Precision breeding with genomic tools: A decade long journey of molecular breeding in rice

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Abstract

Plant breeding helps in recombining and fixation of desirable alleles which enables the development of improved varieties with better productivity. Though classical plant breeding has contributed enormously in achieving genetic gains in different crops including rice, the challenges imposed by resource constraints as well as greater anticipated demand need better tools to meet the expectations. Molecular breeding which uses modern genomic tools integrated with plant breeding offers enormous potential for improvement of crop varieties. The present review focuses on the achievements of ICAR-IARI in molecular breeding in rice wherein as many as eight rice varieties were incorporated with genes governing resistance/tolerance to different traits and released for commercial cultivation in India. The various factors which enabled the successful integration of molecular breeding including development of infrastructure, human resources and the genetic and genomic resources have been discussed in detail. All the examples of translational research are from the marker assisted backcross breeding strategy, which limits the scope for achieving yield gains. In the foregoing discussion, the challenges and the opportunities for implementation of molecular breeding in rice to realize the full potential of these tools in plant breeding are discussed based on the decade long experience, which may be valuable for other crops as well.

Key words: Molecular breeding, climate-smart varieties, stress tolerance, grain quality, rice

Introduction

Plant breeders through the ages have created, recombined, fixed desirable allelic combinations and selected superior genotypes thereby enabling the evolution and improvement of crop cultivars for productivity, quality as well as for other adaptive and commercially important traits. Classically, plant breeding involves selective mating of genotypes to provide chances for genetic recombination and to create an opportunity to select desirable recombinants primarily based on the expressed phenotypes. Improving the productivity of different crops in India has been made possible by virtue of strong foundation in plant breeding based on genetic principles, ably supported by the developments in complimentary agricultural technologies from other disciplines of agriculture. This has helped not only making India self-sufficient but also an exporter of agricultural products including rice. However, there is a need to improve productivity at an accelerated pace to address the impending challenges imposed by climate change, shrinking resources and increased food demand from the burgeoning population.

Basmati rice is popular across the world for its exquisite grain and cooking quality. Classical pedigree breeding based Basmati rice research at ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi spanning over two decades led to development of world’s first semi-dwarf high yielding Basmati rice cultivar released for cultivation in 1989 (Singh and Krishnan, 2016). The release of Pusa Basmati 1121 was another milestone in terms of improving cooked kernel elongation in Basmati rice (Singh et al. 2018b) which was followed by release of Pusa Basmati 6. The release of a climate smart Basmati rice variety, Pusa Basmati 1509 improved the net returns of farmers due to its short duration (120 days) and higher

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Marker assisted breeding in rice – a precursor for integrating genomics-assisted breeding

In Asia, the early efforts for implementing marker assisted breeding in crops such as rice and maize were started with the inception of Asian Rice Biotechnology Network (ARBN) and Asian Maize Biotechnology Network (AMBIONET) wherein a network of institutes across India were involved in developing the infrastructure and human resources needed for molecular breeding based on marker assisted selection (MAS). The initial efforts in rice was aimed at transferring major gene(s) governing resistance to bacterial blight (BB) disease namely \(Xa4, xa5, xa13\) and \(Xa21\), for which reliable polymerase chain reaction (PCR) based markers and donors were available in the form of pyramided lines with two, three and four gene combinations (Huang et al. 1997). Under the ambit of this programme, several institutions across India working on rice improvement namely Central Rice Research Institute (presently ICAR-National Rice Research Institute), Cuttack; Directorate of Rice Research (presently ICAR-Indian Institute of Rice Research), Hyderabad in collaboration with CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad; Punjab Agricultural University (PAU), Ludhiana and ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi in collaboration with National Research Centre on Plant Biotechnology (presently ICAR-National Institute of Plant Biotechnology), New Delhi studied the effectiveness of these gene(s) and gene combinations against the prevailing isolates of BB pathogen, \(Xanthomonas oryzae pv. oryzae\) and accordingly initiated marker-assisted improvement of the popular rice varieties, which were highly susceptible to BB. This enabled the establishment of much needed infrastructure and development of human resources for implementation of molecular breeding in the country. As a result, several gene-pyramided lines were developed through backcross breeding, wherein MAS was integrated for selecting genes governing BB resistance (foreground selection) and for hastening the recurrent parent genome (RPG) recovery (background selection). Popular \textit{indica} rice cultivars, such as PR106 (Singh et al. 2001), Basmati rice variety, Pusa Basmati 1 (Joseph et al. 2004) and medium slender grain rice variety, Samba Mahsuri (Sundaram et al. 2008) were used in the initial phase of improvement, leading to the commercial release of “Improved Pusa Basmati 1” possessing two BB resistance genes namely, \(xa13\)
and Xa21, the first improved rice variety developed through MAS in India (Gopalakrishnan et al. 2008) by ICAR-IARI. Subsequently, the availability of an array of genes and QTLs governing resistance/tolerance to different biotic (BB, blast, sheath blight, bakanae, brown plant hopper) and abiotic (salt, drought, low phosphorus and submergence) stresses which have been mapped/cloned, their linked/gene based markers as well as the donors from international rice nurseries possessing these genes/QTLs catalysed the wide-spread adoption of molecular marker assisted breeding in India. This was made possible by continued support for molecular breeding in different crops through different network projects from Indian Council of Agricultural Research (ICAR) namely Gene Pyramiding project, CRP on molecular breeding, and Generation Challenge Project (GCP) on molecular marker assisted breeding as well as different projects supported by Department of Biotechnology, Government of India. Marker assisted backcross breeding (MABB) has led to successful development and central release of 14 improved rice cultivars by Central Sub-Committee on Crop Standards, Notification and Release of Varieties (CSCSN&RV) possessing resistance to biotic stresses (BB, blast), tolerance to abiotic stresses (drought and submergence) in the background of popular rice varieties across India. Among these, the contribution of ICAR-IARI developed varieties (57%) have been substantial (Table 1). A glimpse of the two recently developed improved rice varieties released for commercial cultivation by ICAR-IARI is presented in Fig. 2. Figure 3 presents a timeline of the major milestones in the application of molecular breeding for rice

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Improved varieties</th>
<th>Year of recurrent parent release</th>
<th>Recurrent parent</th>
<th>Donor parent</th>
<th>Trait</th>
<th>Gene(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Improved Pusa Basmati 1</td>
<td>2007</td>
<td>Pusa Basmati 1</td>
<td>IRBB55</td>
<td>Resistance to Bacterial Blight (BB)</td>
<td>xa13 + Xa21</td>
<td>Gopalakrishnan et al. (2008)</td>
</tr>
<tr>
<td>2</td>
<td>Pusa 6 (Pusa 1612)</td>
<td>2013</td>
<td>Pusa Sugandh 5</td>
<td>Tetep</td>
<td>Resistance to blast disease</td>
<td>Pi2 + P54</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Pusa Basmati 1609</td>
<td>2015</td>
<td>Pusa Sugandh 5</td>
<td>Tetep and C101A51</td>
<td>Resistance to blast disease</td>
<td>Pi2 + P54</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pusa 1460</td>
<td>2015</td>
<td>Pusa Basmati 6</td>
<td>SPS 97</td>
<td>Resistance to blast disease</td>
<td>x3 + x54</td>
<td>Singh et al. (2017a)</td>
</tr>
<tr>
<td>5</td>
<td>Pusa Basmati 1728</td>
<td>2016</td>
<td>Pusa Basmati 1121</td>
<td>SPS 97</td>
<td>Resistance to blast disease</td>
<td>x3 + x54</td>
<td>Singh et al. (2017b)</td>
</tr>
<tr>
<td>6</td>
<td>Pusa Basmati 1718</td>
<td>2017</td>
<td>Pusa Basmati 1</td>
<td>IRBL9W</td>
<td>Resistance to blast disease</td>
<td>Pi9</td>
<td>Singh et al. (2017c)</td>
</tr>
<tr>
<td>7</td>
<td>Pusa Samba 1850</td>
<td>2018</td>
<td>Samba Mahsuri</td>
<td>DMSASQ164-2b</td>
<td>Resistance to blast disease</td>
<td>Pi54 + P1 + Pita</td>
<td>Krishnan et al. (2019)</td>
</tr>
</tbody>
</table>

Fig. 2. Photographs showing the field view, grain and cooked rice of two recently released MAS-derived improved rice varieties namely Pusa Basmati 1718 and Pusa Basmati 1728 developed at ICAR-IARI, New Delhi
improvement by ICAR-IARI, New Delhi. Besides this, there have been a large number of MAS derived rice varieties released at state level.

Factors that enabled successful integration of MAS in rice breeding

Several factors contributed to the successful integration of MAS in crop improvement in general and rice improvement in particular, which included constant refinement of the strategy of MAS (Fig. 3). While in the early stages, the concept of marker assisted selection proposed use of markers alone for selection without taking into consideration of the plant phenotype in the early stages of breeding. This practice had met with limited success in the practical breeding for improved cultivars. Addressing this critical gap, we have developed a modified breeding strategy involving combined phenotypic and molecular marker assisted selection while developing Improved Pusa Basmati 1. Development of Improved Pusa Basmati 1 involved transfer of two genes for bacterial blight resistance (\textit{xa13}, \textit{Xa21}) from a non-Basmati source, IRBB55 into a popular semi-dwarf, high yielding Basmati rice variety, Pusa Basmati 1 (Joseph et al. 2004). Basmati rice is uniquely known for its grain and cooking quality (Singh et al. 2018b), due to which there is a potential risk of impairing the grain and cooking quality especially when non-Basmati rice genotypes were used as donors for gene(s)/QTLs governing the target traits. In our programme, to recover the Basmati traits we have incorporated phenotypic selection focussing on grain and cooking quality recovery. This modified MABB strategy proved to be highly successful and was then on utilised not only for recovering the grain quality but also for other agronomic traits as well. This is a standard protocol in MABB for recovery of the recurrent parent phenome and had been successfully employed in introgressing genes governing resistance to BB and blast diseases and also for QTLs governing salt tolerance in Basmati rice varieties (Singh et al. 2012a; Ellur et al. 2016a,b; Khanna et al. 2015 a,b; Babu et al. 2017; Singh et al. 2018a; Sagar et al. 2018).

Initially, random dominant markers such as Amplified Fragment Length Polymorphism (AFLP) markers were utilized for background selection, which were then substituted by more robust co-dominant simple sequence repeat (SSR) markers distributed uniformly across the genome. Dominant markers had the inherent demerit of not being able to resolve the heterozygotes from the homozygotes. Consequently, SSRs were used for hastening the recovery of RPG through easy identification of backcross progenies with higher recovery percentage (Gopalakrishnan et al. 2008; Singh et al. 2012a,b; Ellur et al. 2016a,b). Further, it could enable a reductive screening, by progressively reducing the number of background markers screened, thereby recovering more of the homozygous recurrent parent allele from the segregating progenies (Singh et al. 2018a). If any heterozygosity is observed for the recurrent parent allele, the corresponding homozygotes were selected from the progenies of the segregating generations. Furthermore, the combined phenotypic selection often resulted in high RPG recovery. While phenotypic selection for grain and cooking quality was of prime importance during the early years of marker assisted breeding, the later years also involved selection for various agro-morphological traits such as plant height, days to flowering, etc. from the backcross generations, which enabled faster recovery of the recurrent parent
phenome as well (Singh et al. 2012a, b; Singh et al. 2013). Employing this strategy not only helped in better precision in breeding but also saved considerable resources and valuable time. MAS utilised in a modified backcross pedigree breeding approach could help in developing improved Basmati rice cultivars such as Improved Pusa Basmati 1 with just one backcross (Joseph et al. 2004; Gopalakrishnan et al. 2008), while a modified backcross strategy with only two backcrosses could help in developing useful near-isogenic lines (Singh et al. 2012a). More recently, background selection using single nucleotide polymorphism (SNP) markers in MABB was found to provide precise estimates of RPG recovery as compared to the use of SSR markers (Khanna et al. 2015).

Another major factor, which promoted the large-scale deployment of marker assisted breeding in rice across the country is the landmark decision on the development of criteria for nomination and evaluation of near isogenic lines (NILs). As per current guidelines of the All India Coordinated Rice Improvement Project (AICRIP), the backcross derived NILs with a minimum RPG recovery of 90% could be nominated directly into Advanced Varietal Trial – NILs for testing in multi-location trials, thereby facilitating the release of promising MAS derived NILs with only two years of testing (essentially derived varieties) as against three years of testing mandatory for genotypes developed through classical breeding approaches (independently derived varieties). The three-year system includes Initial Varietal Trial (IVT), Advanced Varietal Trial 1 (AVT1) and Advanced Varietal Trial 2 (AVT2). This is based on the logic that, NILs are improved versions of already released popular cultivars that have been subjected to the mandatory three years of testing under the AICRIP before their release. Therefore, there was no need for the improved NILs to undergo yet another three years of testing in the multi-location trials since the MABB was only targeted to improve a specific trait such as susceptibility to disease caused due to its cultivation over a large area for a number of years. Taking this anomaly into consideration, a committee constituted in the 44th Annual Rice Research Group Meeting held at ANGRAU, Hyderabad in 2009 deliberated upon the issue of separating the evaluation of the MAS derived NILs from the normal ecosystem based evaluation to a more specific evaluation. Being a NIL, the evaluation needs to focus only for the general superiority in productivity as well as for the improvement on the targeted trait in relation to the recurrent parent. These discussions enabled the development of a new set of guidelines for nomination and evaluation of the NILs for testing in AICRIP trials. One of the major criteria in the guidelines was the provision for entering the NILs directly in the Advanced Varietal Trial – Near Isogenic Lines (AVT-NILs), and evaluation only under the ecology where the recurrent parent was originally released, thereby enabling to save valuable time in testing the NILs. The evaluation mainly takes into account of the significant superiority of the NILs over the recurrent parent for the target trait (biotic stress resistance/abiotic stress tolerance), taken together with other criteria such as RPG recovery, trait validation and agro-morphological similarity. The salient features of the recurrent parents and the MAS derived rice varieties developed at ICAR-IARI, New Delhi is presented in Table 2, which shows significant recovery of recurrent parent pheno-mene in the MAS derived NILs. Subsequently, these criteria have been adopted in other crops such as wheat and maize enabling the integration of marker assisted breeding in other crops as well. As there is constant refinement in the marker assisted breeding strategy, the criteria have also been refined during 52nd and the 54th Annual Rice Research Group Meeting in 2017 and 2018 respectively, more specifically to include wider array of target traits, to streamline the process of nomination and evaluation of NILs.

Most important scientific factor for the successful adoption of MAS-derived rice varieties has been the appropriate after-release follow-up, especially in maintenance breeding. Unlike other rice varieties, the maintenance breeding of MAS-derived rice varieties involves testing of genetic purity of the seeds through gene-based/ gene-linked markers for the homozygosity of the target allele(s) for stress resistance or tolerance (Fig. 4). This follow-up step is very important not only at the nucleus and breeder seed production levels but also in the certified seed production as it will ensure the maintenance of the seed quality.

**Challenges and opportunities in molecular breeding**

Notwithstanding the successful integration of marker-assisted breeding in rice varietal improvement, there are several challenges to be addressed for its more effective use. These challenges also present valuable opportunities to improvise and innovate new tools in crop improvement. The first challenge is the constraint in sharing the valuable donors and other related resources not only between countries but also between
Table 2. A comparison of agronomic, grain and cooking quality traits and traits of essential derivation of the MAS derived rice cultivars developed by ICAR-Indian Agricultural Research Institute, New Delhi released for commercial cultivation.

<table>
<thead>
<tr>
<th>Trait</th>
<th>PB 1</th>
<th>Imp. PB 1637</th>
<th>PB 1121</th>
<th>PB 1718</th>
<th>PB 6</th>
<th>PS 5</th>
<th>Pusa 1592</th>
<th>Pusa 1612</th>
<th>Pusa 5204</th>
<th>BPT 1850</th>
<th>PS 1609</th>
</tr>
</thead>
<tbody>
<tr>
<td>TI</td>
<td>BB</td>
<td>BL</td>
<td>BB</td>
<td>BB</td>
<td>BL</td>
<td>BL</td>
<td>BB</td>
<td>BL</td>
<td>BL</td>
<td>BL</td>
<td></td>
</tr>
<tr>
<td>BB/BL</td>
<td>6.3</td>
<td>4.3</td>
<td>2.8</td>
<td>7.3</td>
<td>2.2</td>
<td>7.0</td>
<td>2.8</td>
<td>7.0</td>
<td>4.3</td>
<td>4.4</td>
<td>6.6</td>
</tr>
<tr>
<td>PHT</td>
<td>101.0</td>
<td>99.0</td>
<td>103.0</td>
<td>115.0</td>
<td>115.0</td>
<td>100.0</td>
<td>104.0</td>
<td>105.0</td>
<td>104.0</td>
<td>113.0</td>
<td>91.0</td>
</tr>
<tr>
<td>DFF</td>
<td>108</td>
<td>111</td>
<td>109</td>
<td>105</td>
<td>103</td>
<td>115</td>
<td>115</td>
<td>97</td>
<td>97</td>
<td>96</td>
<td>115</td>
</tr>
<tr>
<td>YLD</td>
<td>4165</td>
<td>4290</td>
<td>4227</td>
<td>4401</td>
<td>4641</td>
<td>4108</td>
<td>4182</td>
<td>4724</td>
<td>4726</td>
<td>4916</td>
<td>4603</td>
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<td>HRR</td>
<td>54.2</td>
<td>50.0</td>
<td>52.0</td>
<td>53.5</td>
<td>54.4</td>
<td>52.2</td>
<td>51.8</td>
<td>57.9</td>
<td>58.2</td>
<td>53.0</td>
<td>68.8</td>
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<tr>
<td>KL</td>
<td>7.5</td>
<td>7.4</td>
<td>7.3</td>
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<td>7.3</td>
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<td>1.8</td>
<td>1.7</td>
<td>1.8</td>
<td>2.0</td>
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<tr>
<td>LBR</td>
<td>4.1</td>
<td>4.1</td>
<td>4.7</td>
<td>4.8</td>
<td>4.6</td>
<td>4.6</td>
<td>4.8</td>
<td>4.4</td>
<td>4.5</td>
<td>4.5</td>
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<tr>
<td>KLAC</td>
<td>13.8</td>
<td>13.7</td>
<td>13.8</td>
<td>17.2</td>
<td>17.0</td>
<td>15.1</td>
<td>14.6</td>
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<td>14.0</td>
<td>15.0</td>
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<tr>
<td>ER</td>
<td>1.9</td>
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<td>1.9</td>
<td>2.1</td>
<td>2.1</td>
<td>2.0</td>
<td>2.0</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>-</td>
</tr>
<tr>
<td>ARO</td>
<td>3.0</td>
<td>3.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
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<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>1.0</td>
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<tr>
<td>AMY</td>
<td>23.6</td>
<td>23.5</td>
<td>22.8</td>
<td>21.9</td>
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<td>23.4</td>
<td>22.8</td>
<td>24.3</td>
<td>24.4</td>
<td>24.5</td>
<td>23.9</td>
</tr>
</tbody>
</table>

BB = Bacterial blight resistance; BL = Blast disease resistance; TI = Trait improved; BB/BL = Susceptibility indices of BB/BL; PHT = Plant height in cm; DFF = Days to 50% flowering; YLD = Grain yield in kg/ha; HRR = Head rice recovery in %; KL = Kernel length in mm; KB = Kernel width in mm; LBR = Length-breadth ratio; KLAC = Kernel length after cooking in mm; ER = Elongation ratio; ARO = Aroma score, 1.0-1.9 No scent, 2.0-2.9 Mild, 3.0-3.9 Optimum, 4.0 Strong; AMY = Amylose content in %. Shaded columns represent recurrent parents,

PB1 = Pusa Basmati 1; Imp. PB1 = Improved PB 1; PB 1637 = Pusa Basmati 1637; PB 1121 = Pusa Basmati 1121; PB 1718 = Pusa Basmati 1718; PB 6 = Pusa Basmati 6; PS 5 = Pusa Sugandh 5; PB 1728 = Pusa Basmati 1728; BPT 5204, Samba Mahsuri; PS 1850 = Pusa Samba 1850; PB 1609 = Pusa Basmati 1609,

*MAS derived varieties released through normal three years of testing in AICRIP. Rest of the improved varieties were released through evaluation in NILs trial

Fig. 4. A representative gel image showing the amplification profile of the Pi9 based marker in the (a) panicles of the nucleus seed of Pusa Basmati 1637, the yellow arrow in 9 and 10 column indicates the panicles which possess the susceptible allele of Pi9 based marker Pi9 STS-1, which needs to culled out during maintenance breeding. (b) and (c) shows the amplification profile of the panicles of the nucleus seed of Pusa Basmati 1728 for the presence of the resistance alleles of xa13 and Xa21 using gene-based markers, xa13 prom and pTA248

researchers within the National Agricultural Research and Education System (NARES) under the present intellectual property (IP) regime. This may pose a severe bottleneck in cases where there is limited variability available in the germplasm for the target traits. Even if there is phenotypic variability available in the germplasm accessible to the breeders, it needs to be validated not only for the phenotype but for the marker alleles and also in the segregating population developed by crossing contrasting genotypes, before using it for MAS programmes. This is a time-consuming effort, which may lead to loss of opportunity for genetic improvement in case of any unforeseen stress eventuality.

The second challenge is the limited population size in the segregating generations due to poor seed set especially observed in leguminous crops, more specifically in MABB, which may impede the rapid recovery of genome as well as phenotype. This can be overcome by rapid generation advancement (RGA) or employing techniques such a speed breeding thereby increasing the number of backcrosses to enable development of NILs. Through appropriate planning,
strategies for overcoming the limitations imposed by these factors can be managed. Further, in crop species where techniques such as speed breeding are not standardized, this may result in loss of time.

The third and the most important challenge is the limited scope of crop improvement success in MABB programmes, which is limited to rectifying a specific defect in an otherwise popular variety. While, this may help in achieving the potential yield of the variety under a specific stress thereby helping stabilization of the yield, the real test of the success of the marker-assisted breeding will be gauged by its ability to achieve larger yield gains in crops, which has not been demonstrated in crops including rice. This presents an opportunity to develop novel breeding strategies and models, which can not only help capture the additive effects but also account for the dominance and epistatic interactions, thereby enabling the use of MAS for achieving higher yield gains in crops. ICAR in collaboration with Bill and Melinda Gates Foundation (BMGF) has initiated a mega-network project targeting yield gains in eight important crops namely, rice, wheat, maize, sorghum, pearl millet, pigeon pea, chickpea and potato.

The fourth challenge in deploying marker-assisted breeding is the bottleneck imposed by genotyping, wherein there is a need for specific polymorphic markers for different crosses in order to improve the efficiency of MAS. This can be overcome by having a larger pool of markers which can be utilized in different crosses with cost-effective genotyping technologies and offers flexibility to select the markers based on the polymorphism between the parents and the number of samples to assayed. Additionally, in order to make MAS an integral component of plant breeding, there is a need to provide opportunities to the breeders located even in remote areas of the country. The much-needed access to the modern MAS technologies can be provided through the development of genotyping service centres, such as the National Genomics and Genotyping Facility recently inaugurated at the National Institute of Plant Genome Research, New Delhi with enabler hubs which will facilitate faster analysis and outturn of the samples.

The fifth major challenge is the need for a robust high throughput phenotyping system which can help in phenotyping a large number of recombinants in a molecular breeding programme. Such a phenotyping system should also be possibly non-destructive and have the capability to capture the real-time genotype x environment interactions. There is also a need for development of prediction models which can help the breeders in identifying the potential genotypes for specific target environment.

Yet another challenge in successfully deploying a molecular breeding system is the non-availability of robust markers for every target trait, especially for QTLs (Platten et al. 2019). Although a great leap has been made in integrating resistance to key diseases, there are several other major pest and diseases that have no significant sources of resistance in rice. And in several cases where the ultimate phenotype is determined by quantitative genes, their interactions and environmental influence make molecular breeding a poor choice for improvement. It is therefore essential to identify potential sources for resistance to such stresses. In several cases, however, major effect QTLs often remain rare and more common alleles of tolerance may have low individual effect. If there are several QTLs to be transferred for a single target trait, MAS may pose challenges of severe linkage drag as several foreground targets are to be focussed during the selection process. In such cases, SNPs could be a better choice over SSR markers, as they can help in reducing the linkage drag and improve RPG recovery. To have successful MAS, it is most desirable that the candidate genes/QTLs are free from epistatic and environmental interactions.

The final challenge is the handling of big data from genomics and phenomics, wherein there is a need for developing both infrastructures in terms of hardware as well as software for analysing, archiving and retrieval. One of the objectives of the ICAR-BMGF funded mega project launched recently in India is addressing this issue. Designing artificial intelligence (AI) interface for pattern recognition and decision making will also enable handling of big data in a most desirable manner. Finally, there are research labs which has successfully integrated modern genomic tools in their breeding programmes. Such labs need to establish a streamlined workflow to enable training of next generation breeders in these areas. Streamlining of the molecular breeding workflow and constant sharing of innovations between successful labs and the plant breeders to refine the workflow from time to time will enable not only achieve the challenging targets for improving productivity but also sustain the gains achieved by the breeders.
Future perspective

Marker assisted breeding offers unprecedented opportunities for contemporary plant breeders to enable them to breed futuristic crop varieties. In a typical pedigree breeding, if we can advance two generations per year as practiced in rice breeding, the breeding funnel is narrow and long, with limited number of progenies in an $F_2$ population and a minimum of ten years between the parental cross and varietal release (Fig. 5). Significantly, in the breeding funnel for varietal population helps in effective selection of plants with better RPG recovery, by combining both MAS and phenotypic selection. This helps in rapid RPG gain, thereby reducing the time taken for varietal release. In order to sustain the productivity of the popular rice varieties by imparting stress resilience, targeted introgression of gene(s)/QTLs governing different biotic (BB, Blast, Bakanae, BPH, weeds) and abiotic stresses (drought, salt, low P) through MABB is being undertaken at ICAR-IARI, New Delhi, which are at different stages of development/testing (Table 3).

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Improvement through MABB, the time period has been reduced to five years through the use of molecular tools. In the MABB strategy to introgress major gene(s)/QTLs for rectifying a specific defect in an already popular rice variety, use of a large backcross Fig. 5. The breeding funnel in different breeding schemes (a) long and narrow in classical pedigree breeding, (b) wider and short in MABB, where the target is introgression of gene(s)/QTL(s) to rectify specific defects in popular rice varieties, (c) Wider and short in genomics selection based breeding. *For rapid generation advancement (RGA) (commonly used in rice at present) including techniques such as speed breeding, it takes about 2 years (@ 3 crops/year) whereas for doubled haploids (DH) takes one season to generate the fixed lines for exercising genomic selection. The numbers in parenthesis is the number of entries handled

However, the temporal breeding gain in MABB is limited to essentially derived varieties, which means that development of a new independent cultivar still requires a minimum of ten years. Fortunately, with the availability of high throughput markers such as
SNPs and options for rapid fixation of lines through production of doubled haploids (DH)/RGA will help to reduce the breeding cycle time to just five years as compared to a minimum of ten years in classical pedigree breeding. The availability of relatively large fixed population within a short span of time (ideally one season for DH and upto six seasons/two years for speed breeding) for employing genomic selection will help in drastically improving the genetic gain for yield improvement in rice. RGA systems such as speed breeding can handle large number of populations as well as several individuals per population, because of the significantly reduced area and time required to handle generations. Therefore, the breeding funnel of genomics assisted breeding is wider and shorter as compared to long narrow funnel in case of pedigree breeding. This will help in achieving an accelerated accrual of the genetic gain in every breeding cycle, thereby bringing out several tailor-made improvised cultivars to meet future breeding challenges. The future of rice breeding seems bright, taken together with MABB, the genomics assisted breeding can redesign the paradigm of rice improvement to newer standards showcasing tailor-made climate-smart cultivars and to address future challenges of climate change and consumer preference.

**Declaration**

The authors declare no conflict of interest.

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References


