

Review

Marker assisted selection in plant breeding

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Accepted 18/07/2020

Abstract

Marker assisted selection (MAS) is 'smart breeding' or fast track plant breeding technology. It is one tool utilized in breeding companies and research institutes for fast development of improved varieties, giving possibility to select desirable traits more directly using DNA markers. In this review, we discussed the use of MAS in biotic, abiotic, quality and other agronomic traits. Besides, we emphasized the importance of MAS at ICARDA and underlined the successful application of MAS in the last 10 years. The use of molecular markers makes the process of selecting parental lines more efficient based on genetic diversity analysis. It can aid the conventional breeding, especially for certain biotic and abiotic traits laborious to manage. Still, MAS contributed very little to the release of improved cultivars with greater tolerance to abiotic stresses, with only a few exceptions. MAS was extensively used to improve rice varieties, mainly resistant to bacterial blight and blast disease and was applied in drought tolerance along with GPC (Grain protein content) in quality traits. MAS at ICARDA is used to characterize new parental materials for disease resistance genes as well as in screening advanced lines with a focus on association mapping and identification of new QTLs. The application of MAS increased in the last decade. It is more and more used in different crops. However, rice is still the dominant crop in terms of number of publications using MAS.

Keywords: marker assisted selection, plant, biotic stress, abiotic stress, quality, ICARDA

INTRODUCTION

Wheat breeders continuously seek for new techniques which can be used for assembling target traits into new wheat cultivars and achieve the same breeding progress in a much shorter time than through conventional breeding. The main goals of wheat breeding are increasing the yield, improving the resistance to abiotic and biotic stresses, improving the quality. While simple traits can easily be detected, other complex traits such as disease resistance or drought tolerance are much more difficult to determine for the breeder. Young (1999) wrote: "Before the advent of DNA marker technology, the idea of rapidly uncovering the loci controlling complex, multigenic traits seemed like a dream". Now with DNA marker technology, this dream became reality. The capacity of DNA markers to detect allelic variation in the genes underlying traits offers a great promise for plant breeding. By using DNA markers to assist in plant breeding, efficiency and precision could be greatly increased. The use of DNA markers in plant breeding is called marker-assisted selection (MAS).

Definition of MAS

Marker assisted selection (MAS) is 'smart breeding' or fast track plant breeding technology. It is one tool utilized in breeding companies and research Institutes for fast development of improved varieties, giving possibility to select desirable traits more directly using DNA mark-

ers. The molecular markers can then be used to assist breeders track whether the specific gene or chromosome segment(s) known to affect the phenotype of interest is present in the individuals or populations of interest. The potential of MAS, thus, moving from phenotype based towards genotype based selection using markers linked to gene of interest. Thanks to the advent of DNA markers in the late of 1970s, it has now become possible to directly target genomic regions that are involved in the expression of traits of interest.

The history of Marker assisted selection

The idea of MAS begins with the theory of quantitative trait loci (QTLs) mapping described by Sax (1923), when he observed an association between monogenic trait (Seed coat pigmentation) and polygenic trait (seed size). This concept was further elaborated by Thoday (1961), who suggested mapping and characterizing all QTLs involved in complex traits using single gene marker. The first DNA-based genetic markers were restriction fragment length polymorphisms, RFLPs (Botstein *et al.*, 1980). Permit to construct the first map for tomato using 57 RFLPs in 1986 (Bernatzky and Tanksley, 1986). Beckmann and Soller (1986) described the first use of restriction fragment length polymorphism (RFLP) markers in crop improvement including theoretical issues related to marker-assisted backcrossing (MABC) for improvement of qualitative traits. Tanksley *et al.* (1989) published the use of RFLP as tool to select desir-

able lines. He reported the possibility to analyzing plants at the seedling stage, screening multiple characters that would normally be epistatic with one another, minimizing linkage drag, and rapidly recovering a recurrent parent's genotype. At that time, the idea of selection of target genes based on genotypes rather than phenotype was extremely attractive to plants breeders (Young, 1999). All those initiatives open the door to marker technology and development of simpler DNA marker involving PCR techniques such as Random-Amplified Polymorphic DNAs, RAPDs (Williams *et al.*, 1990), Amplified Fragment Length Polymorphisms, AFLPs (Vos *et al.*, 1995), Simple Sequence Repeat, SSR also known microsatellites (Powell *et al.*, 1996) and Single Nucleotide Polymorphisms, SNPs (Gupta *et al.*, 2001). Along with, the research boost in DNA marker technology and produce specific markers like Sequence Characterized Amplified Region, SCAR (Paran and Michelmore, 1993), Cleaved Amplified Polymorphic Sequence, CAPS (Maeda *et al.*, 1990), Sequence Tagged Site, STS (Olsen *et al.*, 1989), Expressed Sequence Tags, EST (Jongeneel, 2000), and most recent marker Diversity Arrays Technology, DArT (Jaccoud *et al.*, 2001).

The application of molecular marker in parental selection and predicting heterosis

The plant breeders seek ways of facilitating the use of available germoplasm effectively for plant improvement. One hand, the use of molecular markers makes the process of selecting parental lines more efficient. Based on genetic diversity calculated from fingerprinting data, plant material can be classified into genetic pools. This information can be extremely helpful for identifying the most appropriate parental lines to be crossed. Lombardi *et al.* (2014) reported that a selection of divergent parental genotypes for breeding should be made active on the basis of systematic assessment of genetic distance between genotypes, rather than passively based on geographical distance. In other hand, classify parental lines into heterotic groups for the creation of predictable hybrids (Acquaah, 2012). The concept of heterotic groups was developed by Maize research using RFLP-based genetic distances of inbreds for the prediction of hybrid performance and heterosis of single crosses in maize has given different results (Melchinger, 1993). The genetic distance estimates based on molecular marker estimates have been effective in grouping related germplasm (Melchinger *et al.*, 1998). Martin *et al.* (1995) used both pedigree records and Sequence Tagged Sites (STS) molecular markers to determine the relationship between genetic diversity and agronomic performance of the hybrids and they found significant associations between genetic distance based on pedigree and kernel weight and protein concentration of the heterosis. Zhao *et al.* (2008) and others also suggested that genetic distances revealed by molecular markers were highly and positively correlated with heterosis in rice. However, the relationship between parents and genotypic variance components in their progenies has been reported as weak or non-significant across many studies (Helms *et*

al., 1997; Burkhamer *et al.*, 1998; Melchinger *et al.*, 1998; Bohn *et al.*, 1999; Gumber *et al.*, 1999; Brachi *et al.*, 2010; Hung *et al.*, 2012).

MAS in disease resistance breeding

Plant diseases are the result of infection by other organisms that adversely affect the growth, physiological functioning and productivity of a plant. Plant diseases can drastically affect a country's economy. Therefore, disease management has always been one of the main objectives of any crop improvement program. There are at least 50000 diseases of economic plants and new diseases are discovered every year (Lucas, 1992). Plant diseases are sometimes grouped according to the symptoms they cause (root rots, wilts, leaf spots, blights, rusts, smuts), to the plant organ they affect (root diseases, stem diseases, foliage diseases), or to the types of plants affected (field crop diseases, vegetable diseases, turf diseases, etc.) (Agrios, 2004). Using plant resistance genes for developing disease-resistant varieties are a convenient alternative to other measures like pesticides or other chemical control methods employed to protect crops from diseases (Gururani *et al.*, 2012). That is the objective of plant breeding, the identification of resistant plants, which are then crossed with agronomically acceptable but susceptible plants. A program of backcrossing to the susceptible parent and selection of resistant phenotypes leads to the production of plants that are similar to the susceptible parent but having the required resistance. Breeders have successfully developed lines resistant to diseases by integrating R-genes into their cultivars. However, it is not always the case due to the time-consuming by conventional breeding process that take around 10 years, and by this time, in some instances, the pathogen has already evolved a variant that is not recognized by the improved cultivar, leading to susceptibility. DNA markers have enormous potential to improve the efficiency and precision of conventional plant breeding via marker-assisted selection (MAS) by reducing the reliance on laborious and fallible screening procedures. Especially for durable resistance or no specific, that becomes a challenge and the best way to overcome the new races pathogen evolution. The use of molecular markers in selection can aid the conventional breeding, especially for certain traits laborious to manage it. Xu and Crouch (2008) specify four kinds of traits which DNA markers should be helpful. (i) traits that are difficult to manage through conventional phenotypic selection because they are expensive or time-consuming to measure, have low penetrance or complex inheritance; (ii) traits whose selection depends on specific environments or host developmental stages; (iii) maintenance of recessive alleles during backcrossing or for speeding up backcross breeding in general; and (iv) pyramiding multiple monogenic traits or several QTL for a single disease resistance with complex inheritance. Several studies reported the application of molecular markers as a tool to assist phenotypic method to improve concerned traits. For example, Miklas *et al.*, (2006) reported in bean that the most effective strategy to improve bean host plant resistance

to common bacterial blight was a combination of MAS with periodic phenotypic selection, because it allows the retention of minor QTL and selects epistatic interactions that contribute to improved disease resistance. Wilde *et al.* (2008) noted the efficiency of MAS with phenotypic selection combination in improving resistance against *Fusarium* head blight. One of the successful applications of MAS in breeding disease resistance was in Indonesia, and the release of two rice varieties 'Angke' and 'Conde', which are resistant to bacterial leaf blight infection (Bustamam *et al.*, 2002). Also, Zhao *et al.* (2012) succeed in introgression of qHSR1, which is a QTL related to head smut in head smut-susceptible lines via marker-assisted selection, which has significantly reduce disease incidence over time in maize.

MAS in abiotic stress breeding

Abiotic stress is defined as environmental conditions that reduce growth and yield below optimum levels. Plant responses to abiotic stresses are dynamic and extremely complex (Cramer, 2010; reviewed by Cramer *et al.*, 2011). Boyer (1982) indicated that environmental factors may limit crop production by as much as 70%. Many genes affect stress tolerance, but few of the identified genes have proven useful in the field. The genomics era has allowed dissection of the physiological and molecular traits underlying stress tolerance mechanisms to an unprecedented level. Integrated omics analyses have markedly increased our understanding of plant responses to various stresses. These analyses are important for comprehensive analyses of abiotic stress responses, especially the final steps of stress signal transduction pathways (Cramer *et al.*, 2011). The application of omics technologies has contributed to the development of stress-tolerant crops in the field. Several genes are identified to have a great role in abiotic stress tolerance. For instance, SNACs were characterized as factors that regulate expression of genes important for drought and salinity tolerance in rice (Hu *et al.*, 2006; reviewed by Todaka *et al.*, 2012). DREB1/CBF regulon involved in cold-stress-responsive gene expression, and DREB2 involved in osmotic-stress-responsive gene expression (Yamaguchi-Shinozaki and Shinozaki, 2006). The reviews of Nakashima *et al.* (2009) and Todaka *et al.* (2012) discussed more about different abiotic stress genes identified in transcriptomic analyses. This comprehensive knowledge about the genes involved in stress response and tolerance will further allow a more precise use of MAS and transgenics (Dita *et al.*, 2006). However, still MAS contributed very little to the release of improved cultivars with greater tolerance to abiotic stresses, with only a few exceptions (LeDeaux *et al.*, 2006; MacMillan *et al.*, 2006; Ribaut and Ragot, 2007; Welcker *et al.*, 2007). The marker assisted selection was applied especially in drought tolerance. For instance, Courtois *et al.* (2003) used MAS to transfer a number of QTLs related to a deep rooted character from the japonica upland cultivar "Azucena" to the lowland indica variety "IR64". MAS selected lines showed a greater root mass and higher yield in drought stress. Steele *et al.* (2004) made novel method

termed Marker-evaluation selection in rice crop. This approach used a very large segregating population derived from a wide cross between the upland variety Kalinga III and the irrigated variety IR64. The population was selected for overall agronomic performance in several target stress environments over many generations and the products from the selection were evaluated with markers. Varieties developed through MABC (e.g. Ashoka 228) have better drought resistance as they yield more than parent Kalinga III. Similarly, Steele *et al.* (2006) used marker assisted breeding program to improve some root traits related to drought tolerance in an Indian rice cultivar Kalinga III. They introgressed five QTL regions associated with root traits from Azucena into Kalinga III. The target QTL on chromosome 9 (RM242-RM201) significantly increased root lengths under drought stress.

MAS in improving agronomic and seed quality traits

Development of cultivars with high agronomic performance and good quality is preminent in crop breeding programs. Several agronomic and quality traits are polygenic trait controlled by many QTL/genes with smaller effects, such as yield and GPC, seed size seed oil content, days to flower and to maturity, fiber length and strength, etc.; or by few QTL/genes with major effects such as kernel color, flower color, stem color, etc. Those traits cannot be found through phenotypic evaluation alone because they are highly sensitive to environmental changes. In addition, it is difficult to produce ideal cultivars with high yield and good quality due to the existing negative correlation between those traits (Barnard *et al.*, 2002; Chung *et al.*, 2003; Yagdi and Sozen, 2009; Sourour *et al.*, 2018; Ma *et al.*, 2012). Therefore, Molecular detection and genetic tracking of quantitative trait loci (QTL) for agronomic and quality traits will affect positively in manipulation of those traits, and will increase the accuracy of selection. Hence, the identification of QTLs related to quality and agronomic traits is important as an entry point for marker assisted selection. Nowadays, the studies are focusing on desiccation of stable QTLs responsible for agronomic and quality traits in major crops using genome wide association mapping (GWAS), linkage mapping and single nucleotide polymorphism (SNPs). Chen *et al.* (2016) identifies useful QTL qGW4.05 related to Kernel weight and kernel size in Maize. The agronomic and quality traits of *Brassica napus* has been dissected using Genome wide association mapping and using a 6K single nucleotide polymorphism (SNP) array (Körber *et al.*, 2016). New QTLs associated with protein and oil content were identified (Cao *et al.*, 2017; Karikari *et al.*, 2019).

The MAS was extensively used for improving GPC. The selection and introgression of a high GPC allele of *Gpc-B1* has been achieved in several of the released wheat cultivars (DePauw *et al.*, 2005; Humphreys *et al.*, 2010; Randhawa *et al.*, 2013) using molecular markers. A successful example of an integrated approach of combining phenotypic selection with marker assisted backcross breeding in wheat for introgression of *Gpc-B1* in Indian

wheat cultivar HUW468 (Vishwakarma *et al.*, 2016). MAS was adopted for studying the genome composition of winter cultivars Zhengmai 7698 using closely linked or functional markers for gluten protein quality, grain hardness and flour color (Li *et al.*, 2018). It was used to improve oil content in sunflower, and the Marker F4-R1 was validated and proved to be the most efficient in detecting high oil content in sunflower (Dimitrijević *et al.*, 2017). Besides, The MAS was frequently used in the most important trait, yield. Liang *et al.* (2004) developed a new stable improved line '9311xOryza rufipogon' with yield-enhancing genes and high yield potential using SSRs tightly linked markers. Kumar *et al.* (2018) combined grain yield and genotypic data from different generations (F3 to F8) for five marker-assisted breeding programs for analyzing the effectiveness of synergistic effect of phenotyping and genotyping in early generations. They found genotyping and phenotyping cost savings of 25–68% compared with the traditional marker-assisted selection approach.

Marker assisted selection at ICARDA

Crop improvement at ICARDA aims to conserve agricultural biodiversity in dry areas and to use these resources to improve food crops through breeding. It covers durum and bread wheat, barley, chickpea, lentil, faba bean, grasspea, and forage and pasture crops. ICARDA's approach combines conventional and biotechnology research to identify molecular markers and to use it. Identification and utilization of molecular markers for marker assisted selection would enhance the development of widely adapted and high yielding varieties with resistance/tolerance to abiotic and biotic resistance and acceptable level of end use quality. The benefit of this 'marker-assisted selection' is that it will make the breeding process faster and more precise. As a result, breeders and farmers will see rapid improvements in crop production, enabling them to improve livelihoods and boost food security.

MAS at ICARDA is used to characterize new parental materials for disease resistance genes (stripe rust, leaf rust, stem rust, nematodes); insect resistance (Hessian fly and Russian Wheat Aphid), phenological traits such as photoperiodism (Ppd), vernalization requirement (Vrn); plant height (Rht), grain hardness and other desirable genes (Tadesse *et al.*, 2012 and 2016). Molecular markers are also used for pyramiding different resistance genes and developing multi-line cultivars targeting for durable resistance to the disease. It helps of screening real hybrids F1, F2, BC1F1 populations. The use of molecular markers and MAS started at ICARDA since long, by identifying and mapping gene resistance to lentil, pea and chickpea pathogen (Baum *et al.*, 2000). The use of molecular techniques and biotechnology tools have expanded considerably, the techniques are applied to almost all crops and concentrated on the development of marker-assisted selection and characterization and identification of fungal pathogens and nematodes. ICARDA has focused on the propagation of the molecular techniques and their application in crop improvement by organizing extensive training to young researchers, students,

junior level scientists, and also technicians (Ryan *et al.*, 2012). CIMMYT, Biodiversity, International Centre for Agricultural Research in the Dry Areas (ICARDA), and IRRI have partnered with national research organizations from 13 countries in Africa and South Asia to co-generate and share technologies for genetic characterization and marker-assisted improvement of wheat, barley, and rice, focusing on traits and alleles that are important for the crops adaptation to climatic changes (Halewood *et al.*, 2018). Several works done by ICARDA scientists and students on MAS were published. (Halewood *et al.*, 2018) discriminates between resistant and susceptible chickpea genotypes using two codominant markers associated to Ascochyta blight. Molecular marker associated with grain yield under drought conditions such as the CID, are actively and effectively used in the ongoing breeding program (Nachit, 1998; Nachit and Elouafi, 2004). Dura *et al.* (2012) identified potential targets for MAS of grain yield improvement in durum wheat in ICARDA laboratory. Recently, the markers assisted selection has been successfully used to enhance tolerance against Barley scald (Sayed & Baum, 2018). Nowadays, ICARDA is focusing on Association mapping (AM) using phenotypic and genotypic data of association panels, due to the importance of this approach in identifying molecular markers (QTLs) linked to traits of interest for potential use in marker assisted selection. In barley, association mapping was undertaken to identify QTL effective against Psh individual races at seedling stage and QTL for quantitative resistance to barley stripe rust at seedling and adult plant stages (Visioni *et al.*, 2018). In wheat, genome-wide association mapping (GWAM) was employed using DArT markers technology and ICARDA's elite wheat genotypes to identify markers linked to stripe rust resistance genes in wheat for possible use in MAS (Tadesse *et al.*, 2014; Jighly *et al.*, 2015) employed genome-wide association mapping (GWAM). In pulse, the association mapping was designed to determine the genetic basis of seed Fe and Zn concentration in lentil by using single-nucleotide polymorphism (SNP) array derived from cultivated lentil sequences (Singh *et al.*, 2017).

Successful application of MAS in last decade

The MAS of smart breeding method is the method of choice for all breeders. It has been implemented in different crop programs. Several publications declare the application of MAS in crop improvement. But still the number of successful application of this method is less compared to the number of QTLs mapped or markers developed. Moreover, most marker associations are not robust enough for successful marker assisted selection (Young *et al.*, 1999). By using Harzing's Publish or Perish software (Harzing, 2007) and using the query 'Marker assisted selection' in Google scholar and in Scopus between 2010 and 2019, around 571 publications were retrieved in which the title included 'Marker assisted'. At first sight it was often difficult to distinguish from the title whether a publication is actually reporting a MAS application or if only potential MAS applications of the actual research outputs are discussed. Therefore, the publications were

selected by reading the abstracts and sometime the material and methods to distinguish the real application of MAS. The results mentioned in table 1 is the number of publications harvested using MAS keyword. Among 571, only 189 publications were the real applications of MAS. Whereas, others publications were reviews of MAS (163 publications), QTL mapping or identification and/or marker development and validation (149 publications), Characterization and genetic diversity (47 publications) or genomic selection (23 publications). The MAS practical publications were dominant in rice with 87 publications (Figure 1), 29 of them are on bacterial blight diseases. The number of publications in other cereals was limited to 39 publications (18 publications in wheat, 18 publications in Maize and 3 publications in Barley). Marker assisted backcrossing (100 publications, Figure 2) has been most widely and successfully used

Table 1: Total number and type of publications retrieved from Harzing’s Publish from 2010 to 2019 (Harzing, 2007)

Type of publications	Number of publications
MAS articles	189
Reviews	163
Characterization or genetic diversity	47
Mapping or marker development	149
Genomic selection	23
Total	571

up-to-date, compared to other methods such as pedigree method (40 publications), pyramiding (45 publications) and MARS (4 publications). It has been applied to different crops, e.g. rice, wheat, maize, barley, pear millet, soybean, tomato, etc.

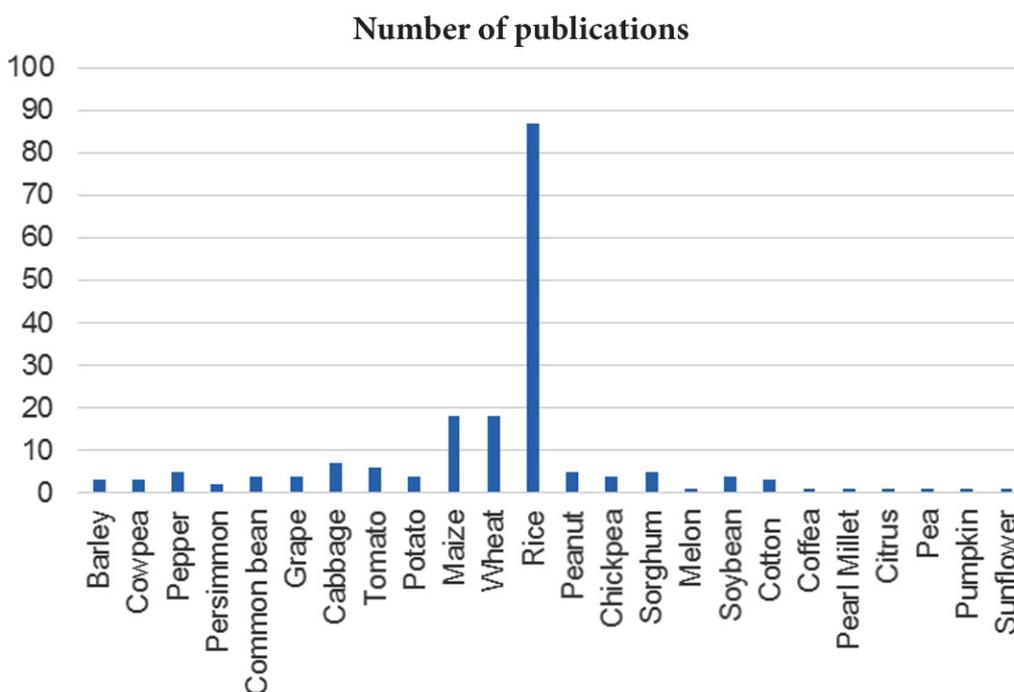


Figure 1: Number of MAS publications applied to different crops in the last 10 years

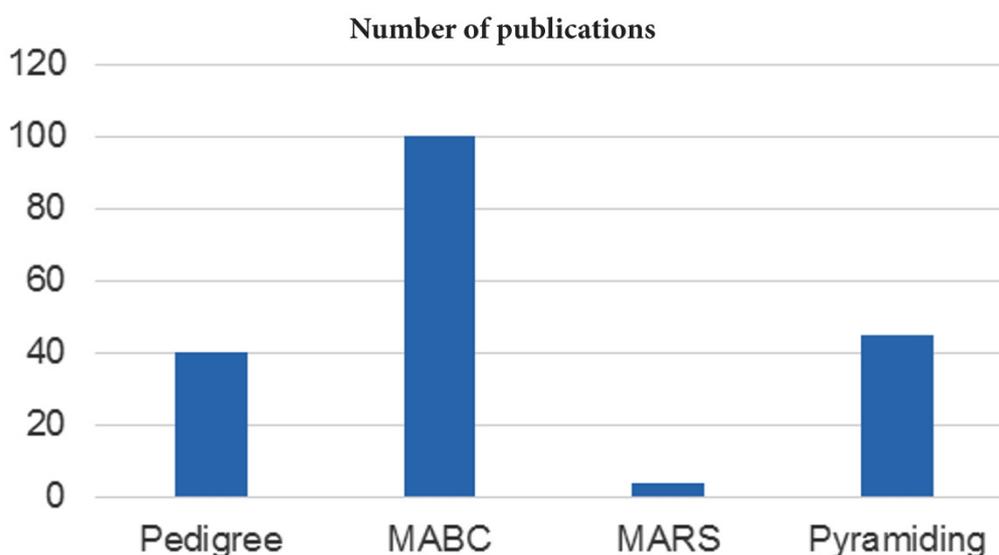


Figure 2: Number of publications for different types of MAS collected during the last 10 years

Table 2: Examples of successful use of marker assisted selection in different crops for the last 5 years

Target trait	Gene (s)/QTL(s)	Type of Marker used	Name of marker used	Crop	Reference
Blast	Pi2	STS	Pi2-4, HC28	Rice	Yang <i>et al.</i> , 2019
Bacterial blight and aroma	Xa21, xa13, xa5, fgr	STS	pTA248, RG136, RG556, BAD2	Rice	Baliyan <i>et al.</i> , 2018
Quality protein	Opaque2 (o2)	SSR	<i>umc1066</i> and <i>phi057</i>	Maize	Hossain <i>et al.</i> , 2018; Pukalenty <i>et al.</i> , 2019
Scald	Rrs1	SSR/SCAR	Ebmac0871-SSR, HVS3-SCAR, Bmag0006-SSR	Barley	Sayed and Baum, 2018
HMW, Grain hardness, Lipoygenase, Yellow pigment content, Polyphenol oxidase, Powdery mildew, Yellow rust, Pre-harvest sprouting		SSR/STS, allele specific	UMN19, Bx7, ZSBy8, ZSBy9a, UMN25, Dx5, UMN26, Pinb-D1a, LOX16, LOX18, YP7A, YP7B-1, YP7D-1, PPO18, PPO19, PPO29, Pm2, Pm4b, Pm8, Xgwm582, Xcfa2040, PHS1, PHS-4AL	Wheat	Li <i>et al.</i> , 2018
Blast	Pi54, Pi1 and Pita	STS, SSR	Pi54MAS, RM224, YL155/87	Rice	Khan <i>et al.</i> , 2018
Bacterial blight	Xa38, Xa21, Xa13 and Xa5	Gene specific markers/STS	Os04g53050-1, pTA248, xa13-Prom, 10603-T10Dw	Rice	Yugander <i>et al.</i> , 2018
Bacterial blight	Gm1, Gm4, xa13 and Xa21	SSR	RM1328, RM22550, xa13 prom and pTA248	Rice	Krishnakumar and Kumaravadi-vel 2018
Mosaic virus	RSC4, RSC8, and RSC14Q	SSR	BARCSOYSSR_14_1413, 4 BARCSOYSSR_14_1417, BARCSOYSSR_14_1418, BARCSOYSSR_02_0606, BARCSOYSSR_02_0610, BARCSOYSSR_02_0616, BARCSOYSSR_02_0618, Satt334, Sct_033, MY750	Soybean	Wang <i>et al.</i> , 2017
Rust and coffee berry	SH3, SH?, Ck-1	SCAR/SSR	SP-M16-SH3, BA-124-12K-f, Sat244, BA-48-21OR, CaRHvII 2, CaRHvII 3, CaRHvII 5, Sat 207, Sat 235	Coffea	Alkimim <i>et al.</i> , 2017
Striga	SG1, SG3, and SG5	SSR	61RM2, SSR-1 and C42-2B	Cowpea	OMOIGUI <i>et al.</i> , 2017
Rust	Lr19 and Lr24	SCAR/SSR	Xwmc221 and SCS1302	Wheat	Singh <i>et al.</i> , 2017
Rust	Lr24 and Lr28	SCAR/SSR	SCS719, SCS1302607, SCS421570 and Xwmc313	Wheat	Kumar <i>et al.</i> , 2017
Drought, <i>Striga hermonthica</i>	SNPs	KASP markers	233 SNPs with KASP assay	Maize	Abdulmalik <i>et al.</i> , 2017
Bacterial blight, Blast	Xa21 and xa13, Pi54	STS	xa13 prom, pTA 248 and Pi54 MAS	Rice	Arunakumari <i>et al.</i> , 2016
Quality protein	opaque2	SSR	phi057 and umc1066	Maize	Kostadinovic <i>et al.</i> , 2016
Grain protein content, Thousand grain weight	GPC-B1 and TGW	SSR	Xucw108, Xgwm297	Wheat	Vishwakarma <i>et al.</i> , 2016 and 2014
<i>Fusarium</i> head blight	Fhb7, Fhb1	SSR	XsdauK66 and Xcfa2240 (Fhb7), Xgwm493 and Xgwm533 (Fhb1)	Wheat	Guo <i>et al.</i> 2015
Leaf curl disease	Ty-2, Ty-3, Ty-5	Linked markers	Ty-2, Ty-3, Ty-5, qTy10.1	Tomato	Prasanna <i>et al.</i> , 2015
Blast and bacterial blight	Pi9(t), Xa23, tms5	SCAR/EST/Indel marker	Pb8, C189, IDtms5	Rice	Ni <i>et al.</i> , 2015
Rice tungro disease	RTSV	SSR	RM336	Rice	Shim <i>et al.</i> , 2015

The successful publications using MAS in last 5 years is resumed in table 2. One successful example of marker assisted backcrossing and pyramiding is the introgression of three BB resistance genes (Xa21, xa13 and xa5) from BB-resistant donor variety IRBB-60 into the BB-susceptible Basmati variety CSR-30 (Baliyan *et al.*, 2018). A successful introgression of Shoot Fly (*Atherigona soccata* L. Moench) Resistance QTLs into Elite Post-rainy Season Sorghum varieties (Gorthy *et al.*, 2017). An example of a successful application of MAS in breeding new cultivars is the development of “Mura Salad” a new fresh pepper cultivar (*Capsicum annum*) containing capsinoids, low-pungent capsaicinoid analogs using dCAPs and SCAR markers (Tanaka *et al.*, 2014). In legumes, a successful application of marker assisted backcrossing in chickpea and specific markers for *Fusarium* wilt-resistance generate the development of new cultivars Super Annigeri 1 and improved JG 74 with enhanced resistance and improved yielding (Mannur *et al.*, 2019). MAS were successfully applied in wheat to improve *GPC-B1* (84-60) and also in Barley to transfer a thermostable β -amylase gene (Xu *et al.*, 2018) scald (*Rhynchosporium commune* L.) resistance gene (Sayed and Baum, 2018).

CONCLUSION

Marker assisted selection is a technology that has already proved its value. Due to the number of QTLs, genes and markers identified the MAS is likely to become more valuable. Many organizations and private sectors succeed in implementing MAS and produced new lines with desirable traits. But still reduced cost and optimized strategies for integrating MAS with phenotypic selection are needed before the technology can reach its full potential.

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