INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 8, No. 1, p. 71-80, 2016

RESEARCH PAPER OPEN ACCESS

An overview of marker assisted selection and QTL mapping in cotton

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Article published on January 25, 2016

Key words: Cotton, molecular markers, marker-assisted selection, QTL mapping.

Abstract

DNA markers are rapidly being developed for almost all the major crops. The most important markers are restriction fragment length polymorphisms (RFLPs), polymerase chain reaction (PCR) based markers such as random amplified polymorphic DNA (RAPD), and fingerprinting markers. DNA markers can supplement isozyme markers for monitoring trait improvement activities such as estimating genetic diversity in breeding populations, germplasm identification, verifying controlled crosses, and estimating seed efficiencies. As the number of DNA markers is potentially limitless, it should be possible to map individual quantitative trait loci (QTL) by linkage analysis with high-density maps. Twenty-first century agriculture will face frightening challenges to provide mankind with an appropriate level of food security while enhancing the sustainability of agricultural practices, lowering their environmental impact and preserving the remaining biodiversity. Marker assisted selection (MAS) have been widely adopted to improve resistance to biotic stresses, more modest results have been reported for the improvement of resistance to biotic constraints particularly drought and yield, mainly due to the elusive nature of the applicable quantitative trait loci (QTLs) and the unpredictability of their effects. In this article, what Marker assisted selection (MAS) is and why it is a good idea is described. MAS will probably exhaust genetic variation more hurriedly than phenotypic selection because many more cycles of selection are possible in a given time period using genomic compared to phenotypic selection.

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Introduction

Cotton (*Gossypium* spp.) is the most extensively used natural fiber in textile manufacturing and most abundantly grown oilseed crop (Campbell *et al.*, 2010). Globally, cotton production has been relatively stable for the last many years, and, although it is native to the tropics and subtropics, including America, Africa and Asia. China, USA, India and Pakistan are the top four cotton-growing nations.

Development in genetic has delivered new tools for exploring and labeling novel alleles and genes. These tools can increase the proficiency of breeding programmes by utilizing these in marker assisted selection. Molecular markers are the biochemical constituents that play a vital role in taxonomy, physiology, embryology, plant breeding etc. Molecular markers should not be considered as normal genes, as they usually do not have any biological effect. Due to presence of diverse molecular techniques and differences in their protocols require careful consideration in the selection of one or more of such marker types (Semagen *et al.*, 2006). In conventional breeding, a breeder generally aims to agronomically related and interested traits through hybridization in different parental lines and their wild relatives and also it depends on precise screening methods and availability of inbred lines. While, through molecular marker techniques it would be possible to accelerate the introgression of desirable genes among different varieties. It would also be feasible to create genetic relationship between sexually incompatible species. The advancement in molecular makers for plant breeding was fist popularized in early 1980s when isozymes were used to speed up introgression of monogenic traits (Tanksley and Rick, 1980). In cotton, about 145 morphological markers have been identified but their utilization in breeding programmes is limited because of their deleterious effects. Marker assisted selection mainly emphasize the traits which are difficult to manage through conventional phenotypic selection due to expensive and time (Young and Tanksley, 1989).

In genetics, relative distance of genes on the genome is mapped on the basis of the frequency of recombination between genes estimated from scoring genotypes of progeny of a cross. Mapping quantitative traits is difficult because the genotype is never unambiguously determined from the phenotype. Almost all QTL studies, regardless of the crop or trait to which they are applied, have come to support a main result of the first study that even for highly complex traits, a small number of QTLs explained a large part of the genetic variability. Two complementary uses of the QTL approach have emerged: the fundamental and the applied (Prioul *et al.*, 1997). The first one, which is of interest for physiologists, targets QTLs by determining their contribution to physiological components of macroscopic traits. The second use of the QTL studies is marker-assisted breeding (MAB) that is used for tagging QTLs of interest so as to pyramid favorable QTL alleles and break their linkage with detrimental alleles (Lee 1995; Ordon *et al.*, 1998; Ribaut and Hoisington 1998). A major challenge for MAS is to deal with parameters controlled by multiple interactive and environment-dependent QTLs, such as yield and yield-relating traits that often have low h^2 (Ribaut and Hoisington, 1998).

This review article deals on the basic principles, advantages, disadvantages of marker assisted selection programme, marker traits association studies and as well as QTL mapping in cotton. We will also discuss the technical issues that play important role for translation of promising markers into effective marker assisted selection programs.

Conventional Breeding and status of Molecular Marker technology in Cotton

Modern crop varieties have been developed from plant populations that have shown genetic variability. Selection, as a systematic process, consists of identification and use of superior individuals coexisting within the population. In other words, the selection changes the genetic structure of the populations as a result of preserving superior alleles and discarding the undesirable ones. Cotton was

among the first species to which the Mendelian principles were applied and has a long history of improvement through breeding with sustained long-term yield gains. A narrow genetic base especially among agriculturally elite types is restraining their utilization, and it is a bottleneck for cotton breeding and cultivar improvement. Further, cotton fiber productivity and quality aspects are genetically and physiologically complex. Breeding for such traits is time consuming and difficult because of the polyploid nature of cotton.

The first hindrance faced by researchers was to develop a standard extraction protocol to produce high quality DNA appropriate for molecular analysis. As cotton tissues include high level of polysaccharides and polyphenolic compounds which intermingle with proteins and nucleic acids, it poses problem in extraction of high quality DNA for molecular scrutiny. The second challenge is the low level of polymorphism found within best upland cultivars. Most genetic studies in cotton have found a narrow genetic base in upland cotton (Van Esbroeck *et al*., 1998; Saha *et al*., 2003). Third complicating aspect is the allotetraploid genome of the major cultivated cottons *G. hirsutum* and *G. barbadense* which means that most "single copy loci" will actually exist as two unlinked loci and the equivalent loci from each subgenome must be determined. Further genetic redundancy makes it difficult to develop polymorphism based DNA markers. The physical size of the cotton genome is relatively large in a range of 2702 to 2246 Mb (Michaelson *et al*., 1991). Moreover, several thousand microsatellite markers are now available to saturate genetic maps of cotton. The first public microsatellite markers were developed at Brookhaven National Laboratory. They identified more than 500 microsatellite clones containing (GA)n and (CA)n repeats, the primer pairs were designated BNL. Liu *et al*. (2000) assigned several BNL microsatellites to the chromosomes using cytogenetic aneuploid stocks. An additional 150 (GA) n repeat loci designated CM were isolated at Texas A and M University (Connell *et al*., 1998).

Reddy *et al*. (2001), with the help of SSR gemomics libraries identified 300 SSR markers (JESPR). Dow Agro Science Seed Company has reported more than 1,200 SSRs. In addition to generation of replicate enriched libraries for development of SSR markers, sequence databases represent a valuable resource for identification of microsatellites. The rapid amplification in cotton sequences (ESTs, BAC end sequences and STCs) deposited in public databases has also allowed the identification of microsatellite DNA containing regions from *G. hirsutum* and *G. arboreum* which would be used to develop markers. Saha *et al*. (2003) have shown that SSR loci can be identified from data mining of growing number of cotton EST sequences in databases with an efficiency of 1 to 4.8% of sequenced ESTs. They identified over 18,000 SSR-ESTs in different plant species including cotton. Han *et al*. (2004) developed 544 SSR markers (NAU) from fibre ESTs from *G. arboreum*. Qureshi *et al*. (2004) utilized 9,948 ESTs belonging to *G. hirsutum* and designed 84 primer pairs (MGHES). Park *et al*. (2005) developed Simple Sequence Repeats (MUSS) and Complex Sequence Repeat (MUCS) primers from a total of 1232 ESTs.

Different DNA marker techniques used in cotton

In cotton, diverse molecular techniques have been used and RFLP was the first DNA marker that was used in crop improvements. Meredith (1992), in a study of heterosis and varietal origins investigated the first RFLP evaluation in upland cotton. RFLP analysis, in its original form, consists of DNA digestion with a restriction enzyme, separation of the restriction fragments by agarose gel electrophoresis, transfer of separated restriction fragments to a filter by southern blotting and detection by autoradiography. The differences in sizes of DNA fragments may result from base substitutions, additions, deletions, or sequence rearrangements within restriction enzyme recognition sequences. The major disadvantage of this technique is that a large amount of DNA is required for each reaction and it is labour intensive to apply to numerous samples. While, RAPD, the oldest PCR-based depends on use of short, random PCR primers to intensify random

portions of the genome technique (Williams *et al.*, 1990). RAPD has many advantages over RFLP technique as no preceding sequence information for a genome is necessary. Several factors may influence reproducibility of RAPD profile within and between laboratories including DNA concentration, reproducibility of thermocycler profiles, primer quality and concentration, choice of DNA polymerase, and pipetting accuracy (Rafalski, 1997). RAPD technique has been employed for marker assisted selection in the interspecific population concerning *Gossypium sturtianum* and other species for getting glandless seed and glanded plant type (Mergeai *et al.*, 1998). AFLP technique has been employed for estimating genetic diversity in cotton by Zhang *et al.* (2005). Amplified fragment length polymorphism technique was developed by Vos *et al.* (1995). It involves digesting DNA with two different restriction endonucleases (usually a four bases and six bases recognition sequence) and ligating adapters to the sticky fragment ends created by enzymes. PCR amplification is then performed, which increases reproducibility of the technique. Polymorphism is detected where there is an addition or loss of restriction site.

SSRs characterize a new class of genetic markers which accelerated cotton genome mapping work. Liu *et al.* (2000) used 65 SSR primer pairs to amplify 70 marker loci restricted to a specific cotton genome. They are highly abundant in eukaryotic genome but also occur in prokaryotes at lower frequencies. The regions flanking the microsatellites are generally conserved and PCR primers relative to the flanking regions are used to amplify SSR- containing DNA fragments. The length of the amplified fragment will vary according to the number of repeated residues (Ellegien, 1993). SSRs are now considered as the marker of choice for self-pollinated crops with little intraspecific polymorphism (Roder *et al.,* 1998). Inter Simple Sequence Repeats (ISSR) technique uses primers that are complimentary to a single SSR and anchored at either the 5' or 3' end with a one- to three-base extension. As a result, the high complex banding pattern obtained will often differ greatly between genotypes of the same species. Li and Quiros (2001) introduced this new marker technique; Sequence related amplified polymorphism (SRAP) which involves preferential amplification of ORFs by PCR. This technique combines simplicity, reliability, moderate throughput ratio and facile sequencing of selected bands. Further, it targets coding sequences in the genome and results in a moderate number of codominant markers. However, these techniques use no prior sequence information, and the markers generated are randomly distributed across the genome. In cotton this new marker technique is being used along with other markers (Lin *et al.,* 2005; He *et al.*, 2007) for saturating the genome.

Introgression of Novel Alleles from Cotton Germplasm

Currently, new molecular tools as the molecular markers have started to express their usefulness into practical plant breeding by facilitating the identification, characterization, and manipulation of the genetic variation on important agronomic traits *via* quantitative trait loci (QTL) mapping. These molecular markers have been applied to unravel many biological questions in gene mapping, population genetics, phylogenetic reconstruction, paternity testing, forensic applications, comparative and evolutionary genomics, and map-based gene cloning. The main reasons supporting the utilization of molecular markers in cotton breeding programs are the 100% heritability of the markers and their lower cost. Hence, molecular markers are extensively being engaged in selection of traits with low heritability, identification, and introgression of complex fiber productivity and quality traits from native or exotic germplasm into elite cultivar *via* MAS. In plant breeding, MAS is a relatively new concept, nevertheless the original selection concept *per se* has not changed; that is, the purpose of the selection is to search and preserve the best genotypes, but using molecular markers. At the same time, it is necessary to consider effectiveness and cost of MAS (which is greatly influenced by the marker system used) besides polymorphism, technical feasibility,

and so forth. A highly saturated marker linkage map is necessary for effective MAS.

Applications of Molecular Marker Technology in Cotton

Plant breeders wish to have their new varieties distinct, uniform and stable. So, DNA markers have been employed as a promising method of finger printing. High resolution of molecular markers compared with other markers makes them a valuable tool for varietal and parental identification for protection of cotton breeder's rights. Similar to other crops, an understanding of the evolutionary and genomic relationships of cotton species and cultivars is critical for further utilization of extant genetic diversity in the development of superior cultivars (El-zik and Thaxton, 1989). Apart from this, molecular markers are employed for genetic diversity estimation in place of morphological markers as number of morphological descriptors in various crops. Initially isozymes were applied to *Gossypium* as a taxonomical tool to distinguish differences at species level. Cherry *et al.* (1972) observed minor differences in isozyme banding pattern of A and B genome species whereas greater band variation between the more distantly related species in the C, D and E genome. Brubaker and Wendel (1994) examined genetic diversity in upland cultivars using RFLP markers. Their study investigated that despite surveying 205 loci from 23 upland cultivars, only 6 had unique, multilocus genotypes again suggesting limited diversity. RAPD marker technique has been used to differentiate cultivars resistant to aphids, mites and jassids (Geng *et al*., 1995). Wajahatulla and Stewart (1997) studied the genomic similarity among some of the wild species by utilizing random primers. They reported that there are high intraspecific genetic similarities for *Gossypium nelsonii, Gossypium australe, Gossypium sturtianum* and *Gossypium bickii.* Genetic similarity of *Gossypium nandewarense* with two accessions of *Gossypium sturtianum* was high and median in placement, indicating that it should not be considered as a separate species. Pillay and Meyers (1999) utilized variation in ribosomal RNA genes (rDNA) to distinguish New and Old world cottons using AFLP markers to establish the extent of genetic diversity, they reported that AFLP technique differentiated two diploid species (*G. arboreum* and *Gossypium herbaceum*) from each other and as well as from tetraploid (*G. hirsutum* and *G. barbadense*). Genetic diversity of 31 available *Gossypium* species, three sub-species and one interspecific hybrid was studied by Khan *et al.* (2000). They screened these genotypes with 45 RAPD primers to distinguish the genotypes. The result indicated interspecific genetic relationship of several species as related to their centre of origin. Abdalla *et al.* (2001) determined intra and inter specific genetic relationships of *G. herbaceum, G. arboreum, G. raimondii, G. hirsutum* and *G. barbadense* using AFLP technique. They showed that AFLP technique is useful for estimating genetic relationships across a wide range of taxonomic levels, and for analyzing the evolutionary and historical development of cotton cultivars at the genomic level. Kumar *et al.* (2003) studied genetic diversity of 30 elite cotton germplasm lines including 20 *G. hirsutum*, seven *G. arboreum*, one each of *G. herbaceum, G. thurberi* and *Gossypium klotzschianum* using RAPD markers and morphological characters. They reported one 1100 bp *G. arboreum* specific band. *G. klotzchianum* was reported to exhibit the least similarity coefficient of 0.5 with all other species studied.

In plant genome analysis of cotton through MAS is adding new dimensions to evolutionary theories. Through molecular markers mapping and localization of genes providing breeders information for polygenic analysis of cotton. Assortment of exotic germ plasm has opened new doors to genetic variation. Because genetic variation in cotton is constantly restricted for some special traits like yield, thus translating them to more vulnerable to disease and insects spates and endangering the potential for constant genetic improvement over a large span.

Basic limitations that affects marker assisted selection

To evaluate the hurdles, substantial investments have been made by the private sector for the development

of genomics tools for crops of greatest commercial interest including maize (*Zea mays* L.), soybean (*Glycine max* L.) canola (*Brassica* spp), cotton (*Gossypium hirsutum* L.) and sunfl ower (*Helianthus annuus* L.). This has included simultaneous MAS for multiple traits such as yield, biotic and abiotic stress resistance, and quality attributes, most of these are polygenic in nature. Marker-assisted selection has also been used in public breeding programs for gene introgression and gene pyramiding, particularly for major gene-controlled disease resistance in primary crops but also in crops of less interest to the private sector. Using these approaches, commercial breeding programs have reported twice the rate of genetic gain over phenotypic selection with these and other MAS breeding programs in the public sector worldwide, it is surprising that there are still very few documented releases or registrations of new varieties resulting from MAS. The limited success in developing finished breeding products using MAS is further illustrated by the numbers of publications that have been generated on QTL mapping versus MAS since the discovery of the first generation of DNA markers (Miklas *et al.*, 2003). This is reflected in the annual number of articles with the term "marker-assisted selection," which has consistently lagged behind the number of articles with the term "quantitative trait locus" or "quantitative trait loci" by a factor of three for the past decade. Next, it is necessary to develop simple, quick, and cheap technical protocols for tissue sampling, DNA extraction, genotyping, and data collection that remain reliable and precise when routinely applied in large-scale systems. Molecular breeders must also develop tailored sample and data tracking and management systems to ensure effective integration of genotyping into breeding programs. Although MAS has been successfully applied in cultivar development in the private sector. Some limitations in assimilation of useful genomics create hindrance in Qtl mapping and marker assisted selection breeding programs. Therefore, further studies are necessary to understand the number of copies of genes, their regulation and their expression.

QTL mapping and its utilization in cotton (Gossypium hirsutum L)

Genetic maps are also essential to locate the genes that are involved in the expression of traits. This can easily be done for simple heritable traits based on one gene, but is also possible for complex traits that are based on more genes (QTL). In the latter case, large segregating populations (n >100) are required to unravel the number of loci involved in the trait (Jeuken *et al.*, 2001). In cotton, the contribution of new markers to generate a more saturated Upland cotton linkage map will enhance our understanding of its genetics and improve cotton breeding efficiency, especially when quantitative traits are implicated. Most agronomically important characteristics of crops are inherited quantitatively and are under the influence of both the environment and the genetic factors determined by QTL (Gelderman, 1975). Since it is not practical to infer an individual's genotype from its phenotype, it is difficult task to identify and characterize the QTL. Information on QTL analysis has accumulated quickly, and will eventually help the manipulation of the complex traits in cotton breeding (Tanksley, 1993). In cotton, 24 several QTL studies have been conducted using both intra- and inter-specific crosses mainly using RFLPs as markers to construct linkage maps. There are a large number of different methods for identifying the QTL segregating in a mapping population. These methods can be divided into methods that model a single QTL at a time (single QTL methods) and methods that model the effect of several QTL at once (multiple QTL methods). Single QTL methods include analysis of variance (t test or F test) (Soller *et al.*, 1976), interval mapping (maximum likelihood using flanking markers and regression mapping (an approximation to interval mapping).

Mapping QTLs of traits contributing to yield

The availability of molecular markers has provided different markers suitable for use in cotton research. For this reason, many intraspecific maps of *G. hirsutum* were also constructed (Zhang *et al.*, 2005). In recent years, more and more recombination inbred lines developed from previous temporary populations

have been used for QTL mapping. Although many QTL studies have been completed on interspecific and intraspecific populations in upland cotton, few have been performed in *G.* barbadense. In order to determine the genetic basis of economic traits of *G. barbadense*, a complete genetic map comprising SSRs, EST- SSRs , SRAPs, and SSCP-SNPs for yield and yield components – including lint index (LI), seed index (SI), lint yield (LY), seed cotton yield (SCY) and number of seeds per boll (NSPB). Coupled with polymerase chain reaction-based markers, which are efficient, economic, and easy to handle, QTL mapping of yield and yield components would benefit marker-assisted selection, map-based cloning, and yield manipulation. QTL mapping in cotton was first reported in 1996 .However, this QTL mapping was not reliable because of a computer coding error in the QTL data. After that, on the basis of the genetic map with 31 linkage group constructed by Shappley *et al.* (1996b, 1998b), a total of 100 QTLs were identified by means of mixed linear model running in QTLMaper software. Meredith (2000) localized 26 QTLs on a restriction fragment-length polymorphism (RFLP) linkage map with 81 marker loci, of which two QTLs conferred LY and two QTLs controlled seed weight. However, the genetic effects were not analyzed for these QTLs.

Other researchers, such as Saranga *et al.* (2001) have also mapped QTLs that influence yield and yield-related traits, but these data were not comparable because they were based on different linkage maps. The cotton yield breeding program paid attention to the improvement of LY, but the other yield components that affected LY were not ultimately goals. The previous results are available to cotton breeders and impel cotton genetic manipulation, but limitations on application are still existing because of the complexity of molecular markers (RFLP), which were not available for marker-assisted selection (MAS), low reliability because of the remote generation for traits from the generation for genetic mapping; low marker density and low marker coverage of the entire genome; no genetic effects characterized; and so on. This is why further studies on QTL mapping of yield traits are still required.

Conclusion

Marker-assisted selection becoming successful for incorporation and pyramiding major genes, however many objections remain to be resolved before MAS can routinely provide added value for breeding very complex traits. In the private sector, MAS has been much more dramatic, but it continues to be dominated by transgene introgression programs and to a lesser extent backcross conversion programs for simple traits. In other sense, it is expected that the greatest growth in public sector MAS will be for mono- and oligogenic traits that are difficult or expensive to screen using conventional phenotyping methods. Two fundamentals for implementing marker aided selection in breeding programs are: 1. A firmly associated marker to the gene concerned and 2. Population which is polymorphic for the marker and the gene which are in great linkage disequilibrium. A new field of genetics focusing on global gene expression has emerged based on extrapolating traditional techniques of linkage and association analysis to the thousands of transcripts measured by microarrays. The future involvement will depend on our capability to map QTLs and their effective incorporation in to marker assisted breeding programs. Transgenic approach will have its responsibility in future as through this we can get substantial improvement in traits concerned in shortest time. MAS greatly enhance the productivity and effectiveness in plant breeding programs as compared to conventional breeding methods. Through cotton genomics research, potentially new tools like microarrays, ESTs and proteomics are being introduced allowing the identification of multiple genes and QTLs in cotton. With the accessibility of such information and tools, the early stages of plant breeding programs will become much more efficient in a design-led way. However, there will continue to be no replacement for multi location replicated evaluation trials for screening elite breeding lines for the selection and confirmation of finished products

before distribution to local breeding companies and farmers' fields.

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