and comparing discoveries within and between species (http://www.animalgenome.org/cgi-bin/QTLdb/index).

Applying MAS in developing countries

In the debate on the value of MAS as a potential tool for genetic improvement in developing countries, some of the factors that should be considered are described below:

Economy

According to Dekkers and Hospital (2002), "economics is the key determinant for the application of molecular genetics in genetic improvement programs. The use of markers in selection incurs the costs that are inherent to molecular techniques. Apart from the cost of QTL detection, which can be substantial, costs for MAS include the costs of DNA collection, genotyping and analysis." There is a difference between development costs (*e.g.* identifying molecular markers on the genome, detecting associations between markers and the traits of interest) and running costs (typing individuals for the appropriate markers in the selection programme) of MAS. Development costs can be high, so developing countries need to consider whether to develop their own technology or to import the technology. Another aspect to be considered is how to evaluate the economic benefits of MAS.

MAS versus conventional methods

Although conventional breeding programmes have limitations, they can be highly successful. The potential benefits of MAS need to be compared to those achieved or expected from conventional breeding programmes. There seems to be general consensus that the success of MAS compared to conventional breeding may depend on the kind of trait (or traits) to be improved. If the trait is difficult to record or is not routinely recorded in conventional programmes, MAS will offer advantages. Similarly, if the trait is sex-limited or can only be measured late in life then MAS is favoured, as marker information can be used in both sexes and at any age. Moreover, marker-assisted genomic selection reduces Generation Interval. Fig. 1 shows the timeline for a traditional progeny-testing scheme, which has a generation interval for the SM pathway of approximately 63 months.

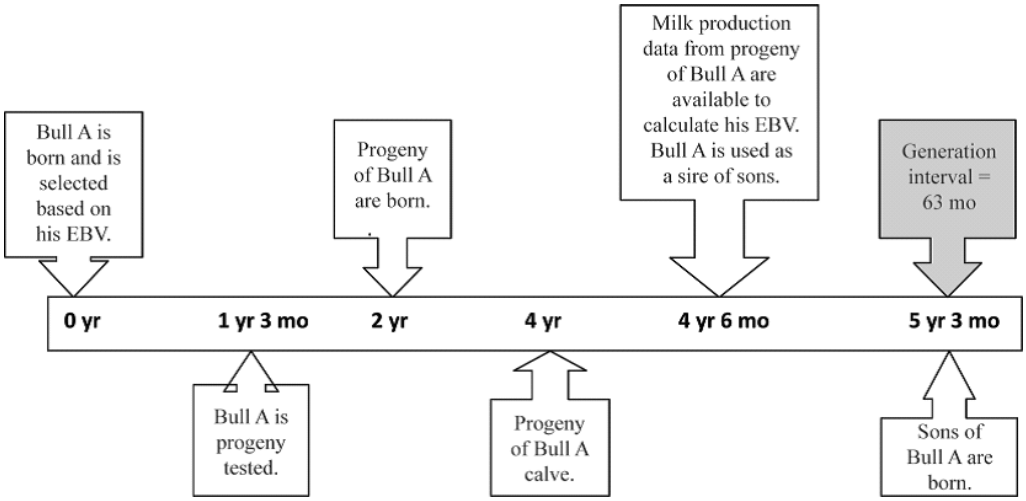

Fig. 1. Timeline of a traditional artificial insemination breeding programme based on progeny testing. EBV = estimated breeding value (Schefers and Weigel, 2012)

On the other hand, MAS allows AI companies to make decisions based on genomic estimated breeding value (GEBV), which are available at a very young age. Therefore, younger bulls can be used, limited only by their sexual maturity. Instead of waiting a minimum of 4.5 years to use progeny-tested bulls, AI companies can use bulls by roughly 1 year of age. This drastically reduces the generation interval and, as noted by Schaeffer (2006), it could lead to doubling of the rate of genetic gain. Fig. 2 illustrates the timeline for an aggressive AI breeding programme based on using genomic bulls as sires of sons. The generation interval for the SM pathway can be reduced to 21 months.

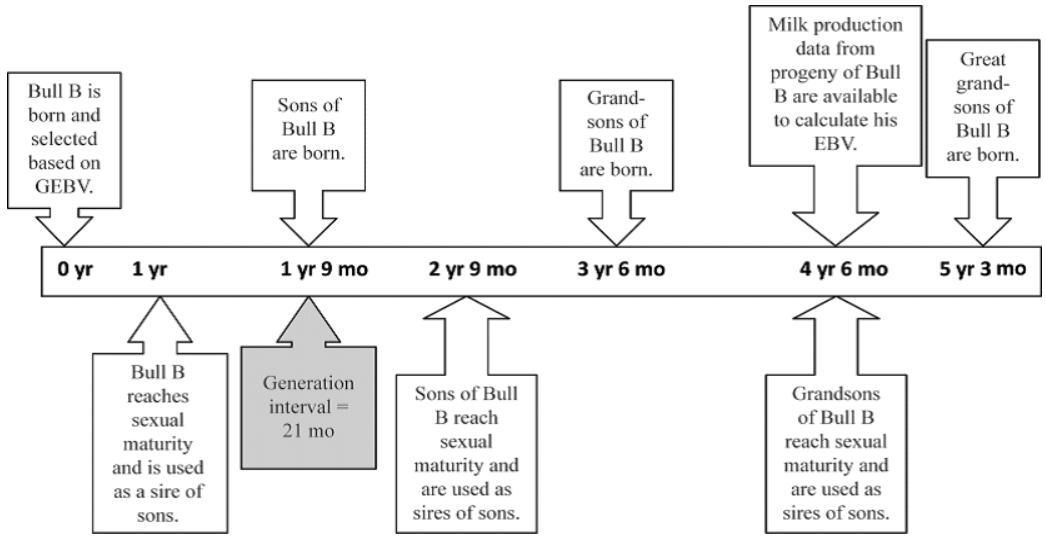

Fig. 2. Timeline of an aggressive artificial insemination breeding programme based on the use of genomic bulls as sires of sons. GEBV = genomic estimated breeding value; EBV = estimated breeding value (Schefers and Weigel, 2012)

In a progeny-testing programme, the accuracy of selection depends largely on the number of offspring per sire and, hence, on the number of cows in progeny test herds available for mating to young unproven bulls. With genomic selection, accuracy is primarily a function of the size of the reference population that is used to estimate single nucleotide polymorphism effects, which in turn are used to compute GEBV of selection candidates. This reference population may consist of genotyped females, genotyped males that have daughters, or a combination of the two. At present, the reliabilities of GEBV for production traits are often 70% or greater in North American Holsteins (Vanraden *et al*., 2009), which is twice the level of reliability associated with traditional parent averages computed from pedigrees.

MAS versus other biotechnologies for genetic improvement

The relative costs and benefits of applying MAS should be compared not only with conventional breeding but also with other new technologies. These include tissue culture in plants; reproductive technologies (e.g. embryo transfer or cloning) in livestock and triploidisation or sex-reversal in farmed fish. These also include genetic modification (GM), a technology that can be applied to all sectors but does not always command acceptance by the public.

Genomic Selection (GS) and MAS

Genomic selection is a form of marker-assisted selection. The markers used for MAS can be linked to the QTL but in linkage equilibrium with it; in linkage disequilibrium (LD), the QTL or the marker can actually be the QTL (Dekkers, 2004). If the marker is in linkage equilibrium with the QTL, all QTL alleles in founder animals are considered to be different and hence the number of QTL alleles whose effects must be estimated is further increased. Despite these difficulties, Boichard *et al*. (2006) show how gains can be made, although a very large amount of genotyping was necessary. To overcome these difficulties, Meuwissen *et al*. (2001) proposed a variant of MAS called genomic selection. The key features of this method are that markers covering the whole genome are used so that potentially all the genetic variance is explained by the markers; and the markers are assumed to be in LD with the QTL so that the number of effects per QTL to be estimated is small. Using simulation, they showed that the breeding value could be predicted with an accuracy of 0.85 from marker data alone.

Many countries have adopted the use of genomic information in their genetic evaluations. Canada collaborated with the United States in developing genomic evaluations based on BovineSNP50 genotypes and released official genomic evaluations in 2009. France first used microsatellite markers in a marker-assisted selection program in 2001 and then began using a relatively small number of SNP for unofficial evaluations in 2008. The Netherlands uses SNP from a customized Illumina chip as well as the BovineSNP50 BeadChip. New Zealand was an early adopter of the BovineSNP50 chip for its evaluation system and has encouraged widespread use of

young genomically evaluated bulls. Australia, Germany, Italy and Switzerland have implemented a genomic evaluation system by 2011 (Wiggans *et al.*, 2011).

In developed countries, phenotypes and pedigrees have been recorded for certain species, such as dairy cattle, for more than 100 years. Progeny testing has been implemented for nearly 50 years. Developing countries are often limited by the absence of programs that record phenotypes on pedigreed animals and the lack of evaluation or national testing programs to assess the genetic value of germplasms. Genomic approaches should help in identifying critical populations for preservation together with some local well-adapted breeds that could be further utilized to breed valuable animals through a combination of selection and cross-breeding. Of course, as with genomics, you can manage only what you can measure, and collecting a minimum number of phenotypes in the field will remain one of the critical and challenging steps to further deployment of genomic selection in developing countries.

Intellectual property rights

The issue of intellectual property rights (IPRs) is playing an ever greater role in food and agriculture in developing countries. It is reducing the quality of agricultural research and the nature of research collaborations between the public and private sector and between developing and developed countries. IPRs may also impact MAS in developing countries. The impact may be felt at a number of steps involving development and application of markers for genetic improvement. For example, Amplified Fragment Length Polymorphism molecular marker mapping technique is patented. Molecular markers can be patented, although this can often be overcome by using other markers near the gene of interest. Individual genes can also be patented. There is then public disclosure of the invention or information. Non-disclosure of information, where patents are not sought but the information on markers or detected QTLs is kept secret, can deny access to potentially useful information.

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