Society for Advancement of Wheat and Barley Research
ICAR-Indian Institute of Wheat & Barley Research
Karnal - 132 001, India

Journal of Cereal Research

12(2): 85-102

Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

Review Article

Marker assisted breeding in cereals: Progress made and challenges in India

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Article history
Received: 1 July., 2020
Revised: 5 Aug., 2020
Accepted: 14 Aug., 2020

Citation
Kamboj D, S Kumar, CN Mishra, P Srivastava, G Singh and GP Singh 2020. Marker assisted breeding in cereals: Progress made and challenges in India. *Journal of Cereal Research* **12(2)**: 85-102. http://doi.org/10.25174/2582-2675/2020/104208

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Abstract

An increasing global population requires increased crop production but crop yield rates are currently declining, hence yield, stability, and sustainability traits should be a major focus of plant breeding. Crop characteristics such as resistance to disease, tolerance to biotic stress, abiotic stress and flexibility in the use of nutrients and water have acquired importance. The use of DNA markers is called marker assisted selection (MAS) in plant breeding. MAS has been an important part of improving the germplasm. A large number of genes / QTLs have been tagged with different molecular markers to enhance the trait improvement. Identification and molecular cloning of the quantitative trait loci (QTL) genes provides the possibility to investigate the naturally occurring variability in the alleles for each gene complexity. In order to improve productivity, new alleles, identified by functional genomics can enrich the genetic base of crops grown. Advances made in recent years in cereal genomics research thus give opportunities for improving the prediction of phenotypes from genotypes. This review provides an overview of the latest developments in MAS, QTL detection, gene pyramiding and discusses some of the specific problems that have arised in the application of molecular techniques for cereal breeding.

Keywords: MAS, markers, QTL, gene, gene pyramiding

1. Introduction

Cereal grains consumption accounts for over 50 % of the global daily caloric intake (Awika, 2011). Key food source and a significant quantity of protein, minerals (potassium and calcium) and vitamins (vitamins A and C) are provided by cereals. Globally, food and feed are primarily supported by major cereals including rice, wheat and maize. The annual cereal supply needs to be increased in order to meet potential requirements for an estimated global population of 9.7 billion people in 2050 (Wani and Sah, 2014). Together with the challenges of worsening quality of arable land and water, the growing population of the twentieth-century has provided plant breeders with a major challenge in order to satisfy human needs for food in errant conditions. Urbanization has reduced access to land, and climate change, along with population growth, is threatening global food security (Chaudhary *et al.,* 2019).

The latest FAO world trade outlook for cereals for 2020/21 is 435.0 million tons, a rise of 9.0 million (2.1 percent) tonnes, compared to a previous high record point in the 2019/20 financial years (give value). The forecast on world's cereal usage has also been increased by 2735 million tons in 2020 as compares with43 million tons (1.6 per cent) during 2019/20 (FAO, 2020). India has produced 101.20 million tons of wheat in 2018-19 and has therefore retained its position as the second largest wheat producer in the world (Anonymous, 2019).

One of the key factors that restrict crop production is stress. Climate stress such as drought, salt, heavy metal

Table 1: World Area, production and yield of the three major cereals in world (FAO,2020)

Crop	Year	Area (million hectares)	Production (million tonnes)	Yield (tonne/ha)
Rice	2015	162.63	745.90	45685
	2016	162.98	751.88	46133
	2017	166.08	769.82	46352
	2018	167.13	782	46789
Wheat	2015	223.47	741.64	33187
	2016	219.09	748.39	34158
	2017	218.42	773.47	35412
	2018	214.29	734.04	34254
Maize	2015	190.57	1052.12	55208
	2016	195.60	1126.99	57616
	2017	197.46	1164.40	58967
	2018	193.73	1147.62	59237

Table 2: Area, production and yield data of the three major cereals in India

Crop	Year	Area (million hectares)	Production (million tonnes)	Yield (Kg/ha)
Rice	2015	44.1	105.48	2391
	2016	43.5	104.41	2400
	2017	44.0	109.70	2494
	2018	43.8	112.76	2576
	2019	43.8	116.42	2659
Wheat	2015	31.47	86.53	2750
	2016	30.42	92.29	3034
	2017	30.79	98.51	3200
	2018	29.65	99.87	3368
	2019	29.14	102.19	3507
Maize	2015	9.19	24.17	2632
	2016	8.81	22.57	2563
	2017	9.63	25.90	2689
	2018	9.38	28.75	3065
	2019	9.18	27.23	2965

Source: Department of Agriculture, Cooperation and Farmers Welfare, Govt. of India, Ministry of Agriculture and Farmers Welfare and water submergence greatly affect the crop yield (Ghatak et al., 2017). Climate and agriculture are inter-linked in various ways, as both biotic and abiotic stresses have an adverse impact on agriculture (Raza *et al.* 2019).

Rapid progress in genomic and molecular biology research has led to the development of accurate, effective and reliable molecular markers for the fast development of new cultivars (Randhawa *et al.,* 2009). Genetic variation in different yielding crops have led to the development of tolerant cultivars but still a great deal of effort for finding unique molecular markers is required. Similarly, related and gene-based markers have become available in wheat for several quality traits. The combination of Marker-Assisted Selection (MAS) with traditional phenotypic selection can therefore improve the efficiency of breeding and the precise transition of the target allele into the advanced progenies and sometimes in relatively shorter time. A major increase has been achieved in the production of improved climate-resilient crop varieties by novel molecular biology approaches when applied to conventional plant breeding methods (Wani *et al.,* 2018). The rapid advances in marker and sequence technology have occurred in recent decades which have reduced genotyping costs (Varshney *et al.,* 2016)and have led to numerous genetic mapping and identity studies for various major and minor Quantitative Loci (QTLs) for specific abiotic and biotic stresses. The reproduction methods to map and introgress QTLs by means of marker-assisted selection for the development of elite cultivars or the improvement of plant populations is called as QLT breeding (Chaudhary *et al.,* 2019). Molecular markers not only promote the development of new varieties by reducing the time taken to detect specific traits in progeny plants, but also fasten the identification of desirable genes thereby accelerates the efficient reproduction of desired traits by MAS into a cultivar. MAS relies on identifying marker DNA sequences which over the first few generations are inherited alongside a desired trait. Subsequently, plants carrying the traits can be picked easily by looking for the marker sequences, allowing several breeding rounds to run in rapid succession (Kumar *et al.,* 2007).MAS has been used effectively in the pyramiding of known genes in short cycle via foreground and background selection, thereby adding resistance to each crop's existing cultivars (Prabhu *et al.,* 2009). It is the use of molecular markers to monitor where genes of interest in a breeding program are located (Khan *et al.,* 2011).

In order to harvest food consistently, our crops must be resilient to the changing environment, especially against abiotic stress. Numerous alleles of resistance or tolerance to abiotic stresses have been extensively studied in manycrop germplasms and, in all cases, these alleles have been identified in neglected crop genotypes, whether landraces, wild relatives or progenitors, which are generally poor yielders and are therefore ignored by the farming community. The result of the hybridization and selection process will be a high yielding genotype under stress. Traditionally, breeders have relied on visible traits to select improved varieties however; MAS rely on identifying marker DNA sequences that are inherited alongside a desired trait during the first few generations. Molecular markers are also considered as useful tools for pyramiding of different resistance genes and developing multi-line cultivars targeting for durable resistance to the disease. With the development of methodologies for the analysis of plant gene structure and function, molecular markers have been utilized for identification of traits to locate the gene(s) for a trait of interest on a plant chromosome and are widely used to study the organization of plant genomes and for the construction of genetic linkage maps. Breeders used molecular markers to increase the precision of selection for best trial combinations.

2. Marker assisted selection in India: Achievements from major cereal crops

The MAS has played an important role in crop improvement over the past few decades, with numerous success stories for various abiotic and biotic stress. There is genetic variation for drought tolerance in cop cultivars and it is possible to achieve improved adaptation response in cereals by implementing appropriate crossing and selection strategies. MAS is most useful for traits that are difficult to select e.g., disease resistance, salt tolerance, drought tolerance, heat tolerance, quality traits (aroma of basmati rice, flavor of vegetables). The approach involves selecting plants at early generation with a fixed, favorable genetic background specific loci, conducting a single large scale marker assisted selection while maintaining as much as possible the allelic segregation in the population and the screening of large populations to achieve the objectives of the scheme.

2.1 Rice

To more than half of the world's population, rice (*Oryza sativa* L.) is the main source of food. Rice is grown in a wide variety of circumstances from wetlands flooded to dryland flooding. Irrigated rice, representing 55% of the world rice region, accounts for 75% of world rice production and consumes around 90% of the agricultural freshwater resources (Sandhu *et al.,* 2013).

Drought, salinity and submergence is a difficult aspect of traditional breeding methods but recent developments in genomic techniques have resulted in a precise and selective recognition of the underlying mechanism for these stresses. Combining customary breeding progress with advanced genomic tools, genomics assisted breeding became common. Association mapping (AM) and Quantitative Trait Loci (QTL), have helped to reliably classify some minor genes and some significant stress tolerance genes for significant grains. For crop improvement efforts, GAB programs use molecular markers correlated with the desired trait / QTL. Various MAB techniques exist, including Marker Assisted Selection (MAS), Marker Assisted Backcross Breeding (MABB), Marker Assisted Recurrent Selection (MARS), Genome Wide Association Studies (GWAS) and Genomic Selection (GS) for the transfer of genes or introgression features (Wani *et al.,* 2018). A mapped locus can also be efficiently used for improving the drought tolerance of mega varieties or common cultivars good in their quality but susceptible to drought stress (Sandhu *et al.,* 2018; Muthu *et al.,* 2020). A variety of QTL mapping studies conducted for drought tolerance for rice are given in Table 3. The drought tolerance in rice is regarded as a quantitative attribute despite its dynamic nature. The first drought tolerant aerobic rice developed through MAS was MAS 946-1 by Gandhi (2007).

There are several reasons why some drought tolerance genes cannot be mapped in a breeding population of which the environmental impact and low heritability are among the key factors (Vinod *et al.,* 2019). Drought responsive QTLs in rice for shoot and root traits using GBS-based SNP map were identified by Bhattarai and Prasanta (2018). Fourteen QTLSs related to shoot length, root length, number of tillers, dry root mass and dry soot mass were recognized using a RIL population (Cocodrie x N-22). These QTLs can be introgressed into high-yielding rice varieties to produce drought tolerant rice variety. Three QTLs (RM8085, I12S and RM6836) have been mapped by Prince *et al.* (2015) to cover the physiological and yield characteristics using the RIL population of the cross between Nootripathu with IR20. These QTLs can be utilized effectively to reach the drought affected areas in elite row. Barik *et al.* (2018) have used two contrasting parents (Krishnahamsa and CR 143-2-2) for the production of recombinant inbred lines (RILs) for drought tolerant QTL associated with Relative Water Content (RWC) in rice. QTL, qRWC9.1 associated with RWC was mapped on chromosome 9 using seventy-two polymorphic SSRs after genotyping the RILs. Meanwhile, Barik *et al.* (2019) observed five QTLS pertaining to relative water content, leaf rolling and drying, spikelet fertility from a mapping population of F7 RILs developed from the cross between CR 143-2-2 with Krishnahamsa. In total, 401 SSR primers were utilized for parental polymorphisms, of which 77 were polymorphic. The four QTLs out of the five were novel and can prove blessing in MAS approach to develop drought tolerant rice. Shamsudin *et al.* (2016) used three QTLs of drought yield, qDTY2.2, qDTY3.1, and qDTY12.1, consistently impact grain yields under the gene pyramiding of a Malaysian rice farming elite under reproductive drought stress. In each of their breeding generations these three QTLs performed successfully in the first selection. Many QTLs and genes had been identified for drought (Sabar *et al.,* 2019; Barik *et al.,* 2018; Singh *et al.,* 2016; Donde *et al.,* 2019) salinity and submergence tolerance (Muthu *et al.,* 2020; Babu *et al.,* 2014; Krishnamurthy *et al.,* 2014) in rice that can be used in breeding programmes to develop high yielding and abiotic stresstolerant rice varieties.

Enhancing the resistance of host plants is one of the best environmental and ecological approaches to deal with various rice-related biotic stresses. Rice crops and hybrids that are resistant to several biotic stresses must be produced. Multiple pest / disease resistance breeding was not a new concept, but the emergence of reliable PCR-based markers made it possible for genes to combined rapidly (i.e. gene pyramiding) and easily with MAS. *Xanthomonas oryzae pv oryzae* (Xoo) causing bacterial blight (BB) disease is a threat to rice plants in irrigated and rain-fed regions. Numerous diagnostics, management and disease control trials have been carried out. Improving rice genetic resistance has shown to be the most effective form of disease control. PCR-based molecular markers were used by Sundaram *et al.* (2008) to introduce three bacterial blight genes viz., *Xa21, Xa13 and Xa5* from donor SS113 to popular rice cv. Sambha Mahsuri. Similarly, the four BB resistant genes (*Xa4, xa5, xa13, Xa21*) were pyramided into rice cultivar Mahsuri and two parental lines, KMR3

Table 3: Marker assisted studies in rice conducted by different researchers in India

Trait	Gene/QTL	Marker	References
Drought	*qSL1.38* *14 QTLs*	SI-38023681 SI-38286772 263091 SNPs	Bhattarai and Subudhi, 2018
	qTGW1, *qGW3-2,* *qGW3-1,* *qPW8*	RM302-RM529 RM16-RM130 RM563-RM16 RM337-RM556	Sangodele *et al.,* 2014
	qDTY1.1	RM11943-RM12091 RM431	Vikram *et al.,* 2011
	qGY6.1, *qEVV9.1,* *qGY10.1* *qDTY2.1*	Id6010515-id6015531 K-id1024836-id1026726 Id10005369-id10006378 RM154-RM324, and RM263 RM573	Sandhu *et al.,* 2014 a, 2014 b
	qDTY3.2 and *qDTY12.1*	RM231, RM28099 and RM28199	Dixit *et al.,* 2017a, 2017b
	qDTY3.1 *qDTY6.1*	RM168 and RM468 RM586-RM217	Dixit *et al.,* 2014
	qDTY2.2	RM236,RM279 and RM555	Swamy *et al.,* 2013
	qDTY12.1	RM28166	Mishra *et al.,* 2013
	QTL	SSR markers	Suji *et al.,* 2012
	3 QTLs	SSRs	Prince *et al.,* 2015
	1 QTL	SSR	Barik *et al.,* 2018
	5 QTLs	SSR	Barik *et al.,* 2019
	3 QTLs	SNP	Yadav *et al.,* 2019
	21 QTLs	SSR	Sabar *et al.,* 2019
	4 QTLs	SSR	Ramchander *et al.,* 2016
Drought and flood tolerance	*QTLs*	SSR	Sandhu *et al.,* 2019
Salinity tolerance	*Saltol QTL*	SSR	Babu *et al.,* 2014
		Saltol markers	Krishnamurthy *et al.,* 2014
	qPH1.1	RM128-RM472	Hossain *et al.,* 2015
	QTL	?	Mishra *et al.,* 2019
Submergence tolerance	*Sub1 QTL*	SSR	Neeraja *et al.,* 2007
Submergence & salinity tolerance	*Sub1 & Saltol*	SUB1BC2 RM10745	Das and Rao, 2015
Biotic & abiotic stress	*QTL*	SSRs	Akula *et al.,* 2020
	bph5	SSR	Deen *et al.,* 2017
	bph34	SNP & SSR markers	Kumar *et al.,* 2018
	qBph4.3 & qBph4.4	SSR	Mohanty *et al.,* 2017
	QTL	SSR	Kumar *et al.,* 2020
Bacterial blight resistance	*Xa13,Xa 21*	SSR	Singh *et al.,* 2011 b
		RAPD, SCAR	Singh *et al.,* 2011 a
	Xa21 & Xa38	SSRs	Yugander *et al.,* 2018
	Xa38	SSR	Yugander *et al.,* 2019
	Xa21,Xa13& Xa5	CAPS & STS marker	Sundaram *et al.,* 2008
	Xa5 & Xa 13	CAPS & STS marker	Sundaram *et al.,* 2009

	Xa5,xa13 & Xa21	CAPS & STS marker	Singh *et al.,* 2001
	Xa4,xa5,xa13, xa21	SSR	Guvvala *et al.,* 2013
	Xa4,xa8, xa13 & xa21	STS, SSR & AFLP marker	Joseph *et al.,* 2004
	Xa21 & Xa13	SSR markers	Pandey *et al.,* 2013
	Xa13 & Xa 21	CAPS & STS marker	Gopalkrishnan *et al.,* 2008
	Xa5		Iyer *et al.,* 2004
Bacterial blight & gall midge resistance	*Gm4, Gm8, Xa21*	SSRs	Kumar *et al,.* 2017
Insect and disease resistance	*T1p, Xa 21, gna*	SSRs	Rajesh *et al.,* 2020
Blast resistance	*Pi9 and Pita*	SSRs	Khanna *et al.,* 2015
	Pitp, Pi1, Pi 2, Pi 9 and Pi 54	Gene specific markers	Azameti *et al.,* 2020
	Pi-1 and Piz-5	SSRs	Gouda *et al.,* 2013
	Pi54	SSRs	Ramkumar *et al.,* 2010
	Pi5 and Pi54	SSR markers	Singh *et al.,* 2012
	Pi40 , Pi42 (t)	DNA based marker	Akhtar *et al.,* 2010
Bacterial blight and blast resistance	*Piz5 + Xa 21*	SSRs	Narayanan *et al.,* 2002
Gall maidge resistance	*Gm8*	SSR markers	Sama *et al.,* 2012

lines, KMR3 and PRR78 by Guvvala *et al.,* (2013) by utilizing the approach of MABB. The pyramided families were evaluated both in natural and artificial environments. No adverse effect on the agronomic efficiency of any pyramids was observed by pyramiding resistance genes. Meanwhile, two dominant BB resistant genes, Xa21 and Xa38 were pyramided into rice maintainer line, APMS 6B by MABB approach by Yugander *et al.,* (2018). Also Yugander et al. (2019) introgressed Xa38 gene into rice line APMS 6B by marker-assisted backcross breeding. Such introgressive lines showed high BB resistance to different Xoo strains at BC2F6 generation in relation to different agro-morphological characteristics and were identical to APMS 6B. The rice blast is caused by fungus *Magnaporthe oryzae,* a haploid filamentous Ascomycete with a fairly low genome of ~40 Mb with seven chromosomes. While several resistance species have been identified, there are continued threats to the efficacy of the cultivars produced because of genetic plasticity of the pathogen genome. Using MABC approach, Gouda *et al.,* (2013) introduced two blast resistance genes Pi-1 and Piz-5 into PRR78 line of rice. Markers RM5926 and AP5659-5 tightly linked to these genes, were used for foreground selection. These pyramided lines were tested for disease reaction, agronomic performance and cooking quality traits and were found to superior than PRR78 in yield. Khanna *et al.,* (2015) intercrossed two NILs Pusa 1637-18-7-620 and Pusa 1633-8-8-16-1 having gene Pi9 and Pita by MAS to develop pyramided lines. On evaluation under artificial and natural environments, these pyramided lines show resistant against three virulent pathotypes, *Mo-nwi-kash 1, Mo-nwi-lon 2 and Mo-ei-ran 1.*

Brown plant hopper (BPH), *Nilaparvata lugens* (Stål) are among the insects that are one of the most harmful rice insect pest. The effect of the suction of the phloem sap, reduces the production, strength and number of productive tilers, leads to direct damages to the rice crop. It triggers dynamic wound response hopper burn under serious infestation which make the crop looks decolored and dehydrated. A rice population derived from cross between ARC10550 with Taichung Native 1 was mapped for BPH resistance by Deen *et al.,* (2017) utilizing SSR markers. They observed five QTLs governing BPH resistance (two for days to wilt, one for damage score and other two for nymphal preference). Mohanty *et al.,* (2017) developed RIL population for BPH resistance. After mapping of population, two new QTLS were observed for BPH resistance (qBph4.3and qBph4.4). These QTLs were introduced into two rice cultivars, Samba Mahsuri and Pusa 44. Fine mapping of the established QTLs resulted in effective transmission of QTLs in the germplasms of the cultivars. Meanwhile, Kumar et al. (2018) mapped Bph34, a novel locus for BPH resistance by using F2 population derived from cross between PR122 with IRGC104646. A linkage map was constructed using SNP as well as SSR markers. These markers proved to be helpful in marker aided transfer into rice cultivars for further use in rice

2.2 Wheat

Renewed studies are under way to examine the genetic basis of several important features of wheat through the production of AFLP and microsatellite marker systems. Future challenges include the development of cost reduction strategies per test, the acquisition of more desirable indicators that complement the efforts of wheat producers and the evaluation of new technologies in order to increase cost throughput. There is no doubt the low degree of polymorphism among the elite variants, coupled with the hexaploid character of the crop, is an important obstacle for molecular markers to grow and use in genetic trials. Advances in MAS were hampered by the limited supply of wheat genome data, but advances until recently in the techniques of genotyping and DNA; genome datasets used to develop single sequence repeats (SSRs) and SNP markers have been produced in sequencing (Srivastava, 2019). The details on the wheat genome sequence can be analyzed to classify the candidates for complex agronomic significance genes for the purpose of speeding up the programs to develop wheat. For effective phenotyping for abiotic stresses, the state of the art aerial vehicle technology with high-throughput imaging systems can be integrated. The wheat breeding programs in India have been mainly focused on yield and disease resistance to develop varieties to feed the ever-growing population (Rai *et al.,* 2019). In this line, first Indian wheat variety to be developed through marker assisted breeding was PBW723 having stripe and leaf rust resistance genes (Anonymous, 2016). The variety was released for cultivation in 2016 in the North Western Plains of India. The details of various studies on marker assisted selection in wheat are given in Table 4.

Rai *et al.,* (2018) developed five wheat lines with inbuilt capacity to tolerate drought conditions using three linked quantitative trait loci (QTLs) in BC1F1 population of 516 plants. Meanwhile, Mujtaba *et al.,* (2018) tested 26 wheat genotypes under drought stress to evaluate potential for desiccation tolerance. They found that six genotypes (MAS-2/2014, MAS-3/2014, MAS-8/2014, MAS-12/2014, MAS-18/2014 and MAS-20/2014) exhibited greater tolerance under conditions of drought, making them ideal for increasing the productivity of rainfed and arid regions. Gautam *et al.,* (2020a/b) introduced yield QTL (Qyld.csdh.7AL) into four wheat cultivars *viz.* HUW468, HUW234, DBW17 and K307 to generate high-yielding drought tolerant genotype. After phenotypic selection, 55 advanced lines were identified which were further evaluated under rainfed and irrigated conditions at two different locations having different climatic conditions for two crop seasons. The advanced line gave higher yield in rainfed conditions and also had a low pressure sensitivity index, indicating its ability to tolerate water stress. This study was a perfect example of effective utilization of MAS along with phenotypic selection for creating advanced wheat lines having higher yield in irrigated as well as rainfed conditions.

With climate change, the wheat crop faces numerous threats because of biotic and abiotic stresses which lead to considerable loss of return. Amongst the biotic stresses are known world-wide for the significant loss of wheat yield, rust diseases caused by three types of rust, including leaf rust (brown rust), stem rust (black rust), and stripe rust (yellow rust) are important concern. Puccinia rust fungus is a disease pathogen. *Puccinia graminis* causes "system or black rust," *P. triticina* causes "bladder or brown rust" and *P. striiformis* causes "stripe or yellow rust". The three types of Puccinia need heterogeneous hosts (alternate hosts) that is two separate and distant hosts. Because of leaf rust yield losses of up to 7-30% (Singh *et al.,* 2011), especially in connection with the emergence of Ug99 have been reported. Many genes of rust resistance for the three rusts were deployed in wheat cultivars, although some of these genes were rendered ineffective within 3–5 years after rapid evolution of the new virulent breeds of pathogens (Singh *et al.,* 2015). This requires the discovery, deployment and pyramidization of new rust resistance genes for long-lasting resistance of the three rust pathogens against ever-evolving virulent races. The molecular tools helped in identifying potential candidate genes and QTLs for biotic stress tolerance and their successful use in marker aided breeding (Khan *et al.,* 2011). Gupta *et al.,* (2005) used near-isogenic line (NIL) of HW2055 having leaf rust-resistance gene *Lr9* obtained from *Aegilops umbellulata* and recurrent parent HD2329 to identify the RAPD markers linked with *Lr9* gene. They developed a sequence-characterized amplified region (SCAR) marker SCS5550 from RAPD marker for validating *Lr9* gene. The SCS5550 was validated using 10 resistant NIL pairs carrying gene *Lr9* taken from 10 different Indian wheat genetic backgrounds. Meanwhile, Gupta *et al.,* (2006) have successfully converted three random amplified polymorphic DNA marker (S1302609, S1326615 and OPAB-1388) to SCAR markers to enable gene-specific selection for an *Agropyron elongatum*-derived leaf rust resistance gene *Lr24*. Six RAPD markers co-segregating with Lr24 gene located on wheat chromosome 3DL.

Table 4: Marker assisted breeding studies in Wheat conducted by different researchers in India

Trait	Gene/QTL	Marker	References
Drought	*QRWC2AC*	KSUM-119	Malik and Malik, 2015
	QHt.ccsu-2B	wPt-9423	Gahlaut *et al.,* 2017
	QABA-ww-3B	Barc164-Srap19	Barakat *et al.,* 2015
	4 QTLs	SSR	Fatima *et al.,* 2018
	QTLs	DArTseq SNPs	Sukumaran *et al.,* 2018
	7 QTLs	SSR	Khanna-Chopra *et al.,* 2019
	Qyld.csdh.7A L	Xwmc273.3 marker	Gautam *et al.,* 2020a/b
	3 QTLs	SSR	Rai *et al.,* 2018
	QTL	EST markers	Khan *et al.,* 2011
Drought & heat	*QTLs*	Xwmc89, barc20, gwm368, Xgwm111,gwm397	Jain *et al.,* 2014
Heat tolerance	*HSP20*	SSR	Pandey *et al.,* 2015
Leaf rust resistance	*Lr9*	SCAR & RAPD marker	Gupta *et al.,* 2005
	Lr24	RAPD	Gupta *et al.,* 2006
	Lr19	SSR	Pandey *et al.,* 2015
	PHST QTL & Lr24+ Lr28	*SSR & SCAR*	Kumar *et al.,* 2010
	Lr46, Lr34	SSR	Awan *et al.,* 2017
Leaf and stripe rust resistance	*Lr19 and Yr15*	SSR, SCAR	Pal *et al.,* 2019
Yellow & stem rust resistance	*Yr57, Yr51, Sr26, Sr22 & Sr50*	SSR	Randhawa *et al.,* 2019
	Sr2	Xgwm 533	Vishwakarma *et al.,* 2019
Leaf, yellow & stem rust resistance	*QTLs*	SSR	Gautam *et al.,* 2020 a/b
Stripe rust resistance	Yr10	SSR	Singh *et al.,* 2009
Stripe rust resistance	Yr genes	SSR, CAPS, RGAP, STS, EST-SSR	Rani *et al.,* 2019
Karnal bunt resistance	QTL	SSRs	Kaur *et al.,* 2016
Grain protein content	QTLs	SSR	Prasad *et al.* 2003
	Gpc-B-1 gene	SSR	Kumar *et al.,* 2011
	GpcB1	SSR	Vishwakarma *et al.,* 2014
Grain weight	QTLs		Kumari *et al.,* 2019
Yield & yield related traits	QTL	SSR	Kumar *et al.,* 2007
	tin	SSR	Kumar *et al.,* 2015

The SCAR markers were validated in wheat NILs with *Lr24* for their specificity to the gene. Singh *et al.,* (2009) developed a PCR-based assay for easy selection of stripe rust resistance, *Yr10* gene. Similarly, Kumar *et al.,* (2010) developed leaf rust resistant and pre-harvest sprouting tolerant (PHST) genotypes of wheat via marker-assisted selection. They had introduced PHST QTL, QPhs.ccsu-3A.1 along with two *Lr* genes (*Lr24+ Lr28,* leaf-resistant genes) into an elite PHS susceptible cultivar, HD2329 using MAS approach. Another example of the success and use of MAS for pyramiding genes in wheat and is perhaps the first example of pyramiding of as many as 12 genes/ QTL in wheat was studied by Gautam *et al.,* (2020 a/b). They developed improved wheat lines with amber grains, which carried genes/QTL for grain quality, grain weight, and rust resistance. Two enhanced lines (P_1 and P_2) were crossed having PBW343 as background; these two lines were developed earlier using MAS. Line P1 constitute of *Yr70/Lr76 + Lr37/Yr17/Sr38* while line P2 include *Gpc-B1/Yr36+QPhs.ccsu-3A.1+QGw.ccsu-1A.3+Lr24/ Sr24+Glu-A1-1/Glu-A1-2*. After the F_2, F_3, and F_5 analysis of MAS, 23 lines were selected resistance to all the three rusts in homozygous condition and each of the genes / QTL for the grain quality. Of these one line (CCSU-7) had a substantially higher grain yield and protein content than the cv PBW343, which can be useful to boost grain quality as well as the resilience of resistance against all three rusts in future wheat breeding program.

Prasad *et al.,* (2003) performed QTL interval mapping in bread wheat using a mapping population, available in 100 recombinant inbred lines (RILs) for the grain protein content (GPC). Thirteen QTLs have been detected using three separate approaches (Single Marker Analysis or SMA, Simple Interval Map or SIM and Composite interval mapping and LOD scores ranging between 2.5 and 6.5). Out of 13, only four QTLs (*QGpc.ccsu-2B.1; QGpc.ccsu-2D.1; QGpc.ccsu-3D.1* and *QGpc.ccsu-7A.1*) had been found in many locations using an approach other than CIM. Another QTL (*QGpc.ccsu-3D.2*), has been detected significant in all the approaches. Kumar *et al.,* (2011) introgressed gene Gpc-B1, having high GPC in 10 elite wheat genotypes through MAS. Seven MAS-based progenies were obtained which showed significantly higher GPCs (14.83-17.85%) than the parental genotypes and did not impose any yield penalty. No substantial negative association between GPC (percent) and protein production has been observed in these selected progenies which indicate that GPC can be enhanced unless a yield penalty is imposed. In combination with phenotypical selection, their work thus suggested that MAS is a useful strategy in the production of high-GPC wheat genotypes associated with no yield loss. Similarly, gene Gpc-B1 (high grain protein content) from genotype Glu269 was introduced into wheat cultivar HUW468 through marker-assisted backcrossing (MABC) by Vishwakarma *et al.,* (2014). Foreground selection was done with the marker Xucw108 whose locus was linked to the gene Gpc-B1. Background selection was done using 86 polymorphic SSR markers. Enhanced lines showed 88.4–92.3 per cent of the recurrent parent genome (RPG), with higher GPC.

Eight pairs of near-isogenic lines (NILs) for grain weight were developed via MAS by transferring three QTL for grain weight (*QGw.ccsu-1A.2, QGw.ccsu-1A.3 and QGw. ccsu-1B.1*) by Kumari *et al.,* (2019). Seven pairs had the background of Raj3765 and one pair had the background of K9107. Each NIL pair had a solo QTL. The difference in 1000 grain weight (TGW) for two individual NILs of an individual couple varying between 2.8 and 7.5 g, validates the QTL effect for TGW. QTL, QGw.ccsu-1A.2 has, a total average difference of 2.8 g for TGW in the NILs covering the three QTLs and the NILs involving TGW.

2.3 Maize

Maize (*Zea mays* L.) is a special crop, for food, feed and source of a wide range of industrial products in world agriculture. Drought is the most important constraint in rainfed lowlands and uplands covering approximately 70 percent of maize production. The acid soils, the water logging, the mildew drowning, stalk red, and leaf blight are other abiotic and biotic constraints, which have widespread productive effects and should be of great importance in maize breeding research. Most maize research programs provide molecular methods to improve breeding efficiency and effectiveness. The molecular data volume for diverse populations and breeding lines has accumulated rapidly both within public and in private breeding institutions.With SSR, SNPs and other technologies numerous genes have been cloned which control various aspects of plant growth, biotic and abiotic stress resistance and quality characteristics.

Currently, in maize researchers' SSRs are the most commonly used markers because of their large numbers (Maize GDB; http:/www.maizegdb.org), quick and efficient availability. These gene-dominant PCR-based markers are stable, reproducible, hypervariable, abundant, and uniformly distributed in plant genomes. New and current methods, such as identification of mutation, high-throughput genotypes of gene discovery, omics, micro-arrays and exome sequence, are being must be used more widely. Taking into account the importance of root architectural features in battling abiotic stress, the implementation of smart climate agriculture practices should be a priority to phenotyping platforms visualizing root system architecture (Chaudhary *et al.,* 2019). Different studies regarding use of MAS in maize are given in Table 5.

In India, the first maize variety developed through MAS was Vivek QPM 9 by Gupta *et al.,* (2009). They transferred Opaque 2 gene which had higher tryptophan and lysine content. Muthuswamy *et al.,* (2014) introduced crtRB1 gene by utilizing SSR markers into seven elite maize genotypes, leading to enhancement of kernel -carotene. In crtRB1 introduced inbreds, the -carotene concentration ranged from 8.6 to 17.5 mg/g which was upto 12.6 times the average increase in recurrent parents. Genome Sequencing (GS) based work has gained at traction as a tool to establish causal SNPs for abiotic stress-related characteristics. In GS maize studies, 77 SNPs were established and regulated various functions associated with root growth, hormonal signaling and photosynthesis linked to ten drought-response transcription factors by Shikha *et al.,* (2017). They tested 240 subtropical maize lines phenotyped for drought with 29,619 SNPs in different environments. Maize Streak Virus (MSV) disease is a devastating disease which causes significant yield loss in maize. QTL, qMsv1 governing MSV resistance was mapped on chromosome 1 using F2 population derived from cross between CML206 with CML312 by Nair *et al.,* (2015). Association mapping was done using genotyping-by-sequencing GBS markers. They developed KASP assays for marker screening for MSV resistance. Zunjare *et al.,* (2018) stacked three alleles crtRB1, lcyE and o2 in four maize hybrids (HQPM1, HQPM4, HQPM5 & HQPM7) by utilizing MAS. Background recovery of recurrent parent genome ranged from 89 to 93% among the selected backcross progenies. This is the first study where the three alleles were stacked simultaneously in single genetic background. These biofortified maize hybrids rich in proA, lysine and tryptophan can be used in future for national food and nutritionalsecurity.

3. Challenges and future strategies

India's more than one billion inhabitants rise almost parallel to the annual cereal growth rate at a rate of about 1.8 percent per year. The growing numbers of people in India have alarmed the production of food and attempts have been made to incorporate modern technological instruments into traditional breeding in order to boost key crops like rice, wheat and maize. Another big obstacle is the incorporation of molecular breeding activities into a few regional program partners. One of the key concerns of wheat researchers is making Indian wheat competitive on a global scale and reduce rising costs and increasing farmers' profitability. For the future, scientific researchers should be prepared to build resource efficient varieties with exact combinations of desirable characteristics by providing precise knowledge about the position and role of genes encoding for useful traits. Genetic transformation will still remain a significant method to understand the role of genes and the utility of new sequences.

In the near future, crop varieties will be adapted to suit

Table 5: Marker assisted breeding studies in Maize conducted by different researchers in India

Trait	Gene/QTL	Marker	Reference
Drought tolerance	*QTLs*	SNPs	Shikha *et al.,* 2017
	QTLs	RFLP	Rahman *et al.,* 2011
	QTLs	SSRs	Kaur, 2017
Downy mildew	*QTL*	SSR	Nair *et al.,* 2005
Maize Streak Virus	*Msv1, QTL*	SNPs	Nair *et al.,* 2015
Quality protein maize	*crtRB13' TE*	SSRS	Vignesh *et al.,* 2012
	Opaque 2 gene		Gupta *et al.,* 2009
	Ley E & CrtRB1		Babu *et al.,* 2013
	-carotene hydroxylase allele		Muthuswamy *et al.,* 2014
	-carotene hydroxylase, lycopene- -cyclase & opaque 2 gene		Zunjare *et al.,* 2018
Yield & yield related traits		SNPs & MTAs	Sivakumar *et al.,* 2019

local customer needs as well as regional climate and niche requirements. After the advent of the Green Revolution, ICAR- Indian Institute of Wheat and Barley Research has played a major role in improving the Indian wheat system. In the light of new challenges, Research Institutes should give priority to maintain pace with time and development, focusing on molecular reproductive systems, functional genomics and transgenic deployment for abiotic stresses. While biotechnology's potential has been often over estimated, its use in the improvement of wheat clearly supports a high degree of optimism. Functional genomics, as they are called today, would certainly revolutionize the potential manner in which plant breeding is carried out. Fundamental research has led to a better understanding of the genetic mechanisms that function in a plant in response to the various stresses it has to face and to the overall biomass and grain production. The goal is to use the latest technologies as much as possible for developing countries.

3. References

1. Akhtar S, MA Bhat, SA Wani, KA Bhat, S Chalkoo, MR Mir and SA Wani. 2010. Marker assisted selection in rice. *Journal of Phytology* **2(10)**: 66-81.
2. Akula SH, MA Dass, SK Surapaneni, P Balaravi. 2020. Mapping of quantitative trait loci associated with resistance to brown planthopper in background of Swarna from a traditional variety PTB33. *Euphytica* **216**: 114.
3. Anonymous. 2017. Progress report of AICRP on Wheat and Barley (Crop Improvement) 2016-17, Ed: Tiwari *et al.,* ICAR-*Indian Institute of Wheat and Barley Research, Karnal, Haryana, India*, 249p.
4. Anonymous. 2019. Director's report of AICRP on Wheat and Barley 2018-19, Ed: GP Singh. ICAR-*Indian Institute of Wheat and Barley Research, Karnal, Haryana, India,* 72p.
5. Awan SI, SD Ahmad, L Mur and MS Ahmed. 2017. Marker-assisted selection for durable rust resistance in a widely adopted wheat cultivar "Inqilab-91". *International Journal of Agriculture and Biology.* **19(6):** 1319-1324.
6. Awika JM 2011. Major cereal grains production and use around the world. Soil & Crop Science Dept./ Nutrition and Food Science Dept., Texas A&M University, 2474 TAMU, College Station, TX 77843-2474.
7. Azameti MK, B Vishalakshi, B Umakanth, M Balram, MS Prasad and MS Madhav. 2020. Molecular characterization of popular rice (*Oryza sativa* L.) varieties of India and association analysis for blast resistance. *Genetic Resources and Crop Evolution.* **67(8):** 2225-2236.
8. Babu NN, SG Krishnan, KK Vinod, SL Krishnamurthy, VK Singh, MP Singh and AK Singh. 2017. Marker aided incorporation of Saltol, a major QTL associated with seedling stage salt tolerance, into *Oryza sativa* B *Frontiers of Plant Science* **8**: 41 Pusa Basmati 1121.
9. Babu NN, KK Vinod, SG Krishnan, PK Bhowmick, T Vanaja, SL Krishnamurthy, M Nagarajan, NK Singh, KV Prabhu and AK Singh. 2014. Marker based haplotype diversity of Saltol QTL in relation to seedling stage salinity tolerance in selected genotypes of rice. *Indian Journal of Genetics* **74(1)**: 16-25.
10. Babu R, NP Rojas, S Gao, J Yan and K Pixley. 2013. Validation of the effects of molecular marker polymorphisms in LcyE and CrtRB1 on provitamin A concentration for 26 tropical maize populations. *Theoretical and Applied Genetics* **126**: 389–399.DOI 10.1007/s00122-012-1987-3.
11. Barakat MN, MS Saleh, AA Al-Doss, KA Moustafa, AA Elshafei, AM Zakri and FH Al-Qurainy. 2015. Mapping of QTLs associated with abscisic acid and water stress in wheat. *Biologia Plantarum* **59(2)**: 291-297.
12. Barik SR, E Pandit, SK Pradhan, S Singh, P Swain and T Mohapatra. 2018. QTL mapping for relative water content trait at reproductive stage drought stress in rice. *Indian Journal of Genetics* **78(4)**: 401-408.
13. Barik SR, E Pandit, SK Pradhan, SP Mohanty and T Mohapatra. 2019. Genetic mapping of morpho-physiological traits involved during reproductive stage drought tolerance in rice. *PLoS ONE* **14(12)**: e0214979.
14. Bhattarai U and PK Subudhi. 2018. Identification of drought responsive QTLs during vegetative growth stage of rice using a saturated GBS-based SNP linkage map. *Euphytica* **214(2):** 38.
15. Chaudhary HK, A Badiyal, W Hussain, NS Jamwal, N Kumar, P Sharma, and AD Singh. 2019. Innovative Role of DH breeding in genomics assisted-crop improvement: focus on drought tolerance in wheat. Genomic assisted breeding of crops for aboitic stress tolerance, volume II sustainable development and biodiversity 21: 69–90.

16. Das G and GJN Rao. 2015. Molecular marker assisted gene stacking for biotic and abiotic stress resistance genes in an elite rice cultivar. *Front Plant Sci* **6**: 698.

17. Deen R, K Ramesh, G Padmavathi, BC Viraktamath and T Ram. 2017. Mapping of brown planthopper [Nilaparvata lugens (Sta°l)] resistance gene (bph5) in rice (*Oryza sativa* L.). *Euphytica* **213**: 35 .

18. Divya D, KR Madhavi, MA Dass, RV Maku, G Mallikarjuna, RM Sundaram, GS Laha, AP Padmakumari, HK Patel, MS Prasad, RV Sonti and JS Bentur. 2018. Expression profile of defense genes in rice lines pyramided with resistance genes against bacterial blight, fungal blast and insect gall midge. *Rice* **11**: 40.

19. Dixit S, A Singh, N Sandhu, A Bhandari, P Vikram and A Kumar. 2017a. Combining drought and submergence tolerance in rice: marker-assisted breeding and QTL combination effects. *Molecular Breeding* **37:** 143.

20. Dixit S, A Singh, MTSta Cruz, PT Maturan, M Amante and A Kumar. 2014. Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC Genet* **15:** 16.

21. Dixit S, RB Yadaw, KK Mishra and A Kumar. 2017b. Marker-assisted breeding to develop the drought-tolerant version of Sabitri, a popular variety from Nepal. *Euphytica* **213:** 184.

22. Dixit S, UM Singh, AK Singh, S Alam, C Venkateshwarlu, VV Nachimuthu, S Yadav, R Abbai, R Selvaraj, MN Devi, PJ Ramayya, J Badri, T Ram, J Lakshmi, G Lakshmidevi, JV LRK, AP Padmakumari, GS Laha, MS Prasad, M Seetalam, VK Singh and A Kumar. 2020. Marker assisted forward breeding to combine multiple biotic-abiotic stress resistance/tolerance in rice. *Rice* **13:** 29.

23. Donde R, J Kumar, G Gouda, MK Gupta, M Mukherjee, SY Baksh, P Mahadani, KK Sahoo, L Behara and SK Dash. 2019. Assessment of genetic diversity of drought tolerant and susceptible rice genotypes using microsatellite markers. *Rice Science* **26(4):** 239-247.

24. FAO (2020) http://www.fao.org/faostat/en/#data/QC

25. Fatima S, SK Chaudhari, S Akhtar, MS Amjad, M Akbar, MS Iqbal, M Arshad and T Shehzad. 2018. Mapping QTLS for yield and yield components under drought stress in bread wheat (*Triticum aestivum* L.). *Applied Ecology and Environmental Research* **16(4):** 4431-4453.

26. Gahlaut V, V Jaiswal, S Singh, HS Balyan and PK Gupta. 2019. Multi-locus genome wide association mapping for yield and its contributing traits in hexaploid wheat under different water regimes. *Scientific Reports* **9:** 19486.

27. Gahlaut V, V Jaiswal, BS Tyagi, G Singh, S Sareen, HS Balyan and PK Gupta. 2017. QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. *PLoS ONE* **12(8):** e0182857.

28. Gandhi D. 2007. UAS scientist develops first drought tolerant rice. The Hindu. www.thehindu.com/2007/11/17/stories/2007111752560500.htm

29. Gantait, S, S Sarkar and SK Verma. 2019. Marker-assisted selection for abiotic stress tolerance in crop plants In: *Molecular Plant Abiotic Stress: Biology and Biotechnology:* 335-368. https://doi.org/10.1002/9781119463665.ch18.

30. Gautam T, Amardeep, G Saripalli, Rakhi, A Kumar, V Gahlaut, DA Gadekar, M Oak, PK Sharma, HS Balyan, and PK Gupta. 2020 a. Introgression of a drought insensitive grain yield QTL for improvement of four Indian bread wheat cultivars using marker assisted breeding without background selection. *Journal of Plant Biochemistry and Biotechnology.* https://doi.org/10.1007/s13562-020-00553-0.

31. Gautam T, GS Dhillon, G Saripalli, Rakhi, VP Singh, P Prasad, S Kaur, P Chhuneja, PK Sharma, HS Balyan, and PK Gupta. 2020 b. Marker-assisted pyramiding of genes/QTL for grain quality and rust resistance in wheat (*Triticum aestivum* L.). *Molecular Breeding* **40:** 49.

32. Ghatak A, P Chaturvedi, and W Weckwerth. 2017. Cereal crop proteomics: systemic analysis of crop drought stress responses towards Marker-Assisted Selection breeding. *Frontiers in Plant Science* **8:** 757.

33. Gopalkrishnan S, RK Sharma, K Anand Rajkumar, M Josheph, VP Singh, AK Singh, KV Bhat, NK Singh and T Mohapatra. 2008. Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. *Plant Breeding* **127:** 131-139.

34. Gouda PK, S Saikumar, CMK Varma, K Nagesh, S Thippeswamy, V Shenoy, MS Ramesha and HE

Shashidhar. 2013. Marker-assisted breeding of Pi-1 and Piz-5 genes imparting resistance to riceblast in PRR78, restorer line of Pusa RH-10 Basmati rice hybrid. *Plant Breeding* **132(1):** 61-69.

35. Gupta HS, PK Agrawal, V Mahajan, GS Bisht, A Kumar, P Verma, A Srivastava, S Saha, R Babu, MC Pant and VP Mani. 2009. Quality protein maize for nutritional security: rapid development of short duration hybrids through molecular marker assisted breeding. *Current Science* **96(2)**:____.

36. Gupta PK, HS Balyan and V Gahlaut. 2017. QTL Analysis for drought tolerance in wheat: Present status and future possibilities. *Agronomy***7(5):** ___

37. Gupta PK, S Rustgi and RR Mir. 2013. Array-based high-throughput DNA markers and genotyping platforms for cereal genetics and genomics. In: Gupta PK, Varshney RK (eds) *Cereal Genomics II.* Springer, The Netherlands, pp 11-55.

38. Gupta SK, A Charpe, S Koul, QMR Haque and KV Prabhu. 2006. Development and validation of SAR markers co-segregating with an Agropyron elongatum derived leaf rust resistance gene Lr24 in wheat. *Euphytica* **150:** 233–240.

39. Gupta SK, A Charpe, S Koul, KV Prabhu and QMR Haq. 2005. Development and validation of molecular markers linked to an *Aegilops umbellulata*–derived leaf-rust-resistance gene, Lr9, for marker-assisted selection in bread wheat. *Genome* **48:** 823-830.

40. Guvvala LD, P Koradi, V Shenoy and LS Marella. 2013. Improvement of resistance to bacterial blight through marker assisted backcross breeding and field validation in rice (*Oryza sativa*). *Research Journal of Biological Sciences* **1:** 52-66.

41. Hossain H, MA Rahman, MS Alam and RK Singh. 2015. Mapping of quantitative trait loci associated with reproductive-stage salt tolerance in rice. *Journal of Agronomy and Crop Science* **201(1):** 17–31.

42. Iyer AS and SR McCouch. 2004. The rice bacterial blight resistance gene Xa5 encodes a novel form of disease resistance. *Molecular Plant-Microbe Interactions* **17:** 1348–1354.

43. Jain N, GP Singh, PK Singh, P Ramya, H Krishna, KT Ramya, L Todkar, B Amasiddha, KC Prashant and Vijay P. 2014. Molecular approaches for wheat improvement under drought and heat stress. *Indian Journal of Genetics* **74(4):** 578–583.

44. Joseph M, S Gopalakrishnan, RK Sharma, VP Singh, AK Singh and T Mohapatra. 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Molecular Breeding* **13:** 377-387.

45. Kaur M, R Singh, S Kumar, RP Mandhan and I Sharma. 2016. Identification of QTL conferring Karnal bunt resistance in bread wheat. *Indian Journal of Biotechnology* **15:** 34-38.

46. Kaur K. 2017. Mapping of QTLs for drought tolerance component traits in maize (Doctoral dissertation, Punjab Agricultural University, Ludhiana).

47. Khan MA, M Iqbal, M Jameel, W Nazeer, S Shakir, MT Aslam and B Iqbal. 2011. Potentials of molecular based breeding to enhance drought tolerance in wheat (*Triticum aestivum* L.) *African Journal of Biotechnology* **10(55):** 11340-11344.

48. Khanna A, V Sharma, RK Ellur, AB Shikari, S Gopalakrishnan, UD Singh, G Prakash, TR Sharma, R Rathour, M Variar, SK Prashanthi, M Nagarjan, KK Vinod, PK Bhowmick, H Rajashekhara, NK Singh, KV Prabhu and AK Singh. 2015. Marker assisted pyramiding of major blast resistance genes Pi9 and Pita in the genetic background of an elite Basmati rice variety, Pusa Basmati 1. *Indian Journal of Genetics* **75(4):** 417-425.

49. Khanna-Chopra R, K Singh, S Shukla, S Kadam and NK Singh. 2019. QTLs for cell membrane stability and flag leaf area under drought stress in a wheat RIL population. *Journal of Plant Biochemistry and Biotechnology* **29(2)**: 276-286.

50. Krishnamurthy SL, SK Sharma, V Kumar, S Tiwari, V Batra and NK Singh. 2014. Assessment of genetic diversity in rice genotypes for salinity tolerance using Saltol markers of Chromosome 1. *Indian Journal of Genetics* **74(2):** 243-247.

51. Kumar K, P Kaur, A Kishore, Y Vikal, K Singh and K Neelam. 2020. Recent advances in genomics-assisted breeding of brown planthopper (*Nilaparvata lugens*) resistance in rice (*Oryza sativa*). *Plant Breeding* :1–15.

52. Kumar J, V Jaiswal, A Kumar, N Kumar, RR Mir, S Kumar, R Dhariwal, S Tyagi, M Khandelwal, KV Prabhu, R Prasad, HS Balyan and PK Gupta. 2011. Introgression of a major gene for high grain protein content in some Indian bread wheat cultivars. *Field Crops Research* **123:** 226–233.

53. Kumar J, RR Mir, N Kumar, A Kumar, A Mohan, KV Prabhu, HS Balyan and PK Gupta. 2010. Marker-assisted selection for pre-harvest sprouting tolerance and leaf rust resistance in bread wheat. *Plant Breeding* **129:** 617-621.

54. Kumar N, PL Kulwal, HS Balyan and PK Gupta. 2007. QTL mapping for yield and yield contributing traits in two mapping populations of bread wheat. *Molecular Breeding* **19:** 163–177.

55. Kumar K, PS Sarao, D Bhatia, K Neelam, A Kaur, GS Mangat, DS Brar and K Singh. 2018. High resolution genetic mapping of a novel brown planthopper resistance locus, Bph34 in *Oryza sativa* L. X Oryza nivaraderived interspecifc F2 population. *Theoretical and Applied Genetics* **131:** 1163–1171. https://doi.org/10.1007/s00122-018-3069-7.

56. Kumar S, SS Singh, CN Mishra, M Saroha, V Gupta, P Sharma, V Tiwari and I Sharma. 2015. Assessment of tiller inhibition (tin) gene molecular marker for its application in Marker-Assisted Breeding in wheat. *Natl. Acad. Sci. Lett.* **38(6):** 457–460.

57. Kumar VA, CH Balachiranjeevi, SB Naik, G Rekha, R Rambabu, G Harika, K Pranathi, SK Hajira, M Anila, M Kousik, R Kale, TD Kumar, MS Prasad, AS Prasad, AP Padmakumari, GS Laha, SM Balachandran, MS Madhav, P Senguttuvel, KB Kemparajan, AR Fiyaz, JS Bentur, BC Viraktamath, V Babu and RM Sundaram. 2017. Marker-assisted pyramiding of bacterial blight and gall midge resistance genes into RPHR-1005, the restorer line of the popular rice hybrid DRRH-3. *Molecular Breeding* **37(7):** 86.

58. Kumari S, RR Mir, S Tyagi, HS Balyan and PK Gupta. 2019. Validation of QTL for grain weight using MAS-derived pairs of NILs in bread wheat (*Triticum aestivum* L.). *Journal of Plant Biochemistry and Biotechnology* **28(3):** 336-344.

59. Malik S and TA Malik. 2015. Genetic mapping of potential QTLs associated with drought tolerance in wheat. *Journal of Animal and Plant Sciences* **25(4).**

60. Mishra SK, R Chandra, M Rathore, D Ghosh, S Mahesh and B Kumar. 2019. Screening of weedy rice biotypes for water deficit and salt stress tolerance. *Annals of Plant and Soil Research* **21(1):** 51-57.

61. Mishra KK, P Vikram, RB Yadaw, BPM Swamy, S Dixit, MT Sta Cruz, P Maturan, S Marker and A Kumar. 2013. qDTY 12.1: a locus with a consistent effect on grain yield under drought in rice. *BMC Genet* **14:** 12.

62. Mohanty SK, RS Panda, SL Mohapatra, A Nanda, L Behera, M Jena, RK Sahu, SC Sahu and T Mohapatra. 2017. Identification of novel quantitative trait loci associated with brown planthopper resistance in the rice landrace Salkathi. *Euphytica* **213:** 38.

63. Mujtaba SM, S Faisal, MA Khan, MU Shirazi and MA Khan. 2018. Evaluation of drought tolerant wheat genotypes using morpho-physiological indices as screening tools. *Pak. J. Bot.* **50(1):** 51-58.

64. Muthu V, R Abbai, J Nallathambi, H Rahman, S Ramasamy, R Kambale, T Thulasinathan, B Ayyenar and R Muthurajan. 2020. Pyramiding QTLs controlling tolerance against drought, salinity, and submergence in rice through marker assisted breeding. *PLoS ONE 15(1):* e0227421.

65. Muthusamy V, F Hossain, N Thirunavukkarasu, M Choudhary, S Saha, JS Bhat, BM Prasanna and HS Gupta. 2014. Development of b-Carotene rich maize hybrids through marker-assisted introgression of b-carotene hydroxylase Allele. *PLoS ONE* **9(12):** e113583. doi:10.1371/journal.pone.0113583.

66. Nair SK, R Babu, C Magorokosho, G Mahuku, K Semagn, Y Beyene, B Das, D Makumbi, PL Kumar, M Olsen and PM Boddupalli. 2015. Fine mapping of Msv1, a major QTL for resistance to Maize Streak Virus leads to development of production markers for breeding pipelines. *Theor Appl Genet* **128:** 1839–1854.

67. Nair SK, BM Prasanna, A Garg, RS Rathore, TAS Setty and NN Singh. 2005. Identification and validation of QTLs conferring resistance to sorghum downy mildew (Peronosclerospora sorghi) and Rajasthan downy mildew (*P. heteropogoni*) in maize. *Theor Appl Genet* **110:** 1384–1392.

68. Narayanan NN, N Baisakh, CM Vera Cruz, SS Gnanamanickam, K Datta and SK Datta. 2002. Molecular Breeding for the development of blast and bacterial blight resistance in rice cv. IR50. *Crop Science* 42(6): 2072.

69. Neeraja CN, R Maghirang-Rodriguez, A Pamplona, S Heuer, BCY Collard, EM Septiningsih, G Vergara, D Sanchez, Xu K, AM Ismail and DJ Mackill. 2007. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theoretical and Applied Genetics* **115:** 767-776.

70. Pal D, SC Bhardwaj, M Patial, S Kumar, OP Gangwar, P Sharma and KV Prabhu. 2019. Transfer

of leaf rust and stripe rust resistance genes Lr19 and Yr15 into a susceptible wheat cultivar HS295. *Indian Journal of Genetics* **79(3):** 618-621.

71. Pandey B, A Kaur, OP Gupta, I Sharma and P Sharma. 2015. Identification of HSP20 gene family in wheat and barley and their differential expression profiling under heat stress. *Appl Biochem Biotechnol* **175:** 2427–2446. DOI 10.1007/s12010-014-1420-2.

72. Pandey MK, NS Rani, RM Sundaram, GS Laha, MS Madhav, KS Rao, I Sudharshan, Y Hari, GS Varaprasad, LVS Rao, K Suneetha, AKP Sivaranjani and BC Viraktamath. 2013. Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. *Molecular Breeding* **31:** 239-246.

73. Prabhu KV, AK Singh, SH Basavaraj, DP Cherukuri, A Charpe, S Gopala Krishnan, S Gupta, M Joseph, S Koul, T Mohapatra, JK Pallavi, D Samsampour, A Singh, VK Singh, A Singh and VP Singh. 2009. Marker assisted selection for biotic stress resistance in wheat and rice. *Indian Journal of Genetics* **69(4)** (Spl. issue): 305-314.

74. Prasad M., N Kumar, PL Kulwal, MS Roder, HS Balyan, HS Dhaliwal and PK Gupta. 2003. QTL analysis for grain protein content using SSR markers and validation studies using NILs in bread wheat. *Theoretical and Applied Genetics*. **106:** 659–667.

75. Prince SJ, R Beena, GS Michael, S Senthivel and BR Chandra. 2015. Mapping consistent rice (*Oryza sativa* L.) yield QTLs under drought stress in target rainfed environments. *Rice* **8:** 25.

76. Rahman H, S Pekic, V Lazic-Jancic, SA Quarrie, SM Shah, A Pervez and MM Shah. 2011. Molecular mapping of quantitative trait loci for drought tolerance in maize plants. *Genetic and Molecular Research* **10(2):** 889-901.

77. Rai A, AM Singh, K Raghunandan, TPJ Kumar, P Sharma, AK Ahlawat, SK Singh, D Ganjewala, RB Shukla and M Sivasamy. 2019. Marker assisted transfer of PinaD1a gene to develop soft grain wheat cultivars. *3 Biotech* **9:** 183.

78. Rai N, A Bellundagi, PKC Kumar, RK Thimmappa, S Rani, N Sinha, Harikrishna, N Jain, GP Singh, PK Singh, S Chand and KV Prabhu. 2018. Marker-assisted backcross breeding for improvement of drought tolerance in bread wheat (Triticum aestivum L. em Thell). *Plant Breeding*. **137:** 514–526.

79. Rajesh T, S Maruthasalam, K Kalpana, K Poovannan, KK Kumar, E Kokiladevi, D Sudhakar, R Velazhahan and P Balasubramanian. 2020. Pyramiding insect and disease resistance in an elite indica rice cultivar asd16. *Biologia Plantarum* **64:** 77-86.

80. Ramchander S, M Raveendran and S Robin. 2016. Mapping QTLs for physiological traits associated with drought tolerance in Rice (*Oryza sativa* L.). *Journal of Investigative Genomics* **3(3):** 00052.

81. Ramkumar G, K Srinivasarao, K Madhanmohan, I Sudarshan, AKP Sivaranjani, K Gopalkrishna, CN Neeraja, SM Balachandran, RM Sundaram, MS Prasad, N Shobharani, AM Rama Prasad, BC Viraktamath and MS Madhav. 2010. Development and validation of functional marker targeting an InDel in the major rice blast disease resistance gene Pi54 (Pikh). *Molecular Breeding* **27:** (129-135).

82. Randhawa HS, JS Mutti, K Kidwell, CF Morris, X Chen and KS Gill. 2009. Rapid and targeted introgression of genes into popular wheat cultivars using marker-assisted background selection. *PLoS One*. **4(6):** e5752.

83. Randhawa MS, NS Bains, VS Sohu, P Chhuneja, RM Trethowan, HS Bariana and U Bansal. 2019. Marker assisted transfer of stripe rust and stem rust resistance genes into four wheat cultivars. *Agronomy*. **9:** 497.

84. Rani R, R Singh and NR Yadav. 2019. Evaluating stripe rust resistance in Indian wheat genotypes and breeding lines using molecular markers. *Comptes Rendus Biologies* **342:** 154–174.

85. Raza A, A Razzaq, SS Mehmood, X Zou, X Zhang, Y Lv and J Xu. 2019. Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. *Plan Theory* **8:**34.

86. Sabar M, G Shabir, SM Shah, K Aslam, SA Naveed and M Arif. 2019. Identification and mapping of QTLs associated with drought tolerance traits in rice by a cross between Super Basmati and IR55419-04. *Breeding Science* **69(1):** 169-178.

87. Sama VSAK, K Himabindu, SB Naik, RM Sundaram, BC Viraktamath and JS Bentur. 2012. Mapping and marker-assisted breeding of a gene allelic to the major Asian rice gall midge resistance gene Gm8. *Euphytica*.**187:** 393-400.

88. Sandhu N, A Singh, S Dixit, MTS Cruz, PC Maturan,

RK Jain, A Kumar. 2014a. Identification and mapping of stable QTL with main and epistasis effect on rice grain yield under upland drought stress. *BMC Genet* **15:** 63.

89. Sandhu N, S Dixit, BM Swamy, P Vikram, C Venkateshwarlu, M Catolos and A Kumar. 2018. Positive interactions of major-effect QTLs with genetic background that enhances rice yield under drought. *Scientific Reports* **8(1):** 1-3.

90. Sandhu N, S Dixit, BPM Swamy, A Raman, S Kumar, SP Singh, RB Yadaw, DN Singh, JN Reddy, A Anandan, S yadav, C Venkateshwarllu, A Henry, S Verulkar, NP Mandal, T Ram, J Badri, P Vikram and A Kumar. 2019. Marker Assisted Breeding to develop multiple stress tolerant varieties for flood and drought prone areas. *Rice* **12:** 8. https://doi.org/10.1186/s12284-019-0269-y.

91. Sandhu N, RO Torres, MTS Cruz, PC Maturan, R Jain, A Kumar and A Henry. 2014. Traits and QTLs for development of dry direct-seeded rainfed rice varieties. *Journal of Experimental Botany,* **66(1):** 225–244.

92. Sangodele EA, RR Hanchinal, NG Hanamaratti, V Shenoy and VM Kumar. 2014. Analysis of drought tolerant QTL linked to physiological and productivity component traits under water-stress and non-stress in rice (*Oryza sativa* L.). *International Journal of Current Research and Academic Review* **2(5):** 108-113.

93. Shamsudin NAA, BM Swamy, W Ratnam, MTS Cruz, A Raman and A Kumar. 2016. Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice cultivar, MR219. *BMC Genet* **17(1):** 30.

94. Sharma DK, AM Torp, E Rosenqvist, CO Ottosen and SB Andersen. 2017. QTLs and potential candidate genes for heat stress tolerance identified from the mapping populations specifically segregating for Fv/Fm in wheat. *Frontiers in Plant Science* **8:** 1668.

95. Shikha M, A Kanika, AR Rao, MG Mallikarjuna, HS Gupta and Nepolean T. 2017. Genomic selection for drought tolerance using genome-wide SNPs in Maize. *Frontier in Plant Sciences* **8:** 550.

96. Showmy KS and A Yusuf. 2020. Characterization of disease resistance in nine traditional rice (*Oryza sativa* L.) cultivars and expression of chennellu PR1 gene in response to *Xanthomonas oryzae* pv. *oryzae*. *Indian Phytopathology* **73(2):** 281-291.

97. Singh RP, DP Hodson, Y Jin, ES Lagudah, MA Ayliffe, S Bhavani, MN Rouse, ZA Pretorius, LJ Szabo, J Huerta-Espino, BR Basnet, C Lan and MS Hovmoller. 2015. Emergence and spread of new races of wheat stem rust fungus: continued threat to food security and prospects of genetic control. *Phytopathology* **105:** 872–884.

98. Singh RP, DP Hodson, J Huerta-Espino, Y Jin, S Bhavani, P Njau, S Herrera-Foessel, PK Singh, S Singh and V Govindan. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annu Rev Phytopathol* **49:** 465–481.

99. Singh R, D Datta, Priyamvada, S Singh and R Tiwari. 2009. A Diagnostic PCR Based Assay for Stripe Rust Resistance Gene *Yr10* in Wheat. *Acta Phytopathologica et Entomologica Hungarica* **44 (1):** 11-18.

100. Singh R, Y Singh, S Xalaxo, S Verulkar, N Yadav *et. al.,* 2016. From QTL to variety- harnessing the benefits of QTLs for drought, flood and salt tolerance in mega rice varieties of India through a multi-institutional network, *Plant Science*. http://dx.doi.org/10.1016/j.plantsci.2015.08.008

101. Singh S, JS Sidhu, N Huang, Y Vikal, Z Li, DS Brar, HS Dhaliwal and GS Khush. 2001. Pyramiding three bacterial blight resistance genes (Xa5, Xa13 and Xa21) using marker-assisted selection into indica rice cultivar PR106. *Theor Appl Genet.* **102:** 1011-1015.

102. Singh D, A Kumar, A Sirohi, P Kumar, J Singh, V Kumar, A Jindal, S Kumar, N Kumar, V Kumar, V Sharma, S Gupta and S Chand. 2011a. Improvement of Basmati rice (*Oryza sativa* L.) using traditional breeding technology supplemented with molecular markers. *African Journal of Biotechnology Vol.* **10 (4):** 499-506.

103. Singh AK, S Gopalakrishnan, SVP Singh, KV Prabhu, T Mohapatra, NK Singh, TR Sharma, M Nagarajan, KK Vinod, D Singh, UD Singh, S Chander, SS Atwal, R Seth, VK Singh, RK Ellur, A Singh, D Anand, A Khanna, S Yadav, N Goel, A Singh, AB Shikari, A Singh and B Marathi. 2011b. Marker assisted selection: a paradigm shift in Basmati breeding. *Indian Journal of Genetics and Plant Breeding* **71(2):** 120-128.

104. Singh VK, A Singh, SP Singh, RK Ellur, V Choudhary, S Sarkel, D Singh, SG Krishnan, M Nagarajan, KK Vinod, UD Singh, R Rathore, SK Prashanthi, PK Agrawal, JC Bhatt, T Mohapatra, KV Prabhu and AK Singh. 2012. Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line, through marker assisted backcross breeding. *Field Crops Research* **128:** 8-16.

105. Singh VK, BD Singh, A Kumar, S Maurya, SG Krishnan, KK Vinod, MP Singh, RK Ellur, PK Bhowmick and AK Singh. 2018. Marker-assisted introgression of Saltol QTL enhances seedling stage salt tolerance in the rice variety BPusa Basmati 1. *International Journal of Genomics,* Vol. of 2018. https://doi.org/10.1155//2018/8319879.

106. Sivakumar S, M Dhasarathan, A Karthikeyan, P Bharathi, N Kumari Vinodhana, K Ganesamurthy and N Senthil. 2019. Population structure and association mapping studies for yield-related traits in Maize (*Zea mays* L.). *Current Plant Biology* Vol. of 18.

107. Srivastava, N. 2019. Molecular and biotechnological tools in developing abiotic stress tolerance in wheat. *Wheat Production in Changing Environments Springer, Singapore.* https://doi.org/10.1007/978-981-13-6883-7-13.

108. Suji KK, KSJ Prince, PS Mankhar, P Kanagaraj, R Poornima, K Amutha, S Kavitha, KR Biji, M Gomez and RC Babu. 2012. Evaluation of rice (*Oryza sativa* L.) near isogenic lines with root QTLs for plant production and root traits in rainfed target populations of environment. *Field Crops Research* **137:** 89-96.

109. Sukumaran S, MP Reynolds and C Sansaloni. 2018. Genome-wide association analyses identify QTL hotspots for yield and component traits in durum wheat grown under yield potential, drought, and heat stress environments. *Frontiers in Plant Sciences* **9:** 81.

110. Sundaram RM, MRV Priya, GS Laha, NS Rani, PS Rao, SM Balachandran, GA Reddy, NP Sarma and RV Sonti. 2009. Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety by molecular marker assisted breeding. *Biotechnol J.* **4:** 400-407.

111. Sundaram RM, MR Vishnupriya, SK Biradar, GS Laha, AG Reddy, NS Rani, NP Sarma and RV Sonti. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica.* **160:** 411-422.

112. Swamy BPM, HU Ahmed and A Henry. 2013. Genetic, physiological, and gene expression analyses reveal that multiple QTL enhance yield of rice mega-variety IR64 under drought. *PLoS One* **8:**e62795.

113. Varshney RK, VK Singh, JM Hickey, X Xun, DF Marshall, J Wang, D Edwards and J Ribaut. 2016. Analytical and decision support tools for genomics-assisted breeding. *Trends in Plant Science* **21(4):** 354–363

114. Vignesh M, F Hossain, T Nepolean, S Saha, PK Agrawal, SK Guleria, BM Prasana and HS Gupta. 2012. Genetic variability for kernel -carotene and utilization of crtRB1 3'TE gene for biofortification in maize (*Zea mays* L.). *Indian Journal of Genetics* **72(2):**189-194.

115. Vikram P, BM Swamy, S Dixit, HU Ahmed, MTS Cruz, AK Singh and A Kumar. 2011. qDTY 1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genetics* **12(1):** 89.

116. Vinod KK, SG Krishnan, T Thribhuvan and AK Singh. 2019. Genetics of drought tolerance, mapping QTLs, candidate genes and their utilization in rice improvement. In: Rajpal VR ed. *Genomics Assisted Breeding of Crops for Abiotic Stress Tolerance* **2:** 145-186.

117. Vishwakarma G, RP Sanyal, A Shitre, DA Gadekar, A Saini and BK Das. 2019. Validation and marker-assisted selection of stem rust resistance gene *Sr2* in Indian wheat using gel-based and gel-free methods. *Journal of Crop Science and Biotechnology* **22 (4):** 309-315.

118. Vishwakarma MK, VK Mishra, PK Gupta, PS Yadav, H Kumar and AK Joshi. 2014. Introgression of the high grain protein gene Gpc-B1 in an elite wheat variety of Indo-Gangetic Plains through marker assisted backcross breeding. *Current Plant Biology* **1:** 60–67.

119. Wani SH and SK Sah. 2014. Biotechnology and abiotic stress tolerance in Rice. *Journal of Rice Research* **2:** 2.

120. Wani SH, M Choudhary, P Kumar, NA Akram, C Surekha, P Ahmad and SS Gosal. 2018. Marker-assisted breeding for abiotic stress tolerance in crop plants. In: *Biotechnologies of Crop Improvement* **3:** 1–23

121. Yadav MK, SAU Ngangkham, HN Shubudhi, MK Bag, T Adak, S Munda, S Samantaray and M Jena. 2017. Use of molecular markers in identification and characterization of resistance to rice blast in India. *PLOS ONE* **12(6):** e0179467.

122. Yadav S, N Sandhu, VK Singh, M Catolos and A Kumar. 2019. Genotyping-by-sequencing based QTL mapping for rice grain yield under reproductive stage drought stress tolerance. *Scientific Reports* **9:** 14326.

123. Yadav OP, F Hossain, CG Karjagi, B Kumar, PH Zaidi, SL Jat and BS Dhillon. 2015. Genetic Improvement of Maize in India: *Retrospect and Prospects. Agricultural Research* **4(4):** 325-338.

124. Yugander A, RM Sundaram, K Singh, P Senguttuvel, D Ladhalakshmi, KB Kemparaju, MS Madhav, MS Prasad, AS Hariprasad and GS Laha. 2018. Improved versions of rice maintainer line, APMS 6B, possessing two resistance genes, *Xa21* and *Xa38,* exhibit high level of resistance to bacterial blight disease. *Molecular Breeding* **38:** 100.

125. Yugander A, RM Sundaram, K Singh, MS Prasad, AS Hari Prasad, MS Madhav and GS Laha 2019. Marker assisted introgression of a major bacterial blight resistance gene, Xa38 into a rice maintainer line, APMS 6B. *Indian Phytopathology* **72(1):** 35-41.

126. Zunjare RU, Hossain F, Muthusamy V,Baveja A, Chauhan HS, Bhat JS,Thirunavukkarasu N, Saha S and Gupta HS (2018) Development of biofortified maize hybrids through marker-assisted stacking of -Carotene Hydroxylase, Lycopene- -Cyclase and Opaque 2 Genes. *Frontiers in Plant Science* **9:** 178.