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Marker Assisted Selection and it's use against Genetic Improvement of Biotic and Abiotic Stress Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT: Improving of crop in terms of agronomic characters, yield and also tolerance to stress for feeding the growing population. All though this can be achieved by the conventional breeding but it is time consuming, high cost and presence of linkage drag. To overcome this many researchers started using the molecular techniques like molecular markers. This are tightly linked to the trait of interest which overcomes linkage drag and it can identified during seedling stage only. In this review we discuss about the MAS and its procedure, applications in resistance cultivar development, genetic diversity study and for improvement of quality of crop. How Marker assisted back crossing (MABC) help in improving the cultivars for abiotic and biotic stress tolerance by reducing number of back crossing. All most 70 to 80% yield loss in rice is due to this stress which can be overcome by using the molecular markers (SSR, STS, SNP's etc), Which are tightly linked to the resistance genes like Pi9(blast), Saltol(salinity), Sub1Agene (Submergence) etc are R genes which responsible for tolerance. By gene pyramiding large number of genes are incorporated into the single cultivar which is highly durable resistance than single gene cultivar. About the QTL's and markers which help in MAS in the rice cultivar for stress and donor parents which contributing the resistance gene for crop improvement.

INTRODUCTION

It was predicted that global population reaches to 9 billions by 2050 to feed this population we need to produce surplus of 10 thousand lakh ton's of cereals by end of the 2050 (Alexandratos, 2012). To achieve this a new approach should be combined with the traditional breeding to reduce the time and cost of production. The 2 main objective of breeding is to increase the quantity in terms of yield and quality improvement, tolerance for biotic and abiotic stress, elimination of toxic material, proper water and nutrients use (Collard, 2008).

To achieve the above objectives a new approach named molecular markers are introduced from 1980's which made available of large agronomic characters and diseases tolerance characters in major crops (Phillips and Vasil, 2001). molecular markers are gene or DNA nucleotide sequence with in the known location on chromosomes, this is used as tool for identification of the gene of interest, use in genetic mapping and use for detection of mutated genes which are interest, MABC, population studies etc (Hartl and Jones, 2009). Marker assist the breeders to track the specific gene of interest present in the individual. The use of this DNA markers in breeding programme is known as marker assisted selection (MAS). MAS is one of the smart breeding technique which developed a new era in molecular breeding (Gupta *et al.*, 2010). It is manipulating the genomic region which involved in desired trait through DNA markers, thus it helps in movement from the phenotypic selection to the genotypic based selection by using the markers which are linked for our gene of interest. This are environmentally not affected, so it can detectable in all stages of plants. MAS has became possible for both major genes and QTL's (Francia *et al.*, 2005). A number of breeding companies and research institutes for past decades has developed the improved varieties very fast by using this approach. Various markers like morphological, biochemical and molecular are present but the DNA markers are most extensively used in MAS for many traits (Madina *et al.*, 2013).

Rice is one of the major staple food in the Asian countries which is the cheapest source of food and energy. There is reduction of almost 70% yield due to the biotic and abiotic stress at different stages (Akram, 2019). Biotic includes the diseases, pest and weeds, abiotic includes the climatic condition's, soil salinity, drought etc. Major biotic stress in the rice are Bacterial blight, blast, brown plant hopper. In abiotic stress submergence and salinity. So this has become one of major challenge to the breeders, to overcome this problem and to produce maximum yield varieties MAS

has started. Breeding for the multiple resistance varieties with better quality and quantity needed from many decades (Khush, 2005). As with available of the rice genome sequence and position of genes/ QTL's governing stress open up opportunities for transfer of genes to desired rice varieties. So MAS are mostly used to improve the varieties of rice for the blight, blast and applied for drought tolerance along with grain protein content in quality characters. One of the approach in MAS is using molecular markers to develop the rice varieties with improved quality (Singh *et al.,* 2010). It help in pyramiding of abiotic and biotic stress related R genes into the existing cultivars (Prabhu *et al.,* 2009). The bacterial blight (BB) reduce yield by partial filling of grains (Pradhan *et al.,* 2015). So far 30 BB resistance genes have identified and introduce to high yield varieties (Kumar *et al.,* 2014). In Blast there is 50% yield loss (Babujee, 2004). Gall midge cause by *Orseolia oryzae,* gene pyramiding with 2 or few genes which resistance are introduced to a single variety lead to resistance cultivar (Dutta *et al.,* 2014). Submergence is control by Sub1A gene which located at centromere of 9 chromosome. For this many researches used QTL mapping and map based cloning (Septiningish *et al.,* 2009). Salt stress leads to 50% loss (Molla *et al.,* 2015). So main objective is pyramiding/ stacking of gene / QTL conferring tolerance for creating resistance varieties by using MAS.

MARKER ASSISTED SELECTION

It refer to process of indirect selection of desired plant phenotype based on the linked DNA markers. It infer the presence of a gene by presence of marker which is linked tightly to interest of gene. It help's in avoiding the consequence in the conventional breeding (Tabor *et al.,* 2002) and also helpful to identify the desired dominant or recessive alleles throughout and also in segregating population (Francia *et al.,* 2005).

Properties of MAS:

I. Pre-requisites (Jiang, 2013)

a) Relevant marker system and suitable marker: This two are important for MAS. Suitable marker should posses following characters like ease and low input of use, requires small sequence of DNA, it should be co-dominant, reproducible of results, polymorphism should be high leveled. The DNA markers are predominant then classical markers as they are not affected by the environment. From all the markers SSR are mostly suitable for the use.

b) There should be tight linkage between the DNA marker and the gene of interest.

c) The gene which governing the desired trait should be highly heritable.

d) Fast DNA extraction and high throughput marker detection: for large scale screening of multiple markers and to handle large sample this method used, high throughput like PAGE and AGE are used for marker detection.

e) Genetic maps: It provide framework for detection of marker trait association and help in making the choice of marker which help in the MAS, high-density linkage map is very much used in this method. A desired genetic map should posses the evenly-spaced markers to locate the QTL's (gene) (Babu *et al.,* 2004).

f) Information on marker-trait association: if marker is closely linked to trait the higher is success, so this information we get from QTL analysis, association mapping, bulked segregation analysis etc. Also we came to known whether marker linked in coupling or repulsion to the desired gene of trait.

g) Quick and efficient data processing and management: for this bio-informatics and statistical software helps.

II. Markers used:

It uses various type of the molecular or DNA markers (Miah *et al.,* 2013, Semagh, *et al.,* 2006).

Features	RFLP	RAPD	SSR	Microsatellite	SNPs	AFLP
Quantity of DNA required (micro-grams)	10	0.02	0.05	5	0.05	0.5-1
PCR based	no	yes	yes	yes	yes	yes
Type of polymorphism	Single base pair change, deletion, insertion	Single base pair change, deletion, insertion	Changes in the repeat length	Changes in length of repeats	Single nucleotide change, deletion, insertion	Single base pair change, deletion, insertion
Dominance	Co-dominant	dominant	Co-dominant	Co-dominant	Co-dominant	dominant
Inheritance	Co-dominant	dominant	Co-dominant	Co-dominant	Co-dominant	dominant
Marker index	Low	medium	medium	medium	medium	high
Level of polymorphism	low	Low to moderate	high	high	high	Low to moderate

reproducibility	high	unreliable	high	high	high	high
DNA quality	high	high	moderate	moderate	high	moderate
Developmental cost	low	low	high	high	high	moderate
Detection of alleles	yes	no	yes	yes	yes	no
Part of genome surveyed	Low copy coding regions	Whole genome	Whole genome	Whole genome	Whole genome	Whole genome
automation	low	moderate	low	high	low	moderate

III. **Applications**

It is applicable for both the animals and plant. In plant it is applied for both the self and cross pollinated crop.

a. It is used for the transfer of gene from one species to the other species e g; Bt cotton.
b. It helps in improve quality of food crops, like protein quality in maize, linolenic acid content in soybean.
c. Used in gene pyramiding of different gene's which are responsible for the biotic and abiotic stress tolerance.
d. Help in transfer of the male sterility from different sources to well cultivated genotype.
e. Help in introgression of desirable trait from the wild to the cultivated varieties and also genetic improvement of the tree species.

IV. MAS leads for developing non- transgenic plants.
V. It help in the improvement of both the oligogenic (quality) and polygenic (quantitative) traits.
VI. Speed of progress is more in MAS then conventional breeding which take 8-10 years. As it has capacity to identify allele even in heterozygous conditions.
VII. They have high accuracy as they are not much effected by environmental conditions.

Marker development and procedure of MAS

Marker development procedure (Collard, 2008, Semagn *et al.,* 2006)

a) Population development: parent selection with trait of interest, hybridization and screen population for trait of interest.
b) QTL mapping: Construct linkage maps (Semagn *et al.,* 2006), cross with adequate number of markers to QTL's, phenotypic evaluation, QTL's analysis.
c) QTL verification: Conformity the effect and position of the QTL's, testing them in different genetics backgrounds, fine mapping.
d) Marker validation: Test for applicability and reliability of marker predicating traits, identification of toolbox of polymorphic markers.

Steps in Marker assisted selection:

RFLP are widely used for genetic improvement.

1. Parental selection: Parents with the contrasting characters are used for the selection. They should be homozygous, in cross pollinated species we use the inbreeds as the parental type.
2. Breeding population development: The selected parental lines are crossed to obtain F1 plants which are homogenous but for the RFLP's marker they are heterozygous, so F2 progeny is needed for knowing segregation pattern of RFLP's.
3. DNA isolation: The main advantage of MAS is DNA isolation can be done from the seedling, no need to wait till last stage of crop. DNA isolated with specific protocol are digested with restriction enzyme and subjected to agarose gel and variation of fragments can view under UV light,
4. Scoring RFLP's: The diversification between the parents and recombinants in F2 population is determined by using the probes. ^{32}P is commonly used probe.
5. Correlate with morphological markers: DNA marker are correlated to morphological characters and make indirect selection.

Marker Assisted Assessment of breeding material

Previous to hybridization and development of line, molecular marker are useful for other breeding applications like parental line selection, variety identity, genetic diversity evaluation, hybrid confirmation.

I. **Assessment of purity of cultivar:**

Purity is one major important objective in breeding, it is often mislead by mixing of the different strain during the process of the handling. This can be overcome by using the markers. SSR are used in the hybrid rice to confirm purity other than GOT (Yashitola *et al.,* 2002). For genetic purity of the rice varieties microsatellite (RM247, RM303, RM164, RM108, RM724, RM19) and SSR marker (UBC842, UBC810, UBC808 etc) are found to asses 160 alleles for seed purity analysis (Jitendra Kumar, 2014) genetic purity of seeds of linseed is assessed by set of 38 SSR which located on 30 chromosome out of which 28 are polymorphic for cultivars (Abhinav Sao, 2013).

Evaluation of genetic variance and parental line selection:

Diverse strains are required for the hybridization to produce elite cultivar this is achieved by study of genetic diversity of strains by using molecular marker (Xu *et al.,* 2004). Genetic diversity among the 28 cultivars of pea is done by analyzing 32 SSR marker. The average polymorphism is 0.493, the variation among the cultivar range from 0.11 to 0.73 (Kumari *et al.,* 2013). Genetic diversity in green gram is assayed by the SSR marker. Clustering pattern of SSR marker give information on narrow genetic base of mung bean,

it reveals that out of 15 alleles of generation 13 are found to be polymorphic (Kaur *et al.,* 2018) selection of the parental lines should be based on the systematic assessment of genetic distance between the varieties or genotypes other than geographical distance. For predicting the hybrid, heterotic groups are formed by using the parental lines. Heterotic group are first formed in the maize by using the RFLP marker (Melchinger, 1998).

III. Heterosis study:

The use of molecular markers along with morphological data to select heterotic hybrids are also done. mostly in sorghum and maize for heterosis study this are helpful (Collard, 2008). In maize heterosis effect for yield characters are predicted by the SNP (Single nucleotide polymorphism) and Silico DAoT marker (Agnieszka Tomkowials, 2019).

Marker Assisted Back crossing (MABC):

It is one of the important method in which the one or few gene are transfer from the wild to the most adoptive variety for diseases resistance, abiotic stress tolerance purpose. In this method the elite variety contain all the favorable gene except one or few gene/character which should be transferred from non Reccurent parent (Allard, 1999). It was first started in the year 1922. For this procedure if we use the markers to select the desired cultivar it is known as Marker Assisted Back crossing (Holland, 2004). In foreground selection the marker allele of the donor parent are selected, its aim is to maintain the target locus in heterozygous condition. Then go for selfing of selected plants and obtained progeny are homozygous for donor allele. In background selection the main aim is to avoid the linkage drag by eliminating the gene from the donor parent (Hospital, 2005). Due to double recombination occur on both sides of target region linkage drag is minimized by flanking marker to the target gene. Two back cross generation are need for recombinants selection (Frisch *et al.,* 1999). By the conventional breeding we achieve all character of the recipient parent in 5 to 6 year but by using the markers we achieve with in BC_4 *or even in* BC_2 (Hospital, 1997). Example: for making the AS996 rice variety tolerance to submergence it is crossed with the recurrent parent SUB1 which is 70% tolerance to submergence with 460 markers, they carried out the parental diversity out of which 53 are polymorphic marker. MABC is used after the 3 generation of back cross absorbed BC_3F_1 individual with 100% recipient alleles (Luum *et al.,* 2012).

MAS for the qualitative characters or for major genes

The traits or characters which are mono or oligogenic inheritance are mainly controlled by the major genes/QTL's. The traits include the biotic stress, self - incompatibility, colour, shape, structure of the whole plants etc. (Jiang, 2013). High portion of the phenotypic variance of the quality trait is also govern by one or few QTL genes (Bilyeu *et al.,* 2006). So transferring that gene to well developed cultivar lead for crop improvement.

In rice to develop the aromatic genotype they made cross between the Nemat and 4 Local aromatic varieties. ASA (allele specific amplification) used as marker for fragrance and SSR RM190 marker is used for the amylose content. In F_2 population it showed that 11 lines are homozygous aromatic and 32 lines have best cooking quality (Hajiaqatabar, 2019). As we know SI is one of important for hybrid seed production. In Rape seed (*Brassica napus*) molecular analysis is performed for S locus to detect the SI plants. SLG, SCR, SRK analysis based on molecular marker is used for the selection of SI plants (Žaludová *et al.,* 2013). In soybean the cyst nematode is one of major pathogen it can overcome by the resistance cultivar (Cregan *et al.,*1999) for identification of resistant cultivars it take more time but by using the SSR marker Satl 309 identified the resistance gene rhg1. In rice to produce the thermosensitive male sterile line the two TGMS line EC720903 and EC720904 along with the two male fertile lines Jyothi and Uma, this male sterile and fertile line differ by morphologically and molecular level by using the RM3351 marker (Celine *et al.,* 2014).

MAS for improvement of quantitative traits

Most of traits of agronomy are controlled by the poly genes or QTL. Its improvement is difficult as this gene are effected by the environment and epistasis, here each gene has small effect on phenotype, due to QTL X E the efficiency of MAS is also became less.

MAS is restricted for QTL in breeding programme as the low accuracy of QTL and it need to check the QTL in different genetic backgrounds, high cost for genotyping but present large scale genotyping reduced the cost (Schuster, 2011). In rye grass to find out the nitrogen use efficiency by using the AFLP marker system and find out that 1-5NUE related QTL's are present on 5 chromosomal regions. The study show their is indirect relation between the marker selection on NUE (Dolstra *et al.,* 2003). By using this we can increase the efficiency of the artificial selection on phenotype's then after hybridizing the selected lines this requires a more number of marker loci scored. The major disease in wheat and barley is *Fusarium* head blight which are quantitatively inherited and mainly QTL's are responsible for that (Buerstmayr *et al.,* 2009) 19 pair of Near Isogenic Lines for FHB shown the decrease of severity of diseases upto 27% between pairs there is good success in implementation of MAS for QTL (Anderson *et al.,* 2007).

MAS in disease resistance breeding

Major crop looses in term of yield and quality is the diseases, this effect the economy of the country, so

disease management is one of main objective of agriculture, averagely 50,000 economic plants are affected with disease and every year new disease are discovered (Lucas, 1992). They are grouped into different types based on the parts which they are infected and symptoms . Developing the resistance varieties is main objective of breeders other than using the pesticides. By back crossing R gene are inserted into the well adopted cultivars which lack the diseases resistance but this process is the time taking so this is overcome by the MAS. There are 4 major traits where molecular marker play important role 1. where trait can't be managed by phenotypic selection 2. trait which depend on environmental selection 3. To speed the back cross breeding 4. For pyramiding of gene into a single cultivar.

As wheat have long growing season it is effected by many diseases, the diseases resistance are 2 types complete or qualitative resistance govern by single gene where as partial or quantitative resistance govern by QTL Yellow rust in the wheat is major disease caused by *Puccinia striformis.* Almost 70 yr genes are identified for YR resistance most of gene help to possess the seedling resistance which govern by single gene other govern adult plant resistance which is more durable (Chen *et al.,* 2014). In High temperature adult plant possess the genes which are resistance to YR, genes like Yr5, Yr7, Yr15, Yr78 are confirmed by SSR, SNP, CAPS marker mostly Y36 and GPC-M use in breeding the resistance variety for YR (Singh, 1992). It is reported that bacterial blight resistance in bean is developed by combining the periodic phenotyping selection with the MAS for better improved variety. Introduction of qHSRt QTL gene into the susceptible head smut of maize via a MAS result in decrease of diseases incidence (Zhao *et al.,* 2012).

MAS in abiotic stress breeding

Abiotic stress are dynamic and complex traits for the plants. This are environment conditions which affect the plant growth and yield. Almost 70% of crop production is reduced due to abiotic stress. Omic technology has help for developing many stress tolerant crop varieties (Cramer *et al.,* 2011). Mujtaba *et al.,* (2018) found total 6 genotypes (MAS-2/2020, MAS-3/2014, MAS-8/2014, MAS-12/2014, MAS-18/2014 and MAS-20/2014) out of 26 genotypes are highly tolerant to drought. 4 wheat genotypes like HOW468, HOW234, DBW17 and K307 are introduce with the Qyld.csdn.7AL QTL for drought tolerance (Gautam *et al.,* 2020). In the maize drought tolerance gene are QTL's which are assessed by the SNP's, RFLP, SSR markers (Shikha *et al.,* 2017). For developing the drought tolerance in the Bengal gram the marker assisted back crossing is widely used by introducing 'QTL-hot spot' into well adopted cultivar like JG11, KAK2 total 7SSR marker are included in 1 'QTL -hot spot' (ICM0249, NCPGR127, IAA170, NCPGR21, JR11, GA24, STMS11) this marker are tested for polymorphism in 32 recurrent parent by using the ICC4958 as non recurrent parent out of which 15 showed polymorphism with at least 2 markers (Thudi *et al.,* 2014). but MAS contributed less extensive for release of varieties which are high tolerance to the abiotic stress.

MAS for Biotic and Abiotic stress tolerance in Rice

Biotic and Abiotic stress causes higher yield loss in Rice crop. Abiotic stress includes drought, extreme cold, salinity, submergence. Biotic stress include the pest, pathogen, and weeds mostly Blast and Bacterial blight affect the crop (Hasan *et al.,* 2015). In rice the SNP's marker system is mostly used almost 17 million are identified in the Rice crop.

Abiotic stress

Submergence: Almost 25% land in world is submerged. It is most problem in the flash flood areas (Iftekharuddaula *et al.,* 2015). The gene which responsible for this trait is Sub1 which is located on centromere of 9th chromosome in FR13A Rice cultivar (Manivong *et al.,*2014). The linked markers like SUB1BC2, RM464A, R2698, C1232, RG381, RG345 (Dasand Rao, 2015) which are linked to the gene of interest or resistance gene Sub1 helped to produce the resistance cultivars like Swarna Sub1 (Das *et al.,* 2017). The Donor parents of Sub1 gene are FR43B, Kurkurappan and thavalu (Endang *et al.,* 2009). Sub1BC$_2$ marker is used to know the presence or absence of Sub1 QTL (Das*et al.,* 2017), which assure whether Rice is tolerance or susceptible genotype, if there is 248bp QTL gene it is tolerance and If 230bp it is susceptible, this marker showed almost 38bp polymorphism between the resistance and susceptible genotype, RYC743, purnendy are identified as tolerance genotype. Direct marker like Sub1A203 is better than Sub1BC2 as it is intergenic and adjacent to the gene of interest Sub1A and also combination of marker like (Sub1A203+Sub1BC2) help the genotype to group in systematic manners (Shibani *et al.,* 2020) for foreground and recombinants and Background selection the marker which is linked to Sub1, flanking Sub1 and unlinked to Sub1 are used when a cross between the submergence tolerant as donor parent with Swarna as Recurrent parent. In BC_2F_2 they found submergence tolerance plant with the SSR alleles of the Swarna except on tip where Sub1 locus (Neeraja, 2007).

Backcrossing of Sub1 with the help of markers to increase common variety Swarna had been successfully demonstrated to have a yield advantage for up to 18 days under submerged conditions (Sandhu *et al.,* 2020). After 15 days of submergence and 8 days of de submergence, the genotype FR13A, a well-known donor for submergence tolerance, the recipient did not make it through the submersion tension In contrast, the

pyramid different percentage of lines with Sub1 QTL revealed a different percentage (Das & Rao 2015). The three genotypes RYC743, Purnendu and FR13A were identified as submergence tolerant by using Indel marker Sub1BC2 closely linked with the Sub1 gene (Sinha *et al.,* 2018). IR64 Sub variety conatin the Sub1 gene which is developed from the genotype IR64 (Das *et al.,* 2017).

Drought: Mostly reproductive stage is effected during drought which in turn effects the yield. This trait is complex quantitative in nature (Reynolds *et al.,* 2008). There are many QTL's are reported in Drought (Vikram *et al.,* 2011) the marker RM431 which is present in chromosome 1 which is linked to the qDTY1.1 gene which increase the yield under drought by screening the genotypes they found Vaidehi, Birar and Dudhi presence of 120bp QTL which is tolerance for drought, so this 3 are used as donor in MABC and introgress the gene into the susceptible (Sinha *et al.,* 2018). MAS 946-1 is first drought tolerance rice developed through MAS (Gandhi, 2007), mainly drought effect the shoot and root traits there are almost 14 QTL's related to this they are inserted to high yield cultivars which result in the drought tolerance Rice (Bhattarai & Subudhi 2018). QTL qRWCR.1 which located on the 9th chromosome with the RWC are mapped by using 72 polymorphic SSR's. 4 QTL's are proven best for the drought tolerance by using the SSR's (Barik *et al.,* 2018) many QTL's out of which 3 QTL (qDTY2.1, qDTY 3.1, qDTY 12.1) are used to increase the yield under the drought conditions (Shamsudin *et.al,* 2016), marker like RM302-RM529, RM16-RM130, RM563-RM16, RM331-RM556 are linked to QTL/gene like qTGW1, qGW2-2, qPW8 which are tolerance to the drought. for the expression of the drought tolerance gene DREB transcription factor play major role for enhancement of drought tolerance (Udvardi *et al.,* 2007) Nagina 22 is used as donor parent in the MAB for donating the Dreb1 resistance gene to the susceptible cultivar (Reddy *et al.,* 2009) 15 alleles are detected by using the 10 SSR markers in 34 genotypes (10 parents, 24 hybrids). mainly 2 markers RM201, MR451 are used for drought response in genotypes mostly (Aboulila, 2015). Drought-tolerant versions of IR64 and Vandna were developed at IRRI by using MABC and have been shown to have a 1.0 and 0.5 t/ha yield ad vantage (Sandhu *et al.,* 2020). In the molecular analysis with RM431 marker, among the 12 genotypes, three genotypes Vaidehi, Dudhi and Birar indicated presence of the drought grain yield QTL (Sinha *et al.,* 2018).

Salinity: Almost 150 million hectares are affected by salinity, Seedling and reproductive are most affected stages for salinity stress. By using the microsatellite markers we can find the genes which are tolerance to salinity conditions (Das *et al.,* 2017). transfer of the hst1 gene which is tolerance from the Kaijin to Yukinko-mai variety which is of high yield through SNP MAS. And used speed breeding for the development purpose (Rana *et al.,* 2019). Through MABC transfer of Saltol QTL from F1478 (donor) to the Bacthom 7 (Reccurent), used total 368 SSR marker out of which 88 are applied to analyse the Back cross generation, 8 marker in QTL (Saltol) locus, 84 marker at other loci. The BC_3F_1 are showed the 100% tolerance to salinity (Vu *et al.,* 2012) high association was founded on SSR locus RM223 on the 8th chromosome when cross done between the IR64/OMC 52000 (Nguyen Thilang, 2008). markers like RM8094, RM140, RM10745, RM10772 are linked to resistance gene Saltol (Das & Rao, 2017) for donating this gene there are many donor parents like FL496, FL478, Patnal23, Vytill91 (Reddy *et al.,* 2009). The line FL478, a MAS product with Saltol transferred from Pokkali, was found to be effective against salinity stress, with a score of 1 showing 100 percent tolerance (Das & Rao 2015).

MAS for Biotic stress in Rice

Blast: One of major important disease in the Rice world wide is blast which is caused by *Magnoporthe grisea*. Almost 50% yield loss occur due to it (Babujee, 2000). Through conventional breeding many resistance cultivars are developed by it is not that effective due to instability of the fungus. So MAS is one of important tool Blast tolerance which is encoded by one or few genes (Young, 1996). The R gene is responsible for resistance, by interaction of R gene with A virulence gene it may compatible (Susceptible) or Incompatible (Resistance). WKRY's transcription factor help in the defense system by producing the PR proteins, Secondary metabolites etc (Huckelhoven, 2007). Almost 40 blast genes are identified, 8 genes are cloned to the susceptible cultivar (Lin *et al.,* 2007). Tetep act as donor parent which donate Pi5 gene which resistance to blast (Yi *et al.,* 2004) almost 100 blast gene are identified out this 14 are wide resistance (Pigm, Pi2, Pi1, Pi20, Pi33, Pi40, Pi48, Pi47, Pi39, Pi56, Pi54rh, Pi2t) (Hayashi *et al.,* 2010)

List of traits, Donor parents, QTLs/genes, and markers associated with the formation of climate resilient lines Against the Abiotic tolerance (Sandhu *et al.,* 2020, Das *et al.,* 2017).

Abiotic traits	QTL	Donor parent	Markers
submergence	Sub1	FR13A, Swarna sub1, IR64 sub1, FR43B, Kurkurappan and Thavalu	Sub1: ART5, snpOS0040 (T)
Drought	qDTY12.1	IR74371-46-1-1,Nagina 22	SnpOS00483(G), SnpOS00484(A), RM28099, RM28166, Indel 8,
Cold	qCTS4a, qCTS11.1	IR 83222-8-1-1-1-1-1-1-1, IR 66160-121-4-4-2, HGKN	qCTS11: RM26889, RM21. qCTS4a: RM349, RM17604, RM17623, RM3648, RM2799
Heat	qHTSF4.1	N22/IR64	id4005120, id4011562
Salinity	Saltol	Pokkali/IR29, FL496, FL478, FL378, Pokkali, SR26B, Patnai 23, Vytilla 1	RM3412, RM493, G11A, AP3206.

Among all R gene of blast Pi9 have high broad-spectrum resistance towards the it this introgrossed in IL's that were checked but several problems are faced during Pi9 marker amplification (Dixit *et.al,* 2020). Seven R genes of blast are introgressed into PB1 and found out Pi9 were more effective for the Blast. As a donor parent Abhaya and IRBB60 are used (Khanna *et al.,* 2015). Pi9 which have NBS-LRR cluster and Pita2 on short arm of 12^{th} chromosome showed high resistance. marker like Pi9, Pi9-6591, Y187 (SNP's, Indels, SSR) are linked to Pi9, Pita2 resistance genes which are donated by WHD-15-75-1-127, IPBC9 donor parent RM5926 and AP5659-5 are tightly linked markers to Pi-1 and Pi2-5 which are introduced to PRR78 by using foreground selection (Gouda *et al.,* 2013). Inter-crossing is done between the Pusa 1637-18-7-620 and Pusa 1633-8-8-10-1 which contain the resistance gene line Pi9 and Pita by using the marker assisted breeding (Khanna *et al.,* 2015). Abhaya was found to be resistant to blast (blast SES score ranged from 1 to 3 with different isolates). In the same way, It was also discovered that IRBB60 is moderately immune response (with blast SES score of 3) by using the Pi9 Marker (Dixit, 2020). Improved Variety Pusa RH10 which contain Piz5 + Pi54 resistance gene, PRR78/IRBB60 Variety which contain (*i54 + Piz5 are developed from PRR78 and* Pusa RH 10 genotypes by MAS (Das *et al.,* 2017).The donors C1O1A51 and WHD-1S-75-1-127 were very successful against blast and had a score of 0 for resistant reaction (R), while the recurrent parent had a score of 1. Both the blast resistance genesPi2 and Pi9 are present in ILGP17 and ILGP 19 which demonstrated a high level of blast resistance (Das & Rao 2015).

Bacterial Blight (BB): Bacterial Blight is cause by *Xanthomonas oryzae pv. Oryzae* it may accounted for 80-100% yield loss in the severe infected condition **(**Nohth, 2007). As through conventional breeding Xa21 gene are masked which are resistance to BB. So MAS help in identify this gene. Gene pyramiding is used for high durable resistance (Huang *et al.,* 1997). Almost 28 R gene for resistance are present (Nino-Liu *et al.,* 2006) some of the gene like (Xa1, Xa5, Xa21, Xa26) are cloned to susceptible and make them as resistance cultivars out of this Xa5 and Xa13 are dominant and marker developed by sequencing this gene (Chu *et al.,* 2006). Xa4+Xa5+Xa2 are pyramiding to one cultivated which is strong resistance compared to single gene cultivar (Jeung *et al.,* 2006). In India Pn106 are pyramided with Xa5, Xa13, Xa21 which are R gene for BB which are tightly resistance (Singh *et al.,* 2001). Varieties Angke posses Xa4+Xa5 and Condel with Xa9+Xa7 are released in 2007 mainly gene stacking or pyramiding helps for high durable resistance to Bacterial Blight marker Xa13 prom are tightly linked to Xa13 which located on chromosome 8 are tolerance to BB, Xa4, Xa5, Xa13, Xa21 are find good from other gene combinations (Das and Rao, 2011) marker like RM224, Xa5R, Xa13-promoter are linked to the gene like Xa4, Xa5, Xa13, Xa21, Xa23 which are from donor parent IRBB60 (Yadavs 2020). The presence of the 120bp , 400bp, and 150bp, 500bp and 1000bp indicate the resistance gene like Xa4, Xa5, Xa13, Xa21 presence using molecular marker like Xa4, Xa5, Xa13

prom and PTA248. for MAS selection Xa13prom and PTA248 linked to Xa13 and Xa21 genes (Sinha *et al.*, 2018). Sudha contained both *xa5* and *Xa21* gene and RYC743 have both *Sub1* and *Xa4*. *Sathyam, Pansoradhan, Kagargod conatin a X4 QTL gens* (Sinha *et al.*, 2018).

Brown plant hopper: One of the most important pest is Brown plant hopper (*Nilaparvata lugens*). almost 60% crop loss take place. Bph14 and Bph15 are two genes which are pyramided to Stengdao15 using the SSR and STS by MABC (Xu, 2013). There is no much resistance to BPH by introgressing the Bph3 and Bph17 resistance gene (Jena *et al.,2017) almost 37 R gene of BPH are* find on the six chromosome (Wang, 2017) out of this only 8 gene (Bph3, Bph14, Bph9, Bph26, Bph17, Bph18, Bph32, Bph29) are cloned and this are resistance. Rathu Heenati is first donor cultivar contain R gene (Lkshminarayana, 1997) using the SSR marker found outed a cultivar which is resistance obtained by crossing between ARI10550 and Taichung native (Deen *et al.*, 2017) Two genes like qBph4.3 and qBph4.4 are cloned between PR122 and IRGC104646 of F_2population which have shown Bph34 novel locus. RAPD marker $OPF10_{1200}$ are present in the resistance cultivar, in resistance bulk two RAPD products like $OPF19_{1500}$ and $OPF19_{1300}$ are detected by using the primer OPC19, to select the homozygous resistance population in F_2 RAPD marker are used (Amudha *et al.*, 2000).

List of traits, Donor parents, QTLs/genes, and markers associated with the formation of climate resilient lines against the biotic stress (Sandhu *et al.*, 2020, Das *et al.*, 2017).

Biotic traits	QTL	Donor parent	Markers
Blast	Pi9, Pita2	WHD-1S-75-1-127, Tadukan, IRBL9,LAC23, 5173, Tetep, IRAT13, Moroberekan, Zhiyeqing, C1O1A51, *O. minuta derivative*, Pusa 1602, IRBLZ5-a, DHMAS-70 Q164-2a,z2143,z1671, Os04g0401000	Pi9: Pi9STS2, MSU7_6_10381500 (M492 + M493), M891 (C), Pi9-659T, Pi9-1477GPi9-659T, Pi9-1477G. Pita2: SnpOS00488(G), YL155/ YL87,MSU7_12_9177624 (M535 + M536) , YL153/YL154. HC28
Bacterial leaf blight	Xa4, xa5, xa13, Xa21, Xa23,Gm1, Gm4,Xa38	IRBB60, Kogyku, Tetep, Chogoku 45, IR20, IR1545-339, CAS209, *O. longistaminata*, Pusa 1460	Xa4: snpOS0054 (AG), RM224, MP1 + MP2 xa5: xa5S, xa5R, xa5DRR xa13: xa13-promoter (M478Lm + M479Lm + M480Lm), xa13F_130- 147/xa13 R_1678-1662. Xa21: snpOS0061 (C), U1/I1, M1207 (T), pTA248 ,Xa21s_exon (M769 + M770). RG136, RG556. SSR:RM1328, RM22550, xa13 prom and pTA248. STS:Os04g53050-1, pTA248, xa13- Prom, 10603-T10Dw,
Brown plant hopper	Bph1, Bph2, Bph3, Bph4, Bph5, Bph6, Bph7, Bph8, Bph9, Bph10(t),Bph20(t), Bph21(t) Bph12(t), Bph13(t), Bph14 (Qbp1) and Bph15 (Qbp2)	Rathu Heenati, Mudgo, ASD7, Rathu Heenati, Babawee, ARC10550, Swarnalata, T12, Chin Saba, pokkali, *O. australiensis*, *Oryza latifolia*, *Oryza eichingeri*, *Oryza offificinalis*	RM8213, RM16556, RM586, RM589, RM190, RM7639, RM19311,RM589,RM586,RM190
Gall midge	Gm1, Gm2, Gm4(t), Gm5(t), Gm6(t), Gm7(t), Gm8(t), Gm9(t), Gm10(t), Gm11.	Abhaya, Kavya, Siam 29, Abhaya, ARC5984, Duokang #1, Bhumansan, NHTA 8, Banglei	GM4_LRR-del_F, GM4_LRR-del_R

FUTURE SCOPE AND CONFLICT OF MAS

Despite the relatively low adoption of markers in rice breeding to date, we expect that adoption will increase significantly over the next decade and beyond. The following factors could contribute to a greater adoption of MAS in rice: Many rice breeding institutes in various countries have developed facilities for marker genotyping and staff training, data on genes/QTLs regulating traits currently available (and continuously increasing) and the identification of tightly-linked markers, creation of successful breeding strategies for using markers, the development and maintenance of public databases for QTL/marker data, accessible resource for creating new markers using DNA sequence data derived from rice genome sequencing and functional genomics research. The key challenges for greater acceptance and impact of MAS on rice breeding in the near future are further optimization of marker genotyping methods in terms of cost-effectiveness and a greater degree of integration between molecular and conventional breeding (especially in designing efficient and cost-effective strategies).In future research for sheath blight, two aspects should be emphasised: the production and construction of new SB-resistant rice germplasms, and the fine mapping and evaluation of ShB resistance QTLs. The MAS is the only feasible solution for this. Disease resistant genes can be found and introduced into breeding lines. When SBQTL is used in a breeding programme, another two minor issues should be considered. To reduce linkage drag in the MAS operation, the interval duration of SBQTL should be further reduced. Another issue is whether a specific SBQTL can be used to increase the degree of resistance in certain rice cultivars (Mir *et al.,* 2015). With the aid of advanced genomic approaches, the problem raised by bacterial blight in rice can be overcome. To avoid losses, this would necessitate a thorough understanding of the consequences and their implementation as soon as possible (Hari Kesh and Kaushik 2020). The prohibitive cost of MAS in rice is currently one of the most significant barriers. Despite the fact that there are only a few studies comparing the cost-effectiveness of MAS versus traditional plant breeding in the literature, the cost-effectiveness of MAS versus conventional plant breeding differs significantly between studies. In order to perform a cost analysis, two additional considerations must be considered: (1) Setting up and maintaining a marker lab requires a significant amount of equipment and consumables; and (2) There is a significant upfront cost associated with the development of markers, which is seldom mentioned. Also QTLs with high LOD scores that explain a large proportion of the phenotype can be influenced by sampling bias (especially in small populations) and thus be ineffective for MAS. In addition, the impact of a QTL can be influenced by the genetic history. This highlights the importance of confirming QTL effects and marker reliability (i.e. QTL/marker validation) prior to MAS. The low reliability of markers to determine phenotype is another important factor impeding the effective application of markers for line growth. This is frequently due to the thoroughness with which the primary QTL mapping analysis was conducted. When opposed to traditional breeding, the initial cost of using markers will be higher (Wijerathna, 2015). To popularise MAS in breeding programmes, cost reduction is critical. Prior to use, the DNA extraction methods that result in high-quality DNA must be standardised.

CONCLUSION

For breeding of crop's the conventional breeding is not that convenient and it is time taken process which may not help to meet the demand of population, so using of the molecular marker for improving the agronomic, biotic and abiotic characters, also for the major genes or quantitative genes which are depend on environment conditions MAS is best method as marker are not affected by the climate conditions. MAS used for Assaying genetic diversity, purity of cultivars, for selection of parental lines, mainly MABS and gene pyramiding help in transferring the gene to elite cultivars, for the abiotic and abiotic stress tolerance MABC and gene pyramiding help to produce the resistance cultivars. Sub1A gene are cloned to susceptible cultivar, for drought linked QTL's are highly tolerant, Saltol is R gene for salinity tolerance, For blast Pi9 are most tolerant gene, Bacterial blight mostly combination of gene like X, Xa5, Xa216 are high tolerance then single gene in cultivar this transfer done by gene pyramiding. For BPH Bph14 and Bph15 are R gene. Marker like SSR, RAPD, SNP's, STS, Microsatellite are used for MAS for abiotic and biotic tolerance in Rice.

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