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DEVELOPMENT OF MARKERS AND CROP IMPROVEMENT PROGRAMMES

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Abstract

The development of markers for genetic analysis of different plant species, for monitoring the variations in and among species, to create new source of genetic variations by introducing new and favorable traits from landraces and related species, to develop the evolutionary tree among plant species and more importantly to increase the production of crops is painstaking efforts of plant breeders started from initial years of last century. Improvement in marker detecting systems and in the techniques used to identify markers linked to useful traits -Marker assisted selection (MAS) and Quantitative trait loci (QTL) - has enabled great advancements in recent years and can be utilized for more advanced research in future. Identification of markers linked to useful traits is based on complete linkage maps which helped to understand the basics of epistasis, pleiotropy and heterosis. While morphological markers have been the basics of most work in marker development system but presently the molecular markers like RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), and AFLP (amplified fragment length polymorphism) has generated the valuable marker systems. SNP (single nucleotide polymorphism) and SSR (simple sequence repeat) marker systems have been developed and is predicted to lead advance study by their implementation in breeding programmes. In this review emphasis has been laid on the current stage of marker development and to reveal the potential uses of supplementing the molecular and biochemical marker systems with morphological markers.

Key Words: Markers; QTL; MAS; Plant breeding; Marker development systems.

Introduction

The science of genetics has progressed at a rapid pace since Mendel's (1) re-discoveries of genetic laws and due this fact the significant progress in crop productivity has been made all over the world. The plant breeding paradigm has been enormously successful on global scale, with key examples, like development of hybrid maize, *Zea mays* L., introduction of wheat, *Triticum aestivum* L. and rice, *Oryza sativa* L. varieties, introduction of hybrid varieties of millets and cotton, noblisation of sugarcane, commercialization of transgenic crops (2, 3, 4) and many others lie in this category. The primary goal of plant breeding is typically aimed at improving yield, nutritional qualities and other traits of commercial value (5). These traits (especially quantitative) were analyzed by marker loci and were successfully done for many crops like in tomato, (6, 7) and in maize (8). For marker based procedures, to be effective in improving the quantitative traits, the manipulations are being done to explore the sequence of genomes and to understand the genetic basis underlying the quantitative trait variations. The mapping and sequencing information has led to the increase in evolutionary understanding of crop species (9, 10), genetic measurement of diversity (11), molecular identification of disease resistance genes (12, 13, 14), genetic control of flowering (15) and many other factors. In future more is expected to come by using marker techniques in order to reveal the mysteries and basic concepts of plant breeding programmes for increasing yield in order to fulfill the demands of increasing population.

Basic history and development of morphological markers

The basic concept of development of markers is to associate them with quantitative traits, first proposed by

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Sax (16). Since then a number of scientists have contributed to the general concept and theory by using mapped genetic markers for identifying, locating and manipulating QTLs (like 17, 18, 19, 20, 21). The history reveals that the curiosity arose among the plant breeders after Mendel's work and were keen to monitor, identify, catalogue, induce, map and link genes (Mendalian factors) in many crops for their improvement using different types of markers which are broadly classified as Morphological, Molecular and Biochemical markers (Table 1). The techniques of genetic mapping which is depicted from the linkage analysis were developed by Morgan and Sturtevent in 1923 while studying segregation data of *Drosophila* sp. An experiment by Hutchinson on chromosome 9 of maize by taking recombination frequencies of crossing over into consideration, it was conclusively revealed the genes are organized in a linear order on a cytologically defined structure called chromosomes with a unit, map unit or cM (cM= Centi Morgan, 1 cM= 1map Unit). In Hutchinson,s terminology 1% RF (Recombination frequency)=1 cM (22). Thus laid the foundation of markers as the first genetic map was phenotypic traits scored by visual observation of morphological characteristics. The numerous investigations been conducted on the inheritance of quantitative traits primarily using classical biometric procedures.

Table1. Broad classification of markers and their comparison

Features	1. Morphological	2. Molecular	3. Biochemical
Principle	Phenotypic	Gene segment	Gene product
Another name	Naked eye polymorphism	DNA marker	Protein marker
Biochemical meaning of marker	Consequence of gene action	Genes that are expressed	Arbitrary DNA sequence, may or may not represent genes
Plant material required for detection	Intact plant	Little amount of tissue	Little to medium amount of tissue
Number of different markers per cross	< 10	< 30	Unlimited
Ease of use	Easy	Moderately difficult	Moderately difficult to difficult

Principle of morphological markers

The basics of genetic mapping through morphological markers is the phenomenon of crossing over of chromosome during meiosis where homologous chromosome exchange section of their genes. This identifies marker locus as a point of reference for the chromosomal segments to be followed through appropriate genetic manipulations.

Utilities of morphological markers

In morphological marker systems several statical approaches have been developed for detecting and quantifying the strength of marker associations with traits (23, 24) for its great utility. Examples are: (i) a population that is amenable for mapping QTLs is the segregating generation (i.e., the F_2 population) produced from self fertilizing and intermating plants from the cross (F_1) of two homozygous line (25, 26). The backcross (F_1 x parent) also has been used as the segregating mapping population; (ii) the reports of linkage between quantitative traits and major genes (27,28); and (iii) a converted effort to locate quantitative factors in wheat (29) used on chromosome 7B influencing grain weight, grain number, height and tiller number were identified and mapped with respect to the marker loci.

Concerns and perspectives of morphological markers

Although numerous investigations by morphological markers had been done (using classical biometric procedure), the plant breeders typically got little information on: (i) the number of genetic factors (loci) involved in the expression of the traits; (ii) the chromosomal location of these loci; and (iii) the relative size of the contribution of individual loci to trait expression when morphological markers were being used alone. Until last 30 years, most of the single markers used in higher plant genetics were those affecting morphological characters (30). Common examples are genes causing dwarfism, chlorophyll deficiencies or altered leaf morphology. These markers undoubtedly have served well in various types of basic and applied research but their use in many areas of plant breeding has been limited because recessive alleles of genes for morphological characters may be deleterious due to unfavorable genotypic and phenotypic expression as being environmental dependant (31). Nevertheless, loci such as those affecting easily scored plant characteristics (e.g., glume colour) have been used effectively as markers in barley, *Hordeum vulgare* L. and maize (32, 33).

Advancements in markers: Development of molecular and biochemical markers

The development of molecular and biochemical markers is due to the fact they have relative advantage over morphological markers for most genetic breeding applications (34) and this lead to their wide acceptance. Both types of markers have advanced over large extent in its application and molecular markers has facilitated investigations of QTL over a wide range, for example, the number of genes , genomic distribution and types of gene action in maize. The summarization of both types of markers-Molecular and Biochemical- are given as:-

Molecular markers and its principle

Molecular markers are based on naturally occurring polymorphism in the DNA sequence i.e., base pair deletion, substitution, addition or patterns (35). There are various methods to detect and amplify these polymorphisms and can be used for breeding analysis. To be very much effective molecular markers should fulfill five inherent points (36) like: (i) must be polymorphic in nature; (ii) should be co-dominat; (iii) should be evenly and frequently distributed throughout genome; (iv) should be fast and cheap to detect and highly reproducible; and (v) must show high exchange of data between laboratories.

Utilities of molecular markers

Molecular markers are mainly used for: (i) the characterization of available germplasm through DNA fingerprinting and estimation of genetic diversity;(ii) evaluation of genetic fidelity during long term conservation; (iii) tagging of genes/QTL for qualitative and quantitative trait association for MAS; (iv) preparation of molecular maps; and (v) the dissection of genetic basis of many quantitative traits of economic importance in many crops through linkage (37, 38, 39, 40, 41).Genetic distance between parents, estimated by molecular markers, infact has been proposed as a useful tool for hybrid vigor prediction (42). The molecular markers when closely linked to numerous traits of economic importance (43) have allowed indirect selection for desirable traits in early segregation generation at the seedling stage. Polygenic characters, which previously were very difficult to analyze using traditional plant breeding methods, are easily tagged using molecular markers and help to establish genetic relationships between sexually incompatible crop plants. Various types of molecular markers techniques have been developed which have been broadly classified into three types (Table 2). For the last six to seven years molecular markers have also been used for testing the genetic fidelity during micropropagation/ex-situ conservation on the one hand and for characterization of plant genetic resources on the other. It is due to application of molecular markers the bacterial blight resistant genes in rice have been pyramided (44) by MAS using RFLP and PCR and the genome map of *Sorghum* L. sp. being developed by using sequence tagged technologies (45).

Table 2. Types of molecular markers based on principle methodology applied and their comparison

Features	1. Hybridization based	2. PCR based		3. Sequence based
		Using single primers	Using pair of primers	
Representative marker	RFLP	RAPD	AFLP	SNP
Principle	Restriction digestion	DNA amplification	DNA amplification	Sequence analysis
Types of Polymorphism	Base change (in, del, sub)	Base change (in, del, sub)	Base change (in, del, sub)	Single base change
DNA required (µg)	10	0.02	0.5-1.0	0.05
Number of polymorphs analyzed	1.0-3.0	1.5-50	20-100	1.0
Developmental cost	Low	Low	Moderate	High
Inheritance	Co-dominant	Dominant	Dominant	Dominant
Reproducibility	High	Unreliable	High	High
Cost / analysis	High	Low	Moderate	Low
MAS	+	-/+	+	+

Key to table: in, insertion; del, deletion and sub, substitution

In recent years variety of molecular markers becomes available (46) but they were very expensive and need sophisticated instruments and the efforts are being made to identify the most efficient and cost effective markers that can be used by practicing plant breeders and developing fine genome mapping. The major challenge of using molecular markers research for crop improvement in the developing countries include;(i) mostly molecular markers research are done by those which are not practicing plant breeders;(ii) there is a limitation of physical facilities, infrastructure and skill to plant breeders; and (iii) the plant breeders need to justify the trait for which the cost of MAS can be justified. In order to cope up problems the validation studies for the molecular markers already developed need to be undertaken using near-isogenic lines or other breeding material with known genetic contributions and recently developed high-throughput genome sequencing efforts should be applied with more work on SNPs. The high-throughput genome sequencing molecular marker technique has dramatically increased knowledge of and stability to characterize genetic diversity in the germplasm pool for essentially any crop species. Using maize as one example, surveys of molecular markers alleles and nucleotide sequencing variation have provided basic information about genetic diversity before and after domestication from its wild ancestors teosinte, among geographically distributed landraces and within historically elite germplasm (47, 48, 49).

Biochemical markers and its principle

Isozymes (proteins) are used as biochemical markers in plant breeding programmes. Isozymes refers to a multiple molecular forms of an enzyme sharing a catalytic activity derived from a tissue of single organisms (50) which are extracted and run on denaturing electrophoresis gels. The technique is based on the principle that allelic variation exists from many different proteins (51). For example, alleles of malic dehydrogenase would both perform the correct enzymatic functions, but the electrophoretic mobility of the two may differ, therefore, two alleles would not migrate to the same location in a starch gel. The difference in size, configuration and ionic charges among the isozymes allow them to be detected and resolved by various separation procedures including electrophoresis, which is combined with appropriate histochemical processes and ion exchange chromatography. Others include PAGE (polyacrylamide gel electrophoresis), SDS-PAGE (sodium dodecyl sulfate-PAGE) and isoelectric focusing. Different genes code for isozymes while each gene may have different alleles at the same locus, coding for slightly modified proteins as a sub-class of the

isozyme called allozymes. Polymorphic differences occur on the amino-acid level allowing singular peptide polymorphism to be detected and utilized as a polymorphic biochemical marker (52).

Utilities of biochemical markers

The studies on the detection of isozyme variation using electrophoresis have been done extensively. For example, an isozyme *sdh-l* was used to identify interspecific hybrids between two-bulu rice and their six wild relatives at seedling stage (53) and two other isozymes were also used to identify a differentiate progenies from the two different accessions. One of the great advantages of biochemical marker utilization is that isozymes could be produced from the crude extract of plant tissues such as coleoptiles and young leaves, hence the isozymes technique is simpler than that of molecular markers. A significant difference between the plant groups may be considered as given isozymic locus show linkage with at least one gene locus concerned in the studied trait called QTL. The electrophoretic analysis of protein variation has also created a population genetic data (54) which justifies the contention that alleles which have highly localized distribution, yet in high frequency in some neighborhoods, represent a substantial fraction of the variation. Moreover isozyme variation is revealed in characteristic banding pattern called zymogarm which is detected by proper staining and interpreted genetically.

Concerns and perspectives of biochemical markers

Biochemical markers although superior to morphological marker, in being environmental independent, the problem with isozyme in MAS are that most cultivars are genetically very similar. An isozyme do not produce a great amount of polymorphism and polymorphism in the protein primary structure may still cause an alteration in protein function or expression. Since the analysis of protein structure (isozyme) using electrophoresis is a first approximation analysis of a gene (55) as being a direct product of gene, isozymes would be better markers than of molecular markers that are not the genes of interest.

Future Prospects: Supplementing molecular and biochemical markers with morphological ones

Up to date many morphological, biochemical and molecular markers have been developed and are powerful tools for successful breeding programmes. The utilization of triple test-cross (a morphological marker methodology) design together with QTL detection procedures, allowed us not only to identify QTL contributing to heterosis but also to estimate the principal mode of action of such QTL (56). Likewise, the extensive transcription profiling comparing the inbred lines and their hybrid means of DNA micro-array technology in maize and mouse (57, 58, 59) indicate that transcriptional regulation and transcriptional over-dominance play an important role as molecular mechanisms establishing hybrid vigor. A finer characterization of QTL with high degree of dominance might provide crucial information for solving this long standing controversy regarding over-dominance.

The promise of marker assisted selection in crop breeding still remains but achieving practical benefits is lacking larger than expected. The main reason for this delay is the insufficient quality of markers (regarding their prediction and/or diagnostic value), inadequate experimental design, high costs and complexity of quantitative traits and furthermore the best markers like isozymes are limited. Molecular markers have been used for estimation of genetic diversity for many crops but none of these studies could be utilized for selecting parents for the hybridization programmes for crop improvement. The use of molecular markers for testing genetic purity of germplasm after long-term storage for conservation has its own limitation. Other drawbacks is when the linked marker used for selection is at a distance away from the gene of interest, leading to cross-over between the markers and gene, produces high percentage false positive/negative in the screening processes. Similarly, use of molecular marker despite has been developed for important traits in several crops but there use for MAS in actual plant breeding is not as visible as earlier anticipated. Globally, upto 2000 (A.D.), the only example where MAS has been successfully used in practical plant breeding include breeding for resistance against soybean cyst nematode (SCN) in USA, pyramiding of genes for resistance against bacterial blight in rice at IRRI (International Rice Research Institute) in collaboration with Punjab Agricultural University, Ludhiana, India (60).

Keeping in view the above points of limitation and concerns of each marker early discussed molecular and biochemical marker technology is now integrated into existing plant breeding programmes all over the world. Plant breeding has a long history of integrating the latest innovations in biology and genetics to enhance crop improvement. The work for analyzing the linkage between biochemical and morphological markers of Rye chromosome 1R, 2R and 5R were of great usefulness (61) which helps marker association with quantitative traits widely acceptable in variable populations. A summary of results from number of long term studies of changes in adoptedness in several experimental

population of annual plants (primarily barley sp.) was reported (62) only when different integrations of markers were done into each other. For several types of markers loci, Allard found consistent association between superior reproductive capacity (greater number of kernels per plant) and marker locus alleles. Association of herbicide response with several isozymes and morphological markers were evaluated (63) in 10 populations of Slender wild Oat (*Avena barbata* Pott. ex. Link) and six populations of wild Oat (*Avena fatua* L.). Using a novel backcrossing scheme marker facilitated back-cross of single isozyme-marked segments from wild barley into an elite barley cultivar was a useful approach for identifying QTLs for improving yield (64, 65). Results from such studies established the positive association of biochemical and molecular marker genotypes with performance of complexity inherited traits such as grain yield (a morphological marker).

Modification of the methods employed like combining morphological, molecular and biochemical markers into each other allow examination of the stability of individual gene effects in varying genetic background and environment. Johnson (66) has effectively summarized combining phenotypic data and molecular marker scores to increase selection grains for maize grain yield and resistance to European corn borer (66). Tanksley and his students and colleagues pioneered the area of MAS and proposed methods to maximize the utility in breeding programmes (67). Plants with most useful trait can be advanced for commercial use via conventional breeding programmes.

Molecular marker genotypes that are either within genes or tightly linked to QTL influencing traits under selection can be employed as a supplement to phenotypic observation in selection index (68) and same would be applied in case of protein markers. It is due to the fact that during past 26 years, the continued development and application of plant biotechnology, molecular markers and genomics has established new tools for the creation, analysis and manipulation of genetic variation and the development of improved cultivars (69, 70, 71). Today over 250 million acres are planted to these “biotech varieties” and many additional biotech varieties are in pipeline.

Hindrances in supplementing molecular and biochemical markers to morphological markers

In spite of molecular breeding where the molecular markers and biochemical markers is supplemented into morphological markers considering an essential component of crop improvement efforts of major crops by large companies certain hindrances come in way. The broad applicability of modern molecular approaches to conventional breeding remains the source of debate among some practicing plant breeders in the public sector, particularly for minor crops (72, 73). It has received relatively little attention from majority of plant biologists engaged in basic scientific research. Moose and Mumm (2008) has given at least three difficulties arising in this field: (i) molecular plant breeding lack training and expertise to implant into the traditional one and the educational efforts remains limited to relatively small group of academic institutions with historic strength in plant breeding (74, 75, 76); (ii)the reduced enthusiasm to embrace biotechnological approaches among plant breeders; and (iii) excitement about the potential of molecular plant breeding also stimulated shifts in funding at public institutions.

Discussion

The success of any breeding programmes in the present scenario depends upon the availability of genetic variability of traits of interest and availability of efficient markers for selection of traits. The entire genome cannot be studied on the basis of only one type of markers as it detect only a fraction of genome not whole genome so a supplementation of one marker with other is need of an hour. Within the last 20 years, molecular biology has revolutionized conventional breeding techniques in all areas. Biochemical and molecular techniques have shortened the duration of breeding programmes from years to months/weeks or eliminated the need for them all together. The use of molecular markers in conventional breeding techniques has also improved the accuracy of crosses and allowed breeders to produce strains with combined traits that were impossible earlier (77). Many issues in quantitative genetics and evolution are those which are difficult to address without additional continuous variations. The identification and examination of individual quantitative genes should provide information about the organization of genomes and insight into the relative contribution of “major” and “minor” genes to continuous variation. The ability to identify specific quantitative gene would also lead to more powerful means and more depth in investigating epistasis, pleiotropy and genetic basis of heterosis and other related characteristics. As these aspects of quantitative genetics are increasingly understood, new methods might be developed. Marker facilitated investigations appear to provide a powerful means of examining aspects of genetic control of quantitative traits. Mapping and sequencing of plant genomes would help to elucidate

gene regulation, gene function and their relative expression. Several cytological, isozyme and molecular markers have used to detect variation and/or confirm the genetic fidelity in micropropagated plants (78). Effective collaboration of plant breeders and molecular breeders by laws should be enforced and the research priority should be maintained by providing facility to breeders. Agricultural advancements will depend even more on genetics in the future as we try to produce more food, whole being in harmony with the environment. In this regard the molecular markers should be visualized with the new approaches of genomics and biotechnology which serve the iterative network like of supplementing markers to each other to exploit genetic diversity for crop improvement. The extensive body of literature (79, 80, 81, 82) have been considering the utility of molecular marker assisted selection to be practically explored so to fit with different breeding methods for crop improvement. The genetic tools available today and those to be developed will increase the precision of plant breeding and at least in many instances reduce the time required to respond to an ever-changing environment both natural and social.

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