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REVIEW ARTICLE

Molecular markers Implementation in plant breeding for identification and introgression of gene and gene pyramiding-a review

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Abstract

The application of DNA markers has been completed in the analysis of plant genetic diversity, gene mapping, gene inheritance, and in using molecular marker technology in the development of molecular breeding. Today, molecular markers are used for molecular characterization, gene transfer and pyramid formation especially for those which are agronomically essential genes. However, this new generation has little significance for polymorphically inherited traits. Despite the fast developments in plant molecular genetics, bioinformatics, genomics and the growth of interest to use new technology, there are many factors that hinder the application of latest technology in breeding practice. This evaluation article is especially focused on the use of marker-assisted technology instead of the conventional plant breeding which has many strengths and weaknesses.

Keywords: Molecular markers, plant breeding, marker assisted selection, gene pyramiding

1. Introduction

Plant breeding is a combination of principles and methods of changing the genetic constitutes of a plant to make it more suitable for human use. It is a combination of science and art (Poehlman, 1994). It depends on the ability of a breeder to identify differences in the traits of economic importance plants. Besides to this, it tries to improve these traits with current scientific knowledge. Although plant breeding has been existed for the last 10,000 years, modern plant breeding methods which are based on scientific principles of cytogenetics and genetics were began only with the rediscovery of Mendel's paper that was originally published in 1866 by Mendel (Foolad, 2007). Consequently, genetic markers can increase the efficiency and precision of plant breeding programs through marker-assisted selection (MAS). They can also enhance the speed of plant improvement in the unintentional association with a desirable trait to marker assay (Sivolap,

2013; Roychowdhury *et al.*, 2014). MAS uses as a tool with a varying degrees allowing breeding approaches to be based on the genotype of plants rather than assessing only the phenotype. This method is cheaper and more reliable. MAS has many befits in comparison to others such as the possibilities to "achieve the same breeding progress of a very shorter time", to "pyramid combinations of genes that could not be readily combined with other means" and to "assemble target traits more precisely with less unintentional losses"(Collard and Mackill, 2008; Brumlop, 2010). Up dated journals on this type of issue are not found today. Therefore, this review is important to overcome the gaps.

Marker Types

Marker areas included DNA sequences and/or segments that are closely linked to a gene, locus and/or morphological or other characters of a plant (Schlegel, 2009). Markers are classified into two categories: classical markers and DNA markers (Xu and Crouch, 2008).

Classical Markers

Classic markers consist of morphological or classical/visible markers, cytological markers, and biochemical markers (White *et al.*, 2007).

Morphological/ phenotypic markers are uses of markers as an assisting tool to select plants with different traits. In this category,visible traits included: leaf shape, flower color, pubescence color, pod color, seed color, seed shape, hilum color, awn type and length, fruit shape, and stripe, flesh color, stem length, etc. (Jiang, 2013).

Cytological markers show the structural features of chromosomes by karyotype and chromosome banding. The banding patterns are displayed in color, width, order and position. It reveals the difference in distributions of euchromatin and heterochromatin (Koshland and Strunnikov, 1996; Paterson *et al.*, 2000; Kato *et al.*, 2005).

Biochemical Markers are Chemicals to assay for isozymes or allozymes which marked the beginning of the practical application of molecular markers (Acquaah, 2009).

DNA Markers

A molecular marker is defined as a particular segment of DNA that is representative of the differences in the genome level (González-Chavira *et al.*, 2006). Molecular markers can be grouped in three main categories (Gupta *et al.*, 2001): (1) Non PCR based markers or restriction fragment length polymorphism (RFLP), (2) PCR-based markers such as: random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and micro satellite or simple sequence repeat (SSR), and (3) sequence or chip-based markers which include: single nucleotide polymorphism (SNP), diversity array technology (DArTs) and single feature polymorphism (SFP).

2. Development of Marker for MAS

There are four major steps that are mandatory to implement MAS: (1) Construction of linkage maps (2) QTL analysis, (3) Towards marker-assisted selection, and (4) marker-assisted selection.

Construction of Linkage Maps

According to Paterson (1998), the most important use of linkage maps is to identify chromosomal locations containing genes and QTLs associated with traits of interest. Such maps may then be referred to as 'QTL' maps. 'Genes or markers that are closed together or tightly-linked will be transmitted together from parent to progeny more frequently than genes or markers that are located far apart. In a segregated population, there is a mixture of parental and recombinant genotypes. The frequency of recombinant genotypes can be used to calculate re-combination fractions which may be used to determine the genetic distance between markers. By analyzing the segregation of markers, the relative order and distances between markers can be determined i.e., the lower the frequency of recombination between two markers, the closer they are situated on a chromosome (conversely, the higher the frequency of recombination between two markers, the far away they are situated on a chromosome). Markers that have a recombination frequency of 50% are described as 'unlinked' and assumed to be located far apart on the same chromosome or on different chromosomes. Mapping functions is used to convert recombination fractions of map units called centi-Morgans (cM). Linkage maps are constructed from the analysis of many segregated markers. The three main steps of linkage map construction are: (i) production of a mapping population, (ii) identification of polymorphism and (iii) linkage analysis marker.

Mapping Populations

The construction of a linkage map requires a segregated plant population (i.e. a population derived from sexual reproduction). The parents selected for the mapping population will differ from one another in more traits of interest. Population sizes used in preliminary genetic mapping studies are generally ranged from 50 to 250 individuals (Mohan *et al.*, 1997). However, larger populations are required for high-resolution mapping. If the map is going to use for QTL studies (which is usually the case), then the important point to note is that the mapping population must be evaluated phenotypically (i.e. trait data must be collected) before subsequent QTL mapping.

Generally, in self-pollinating species, mapping populations are originated from parents that are both highly homozygous (inbred). However, in cross pollinating species, the situation is more complicated since most of these species do not tolerate inbreeding. Many crosses pollinating plant species are also polyploid. Mapping populations used for mapping cross pollinating species may be derived from a cross between a heterozygous parent and a haploid or homozygous parent (Wu *et al.*, 2007). Several different populations may be used for mapping within a given plant species with each population type possessing advantages and disadvantages of their own (Paterson, 2010). F_2 populations which are derived from F_1 hybrids, and backcross (BC) populations that are derived by crossing the F_1 hybrid to one of the parents, are the simplest types of mapping populations developed for self-pollinating species. Their main advantages are that they are easy to construct, and they require only a short time to produce. Inbreeding from individual F_2, plants allow the construction of recombinant inbred (RI) lines which consist of a series of homozygous lines. Each homozygous line contained a unique combination of

chromosomal segments of the original parents. The length of time needed for producing RI populations is the major disadvantage, because usually six to eight generations are required. Doubled haploid (DH) populations may be produced by regenerating plants by the induction to a chromosome doubling from pollen grains, however, the production of DH populations is only possible in species that are willing to tissue culture. The major advantages of RI and DH populations are that they produce homozygous or 'true-breeding' lines that can be multiplied and reproduced without genetic change occurring. This allows for the conduct of replicated trials across different locations and years. Thus both RI and DH populations represent perpetual resources for QTL mapping. Furthermore, seed from individual RI or DH lines may be transferred between different laboratories for further linkage analysis and the addition of markers for existing maps, guaranteeing that all collaborators examined identical material (Young, 1994; Paterson, 2010).

Identification of Polymorphism

The second steps in the construction of a linkage map are to identify DNA markers that reveal differences between parents (i.e. polymorphic markers). It is critical that sufficient polymorphism exists on parents in order to construct a linkage map (Young, 1994). In general, cross pollinating species possess higher levels of DNA polymorphism compared to inbreeding species; mapping in inbreeding species generally require selection of parents that are distantly related. In many cases, parents that provide adequate polymorphism are selected on the basis of the level of genetic diversity of parents (Collard, 2005; Yu and Wise, 2000; Andersen and Lübberstedt, 2003). The choice of DNA markers used for mapping may depend on the availability of characterizing markers or the appropriateness of particular markers for a particular species. Once polymorphic markers have been identified, they must be screened for the entire mapping population, including the parents (and F hybrid, if possible). This is known as marker 'genotyping' of the population. Therefore, DNA must be extracted from each individual of the mapping population when DNA markers are used. Examples of DNA markers screened for different populations are shown in Figure 1. The segregation ratios of markers can be easily understood by using Punnett squares to derive population genotypes (Collard *et al*., 2005)

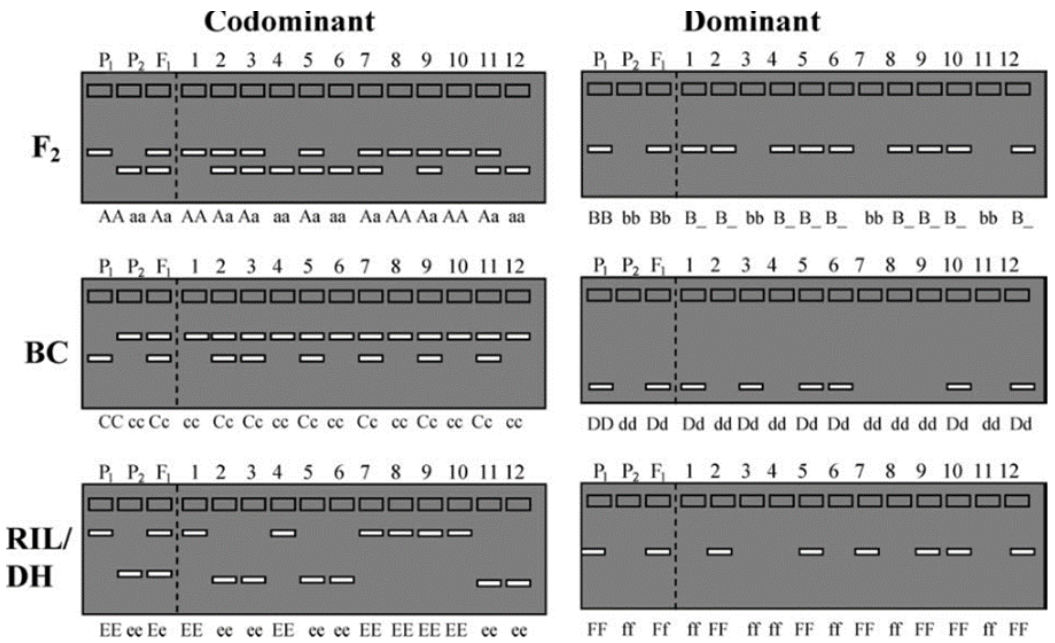

Figure 1. Hypothetical gel photos representing segregated co-dominant markers (left-hand side) and dominant markers (right-hand side) for typical mapping populations. Co-dominant markers indicate the complete genotype of a plant. Note that dominant markers cannot discriminate between heterozygotes and one homozygote genotype in F_2 populations.

Generally, markers can be segregated in a Mendelian way although distorted segregation ratios may be encountered (Sayed *et al.*, 2002; Xu and Crouch, 2008).

Linkage Analysis of Markers

The final step of the construction of a linkage maps is involve code data onto each DNA marker for each individual of a population and conducting linkage analysis using computer programs. Linkage between markers is usually calculated using odds ratios (i.e. the ratio of linkage versus no linkage). This ratio is more conveniently expressed as the logarithm of the ratio, and is called a logarithm of odds (LOD) values or LOD score (Van Ooijen, 1999). LOD values of greater than three are typically used to construct linkage maps. A values of three between two markers indicates that linkage is more likely 1000 times (i.e. 1000:1) than to no linkage. LOD values may be lowered in order to detect a greater level of linkage or to place additional markers within maps constructed at higher LOD values. Commonly used software programs include Mapmaker/EXP (Lander and Botstein, 1989; Lincoln *et al.*, 1993) and MapManager QTX (Manly *et al.*, 2001) which are available free from the internet. Join Map is another commonly-used program for constructing linkage maps (Stam, 2003).

Linked markers are grouped together into 'linkage groups,' which represents chromosomal segments or entire chromosomes Fig 2. A difficulty associated with obtaining an equal number of linkage groups and

chromosomes is that the polymorphic markers detected are not necessarily evenly distributed over the chromosome, but clustered in some regions and absent in others (Paterson, 2010). In addition, the frequency of recombination is not equal to chromosomes (Young, 1994). The accuracy of measuring the genetic distance and determining marker order is directly related to the number of individuals studied in the mapping population. Ideally, mapping populations should consist of a minimum of 50 individuals (Young, 1994).

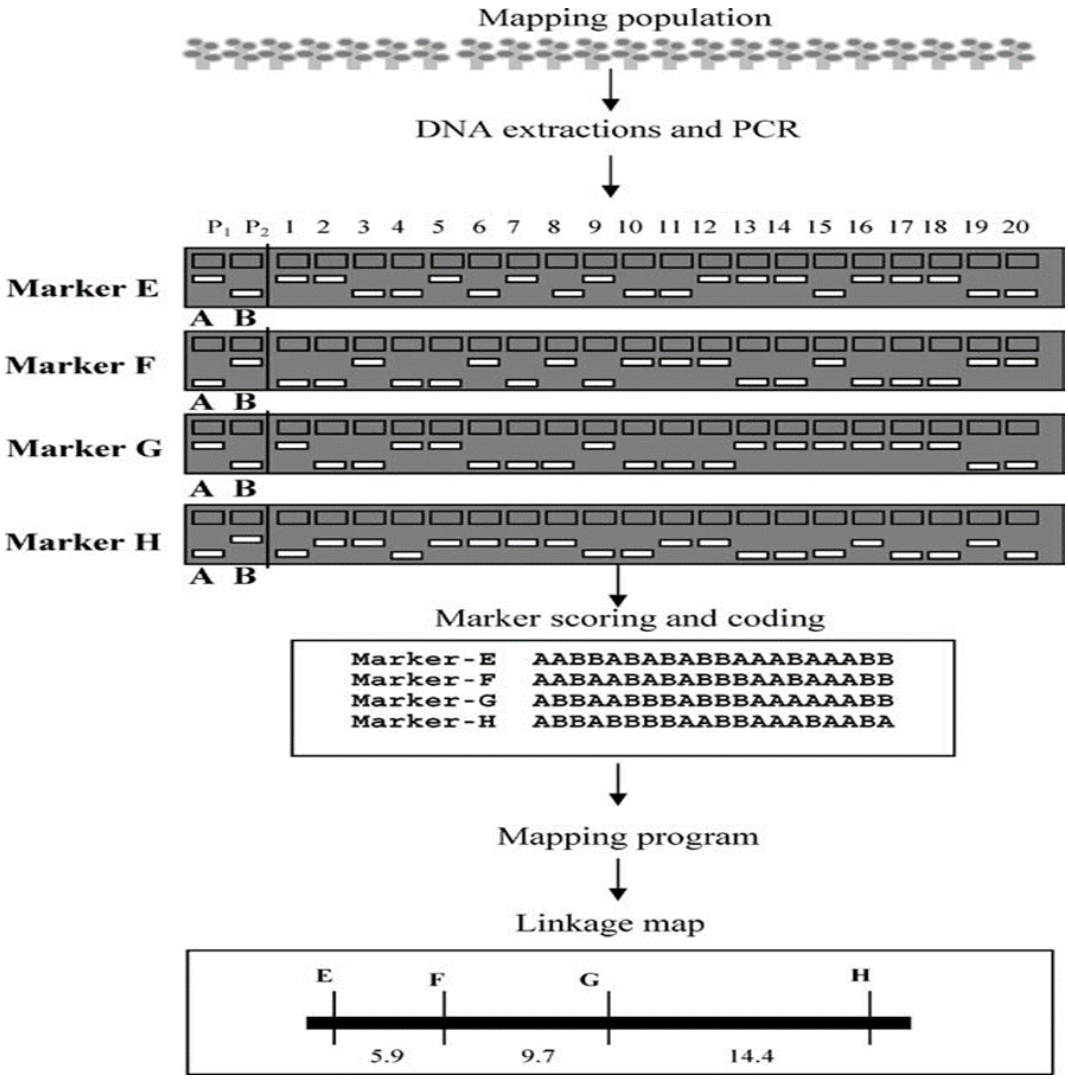

Figure 2. Construction of a linkage map based on a small recombinant inbred population (20 individuals). The first parent (P_1) is scored as an 'A' whereas the second parent (P_2) is scored as a 'B'. Coding of marker data varies depending on the type of population used. This linkage map was constructed using Map Manager QTX (Manly *et al.*, 2001)by applying the Haldane mapping function.

Genetic Distance and Mapping Functions

The greater the distance between markers, the greater the chance of recombination occurs to meiosis. Distance between linkage maps is measured in terms of the frequency of recombination between genetic markers (Paterson, 2010). When map distances are small (<10 cM), the map distance equals the recombination frequency. However, this relationship does not apply for map distances that are greater than 10 cM(Kearsey *et al*., 1997).

QTL analysis

The three most frequently used methods of detecting QTLs are: single-marker analysis, simple interval mapping and composite interval mapping (Liu *et al.*, 1996).

Single-marker to Detect QTLs

Single-marker analysis is the simplest method of detecting QTLs. The statistical methods used for single-marker analysis include: t-tests, analysis of variance and linear regression. Linear regression is most commonly used because the coefficient of determination from the marker explains the phenotypic variation arising from the QTL linked to the marker. Furthermore, this method does not require a complete linkage map and can perform with basic statistical software programs.

Markers that are linked to a gene or QTL and which control a particular trait (e.g. plant height) indicate significant differences when the mapping population is partitioned according to the genotype of the marker. Based on the results in Figure 3, Marker E is linked to a QTL because there is a significant difference between means. Marker H is unlinked to a QTL because there is no significant difference between means. The closer the marker is to the QTL of interest, the lower the chance for recombination between marker and QTL.

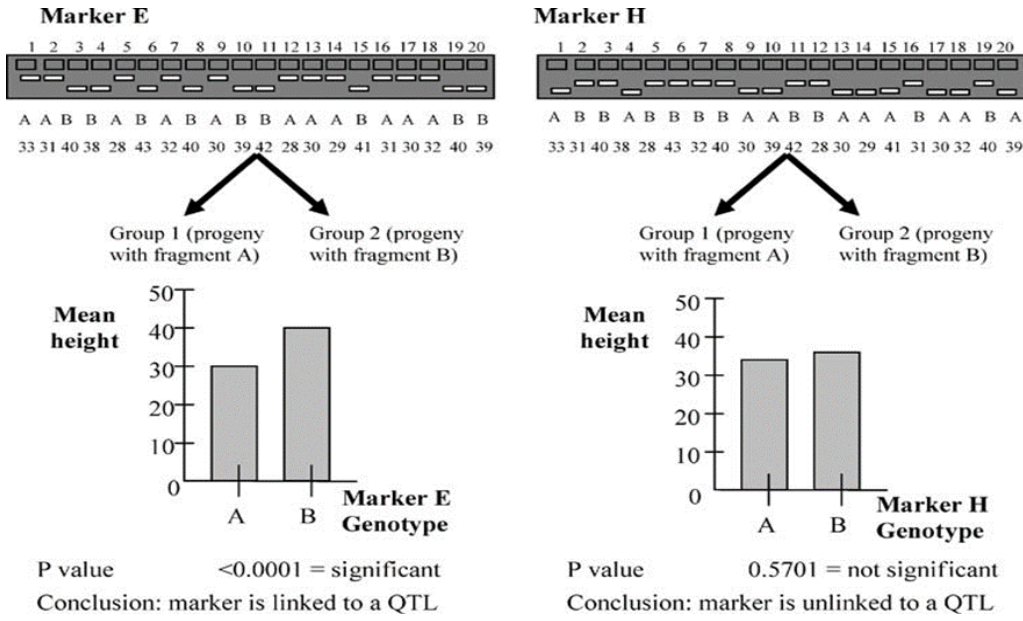

Figure 3. Principle of QTL mapping , adapted from (Young, 1996).

Understanding Interval Mapping Results

Interval mapping method produces a profile of the likely sites for a QTL between adjacent linked markers. In other words, QTLs are located with respect to a linkage map. The results of the test statistic for SIM and CIM are typically presented using a logarithmic of odds (LOD) score or likelihood ratio statistic (LRS). There is a direct one-to-one transformation between LOD scores and LRS scores (the conversion can be calculated by: LRS = 4.6 × LOD) (Visscher and Goddard, 2004). These LODs or LRS profiles are used to identify the most likely position for a QTL in relation to the linkage map which is the position where the highest LOD value is obtained. A typical output from interval mapping is a graph with markers comprising linkage groups on the x axis and the test statistic on the y axis (Fig4).

Before the permutation tests were widely accepted as an appropriate method to determine significance thresholds, a LOD score between 2.0 to 3.0 (most commonly 3.0) was usually chosen as the significance threshold.

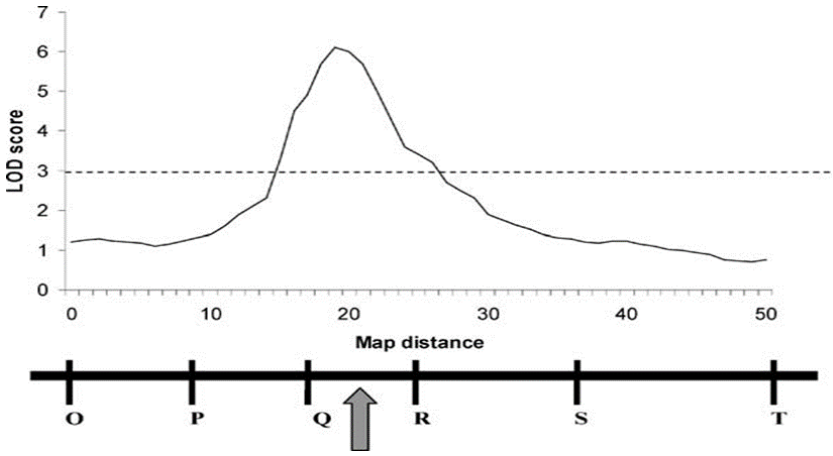

Figure 4. Hypothetical output showing a LOD profile for chromosome 4. The dotted line represents the significance threshold determined by the permutation tests. The output indicates that the most likely position of the QTL is near marker Q (indicated by an arrow). The best flanking markers for this QTL would be Q and R

Confidence Intervals for QTLs

There are several ways through which confidence intervals can be calculated. The two simplest ones are: the 'one-LOD support interval' which is determined by finding the regions on both sides of a QTL peak that correspond to a decrease of 1 LOD score (Hackett, 2002), 'Bootstrapping' which is a statistical method of re-sampling is another method to determine the confidence interval of QTLs (Visscher *et al.*, 1997; Liu *et al.*, 2000), and it can be easily applied to some mapping software programs such as Map Manager QTX (Manly *et al*., 2001).

Number of Markers and Marker Spacing

There is no absolute value for the number of DNA markers required for a genetic map since the number of markers vary with the number and length of chromosomes in the organism.

For detection of QTLs, a relatively sparse 'framework' (or 'skeletal' or 'scaffold') map consisting of evenly spaced markers is adequate, and preliminary genetic mapping studies generally contain between 100 and 200 markers (Mohan *et al*., 1997). However, this depends on the genome size of the species; more markers are required for mapping in species with large genomes. Darvasi *et al.* (1993) reported that the power of detecting a QTL was virtually the same for a marker spacing of 10 cM as for an infinite number of markers, and only slightly decreased for marker spacing of 20 or even 50 cM.

Development of MAS

Generally, the steps required for the development of markers for use of MAS include: high resolution mapping, validation of markers and possibly marker conversion.

High-Resolution Mapping of QTLs

By using larger population sizes and a greater number of markers, more tightly -linked markers can be identified.This process is termed as a 'high-resolution mapping' (also 'fine mapping'). Therefore, high-resolution mapping of QTLs may be used to develop reliable markers for marker-assisted selection (at least <5cM but ideally <1cM away from the gene) to determine reliable markers between a single geneor several linked genes (Michelmore, 1995; Mohan *et al.*, 1997; Stange *et al*., 2013).

There is no universal number for the appropriate population size required to decide high-resolution mapping. However, population sizes that have been used for high-resolution mapping have consisted of>1000 individuals to resolve QTLs to distances between flanking markers for<1cM (Blair *et al.*, 2003; Poczai *et al.*, 2013). The mapping of additional markers may saturate framework maps. High-throughput marker techniques that generate multiple loci per primer combination (e.g. AFLP) are usually preferred for increasing marker density.

Validation of Markers

Generally, markers can be validated by testing their effectiveness in determining the target phenotype in independent populations and different genetic backgrounds. This is referred as 'marker validation' (Li and Quiros, 2001). In other words, marker validation involves testing the reliability of markers to predict phenotype (Ramkumar *et al.*, 2015).

Marker Assisted Selection

Marker assisted selection (MAS) is the breeding strategy in which selection of a gene is based on molecular markers (DNA markers) which are closely linked to the gene of interest rather than to the gene of itself. These markers are used to monitor the incorporation of the desirable allele from the donor source. Selection of a genotype which carry desirable gene via linked marker (s) is called Marker Assisted Selection (Babu *et al.*, 2004) .

Advantage of MAS

According to Boopathi (2012), Lande and Thompson (1990), MAS can theoretically enhance breeder's selection efficiency because of the following reasons: (1) Selection can be carried at seedling stage, (2) Recessive alleles are identified using appropriate linked markers, (3) there is Gene pyramiding or combining multiple genes together, (4) Selected traits are with low heritability, (5) there is elimination of unreliable phenotypic evaluation which are

associated with field trials due to environmental effects, (6) there is testing of specific traits where phenotypic evaluation is not feasible, (7) MAS may be cheaper and faster than conventional phenotypic assays depending on the trait, (8) there is a consideration that may affect cost-effectiveness of MAS since multiple markers can be evaluated using the same DNA sample, (9) Markers can be applied to the choice of parents in crossing programs, and (10) Recessive genes can be maintained without the need for progeny tests of each generation as homozygous and heterozygous plants can be distinguished with the aid of co-dominant markers.

Limitations in MAS

MAS isn't always universally effective, and it cannot be implemented to all of the traits in all of the crops. Some drawbacks of the approach are briefly mentioned hereunder:

1. MAS can be more costly than traditional techniques specifically for start-up and labour costs. Additionally, in sure situations, traditional breeding approach might match properly to fulfill out the breeding objective. A critical drawback for MAS, not regularly reported nowadays is that even though markers can be inexpensive to apply, there's a massive preliminary price of their development.
2. Recombination between the marker and the gene of interest might also lead to false positives. For example, if the marker and the gene of hobby are separated by using five cM, and the choice is primarily based on totally at the marker pattern, there's about 5% threat of choosing the incorrect plant. This is primarily based on the overall tenet that in quick distances, 1 cM of genetic distance is the same to 1% recombination. However, the breeder can determine the mistake rate.This is ideal in the MAS program that mistakes are also normally expected in phenotypic evaluation. To keep away from this affective problem, it could be essential to apply flanking markers on both aspects of the QTL of hobby to growth the chance that the favored gene is selected.
3. Sometimes, markers that have been used to across a locus ought to be come transformed to 'breeder-friendly' markers which might be greatly dependable and simpler to apply. Examples are: RFLP markers want to be transformed to STS markers, and RAPD markers are transformed to SCAR markers for greater reliability.
4. Imprecise estimates of QTL places and results might also bring about additionally slower development than expected. Many QTLs have massive confidence interval of 20 cM or greater or their relative significance in explaining trait inheritance has been over estimated.
5. Markers that are developed for MAS in a single population won't be transferred to different populations because of loss of both marker polymorphism and the absence of a marker–trait association.

Selection of QTLs for MAS

One commonly asked question is that "quantitative traits are controlled by at least several QTLs. How many QTLs are typically selected for MAS?" Theoretically, all markers that are tightly

linked to QTLs can be used for MAS. However, no more than three tightly linked QTLs can be used due to the cost (Ribaut and Betrán, 1999). But Lecomte *et al.* (2004) has reported that up to 5 QTLs can be integrated into tomato via MAS . Even selecting a single QTL via MAS can be beneficial to plant breeding. such a QTL should account the largest proportion of phenotypic variance in the trait (Ribaut and Betrán, 1999). Furthermore, all QTLs selected for MAS should be similar across environments (Hittalmani *et al.*, 2000).

3. Applications of MAS in Plant Breeding

The advantages mentioned above may have a profound impact on plant breeding in the future, and they may alter the plant breeding practice. Currently, the most frequently used MAS breeding methods include: (i) simple screening of populations, (ii) Marker-assisted introgression (MAI) or marker-assisted backcross (MAB), (iii) Gene pyramiding, (iv) Recurrent selection and (5) Selection of an index combining molecular and phenotypic scores.

Marker-Assisted Evaluation of Breeding Material

Prior to crossing (hybridization) and line development, there were several applications in which DNA marker data may be used for breeding. These applications include: cultivar identity, assessment of genetic diversity and parent selection, and confirmation of hybrids.

i. Cultivar identity/assessment of 'purity'

In practice, seeds of different strains are often mixed due to the difficulties of handling large numbers of seed samples used within and between crop breeding programs. Markers can be used to confirm the true identity of individual plants. The maintenance of high levels of genetic purity is essential in cereal hybrid production in order to exploit heterosis.

ii. Assessment of Genetic Diversity and Parental Selection

Breeding program depends on a high level of genetic diversity of achieving progress of selection. Broadening of the genetic base of core breeding material requires the identification of diverse strains on hybridization with elite cultivars (Xu and Crouch, 2008).

iii. Study of Heterosis as a Trait

Heterosis or hybrid vigour describes the most effective performance of heterozygous in F-hybrid plants in terms of increasing biomass, size, yield, speed of development, fertility, resistance to disease and to insect pest, or to resist climatic severities of any kind compared to the average performance of their homozygous parental inbred lines (Lamkey and Edwards, 1999; Hochholdinger and Hoecker, 2007; Barr, 2009). Molecular marker technology was used to identify the genomic regions that contribute to heterosis for a trait of interest. Baranwal *et al.* (2012) identified and characterized quantitative trait loci for seven traits which contribute to heterosis in maize. Heterosis for specific traits in maize can be controlled by dominance, over dominance, or epistasis (Frascaroli *et al.*, 2007). By identtifying the genetic basis of all agronomically important characters and the allelic variation on those loci, the breeder can design superior genotypes 'in silico' called 'Breeding by Design'. This may be

achieved by: (1) mapping loci involved in all agronomically relevant traits,(2) assessment of the allelic variation on those loci and (3) breeding by design (Peleman and van der Voort,2003).

iv. Identification of Genomic Regions under Selection

The identification of shifts in allele frequencies of the genome is important information to breeders to design appropriate breeding strategies (Aversano *et al.*, 2012). Other advantages of identifying of genomic regions under selection for QTL mapping are: the regions under selection can be targeted for QTL analysis or used to validate previously detected marker–trait associations (Ribaut and Ragot, 2007). Ultimately, data on genomic regions under selection can be used for the development of new varieties of specific allele combinations using MAS schemes such as marker-assisted backcrossing (Ribaut and Ragot, 2007).

Marker-Assisted Backcrossing

Backcrossing in plant breeding is used to transfer (introgress) favorable traits from a donor plant to an elite genotype (recurrent parent) (Charcosset, 1997; Stam, 2003; Steele *et al.*, 2006). In most cases, the parent plant used for backcrossing has a large number of desirable attributes, but it is deficient in only a few characteristics (Charcosset, 1997). The use of DNA markers for backcrossing is it greatly increases the efficiency of selection. It comprises a selection of: (1) target locus/foreground selection, (2) limited background/ recombinant selection, and (3) Background selection.

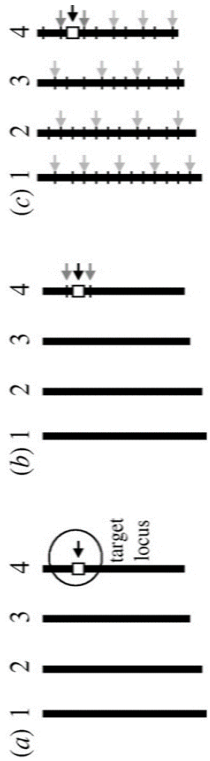

Figure 5. Three levels of selection during marker-assisted backcrossing: a) Target gene/QTL (foreground) selection, b) Recombinant selection, and c) Back-ground selection

In the first level, markers can be used in combination with or to replace screening for the target gene or QTL. This is referred to as 'foreground selection' (Hospital, 2005). This may be particularly useful for traits that have laborious or time-consuming phenotypic screening procedures. It can also be used to select for reproductive-stage traits in the seedling stage allowing the best plants to be identified with backcrossing. With conventional backcrossing, it takes a minimum of five to six generations to recover the recurrent parent. Data on simulation studies are used to suggest that up to four backcross generations can be saved in using markers. Furthermore, recessive alleles can be selected which are difficult to select using conventional methods.

The second level involves selecting BC progeny with the target gene and recombination activities between the target loci and the linked flanking markers which are refer as 'recombinant selection'. The main purpose of recombinant selection is to reduce the size of the donor chromosome segment that contain the target locus (i.e. Size of the introgression). This is important because the rate of decrease of the donor fragment is slower than the unlinked regions, and many undesirable genes that negatively affect crop performance may be linked to the target gene from the donor parent. This is referred as 'linkage drag' (Frisch *et al.*, 1999; Hospital, 2005). Using conventional breeding methods, the donor segment can remain very large even with many BC generations (e.g. more than 10 (Ribaut and Betrán, 1999; Salina *et al.*, 2003). By means of markers that flank a target gene (e.g. less than 5 cM on either sides), it is possible to minimize linkage drags. Since double recombination events which occur on both sides of a target

Table 1: Some examples of MAB

Crop	Target trait	Gene/QTL	Marker(s) used	Reference
Rice	Bacterial blight resistance	(Xa21)	RFLP AFLP	Chen *et al. (2001)*
	Bacterial blight	Xa4,	STS	Shanti *et al. (2001)*
	(BB) resistance	xa5, xa13 &	CAPS	Shanti et al. (2001)
		Xa21	STS Bacterial blight (BB) resistance	Shanti *et al(2001)*
	Bacterial blight	xa5,and xa13,	CAPS	Zhang *et al. (2012)*
	(BB) resistance	Xa21	STS	Zhang et al. (2012)
	+ Blast resistance	Pi25	STS	Zhang et al. (2012)
wheat			RFLP,CAPS	Helguera *et al. (2005)*
	Leaf rust resistance	Lr 21	SSR	Somers *et al. (2005)*
		Lr 47	PCR-based markers	Chicaiza *et al. (2006)*
	Cereal cyst nematode		RFLP	Ogbonnaya(2001)
	Stripe rust resistance	Yr-36	PCR-based markers	Chicaiza et al. (2006)
	Stem rust resistance	sr-38	PCR-based markers	Chicaiza et al. (2006)
	Fusarium head blight (FHB)	8 QTL and 2 genes	SSR	Somers *et al. (2005)*
	durable rust resistance and height	Multiple genes	SSR	Künzel et al. (2000)
Barley	Resistance to boron toxicity		SSR	Emebiri *et al. (2009)*
	Resistance to BaYMVI-III		RFLP	Okada *et al. (2003)*
Tomato	Resistance to tomato wilt virus Spotted		CAPS	Ogbonnaya *et al. (2001))*
	Tomato mosaic virus, spotted wilt virus, and yellow leaf curl virus		SCAR/RAPD/ SNP,	Foolad (2007)
			SCAR/RAPD, and CAPS	Foolad and Panthee (2012)
	Fusaium crown and root rot, fusarium wilt, late blight, leaf mold, powdery mildew, and verticillium wilt		RAPD,SCAR,CAPS;SSR;	Foolad (2007)
			SSR/CAPS; SCAR; and SCAR/SNP respectively	Foolad and Panthee (2012)
	Bacterial canker, speck, spot and wilt.		CAPS; RAPD; SNP ; SCAR/RFLP/CAPS, re-spectively	Foolad and Panthee (2012)

locus are extremely rare, recombinant selection is usually performed using at least two BC generations (Frisch and Melchinger, 2005).

The third level of MAB involves selecting BC progeny with the greatest proportion of recurrent parent (RP) genome using markers that are unlinked to the target locus. This is called 'background selection'. In the literature, background selection refers to the use of tightly linked flanking markers that are used for recombinant selection and unlinked markers are used to select the RP (Frisch *et al*., 1999; Frisch and Melchinger, 2005; Hospital, 2009). Background markers are markers that are unlinked to the target gene/QTL on all other chromosomes. In other words, background markers are markers that can be used to select from the donor genome. This is extremely useful to accelerate RP recovery.

Marker Assisted Pyramiding

Gene pyramiding is defined as a method which is aimed at assembling multiple desirable genes from multiple parents into a single genotype (Suresh and Malathi, 2013). This is possible through conventional breeding, but it is extremely difficult or impossible at early generations. Using conventional phenotypic selection, individual plants must be phenotypically screened for all traits tested. Therefore, it may be very difficult to assess plants from certain population types (e.g. F_2) or for traits with destructive bioassays. DNA markers may facilitate selection because DNA marker assays are non-destructive, and markers for multiple specific genes/ QTLs can be tested using a single DNA sample without phenotyping. The most important use of pyramiding is to combine multiple disease resistance genes in order to develop durable disease resistance Fig. 6.

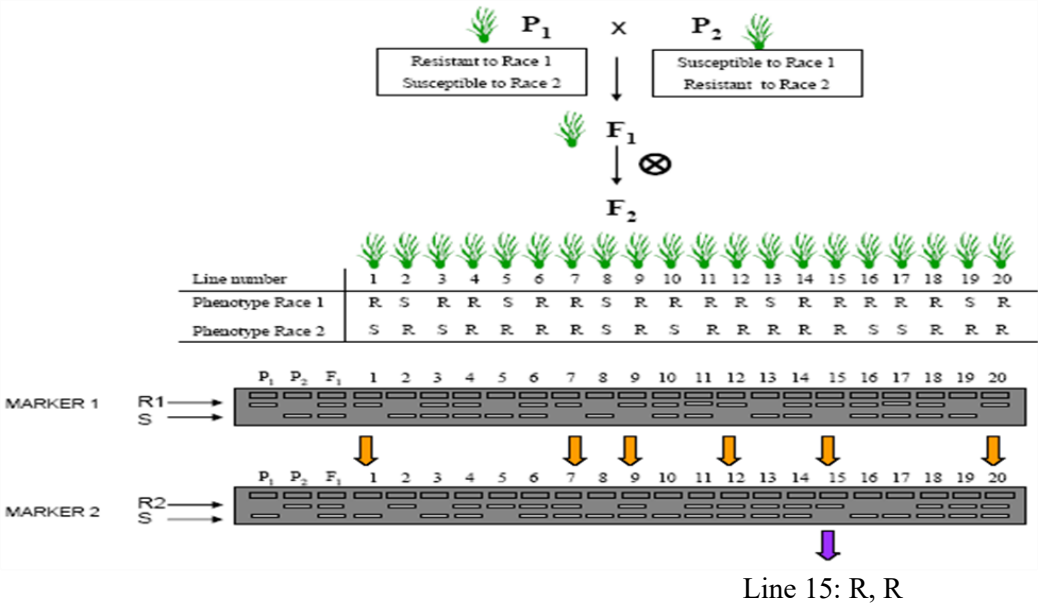

Figure 6. Marker assisted pyramiding of two disease resistance genes. Homozygotes can be selected from the F_2 population.

A Distinct Gene Pyramiding Scheme

The gene pyramiding scheme can be distinguished from two parts (Fig 7). The first part is called a pedigree, which aims at cumulating of all target genes in a single genotype called the root genotype. The second part is called the fixation steps which aims at fixing the target genes into a homozygous state, i.e. to derive the ideal genotype from the one single genotype. Each node of the tree is called an intermediate genotype and has two parents.

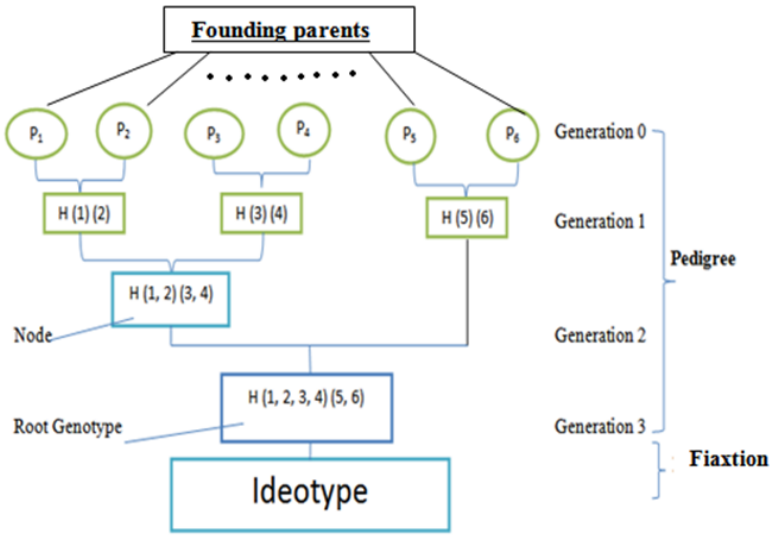

Figure 7. A distinct gene pyramiding scheme cumulating six target genes (Hospital, 2005)

Table 2. Selected examples of MAS based gene pyramiding for important traits in major crops

Crop	Trait	Pyramided genes	Reference
Rice	Blight resistance	Xa4,xa5,xa13,Xa21	Huang *et al.* (1997) Singh *et al.*(2001)
	Blast resistance	Pi(2)t,Piz5,Pi(t)a	Hittalmani *et al.* (2000); Narayanan *et al.* (2004)
Wheat	Leaf rust resistance	Lr41, Lr42, Lr43	Chelkowski and Stepien (2001)
	Powdery mildew resistance	Pm-1, Pm-2	Liu *et al.* (2000)
	Powdery mildew resistance	3gene combinations	Liu *et al.* (2000)
	Powdery mildew resistance Leaf rust resistance	4 genes	Huang and Röder (2004)
		2 genes	Singh et al. (2001)
Cotton	Insect pest resistance	Cry 1Ac, Cry 2Ac	Jackson *et al.* (2003)
Barley	Yellow mosaic virus resistance	rym4, rym5, rym9, rym11	Werner (2005)

Pyramiding of multiple genes/QTLs may be achieved in three different approaches: multiple-parent crossing or complex crossing, back- crossing, and recurrent selection. In the first method, the recurrent parent (RP_1) is crossed with the donor parent (DP_1) to produce the F_1 hybrid, and it can be backcrossed up to third backcross generations (BC_3) to produce the improved recurrent parent (IRP_1). This improved recurrent parent is then crossed with other donor parent (DP_2) to be pyramid with multiple genes. This strategy is less acceptable as it is time taking, but pyramiding is very precise as it involves one gene at one time. In the second strategy, the recurrent parent (RP_1) is crossed with donor parents (DP_1, DP_2, etc.) to get the F_1 hybrids which are then intercrossed to produce improved F_1 (IF_1). This improved F_1 is then backcrossed with the recurrent parent to get the improved recurrent parent (IRP). As such, the pyramiding is done in the pedigree step itself. However, when the donor parents are different, this method is less likely to be used because there is a chance of losing the pyramided gene in the process. The third strategy is an amalgamation of the first two which involves simultaneous crossing of the recurrent parent (RP_1) with many donor parents and then backcrossing them up to the BC_3 generation. The backcross populations with the individual gene are then intercrossed with each other to get the pyramided lines. This is the most acceptable way as in this method not only time is reduced, but a fixation of genes is fully assured.

Combined Approach

The traditional molecular selection index (MSI) employed in marker-assisted selection maximizes the selection response by combining information on molecular markers linked to quantitative trait loci (QTL) and phenotypic values of the traits of the individuals of interest (Cerón-Rojas *et al.*, 2008). A combination of phenotypic screening and MAS approach is useful to maximize genetic gain. When some QTLs are unidentified from QTL mapping, level of recombination between marker and QTL (in other words marker is not 100% accurate), are used to reduce population sizes for traits where marker genotyping is cheaper or easier than phenotypic screening. Marker-directed' phenotyping (also called 'tandem selection') used markers are not 100% accurate. Phenotypic screening is more expensive compared to marker genotyping that can save time and reduce costs especially for quality trait (Han *et al.*, 1997).

4. Molecular Breeding in Developing Countries

Although molecular breeding (MB) has great promises for developing countries (Joshi, 2010), the developing countries are hardly homogeneous in implementing it, however, industrialized countries routinely use and exploit other latest MB applications. Developing countries which are at mid-economic level, are showing interests to apply MB and they are taking initial steps towards adopting MB in day-to-day breeding activities. Unfortunately, many factors still impede adoption practice in these countries. Limited skilled human resources and inadequate field infrastructure are the major challenges. Even though those factors hindered the breeding practice, virtual platforms and modern information and communication technology helped the breeders to have access to genomic resources, advanced laboratory services, and robust analytical and data management tools.

These developments have significant impacts on crop improvements in developing countries.

5. Conclusions and Future Prospects

In order to combat the global challenges caused by the population explosion, the food supply issue, which is a serious

problem to scientists who work in agriculture, has to be strengthened. Advances in molecular biology have sharpened the insights of the breeders, and brighten the prospects of confidence to serve the humanity.

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