Genomics-assisted backcross breeding for infusing climate resilience in high-yielding green revolution varieties of rice


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Abstract

Rice is the most important food crop both in value and volume for the Asian population. Frequent drought, flood and salinity stresses exacerbated by global climate change adversely affect rice production in more than fifty percent of the rice growing areas. Green revolution high yielding varieties carrying Adf dwarfing gene have almost fully replaced the traditional climate resilient landraces and varieties of rice. However, these were bred primarily for yield under high input conditions and therefore are sensitive to adverse climatic conditions. Hence, there is urgent need to combine the high productivity with climate resilience. Knowledge of rice genome and genes for tolerance to different abiotic stresses provided us an opportunity to transfer favorable alleles of these genes into high yielding varieties through genomics-assisted backcross breeding through multi-institutional networks. Six consistent genomic regions (QTLs) for grain yield under drought; namely qDTY1.1, qDTY2.1, qDTY2.2, qDTY3.1, qDTY3.2 and qDTY12.1 have been transferred to flood tolerant versions of mega varieties of rice, Swarna, Samba Mahsuri and IR 64. To address the problem of flash flooding qSUB1 QTL has been transferred to nine popular rice varieties, namely ADT 46, Bahadur, Ranjit, HUR 105, Sarjoo 52, Pooja, Pratikshya MTU 1075 and Rajendra Mahsuri. Further, qSALTOL1 QTL for seedling stage salt tolerance and qSSISFH8.1 for reproductive stage salt tolerance have been transferred to six popular rice varieties, ADT 45, Gayatri, MTU 1010, PR 114, Pusa 44 and Sarjoo 52. We used foreground selection markers for the presence of desired gene/QTL and recombinant selection markers for reduction of linkage drag around these genes. Genotypic background selection was done after BC2F3 stage using a 50K SNP chip on a set of 20 advance lines obtained by phenotypic selection for closeness to the recipient parents. Near-isogenic lines (NILs) with more than 95% similarity to the recipient parent genome have been released and notified for commercial cultivation and are gaining fast popularity. These climate smart rice varieties will provide production stability in the adverse ecologies and support farmer’s income and livelihood.

Key words: Climate change, QTLs, drought, submergence, salinity, MAS, rice

Introduction

Rice (Oryza sativa L.) is the most important staple food crop for the Asian population. Worldwide ninety percent of rice is produced in Asia and China is the largest producer of paddy rice (212.67 million tons) followed by India (168.55 million tons), Indonesia (81.38 million tons) and Bangladesh (48.98 million tons) according to FAOSTAT 2017 (Fig. 1). Due to growing population and rising standards of living, demand for rice is expected to increase further. Global warming induced high temperature and erratic rainfall patterns...
cause unpredictable drought, flooding and salinity stresses that adversely affect rice production in more than fifty percent of the rice cultivation areas. Dry-season irrigated regions of Southern and Central China, Southern and Eastern India and Southeast Asia have experienced shortage of irrigation water at critical growth stages over the last few years and the problem is expected to worsen by 2025 (Tuong et al. 2003). Further, detrimental effects of frequent droughts, flooding and salinity are often compounded by mineral deficiencies (Zn, P) and toxicities (Fe, Al and organic acids) with negative impact on rice productivity across locations and seasons. Rice cultivation in the rainfed regions of Asia is challenged severely by multiple stresses prevailing together or in quick successions. For instance, rainfed lowland ecologies in Bangladesh and India are often affected by submergence and salinity at the early growth stage and drought and salinity at reproductive stage (Wassmann et al., 2009, Asaduzzaman et al. 2010). Considering the threats to rice production stability by global climate change, development of climate resilient varieties has become the focal point of rice breeding programs.

High yielding green revolution (GR) rice varieties carrying \( sd1 \) gene for reduced plant height have almost completely replaced the traditional climate resilient landraces and varieties. However, GR rice varieties are sensitive to drought, submergence and salinity because they were selected primarily for yield under high input conditions. Progress in breeding climate resilient varieties using conventional breeding methods has been slow due to limitations of suitable selection strategies; knowledge of genes involved and unwanted linkage drags. DNA marker-assisted breeding provides accelerated and precise introgression of genes/QTLs for the traits with minimum linkage drag and rapid recipient parent genome (RPG) recovery. Knowledge of reference genome and pan genome of rice and physical location of genes/QTLs for tolerance to different abiotic stresses provides opportunities to transfer favorable genes into widely adapted high yielding mega varieties of rice.

Marker-assisted selection (MAS) has been used extensively in rice breeding for introgression of genes/QTLs of interest in popular rice cultivars. Several studies have targeted genes conferring tolerance to major biotic stresses to successfully develop tolerant lines through MAS (Huang et al. 1997, Hittalmani et al. 2000; Singh et al. 2001; Khanna et al. 2015). Similarly, QTLsgenes underlying GY-related traits and grain quality in rice have also been used for MAS (Joseph et al. 2004; Zhang et al. 2006; Yi et al. 2009; Singh et al. 2018). Well known examples of successful MAS for abiotic stress tolerance in rice are introgression of \( Sub1A \) gene for submergence tolerance (Neeraja et al. 2007) and \( SNORKEL1 \) gene for rapid stem elongation in deep water flooding (Hattori et al. 2009), \( SALTOL1 \) QTL for salinity tolerance (Linh et al. 2012) and \( DTY \) QTLs for drought tolerance (Swamy et al. 2013; Dixit et al. 2017; Shamsudin et al. 2016a,b). Combining tolerance to multiple abiotic stresses through MAS is possible but very rare (Singh et al. 2016; Dixit et al. 2017; Yadav et al. 2019). In India, large areas under rice cultivation suffer vegetative-stage submergence coupled with reproductive-stage drought, where double drought and submergence tolerant lines could prove a boon for yield stability. These lines can also serve as valuable genetic material to be used for further breeding of high-yielding drought, submergence and salinity tolerant varieties in local breeding programs.

Considering the urgent need for developing climate smart rice varieties a large multi-institutional project, “From QTL to Variety: Genomics-assisted introgression and field evaluation of rice varieties with genes/QTLs for yield under drought, flood and salt stress (Q2V)” was initiated in 2010 with funding support from Department of Biotechnology, Government of India in collaboration with IRRI, Philippines. The project now in its second phase involves twelve different research institutes and universities from across India led by ICAR-National Institute for Plant Biotechnology (NIPB), New Delhi (Fig. 2). The aim of the Q2V project is to develop high yielding multi-stress tolerant climate resilient rice varieties for cultivation in different agro-climatic zones of India.
Materials and methods

The Q2V project involved marker-assisted introgression of major QTLs for drought, submergence and salinity tolerance into the backgrounds of widely adapted mega varieties of rice as described earlier by Singh et al. (2016). A list of total 18 highly popular rice varieties used as recipient parents (RP) and respective donor parent (DP) genotypes possessing the QTLs for the traits of interest is provided in Table 1. Six different DTY QTLs for grain yield under drought (drought tolerance) were transferred in to Swarna-Sub1, Samba Mahsuri-Sub1 and IR64-Sub-1 genetic backgrounds either individually or in combination. The qSUB1 QTL for submergence tolerance was transferred to nine regionally adapted popular rice varieties, viz., ADT 46, Bahadur, Ranjit, HUR 105, Sarjoo 52, Pooja, Pratikhya, MTU 1075 and Rajendra Mahsuri. Further, qSALTOL1 QTL for seedling stage salinity tolerance and qSSISFH8.1 QTL for reproductive stage salinity tolerance were transferred into six locally adapted popular rice varieties, viz., ADT 45, Gayatri, MTU 1010, PR 114, Pusa 44 and Sarjoo 52.

For each of the total nine QTLs being transferred one or two peak markers for foreground selection and two QTL flanking markers for recombinant selection were used to ensure the presence of QTL with minimum amount of linkage drag during BC$_1$-BC$_3$ generations. The phenotypic screening of backcross derived lines at different stages of backcrossing under drought, submergence or salinity stress showed the expected co-segregation with the presence of QTLs. The backcross derived lines BC$_3$ onwards were also examined for their phenotypic similarity with the respective recipient parents by planting the recipient parents at regular intervals. After BC$_3$F$_3$ twenty QTL positive NILs for each cross combination with maximum phenotypic similarity to the respective RP and outperforming the RP under corresponding stress were identified for background selection using 50K rice SNP chip (Singh et al. 2015) to identify NILs with >95% RPG similarity. Apart from the development of single QTL NILs, QTL pyramided lines containing multiple QTLs for drought tolerance (qDTY2.1 and qDTY3.1) and submergence tolerance (qSUB1) were identified in the Swarna-Sub1 and Samba Mahsuri-Sub1 backgrounds because of availability of a double QTL donor line with qDTY2.1 and qDTY3.1 (IR 81896-B-B-195). Graphical representation of 50K SNP genotyping based RPG recovery was done using Phenogram software from Ritchie lab, Penn. State University, Pennsylvania, USA (http://visualization.ritchielab.psu.edu/phenograms/plot).

The selected QTL-NILs with significant grain yield advantage over respective RP under stress environment and equal or high yield than control under normal environment identified through station trials were evaluated under multi-location AICRIP AVT1 and AVT2 special trials for drought, submergence and salinity as per the AICRIP guidelines.

Results and discussion

The foreground and recombinant selection markers for each of the total 33 cross combinations, 12 for drought, 9 for submergence and 12 for salt tolerance were validated at ICAR-NIPB and protocols shared with the respective Q2V network partner institutions for use in selection process. For example foreground and recombinant markers validation for transfer of qDTY2.1 into Swarna-Sub1 background at ICAR-NRRI Cuttack is shown in Fig. 3. Apart from the foreground and recombinant markers validation, ICAR-NIPB conducted high throughput genotyping for background selection on upto 20 advance generation lines for each
### Table 1. List of rice mega varieties and their status on introgression of QTLs for drought, submergence and salt tolerance under ‘QTL to Variety’ project

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Recipient variety (trait introgressed)</th>
<th>QTL</th>
<th>Donor line (Original donor)</th>
<th>Lead institution</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Swarna-Sub1 (Submergence and drought tolerance)</td>
<td>qDTY1.1</td>
<td>IR 86918-B-305 (Nagina 22)</td>
<td>IGKV, Raipur</td>
<td>BC3F5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qDTY2.1</td>
<td>IR 81896-B-195 (Apo)</td>
<td>ICAR-NRRI, Cuttack</td>
<td>BC3F4, BC3F5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qDTY3.1</td>
<td>IR 86931-B-6 (Nagina 22)</td>
<td>ICAR-NIPB, New Delhi</td>
<td>BC3F2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qDTY3.2</td>
<td>IR 90019-17-159-B (Way Rarem)</td>
<td>IGKV, Raipur</td>
<td>BC3F3</td>
</tr>
<tr>
<td>2.</td>
<td>Samba Mahsuri-Sub1 (Submergence and drought tolerance)</td>
<td>qDTY1.1</td>
<td>IR 86918-B-305 (Nagina 22)</td>
<td>CAU, Umiam</td>
<td>BC3F4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qDTY2.1</td>
<td>IR 81896-B-195 (Apo)</td>
<td>ICAR-IIRR, Hyderabad</td>
<td>BC3F4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qDTY2.2</td>
<td>IR 87728-367-B-B (Aday Sel.)</td>
<td>CAU, Umiam</td>
<td>BC3F4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qDTY3.1</td>
<td>IR 81896-B-195 (Apo)</td>
<td>ICAR-IIRR, Hyderabad</td>
<td>BC3F4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qDTY3.2</td>
<td>IR 86931-B-6 (Nagina 22)</td>
<td>ICAR-NIPB, New Delhi</td>
<td>BC3F2</td>
</tr>
<tr>
<td>3.</td>
<td>IR64-Sub1 (Submergence and drought tolerance)</td>
<td>qDTY1.1</td>
<td>IR 86918-B-305 (Nagina 22)</td>
<td>ICAR-CRURRS, NRRI</td>
<td>BC3F4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qDTY2.2</td>
<td>IR 87728-367-B-B (Aday Sel.)</td>
<td>Hazaribagh</td>
<td>BC3F4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>qDTY3.1</td>
<td>IR 81896-B-195 (Apo)</td>
<td>BC3F4</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>ADT 46 (Submergence tolerance)</td>
<td>qSUB1</td>
<td>Swarna-Sub1 (FR13A)</td>
<td>PAJANCOA, Karaikal</td>
<td>BC3F3</td>
</tr>
<tr>
<td>5.</td>
<td>Bahadur (Submergence tolerance)</td>
<td></td>
<td></td>
<td>AAU, Assam</td>
<td>BC3F2</td>
</tr>
<tr>
<td>6.</td>
<td>HUR 105 (Submergence tolerance)</td>
<td></td>
<td>IR64-Sub1 (FR13A)</td>
<td>BHU, Varanasi</td>
<td>BC3F4</td>
</tr>
<tr>
<td>7.</td>
<td>MTU 1075 (Submergence tolerance)</td>
<td></td>
<td>Swarna-Sub1 (FR13A)</td>
<td>ANGRAU, Maruteru</td>
<td>BC3F6</td>
</tr>
<tr>
<td>8.</td>
<td>Pooja (Submergence tolerance)</td>
<td></td>
<td></td>
<td>ICAR-NRRI, Cuttack</td>
<td>BC3F3</td>
</tr>
<tr>
<td>9.</td>
<td>Pratikshya (Submergence tolerance)</td>
<td></td>
<td></td>
<td>ICAR-NRRI, Cuttack</td>
<td>BC3F3</td>
</tr>
<tr>
<td>10.</td>
<td>Rajendra Mahsuri (Submergence tolerance)</td>
<td></td>
<td></td>
<td>RPCAU, Samastipur</td>
<td>BC3F2</td>
</tr>
<tr>
<td>11.</td>
<td>Ranjit (Submergence tolerance)</td>
<td></td>
<td></td>
<td>AAU, Assam</td>
<td>BC3F3</td>
</tr>
<tr>
<td>12.</td>
<td>Sarjoo 52 (Submergence tolerance)</td>
<td></td>
<td>IR64-Sub1 (FR13A)</td>
<td>BHU, Varanasi</td>
<td>BC3F4</td>
</tr>
<tr>
<td>13.</td>
<td>ADT 45 (Salt tolerance)</td>
<td>qSALTOL1 and qSSISFH8.1</td>
<td>FL478 (Pokkali) and CSR 27 (Nona Bokra)</td>
<td>PAJANCOA, Karaikal</td>
<td>BC3F3</td>
</tr>
<tr>
<td>14.</td>
<td>Gayatri (salt tolerance)</td>
<td></td>
<td></td>
<td>ICAR-NRRI, Cuttack</td>
<td>BC3F3</td>
</tr>
<tr>
<td>15.</td>
<td>MTU 1010 (salt tolerance)</td>
<td></td>
<td></td>
<td>ANGRAU, Maruteru</td>
<td>BC3F4</td>
</tr>
<tr>
<td>16.</td>
<td>PR 114 (salt tolerance)</td>
<td></td>
<td></td>
<td>ICAR-CSSRI, Karnal</td>
<td>BC3F3</td>
</tr>
<tr>
<td>17.</td>
<td>Pusa 44 (salt tolerance)</td>
<td></td>
<td></td>
<td>ICAR-CSSRI, Karnal</td>
<td>BC3F3</td>
</tr>
<tr>
<td>18.</td>
<td>Sarjoo 52 (salt tolerance)</td>
<td></td>
<td></td>
<td>ICAR-CSSRI, Karnal</td>
<td>BC3F3</td>
</tr>
</tbody>
</table>
of the cross combinations on Affymetrix Gene Titan platform using in-house designed 50K SNP chip ‘OsSNPnks’ (Singh et al. 2015) to ensure the required RP genome recovery in the QTL-NILs so as not to disturb the original productivity, quality and wider adaptability of the mega varieties, which makes them highly popular among the farmers. Apart from its use in background selection for RP genome recovery in the Q2V project, the ICAR-NIPB 50K SNP chip has been used earlier to analyze the Sub1-NILs of mega varieties developed by IRRI, namely Swarna-Sub1, CR1009-Sub1, IR64-Sub1 and Sambha Mahsuri-Sub1 revealing average RP genome recoveries of 97.7%, 91.9%, 91.7% and 78.7%, respectively (Singh et al. 2015).

Pyramiding of drought and submergence tolerance in mega varieties of rice

Drought stress is one of the most severe climatic threats to rice production in the rainfed agro-ecosystems in Asia and Africa. Recent studies have shown increasing frequency of El Nino phenomena and proportional reduction in the number of rainy days has affected rice production in upland (10 Mha) as well as lowland (13 Mha) agro-ecologies in Asia (Pandey et al. 2007). Soil water deficit is an important factor that influences a range of physiological and metabolic processes in the whole plant (Ahmadikhah and Marufinia, 2016). Rainfed lowland rice cultivation areas in great river basins often experience submergence at vegetative stage and drought at reproductive stage in the same field, hence there is felt need to pyramid QTLs for both flooding and drought tolerance in the same popular GR rice varieties grown in these regions. Therefore, it was decided to introgress six consistent major QTLs for grain yield under drought; namely qDTY1.1, qDTY2.1, qDTY2.2, qDTY3.1, qDTY3.2 and qDTY12.1 into three flood tolerant versions of GR mega varieties of rice, namely Swarna-Sub1, Samba Mahsuri-Sub1 and IR 64-Sub1 (Table 1). Ten different crosses were made between RP and DP for production of QTL-NILs with single QTLs, whereas two crosses were made for simultaneous transfer of two QTLs, namely qDTY2.1 and qDTY3.1 into Swarna-Sub1 and Samba Mahsuri-Sub1 back grounds facilitated by availability of dual QTL donor line IR 8196-B-B-195. Most of these NILs developed under Q2V project are presently at the BC3F3 or beyond stage undergoing multi-location field evaluation for drought and submergence tolerance in replicated field trials (Table 1).

A major challenge in the drought tolerance-breeding programme was to break the very tight genetic linkage on rice chromosome 1 between Sd1 gene for plant height and qDTY1.1 locus for drought tolerance. All the semi-dwarf high yielding GR rice varieties have the recessive dwarfing allele (sd1) of the Sd1 gene, which is linked in repulsion with the drought sensitivity allele of the qDTY1.1 locus. Screening of more than 5000 BC1F2 progenies of Swarna-Sub1/IR 86918-B-305 cross at IGKV, Raipur resulted in identification of rare recombinants combining dwarf plant height (sd1) with drought tolerance allele of the qDTY1.1 locus (Fig. 4). Similar dwarf drought tolerant lines resulting from rare recombination between qDTY1.1 and Sd1 loci were also identified earlier by Vikram et al. (2015). However, rice variety containing this recombinant has not been released for commercial cultivation by the farmers until recently.

Recent studies have reported development of dual drought and submergence tolerant lines of rice using marker-assisted breeding but most of these are
not released as varieties for commercial cultivation. Dixit et al. (2017) reported pyramiding of three QTLs for drought tolerance \((qDTY3.1, qDTY6.1\) and \(qDTY6.2)\) with \(SUB1\) for submergence tolerance with the aim to develop drought and submergence-tolerant NILs in the genetic background of TDK1, a popular rice variety in Lao PDR. Further, Yadav et al. (2019) has reported marker-assisted introgression of \(qDTY1.1, qDTY2.1, qDTY3.1\) and \(Sub-1\) in Swarna background. The NILs were evaluated in 60 multi-location trials and study concluded that the rice varieties with pyramided multiple QTLs yield 0.2-1.7 t ha\(^{-1}\) under reproductive stage drought and 0.1-1.0 t per hectare under submergence conditions with no yield penalty under non-stress. However, these lines have not been released for commercial cultivation as yet. ‘DRR Dhan 50’ is the first climate smart rice variety combining \(Sub1A1\) allele for submergence tolerance with tolerant alleles of QTLs \(qDTY2.1\) and \(qDTY3.1\) for drought tolerance in the genetic background of Samba Mahsuri, developed by genomics-assisted backcross breeding (GABB), released and notified by Government of India for commercial cultivation in 2018 (Table 2, Fig. 5). Another variety ‘CR Dhan 802’ with similar genetic composition but in Swarna background was released and notified in 2019. High density genotyping using 50K SNP chip showed that DRR Dhan 50 and CR Dhan 2 have more than 90% background genome similarity with Samba Mahsuri-Sub1 and Swarna-Sub1, respectively, making them very close to the original recipient varieties in terms of productivity, quality and wide adaptability.

Table 2. Climate resilient rice varieties developed by genomics-assisted backcross breeding under “From QTL to Variety” project, released and notified by Government of India for commercial cultivation

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Variety (Release year)</th>
<th>Genetic characteristics</th>
<th>Recommended regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DRR Dhan 50 (2018)</td>
<td>Samba Mahsuri with (qSUB1) QTL for submergence tolerance and (qDTY2.1) and (qDTY3.1) QTLs for drought tolerance</td>
<td>Andhra Pradesh, Telangana, Tamil Nadu, Karnataka, Bihar, Odisha, Chhattisgarh, Eastern part of Uttar Pradesh and Madhya Pradesh</td>
</tr>
<tr>
<td>2.</td>
<td>Ranjit-Sub1 (2018)</td>
<td>Ranjit with (qSUB1) QTL for submergence tolerance</td>
<td>Assam</td>
</tr>
<tr>
<td>3.</td>
<td>Bahadur-Sub1 (2018)</td>
<td>Bahadur with (qSUB1) QTL for submergence tolerance</td>
<td>Assam</td>
</tr>
<tr>
<td>4.</td>
<td>CR Dhan 802 (2019)</td>
<td>Swarna with (qSUB1) QTL for submergence tolerance and (qDTY2.1) and (qDTY3.1) QTLs for drought tolerance</td>
<td>Bihar and Madhya Pradesh</td>
</tr>
</tbody>
</table>
Introgression of Submergence tolerance into popular rice varieties of India

Flash flooding is a major concern to rice production in many countries of South and South East Asia where 10-15 million ha of rice fields are affected by complete submergence for 10-15 days (Dey and Upadhyaya 1996). Rice is a semi-aquatic plant which can tolerate partial submergence but dies within few days of complete submergence costing approximately 1 billion dollar every year (Dey and Upadhyaya 1996). The estimated damage may be even higher in future due to continuous changes in the global climate which alter the weather pattern resulting in heavy storms and erratic rainfalls (Bates et al. 2008; Gornall et al. 2010). Rice survival under flooding depends on depth, duration of submergence, quality of water, light intensities, temperature and pH of soil and water. However, there are traditional varieties e.g., FR13A that can tolerate complete submergence upto 14 days and rice breeders have used this variety as donor for submergence tolerance (Vergara and Mazaredo 1975). The submergence tolerance in rice plant is controlled by a single major QTL qSUB1 located on chromosome 9 (Xu and Mackill 1996, Toojinda et al. 2003). After the public availability of rice genome information (IRGSP, 2005), Xu et al. (2006) was able to sequence the qSUB1 genomic region of FR13A derived lines and identified three putative ethylene response factors encoding genes Sub1A, Sub1B and Sub1C, of which Sub1A-1 allele was identified as the factor imparting submergence tolerance. Map based cloning of the Sub1A gene provided excellent opportunity to understand the molecular mechanism of submergence tolerance as well as to design gene-based marker for precision breeding. In last decade, Sub1A1 allele has been transferred into a number of high-yielding mega varieties of rice e.g. Swarna through marker-assisted backcrossing (Neeraja et al. 2007; Sarkar and Bhattcharjee 2011). There were no significant agronomic differences observed between Swarna and Swarna-Sub1 under non-stress condition while Swarna-Sub1 produced two-fold or higher yield after 10 days of complete submergence in comparison to original parent Swarna (Septiningsih et al. 2009). Swarna-Sub1 was released and notified for commercial cultivation by Government of India in 2009 and has fast replaced the original variety Swarna in many flood prone areas of Odisha, Bihar and West Bengal (Dar et al. 2013).

To address the problem of submergence due to flash floods in major river basins of India, namely Brahmaputra, Cauvery, Ganges, Godavari, Krishna, Mahanadi, Mandovizauri, Narmada and Tapi, it was decided to transfer the Sub1A1 gene on rice chromosome 9 (position 6.35 Mbp) into nine regionally adapted popular rice varieties, namely ADT 46, Bahadur, HUR 105, MTU 1075, Pooja, Pratikshya, Rajendra Mahsuri, Ranjit and Sarjoo 52. In all the cross combinations Sub1B-2 (36 bp InDel, position 6.38 Mbp) located between the Sub1B and Sub1C genes gave clear polymorphism between RP and DP varieties.
and hence was used as foreground selection marker (Fig. 6). Recombinant selection was done using flanking markers RM 23869 (position 6.32 Mbp) and RM 8300 (position 6.65 Mbp), except in the case of MTU 1075 where RM 464 (position 6.50 Mbp) was used in place of RM 8300. Background selection was done using the 50K SNP chip. Submergence tolerant versions of seven of these varieties, namely ADT 46-Sub1, HUR 105-Sub1, Pooja-Sub1, Pratiksha-Sub1, MTU 1075-Sub1, Sarjoo 52-Sub1 and Rajendra Mahsuri-Sub1 are at advance stage of multi-location field submergence trials. Two varieties, Ranjit-Sub1 and Bahadur-Sub1 have been released and notified for commercial cultivation by Government of India in 2018 (Table 2). The RP genome recovery in the Sub-1 derivative of these varieties was more than 90% as revealed by application of 50K SNP chip.

Fig. 6. Parental polymorphism of InDel marker ‘Sub1BC2’ used as peak marker for the selection of Sub1A1 allele for submergence tolerance in the backcross progenies. (1) Swarna-Sub1, (2) IR64-Sub1, (3) CR1009-Sub1, (4) Pooja, (5) Pratiksha, (6) Sarjoo 52, (7) HUR105, (8) Rajendra Mahsuri, (9) ADT39, (10) Ranjit, (11) Bahadur, (12) MTU1075, (13) ADT46, (M) Size markers 50 bp ladder. 1-3 Tolerant allele, 4-13 Sensitive allele

Introgression of salt tolerance into popular rice varieties of India

Due to global climate changes paucity of irrigation water and increasing soil salinity continue to threaten crop productivity and livelihood of nearly 600 million people living in the coastal lowland areas (Wheeler 2011). Broadly salt-affected areas are classified into coastal and inland salinity, while based on the chemical nature of the salt stress the soil can be divided into saline soils (dominated by sodium cations) and sodic soils (high carbonate/bicarbonate). Worldwide 955 million ha of lands are salt affected (Szabolcs, 1994) whereas in India about 8.6 million ha of agricultural land is currently affected by salt stress (Singh 1992). Therefore, development of salt tolerant varieties is a priority to insure rice production stability in the coastal and inland saline and alkaline soils.

Rice is a moderately salt sensitive plant and several studies have illuminated the mechanisms of salinity tolerance in rice. GABB is an efficient approach for introgression of qualitative and quantitative trait from a low yielding donor variety to highly adapted high yielding mega variety of rice as shown above. However, development of salt tolerant rice varieties by introgression of QTLs through MAS has remained slow (Flowers and Flowers 2005). There are a number of salt tolerant landraces and traditional varieties of rice such as Pokkali, Nona Bokra, Azgo, Dular and Damodar but QTLs for salt tolerance have not yet been exploited through molecular breeding (Pandey and Srivastava 1987; De Leon et al. 2017). To develop a salinity tolerant variety, we have targeted introgression of two QTLs into six locally adapted popular rice varieties namely ADT 45, Gayatri, MTU 1010, PR 114, Pusa 44 and Sarjoo 52 through GABB approach. The two QTLs are, (i) SALTOL1 for seedling stage salinity tolerance coming from Pokkali, which is reported to provide selective ion uptake and Na+/K+ homeostasis at seedling stage (Gregorio 1997), and (ii) qSSISFH8.1 for reproductive stage salinity tolerance identified using CSR27/MI48 RILs but coming originally from Nona Bokra (Pandit et al. 2010).

Initially we started our GABB work for salt tolerance with SALTOL1 QTL only. SALTOL1-NILs developed using RM 3412 as peak marker for foreground selection together with G11a, RM 493 markers for recombinant selection are at BC$_2$F$_3$ stage or beyond for all the six cross combinations. Although these NILs provide good seedling stage salt tolerance similar to donor line FL 478, it was realized during multi-location field evaluation of these QTL-NILs that SALTOL1 alone is unable to provide significant yield advantage over salt tolerant check varieties under stress. Therefore, crosses were made to transfer additional QTL qSSISFH8.1 for reproductive stage salinity tolerance using peak marker RM 3395 located on chromosome 8 in an 800 kbp fine mapped region and variable flanking markers polymorphic between individual RP-DP combinations. These introgression lines are lagging behind due to late start and are presently at BC$_2$ stage requiring further backcrossing for sufficient RP genome recovery.

Future prospects

Most breeding programs aimed at improving
productivity in rainfed agro-ecosystems have focused on single abiotic stress in the past. However, recent trends are for pyramiding of multiple QTLs in high yielding mega varieties of rice (Singh et al. 2016; Ali et al. 2017; Dixit et al. 2017; Yadav et al. 2019). These studies have shown that a modified genomics-assisted backcross breeding approach coupled to strong phenotypic selection is an efficient tool for pyramiding multiple QTLs for abiotic stress tolerance present in the primary gene pool for improving multiple abiotic stress tolerance. Maintenance of grain quality, plant type and yield potential of the recipient mega variety is of utmost importance for wider acceptance of these new essentially derived varieties (EDVs). Further, these EDVs must also incorporate genes for multiple biotic stress tolerance and higher yield potential for wider acceptance and yield stability. The GABB approach described here is also suitable for the introgression of these additional genes in the mega varieties of rice.

Author’s contribution

Conceptualization of research (NS); Designing of the experiments (NS, VR, SV, PVS, PS, PKS, RK, TA, JNR, NM, ST, TR); Contribution of experimental materials (NKS, AB, NY, RS, BS, YS, NS, AS, SC, SN, JB, GP); Execution of field/laboratory experiments and data collection (AB, PJ, NY, RS, TA, MGR, PVRR, SLK, DS, SS, N, BM, PS, RKS, DPS, KP); Analysis of data and interpretation (AB, PJ, RS, MR, JK); Preparation and finalization of manuscript (NS, PJ, AB).

Declaration

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