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Marker-assisted selection: A smart biotechnological strategy for modern plant breeding

Selección asistida por marcadores: Una estrategia biotecnológica inteligente para el fitomejoramiento moderno

Shruti Shrestha[1]*; Sudeep Subedi[2]; Jiban Shrestha[2]

*Corresponding author: shrutis2903@gmail.com

https://orcid.org/0000-0002-8954-5538

Abstract

Plant breeders and geneticists use molecular marker-assisted selection also called as MAS as a useful approach for breeding of plant to make selection more efficient and speed up the breeding cycle. MAS can be more efficient, effective, and reliable than phenotypic selection. Molecular markers are useful to identify the economically important traits in the breeding population for further manipulation in a short time. Due to the applicability of markers at the seedling stage ensuring high precision at the reduced level of cost, marker-assisted selection offer the chances to improve responses from selection. The MAS using DNA level polymorphism accelerate the pace of selection. The main marker technologies applied are chiefly co-dominant markers i.e. microsatellite markers/SSR (Simple Sequence Repeats) marker, RFLP (Restriction Fragment Length Polymorphism) marker and SNPs (Single nucleotide polymorphisms). This review overviews the various MAS technologies and their applications in crop improvement programs.

Keywords: *Breeding, Marker Assisted Selection (MAS), Single nucleotide polymorphisms (SNPs)*

Resumen

Los fitomejoradores y genetistas utilizan la selección asistida por marcadores moleculares, también denominada MAS (por sus siglas en inglés), como un enfoque útil para la reproducción de plantas para hacer la selección más eficiente y acelerar el ciclo de reproducción. MAS puede ser más eficiente, eficaz y confiable que la selección fenotípica. Los marcadores moleculares son útiles para identificar los rasgos económicamente importantes en la población reproductora para su posterior manipulación en poco tiempo. Debido a la aplicabilidad de los marcadores en la etapa de plántula, lo que garantiza una alta precisión a un nivel de costo reducido, la selección asistida por marcadores ofrece la oportunidad de mejorar las respuestas de la selección. El MAS que usa polimorfismo a nivel de ADN acelera el ritmo de selección. Las principales tecnologías de marcadores aplicadas son principalmente marcadores codominantes, es decir, marcadores de microsatélites / marcador SSR (repeticiones de secuencia simple), marcador RFLP (polimorfismo de longitud de fragmentos de restricción) y SNP (polimorfismos de un solo nucleótido). Esta revisión describe las diversas tecnologías MAS y sus aplicaciones en programas de mejora de cultivos.

Palabras clave: *Reproducción, selección asistida por marcadores, polimorfismos de nucleótido único (SNP)*

Introduction to MAS

Agricultural researches are being carried out with the primary aim of improving different crop species keeping in mind the desirable traits. Although there are several revolution and more sophisticated process, there is the need of introducing new molecular technology in our breeding scheme like Marker Assisted Selection which is more efficient than conventional breeding schemes (Lema, 2018). Detectable differences are seen due to the presence of markers' specific biomolecules which contain proteins among various species. A molecular marker, used based on naturally occurring DNA polymorphism is a sequence of DNA that can be identified easily. The ideal marker

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[1] Agriculture and Forestry University, Rampur, Chitwan, Nepal
[2] Nepal Agricultural Research Council, National Plant Breeding and Genetics Research Centre, Khumaltar, Lalitpur, Nepal

must be easily reproducible, polymorphic, easy, readily and cheaply detected and must have even distribution throughout the genome (Nadeem et al., 2018).

Molecular marker is a powerful tool found in Quantitative trait loci (QTL) that helps in detection of the genes carrying desirable traits. It consists of a specific molecule that helps to identify different species. A short DNA sequence, like a sequence that surrounds a single base-pair change (single nucleotide polymorphism, SNP), or like mini and microsatellites which are long one (Al-Samarai & Al-Kazaz, 2015). Within genome there exist many regions that contain genes that are associated with a quantitative trait like yield, height, and is known as quantitative trait loci (QTLs). The progress of DNA markers in the 1980s resulted in the selection of QTLs that helps in the representation of quantitative traits (Collard et al., 2005). In agriculture, for the formation of linkage map DNA markers are mainly used for diverse crop species and this linkage map is utilized for determining chromosomal regions that contain genes that control simple traits and quantitative traits using QTL 170 analysis (Mohan et al., 1997). Linkage maps construction and undergoing QTL analysis which helps in defining particular genomic regions that is associated with particular traits is known as QTL /genetic/gene/genome mapping (McCough & Doerge, 1995; Mohan et al., 1997). The process of selecting genes using such markers is referred to as marker-assisted selection (MAS) and is relatively a new discipline of molecular breeding.

There are various types of markers available and the use of particular marker depends upon its availability, objectives of the project, required quantity and quality of DNA, level of polymorphism detecting efficiency, the required time for conducting analysis, cost per unit information, genetic diversity of species under consideration and their utility across the population. Like, for self-pollinated, RAPD (Random-amplified polymorphic DNA) markers are more useful than RFLPs for polymorphism detection within a gene pool. For characterizing other species, RFLPs that is mapped in one population can be used as heterozygous probes. Markers have been elaborated and used for enhancing global food production monitoring its economically important traits. The use of molecular markers has led to the improvement of important crop like rice (Mackill et al., 1999). It has been used for example, in the enhancement of heterosis for the grain yield in the B73xMo17 Elite Single Cross hybrid Maize and also we can find successful example of MABC (Marker Assisted Back Crossing) and Forward crossing in maize (Abler et al., 1991). Even in wheat, Multiparent advanced generation intercross (MAGIC) approach is being used in UK and Australia to develop multi-parent recombinant inbred lines (RILs). For whole genome profiling as well as for background screening, Single nucleotide polymorphisms (SNPs), and diversity array technology (DArT) have also been used widely (Gupta et al., 2010). The improvement has also been made using barley (Thomas, 2003), oilseed (Snowdon & Friedt, 2004), horticultural crops (Mehlenbacher, 1995), and pulses (Kelly et al., 2003). RFLP markers for the cereal cyst nematode have been used in the selection of Cre1 resistance gene in wheat (Ogbonnaya et al., 2001).

This review will give important information and a clear concept about the newly emerging biotechnological interventions using markers. The rice crop is used as an example to show recent advances in MAS.

Characteristics of markers in MAS

Co-dominant markers provide more information than dominant markers as there will be no masking action. So, markers should be co-dominant in MAS approaches. Marker loci should be extensively and evenly distributed so that it can show all resistant genes present of the concerned traits in the chromosome. The detection work of markers should be rapid, easy, and simple and this detection system should be cost-effective and amenable to automation. Markers should be highly reproducible in all cells. The marker system should be highly polymorphic to show differences between genotypes that contain and that do not the target gene. The marker should be reliable in nature which map close to the target gene. Closer the marker to the target gene, lower will be the recombination frequency. Also, rather than using a marker if two markers are used flanking the target gene, there will be higher accuracy of Marker Assisted Selection.

Types of markers techniques

Different kinds of DNA markers have been used based on different polymorphism detecting techniques (southern blotting, northern blotting, PCR – polymerase chain reaction, and DNA sequencing) (Collard et al., 2005). The different molecular marker techniques are given in Table 1.

Procedure of MAS

The general procedure of MAS is given in Figure 1 (Rana et al., 2019). Marker-assisted selection involves the following major methods: (1) screening of populations (e.g., F2, F3, recombinant inbred lines, double haploids, etc.) for genotypes of interest based on molecular markers, (2) marker-assisted backcross, where one or more genes per QTLs of interest are transferred from a donor parent to a recipient parent by repeated backcrossing to improve the target trait, (3) gene pyramiding schemes, where genes (two or more) identified in multiple lines/parents are accumulated into a single genotype, (4) marker-based recurrent selection, a complex scheme used for more loci involving several generations of selection and random mating of selected individuals, (5) selection based on an

Table 1 Different Molecular Marker Techniques

SN	Techniques	References
1	AFLP (Amplified fragment length polymorphism) used for DNA fingerprinting	(Vuylsteke et al., 2007)
2	AP-PCR (Arbitrarily primed PCR) used for genomic fingerprinting	(Welsh & McClelland, 1991)
3	AS-PCR (Allele-specific PCR) used for detection of mutations, polymorphisms, and haplotypes	(Bottema et al., 1993)
4	ASAP (Allele-specific Associated Primers) used for developing resistance in *Pisum sativum* against bean yellow mosaic virus	(Yu et al., 1996)
5	CAPS (Cleaved amplified polymorphic sequences) used for preparation of genetic map	(Shavrukov, 2016)
6	DAF (DNA amplification fingerprinting) used for producing a characteristic spectrum of short DNA products useful for detecting genetic differences	(Caetano-Anollés et al., 1991)
7	ISA (Inter-SSR amplification) used for genome fingerprinting	(Zietkiewicz et al., 1994)
8	RAPD (Random-amplified polymorphic DNA) used for comparing DNA sequences	(Kumar & Gurusubramanian, 2011)
9	RFLP (Restriction fragment length polymorphism) used to characterize the microbial communities	(Schütte et al., 2008)
10	SAP (Specific amplicon polymorphism) for analysis of PCR products amplified from mapped loci of rice genomic DNA	(Williams et al., 1991)
11	SCAR (Sequence characterized amplified region) used for Bdv2 gene's molecular confirmation in wheat germplasm and assessment for resistance against barely yellow dwarf viruses	(Kausar et al., 2015)
12	SPAR (Single Primer Amplification Reactions) for the assessment of diversity in *Jatropha curcas* L.	(Ranade et al., 2008)
13	SSLP (Microsatellite simple sequence length polymorphism) for its characterization in rice	(Panaud et al., 1996)
14	SSR (Simple sequence repeats) for analysis of its polymorphism between N22 and Uma rice varieties	(Waghmare et al., 2018)
15	STS (Sequence tagged sites) for its Generation and validation from diverse genotypes of dioecious Jojoba	(Heikrujam et al., 2014)

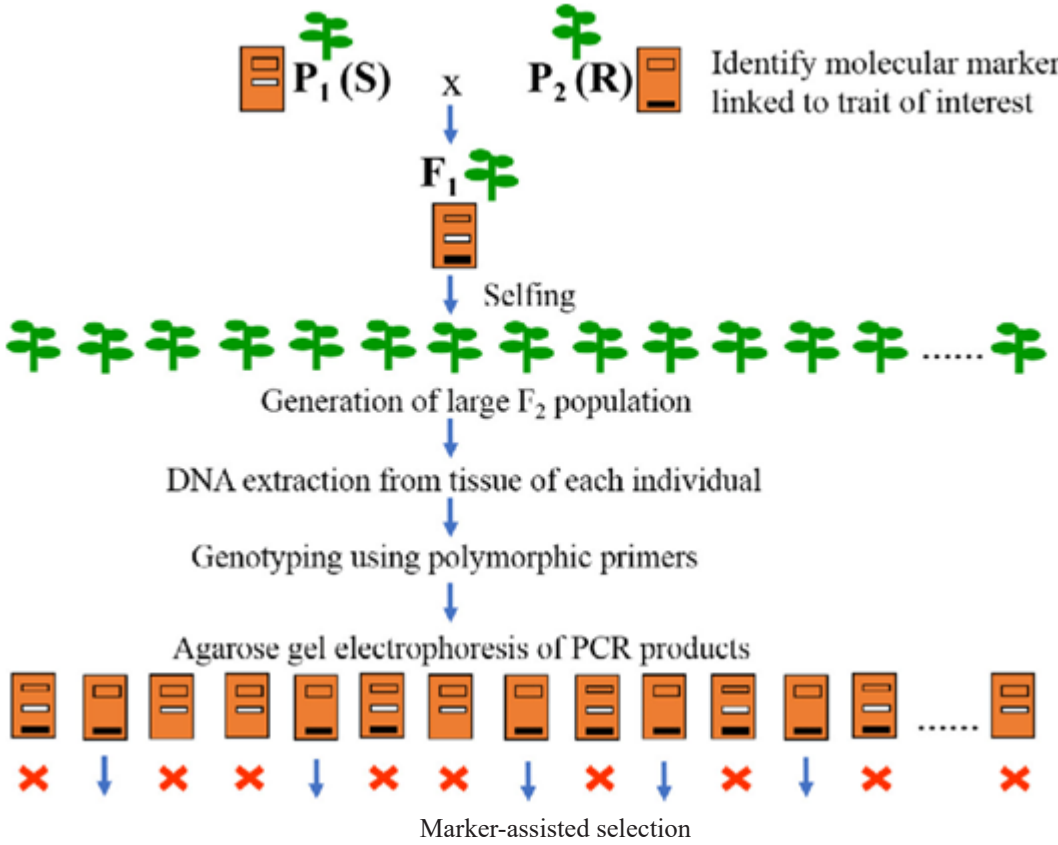

Figure 1. Basic procedure for marker-assisted selection (Rana et al., 2019)

index combining molecular and phenotypic data, and (6) genomic selection, in which genomic estimated breeding value is obtained using information from genome-wide markers.

MAS in gene pyramiding

Gene pyramiding refers to the incorporation of a desirable or resistant gene which has known effects on the target trait from multiple parents to develop superior cultivars. The more resistant gene present, the more challenging it becomes to break the resistance of the plant. Like if one plant has only one resistant gene then it may only survive for 1-2 years but with the application of gene pyramiding it may survive for many years because pathogen requires double or multiple mutation to break resistant in cultivars. Three bacterial resistance genes (*xa5*, *xa13*, and *Xa21*) were introgressed in a rice cultivar Samba Masuri which proved to have durable resistance in rice with no yield penalty (Kottapalli et al., 2010).

Pyramiding is very precise as it includes one gene only at one time. Some important things that are to be considered while selecting such genes are the pathogens should be avirulent to the resistant gene i.e. allele frequency of corresponding Avr gene must be 1 and this is how cultivar remains durable (Joshi & Nayak, 2010). Cultivar with durable and broad spectrum resistance is desired and can be achieved by combing different resistance genes through marker assisted gene pyramiding (Liu et al., 2000). The main advantages of using it are it helps to develop durable resistance, eliminates extensive phenotyping, control linkage drag and breeding duration are reduced. Some examples of application of MAS for gene pyramiding in various crops are presented in Table 2. When the markers are tightly linked to resistant gene, with the help of marker phenotype numbers of the resistant gene carried by progeny can be identified indirectly. It has been found that through the incorporation of multiple genes, durable (broad spectrum) resistance against certain pathogens can be obtained (Kloppers & Pretorius, 1997; Shanti et al., 2001). When qualitative resistance fails, quantitative resistance can assist as an insurance policy as in the single stripe rust gene and two QTLs pyramiding (Castro et al., 2003). We can undergo pyramiding using multiple parents and their number of genes as pyramiding into indica rice cultivar PR106 with the use of three bacterial blight resistance genes (Singh et al., 2001). Pyramiding of genes like *Xa1, xa5, xa13, Xa21, Xa26* and *Xa27* in rice (*Oryza sativa* L.) for resistance to bacterial leaf blight disease has also been reported (Chu et al., 2006; Chukwu et al., 2019; Sun et al., 2004). Marker Assisted Gene Pyramiding has also been done for bacterial blight and blast resistance with the use of marker-assisted backcrossing strategy and pyramiding two Bacterial Blight resistance genes (*Xa21* and *xa13*) and two major blast resistance genes (*Pi54* and *Pi1*) into mega rice variety "Tellahamsa" (Jamaloddin et al., 2020).

MAS in back crossing

Molecular markers are broadly used in improving efficiency of backcrossing to develop high yielding superior cultivars that contributes to the higher yield. In this backcrossing process, the donor's genetic background is removed and that of a recurrent parent is recovered. This process takes longer time and is unreliable and therefore MAS in back crossing aids in transferring the beneficial gene to the recurrent parent determining young plants containing preferred trait and removing all the stray donor genes. Effectiveness of marker-assisted backcrossing depends on each backcross generation population, a distance between the target locus and marker, and the numbers of background markers in use (Hasan et al., 2015). Effective marker backcrossing occurs in three ways (Collard & Mackill, 2008; Holland, 2004). Firstly, Foreground selection which refers to the using of markers that control the gene of interest for its selection used for such qualities having tough or long phenotypic screening procedures, for knowing about the plants' reproductive performance in the early stage of its growth and also for selecting recessive alleles. Secondly, a recombinant selection signifies the selection of progeny from backcross containing the gene of interest and linked flanking markers which help in reducing the undesirable gene containing in the chromosome segment of the donor and thus helps in minimizing linkage drag. Thirdly, background selection denotes the selection of the progeny from backcross that contains recurrent parent's genome that is not linked to the target locus. This helps to recover recurrent parent with less backcrossing (even maybe in BC_2) with an additional gene which is called complete line conversion.

As a combination of methods, Marker assisted backcross-based gene pyramiding can be accomplished in three schemes (Servin et al., 2004) and (Malav & Chandrawat, 2016). Different Schemes of gene pyramiding are given in Figure 2.

In the first scheme, F1 hybrid is produced from the cross between recurrent parent and donor parent which then gives improved recurrent parent when F1 hybrid is backcrossed up to third generation. Then, with the crossing between improved recurrent parent and other donor parent yields pyramid multiple genes. However, this is less acknowledged because it is time-consuming. In the second scheme, F1 hybrid is produced with the crossing between the recurrent parent and donor parents. Then, improved F1 is produced through intercrossing which then gives improved recurrent parent when backcrossing of improved F1 is done with the recurrent parent that may results in the loss of pyramided gene. The third scheme is the combination of the first and second scheme in which instantaneous crossing between the recurrent parent and number of donor parents takes place and the result from this cross is allowed to backcross up to the third generation and finally yields pyramided lines when intercrossed

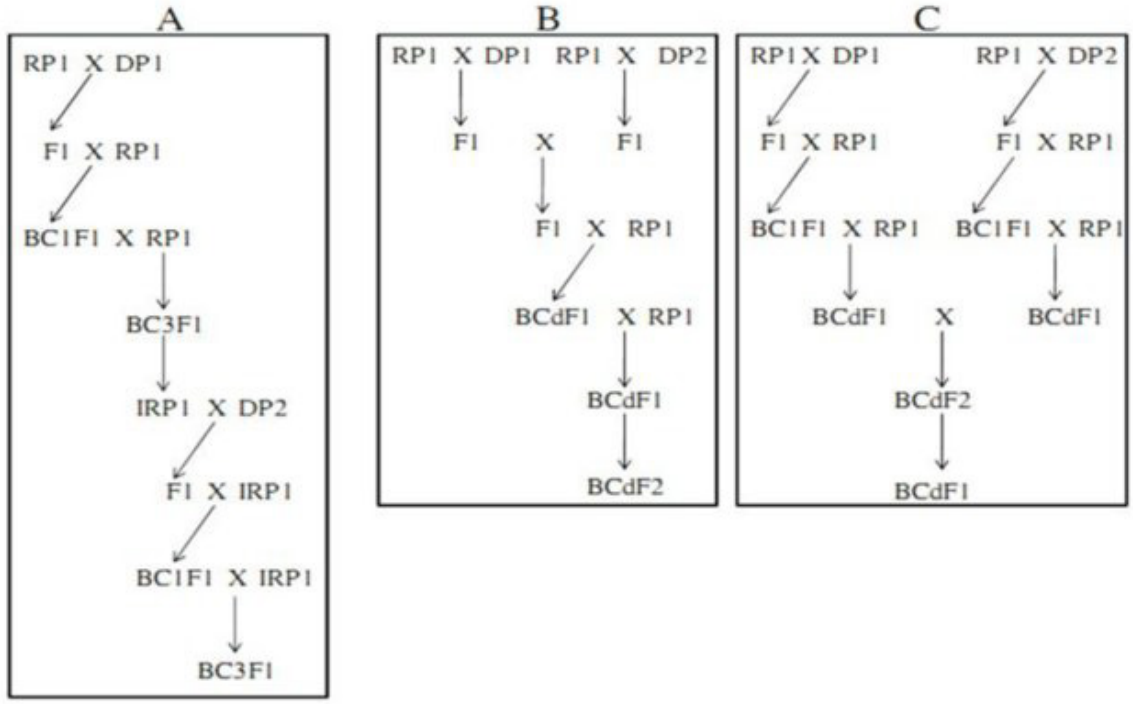

Figure 2: Different Schemes of gene pyramiding. RP= Recurrent parent; DP= Donor parent; BC= Backcross; IRP= Improved recurrent parent. A. Stepwise transfer; B. Simultaneous transfer; C. Simultaneous and stepwise transfer. (Adopted from Malav & Chandrawat, 2016).

Applications of MAS in rice breeding

MAS in rice breeding for bacterial leaf blight

Bacterial blight (BB) is one of the most destructive rice. Twenty-eight genes conferring resistance to bacterial leaf blight (BB) have been reported in rice (Nino-liu et al., 2006). Several genes have been associated with tightly linked DNA markers, and some of them have been cloned (Xa1, xa5, xa13, Xa21, Xa26, Xa27) and used for breeding BB-resistant rice cultivars. With the exception of xa5 and xa13, the BB resistance genes are dominant in nature and the markers are developed from the sequencing information of these genes, which are widely used in MAS (Chu et al., 2006). The resistance genes xa5, xa13, and Xa21 have been pyramided into an indica rice cultivar (PR106) using MAS that expressed strong resistance to BB races of India (Singh et al., 2001).

MAS in rice breeding for blast disease

Blast disease is one of the most serious diseases of rice. Blast resistance is governed by a specific interaction of a particular resistance (R) gene in rice with a particular avirulence gene in the pathogen. Since the initial definition of the plant resistance (R) genes by Flor (1942), many R genes have been identified. The vast majority of the known R genes is composed of proteins carrying nucleotide-binding sites and leucine-rich repeat motifs (NBS-LRR) (Jones & Dangl, 2006). Many R genes have been identified in rice and most code for NBS-LRR genes. About 40 major blast genes have been identified, about 30 genes have been mapped on different rice chromosomes, and tightly linked DNA markers have been developed. The DNA markers have been used effectively to identify resistance genes, and MAS has been applied for integrating different resistance genes into rice cultivars lacking the desired traits. The PCR-based allele-specific and InDel marker sets are available for nine blast resistance genes, and they provide an efficient marker system for MAS for blast resistance breeding (Hayashi et al., 2006).

The breeding works in rice using MAS is given in Table 2.

Application of MAS in other various crops

The efforts and mechanism of MAS in plant breeding in various crops are given in Table 3.

MAS *vs* Conventional breeding

Conventional breeding is the traditional types of breeding which involve the production of cultivars using old tools and techniques and not as sophisticated as modern breeding technology. Marker-assisted selection makes phenotypic evaluation in laboratory relatively easy than conventional breeding. It is very hard to achieve pyramiding with the conventional methods (Collard & Mackill, 2008). The difference between MAS and conventional breeding is given in Table 4.

Table 2. Breeding works in rice using MAS

Crop	Target trait(s)	(Target gene) and Marker type	Reference
(a) Marker-assisted gene pyramiding			
Rice	Bacterial leaf blight resistance	(*Xa1, xa5, xa13, Xa21, Xa26* and *Xa27*); PCR	(Chu et al., 2006; Chukwu et al., 2019; Sun et al., 2004)
	Two Bacterial Blight resistance genes and two Blast resistance genes into mega rice variety "Tellahamsa"	(*Xa21*, *xa13* and *Pi54, Pi1*); pTA248 (*Xa21*), xa13prom (*xa13*), Pi54MAS (*Pi54*) and RM224 (*Pi1*)	(Jamaloddin et al., 2020)
	Bacterial blight and Blast resistance into Indian rice variety MTU1010	(*Xa21*, *xa13* and *Pi54*); SSR	(Arunakumari et al., 2016)
	Bacterial leaf blight resistance	(*Xa21* and *xa13*); (*Xa7* and *Xa14*); R gene pyramid of *(Xa4, xa5* and *Xa21*)	(Arshad et al., 2016)
	Blast resistance	(*Pi1+Piz-5+Pita*); RFLP, PCR-based SAP (*Pi-tq5*, *Pi-tq1*, *Pi-tq6*, *Pi-lm2*); RFLP	(Hittalmani et al., 2000; Tabien et al., 2000)
	Brown plant hopper resistance	(*Bph14* and *Bph15*); SSR and InDel markers	(Hu et al., 2012)
	Gall midge resistance and bacterial blight to RPHR-1005	(*Gm4*, *Gm8* and *Xa21*); SSR	(Kumar et al., 2017)
	Blast resistance and bacterial blight resistance in GZ63S	(*Pi9* and *Xa23*); SCAR	
	Blast resistance genes in Swarna-Sub1	(*Pi1, Pi2*, and *Pi54*)	(Patroti et al., 2019)
	Stripe Disease Resistance and Eating Quality of Wuyujing 3	(*Stv-bi* and *Wx-mq*) ; PCR	(Tao et al., 2016)
(b) Marker assisted backcrossing			
Rice	High-yielding drought-tolerant NILs of Sabitri	2 QTLs (*qDTY3.2* and *qDTY12.1*)	(Dixit et al., 2017)
	Bacterial blight resistance	(*xa5*, *xa13*, and *Xa21*); SSR	(Ramalingam et al., 2017)
	Resistance to blast, gall Midge, submergence, and salinity in a released rice variety CRMAS2621-7-1	blast (*Pi2, Pi9*), gall Midge (*Gm1, Gm4*), submergence (*Sub1*), and salinity (*Saltol*); SSR	(Das & Rao, 2015)
	Bacterial blight resistance	(*Xa23*)	(Ji et al., 2014)
	Bacterial blight resistance in deepwater rice variety, Jalmagna	(*xa5+xa13+Xa21*); STS	(Pradhan et al., 2015)
	Brown plant hopper resistance	(*Bph14* and *Bph15*); SSR and STS	(Xu, 2013)
	Bacterial blight resistance in Improved Samba Mahsuri	(*Xa38*); SSR	(Yugander et al., 2018)
	Blast resistance	(*Pi54, Pi1* and *Pita*); SSR and STS	(Khan et al., 2018)
	Bacterial blight and blast resistance gene into JGL1798	(*Xa21,xa13 and Pi54*); SSR	(Swathi et al., 2019)
	Blast resistance in variety ADT43	(*Pi1, Pi2* and *Pi33*); SSR	(Divya et al., 2014)
	Cooking and eating quality	(*Waxy gene region*); AFLPs	(Zhou et al., 2003)
	Bacterial blight and blast resistance into RPHR-1005	(*Xa21* and *Pi54*); SSR	(Kumar et al., 2016)
(c) Marker-assisted validation			
Rice	Bacterial blight resistance	*(Xa39)*; SSR	(Zhang et al., 2015)
	Bacterial blight resistance	*(Xa40)*; RM27320 and ID55	(Kim et al., 2015)
	Heat resistance	(*qHTSF4.1*); M4	(Nogoy et al., 2016; Ye et al., 2015)
	Deep roots	(QTLs on 1, 2, 7 and 9 chromosomes); RFLP and SSR	(Hasan et al., 2015)
	Heading date	(QTLs Hd1,Hd4, Hd5, or Hd6); RFLP, STS, SSR, CAPS, dCAPs	(Hasan et al., 2015)
	Quality	(Waxy); RFLP	(Hasan et al., 2015)
	Brown plant hopper	*(Bph25, Bph26)* RM6273, RM6775	(Kurokawa et al., 2016)

Table 3. Breeding works on various crops using MAS

Crop	Target trait(s)	(Target gene) and Marker type	Reference
(a) Marker-assisted gene pyramiding			
Wheat	Powdery mildew resistance	(*Pm2+Pm4a*; *Pm2+Pm21*; *Pm4a+Pm21*) ; combinations RFLP	(Liu et al., 2000)
	Leaf rust resistance	(*Lr19* and *Lr24*);SSR and SCAR	(Singh et al., 2017)
	Leaf rust resistance	(*Lr19* and *Lr24*); STS	(Singh et al., 2004)
	FHB resistance	(3 QTL); SSR	(Miedaner et al., 2006)
	FHB resistance and DON content	(3 QTL); SSR	(Wilde et al., 2007)
	Cereal cyst nematode resistance	(*CreX* and *CreY*); SCAR	(Barloy et al., 2007)
	FHB resistance	(3 QTL); SSR	(Wilde et al., 2008)
Maize	Enrichment of lysine and tryptophan	(*opaque2* and *novel opaque16*); umc1066, umc1141 and umc1149	Sarika et al. (2018)
Broccoli	Diamondback moths resistance	(*cry1Ac+cry1c*)	(Cao et al., 2002)
Soybean	Lepidopteron resistance	(*cry1Ac+corn earworm QTL*)	(Walker et al., 2002)
	Soybean mosaic virus resistance	(*RSC4*, *RSC8*, and *RSC14Q*); SSR	(Wang et al., 2017)
	Soybean mosaic virus resistance	(*Rsv1*, *Rsv3*, and *Rsv4*); SSR markers (Sat_154 and Satt510) and a gene-specific marker (Rsv1-f/r)	(Shi et al., 2009)
	Soybean rust resistance	(*Rpp2*, *Rpp3* and *Rpp4*); Markers Satt460 and AF162283	Maphosa *et al.* (2012)
Pea	Powdery mildew resistance	(*er1*,*er2* and *Er3*) RFLP, RAPD/ SCAR and SSR	(Ghafoor & McPhee, 2012)
Mung bean	Powdery mildew resistance	(*PMR1*, *PMR2*); RFLP, AFLP	(Chaitieng et al., 2002; Humphry et al., 2003; Miyagi et al., 2004)
Apple	Apple scab resistant	(*Rvi2*, *Rvi4*, *Rvi5*, *Rvi6*, *Rvi11*, *Rvi12*, *Rvi13*, *Rvi14* and *Rvi15*); SSR and SCAR	(Patocchi et al., 2009)
(b) Marker assisted backcrossing			
Wheat	HMW-glutenins	(*Glu A1* and *Glu-D1* genes); AS-PCR	(De Bustos et al., 2001)
	Fusarium head blight (FHB), orange blossom wheat midge, leaf rust resistance	8 QTL and Sm1 and Lr21	(Somers et al., 2005)
	Powdery mildew	(*Pm1c*, *Pm2*, *Pm4b*, *Pm12*, *Pm13*,*Pm16*, *Pm20*, *Pm21*, *Pm23*, and 13 undocumented genes); AFLP	(Zhou et al., 2005)
	Stripe rust	(1 QTL); SSR	(Chhuneja et al., 2008)
Maize	Southwestern corn borer resistance	(3 QTL); RFLP	(Willcox et al., 2002)
	ProA enhancement in Sweet corn	(*lcyE*); SSR	(Yang et al., 2018)
Barley	yellow dwarf virus resistance	(*Yd2*); PCR based marker	(Jefferies et al., 2003)
(c) Marker-assisted validation			
Wheat	FHB resistance	(1 QTL);SSR	(Pumphrey et al., 2007)
	Scab resistance	(1 QTL); SSR	(Zhou et al., 2003)
	Powdery mildew resistance	(3 QTL); SSR	(Tucker et al., 2006)
	Leaf rust resistance	(*Lr1*, *Lr9*, *Lr24*,*Lr47*); STS, SCAR, CAPS	(Nocente et al., 2007)
(d) Others			
Tomato	Septoria Leaf Spot Resistance	(2 inbred lines *NC 85L-1W* (2007) and *NC 839-2*(2007)-1); RAPD	(Joshi et al., 2015)
	Tomato mosaic virus	(*Tm-1, Tm-2,* and *Tm-22*); PCR-based markers	(Osei et al., 2019)
	Powdery mildew resistance	(*ol-2* gene); RAPD, AFLP	(De Giovanni et al., 2004)
Potato	Potato virus Y resistance	(Ry_{adg} gene); RFLP	(Hämäläinen et al., 1997)
	Late blight of potato resistance	(*R1* gene); RFLP and AFLP	(Meksem et al., 1995)
Rose	Powdery mildew resistance	(Single gene *Rpp1*); AFLPs, RGAs	(Linde & Debener, 2003; Linde et al., 2006)
Barley	Powdery mildew resistance	(*Mlg* resistance locus); RFLP	(Kurth et al., 2001)
Common bean	Anthracnose resistance	(gene *Are*); SCAR, RAPD and RFLP	(Adam-Blondon et al., 1994)
Apple	Powdery mildew resistance	(Single gene *Pl-w*); Isozymes, SCAR, SSR, AFLP, RAPD	(Batlle & Alston, 1996; Evans & James, 2003; Hemmat et al., 1994; Liebhard et al., 2002)

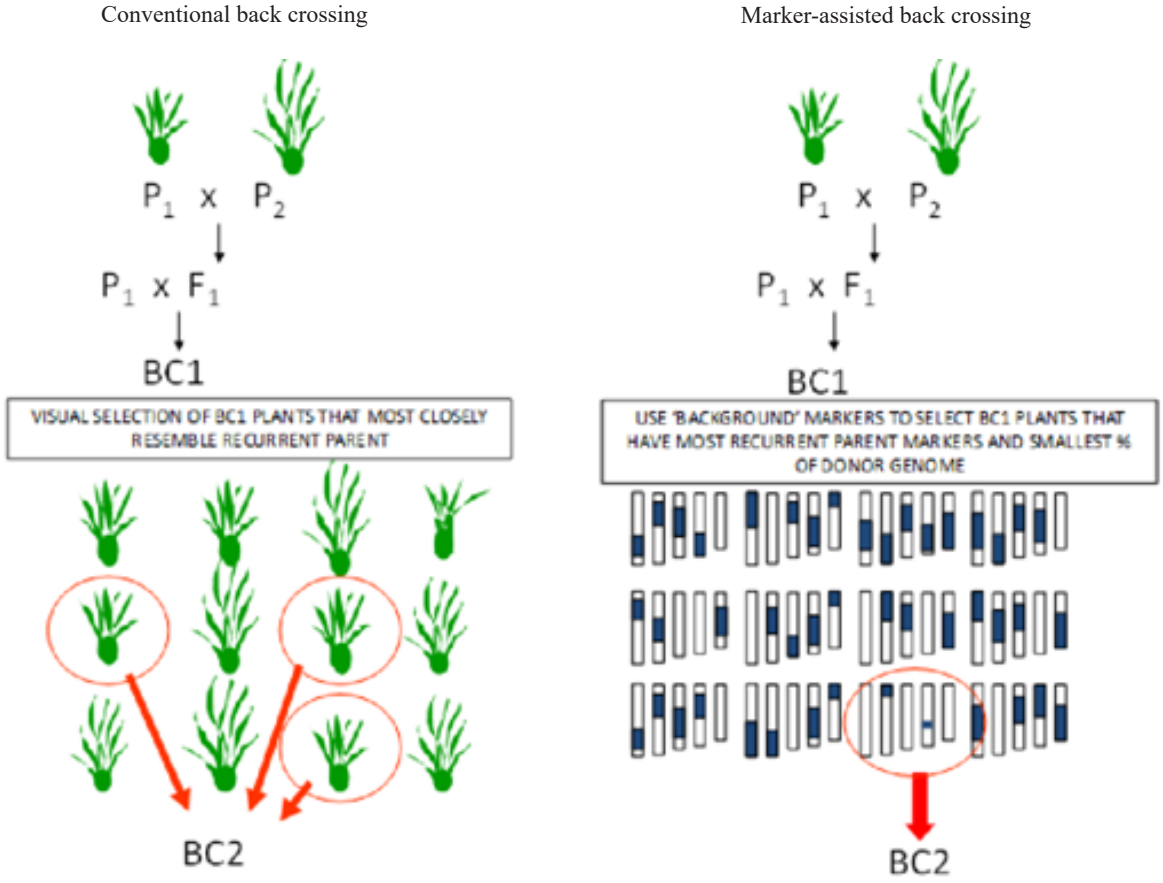

Figure 3: Comparison between conventional backcrossing and background selection during marker assisted backcrossing (Rani et al., 2014)

Table 4. Differences between MAS and Conventional breeding (Dreher et al., 2002)

MAS	Conventional breeding
Marker Assisted Selection aids in determining specific plants with all the resistance alleles imparting more durable multi-genic resistance.	Different allele separately imparts same resistance to particular disease which creates confusion in the multi-genic resistance phenotypic selection.
Marker Assisted breeding provides an opportunity to discard the plants without desirable allele or alleles in the early stage of their growth by observing the banding pattern after running the gel electrophoresis.	Conventional breeding cannot be used to detect desirable trait until the plants' area well established in the field.
Banding patterns can be evaluated for screening the alleles presence that is linked to all those traits.	For the improvement of the plant to make it stress tolerance, insect and disease resistance, screening for each trait must be done through separate trials.
With the aid of markers, the presence or the absence of concerned allele/alleles can be screened without any particular seasonal consideration.	Conventional breeders can improve their cultivar making it cold tolerance only by screening in the cold season that results in lower breeding rate.
It is possible to determine allele or alleles associated with the resistance to a specific pest with the use of the markers.	Conventional breeding does not allow the screening for the resistance of parasitic pest of another country in own country.
Molecular markers aid in undergoing the process of line conversion faster than that of conventional breeding.	The process of line conversion is lagged as conventional breeding is incapable in passing recessive desired alleles into subsequent generation as quickly as with the aid of markers since breeders cannot identify heterozygous plant phenotypically and should undergo selfing several times for its accomplishment.

Table 5. Advantages and Disadvantages of MAS and Conventional breeding (Lema, 2018)

Advantages	Disadvantages
(a) Marker Assisted Selection (MAS)	
It involves genotypic selection of traits of interest of plants through the use of molecular markers.	This method is expensive in genotyping large number of plants.
It helps to maintain high level of genetic purity through cultural identification.	There may be low level of recombination between marker and QTL resulting in the need of flanking markers.
It is useful for genetic diversity assessment and selection of parents.	It is still not widely used due to less researches, published papers, knowledge gap and limited polymorphic markers.
Marker assisted backcrossing helps to reduce linkage drag and parent's genotype can be reconstructed in three generations.	Marker assisted backcrossing is capable of refining only the existing elite genotypes of plants.
Pyramiding of desirable genes is easy, fast and an early stage screening is possible.	There may arise the problem in the exact determination of position and effect of QTL.
Poor heritability and environmental factors do not create problem.	It cannot predict phenotype with 100 percent reliability.
(b) Conventional breeding	
It is being used widely in the development of cultivars.	Its phenotypic selection resulting in longer time to develop superior variety.
It is simple and easy as there is no need of consideration of QTL and target gene.	It requires to undergo 'grow-out tests' for the assessment of purity.
Publications of researches based on it are easily available.	It doesn't deal with the genetic diversity and it is difficult to distinguish homozygous and heterozygous plants just from the phenotype.
It is breeder-friendly.	Recurrent parent genotype reconstruction takes more than six generations.
It is cheap and more reliable method.	Time consuming and hard to test phenotypically the presence of more than one gene.

The comparison between conventional backcrossing and background selection during marker assisted backcrossing is given in Figure 3.

The advantages and disadvantages of MAS and conventional breeding is given in Table 5.

Importance of MAS

It makes efficient use of glasshouse or nursery making the selection possible in the seedling stage as several lines can be discarded early in the breeding scheme which is non-profitable. MAS allows a single selection of plants as screening is carried out using markers that eliminate error due to environmental factors. MAS is not affected by environmental factors and allows for the determination of certain traits (resistance to disease, insect, abiotic stress) independent of the environment as indirect selection of traits is done with the use of markers (Osei et al., 2019). It can even save breeders time, resources, and effort. It also aids in the enhancement of the heterosis, high-density linkage maps construction. Genetic contribution of each parent to its each progeny can also be determined with the aid of marker assisted selection and enables the effective selection for horizontal resistance. RFLP and SSR/microsatellites are co-dominant markers which are technically simple, reliable, robust, and transferable between populations (Kochert, 1994; McCouch et al., 1997; Tanksley et al., 1989). RAPD and AFLP are dominant markers which are quick, simple, a small amount of DNA required and have possibility of multiple loci and generation of the high level of polymorphism respectively (Vos et al., 1995; Welsh & McClelland, 1990; Williams et al., 1991). Marker assisted selection helps in genes pyramiding and also makes backcrossing more efficient. It helps in visualizing the loci for quantitative resistance and compilation of QTLs from different donors into one genotype to promote the level of quantitative resistance. Desirable allele can be recognized in the initial stage as reported for QPM; mutant opaque2 allele can be spotted with the increase in the level of lysine and tryptophan in the kernel in the initial stage of plant growth before the visibility of its reproductive life that will ultimately be economic (Dreher et al., 2002). Stress Resistant and Quality of Rice can be obtained with the aid of marker assisted selection through gene stacking (Das et al., 2017). When recessive alleles governs the trait of our interest but it's challenging enough to detect that alleles from phenotypic evaluation of heterozygous plant and from the traditional backcrossing method as it turns out to be time and resource consuming; MAS makes our work much easier for the detection of recessive alleles with the application of the markers linked with them.

Limitations of MAS

Markers may not be useful for every trait as an effective phenotyping method already exist which is less expensive than MAS. The type of information that is required for conducting QTL validation and mapping has a limited number of published reports. It is an expensive method as it includes large start-up expenses, licensing costs, maintenance costs, etc. There are limited markers with limited polymorphism. Like, the SSR marker in wheat was utilized for indication of the Sr2 gene responsible for stem rust resistance for all except for four Australian cultivars which is susceptible to it (Spielmeyer et al., 2003). Sometimes there is insufficient linkage between markers and genes. Recombination events may occur between the gene of our interest and marker used which may lead to false positive. While conducting MAS, the interaction between quantitative trait loci and environmental effects are not considered. Markers that are developed for MAS may be valid for one population and may not be valid for the other. Knowledge gap between molecular biology experts and the breeder creates the problem in the understanding of the concepts and the language used by the expert (Collard et al., 2005). There is also inadequate coordination between different researchers and plant breeders.

Conclusion

The scope of Marker Assisted Selection is going to be wider as more and more genes are identified and their functions and interactions are annotated. MAS is used to accelerate the recurrent parents' retrieval with the aid of molecular backcrossing. The use of markers that flank a target gene can minimize the number of backcross generations. MAS technology has been successfully utilized for the breeding of disease-resistant crops. Rice yield is subjected to severe losses due to adverse effect of a number of stress factors; utilization of tolerant/resistant cultivars is the most effective method of controlling reduced crop production. Through the process of gene pyramiding, multiple stress resistant genes could be incorporated into a single rice variety in order to develop a rice variety with high yield, biotic stress resistance and abiotic stress tolerance along with enhanced nutritional quality.

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