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

Disha Kamboj¹, Satish Kumar¹*, Chandra Nath Mishra¹, Puja Srivastava², Gyanendra Singh¹ and Gyanendra Pratap Singh¹

¹ICAR – Indian Institute of Wheat and Barley Research, Karnal – 132001
²Punjab Agricultural University, Ludhiana - 141004

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 arisen 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 with 43 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

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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 crop 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 worldwide 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 gains. 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 shoot mass were recognized using a RIL population (Cocodrie x N-22). These QTLs can be introgressed into high-yielding rice varieties. 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 CR143-2-2) 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 CR143-2-2 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 stress tolerant 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 rainfed 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, Xa27) 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                    | SI-38023681 SI-38286772 263091  | Bhattarai and Subudhi, 2018     |
|                                 | 14 QTLs                    | SNPs                            |                                 |
|                                 | 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   | Id6010515-id6015531 K-id1024836-id1026726 Id10005369-id10006378 | Sandhu et al., 2014 a, 2014 b   |
|                                 | qDTY2.1                    | RM154-RM324, and RM263 RM573    |                                 |
|                                 | qDTY3.2 and qDTY12.1       | RM231, RM28099 and RM28199      | Dixit et al., 2017a, 2017b     |
|                                 | qDTY3.1                    | RM168 and RM468 RM586-RM217     | Dixit et al., 2014             |
|                                 | qDTY6.1                    | 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                        | SSR                             | 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          |
| Resistance Type                        | Markers/Genes                                                                 | Resources                        |
|----------------------------------------|------------------------------------------------------------------------------|----------------------------------|
| Bacterial blight & gall midge resistance | Gm4, Gm8, Xa21                                                               | Kumar et al., 2017               |
| Insect and disease resistance          | T1p, Xa 21, gna                                                               | Rajesh et al., 2020              |
| Blast resistance                       | Pi9 and Pita                                                                 | Khanna et al., 2015              |
|                                        | Pi0p, Pi1, Pi 2, Pi 9 and Pi 54                                               | Azameti et al., 2020             |
|                                        | Pi-1 and Pi 5z                                                                | Gouda et al., 2013               |
|                                        | Pi5 and Pi54                                                                 | Ramkumar et al., 2010            |
|                                        | Pi40 , Pi42 (t)                                                               | Akhtar et al., 2010              |
| Bacterial blight and blast resistance  | Pi5 + Xa 21                                                                  | Narayanan et al., 2002           |
| Gall midge resistance                  | Gm8                                                                          | 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 P9 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-nui-kash 1, Mo-nui-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 (qBph4.3 and 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.csw-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               |
|                              | QTLs             | Xwmc89, barc20, gwm368,       | Jain et al., 2014               |
|                              |                  | Xgwm111, gwm397               |                                 |
| Drought & heat               | QTLs             | SSR                           | Pandey et al., 2015             |
|                              | HSP20            | SSR                           | Gupta et al., 2005              |
|                              | Lr9              | SCAR & RAPD marker            | Gupta et al., 2006              |
|                              | Lr24             | RAPD                          |                                 |
|                              | Lr19             | SSR                           | Pandey et al., 2015             |
|                              | PHST QTL &       | SSR & SCAR                    | Kumar et al., 2010              |
|                              | Lr24+ Lr28       |                               |                                 |
|                              | Lr46, Lr34       | SSR                           | Awan et al., 2017               |
| Leaf rust resistance         | Lr19 and Yr15    | SSR, SCAR                     | Pal et al., 2019                |
|                              | Yr57, Yr51, Sr26, Sr22 & Sr50 | SSR                       | Randhawa et al., 2019          |
|                              | Sr2              | Xgwm 533                      | Vishwakarma et al., 2019        |
| Yellow & stem rust resistance| QTLs             | SSR                           | Gautam et al., 2020 a/b         |
| Leaf, yellow & stem 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             | SSR                           | 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 \textit{et al.} (2009) developed a PCR-based assay for easy selection of stripe rust resistance, \(Yr10\) gene. Similarly, Kumar \textit{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 \textit{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 \((P_1\text{ and }P_2)\) were crossed having PBW343 as background; these two lines were developed earlier using MAS. Line \(P_1\) constitut 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 \(F_4\) 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 threerusts in future wheat breeding program.

Prasad \textit{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 \textit{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 \textit{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 \((QGw.ccsu-1A.2, QGw.ccsu-1A.3 \text{ and } QGw. ccsu-1B.1)\) by Kumari \textit{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. QTLs, 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 (\textit{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 up to 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

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**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 overestimated, 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.

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