Sub-Project ID - 010

Program Based Research Grant (PBRG)

Sub-project Completion Report

on

DNA marker-assisted breeding for producing highly stress tolerant elite rice varieties for coastal Bangladesh by introgression of multiple salt tolerance loci (QTLs) into commercial cultivars

Sub-project Duration

May 08, 2018 to October 31, 2021

Coordinating Organization

Plant Breeding Division
Bangladesh Rice Research Institute (BRRI)
Joydebpur, Gazipur-1701

Project Implementation Unit
National Agricultural Technology Program-Phase II Project
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

September 2021

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Implementing Organization

Plant Biotechnology Laboratory
Department of Biochemistry and Molecular Biology
University of Dhaka, Dhaka-1000

Pant Breeding Division and Plant Physiology Division
Bangladesh Rice Research Institute (BRRI)
Joydebpur, Gazipur-1701

Project Implementation Unit
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Citation:

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New Airport Road, Farmgate, Dhaka – 1215
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Abbreviation and Acronyms

BARC	=	Bangladesh Agricultural Research Council
BRRI	=	Bangladesh Rice Research Institute
BLAST	=	Basic Local Alignment Search Tool
BC_1F_1	=	First Backcross generation
BC_2F_1	=	Second Backcross generation
BC_2F_2	=	2^{nd} filial generation Second Backcross generation
CTAB	=	Cetyl trimethyl ammonium bromide
DNA	=	Deoxyribonucleic acid
dS/m	=	deciSiemens per metre
DU	=	Dhaka University
DArT	=	Diversity arrays technology
EDTA	=	Ethylenediamine tetraacetic acid
EC	=	Electrical conductivity
FAM	=	Fluorescein amidites
FGN	=	Filled grain number
F_1	=	First filial generation
F_7	=	Seven filial generation
FRET	=	Fluorescence resonance energy transfer
HEX	=	Hexachloro-fluorescein
IDT	=	Integrated DNA Technologies
IUPAC	=	International Union of Pure and Applied Chemistry
KASP	=	Kompetitive Allele Specific PCR
K^+	=	Potassium
MABC	=	Marker Assisted Backcrossing
Mha	=	Million hectares
PAGE	=	Polyacrylamide Gel Electrophoresis
PCR	=	Polymerase Chain Reaction
PIU	=	Project Implementation Unit
QTLs	=	Quantitative trait loci
RILs	=	Recombinant Inbred Lines
RL	=	Root length

SF	=	Spikelet fertility
SNP	=	Single-nucleotide polymorphism
SSRs	=	Simple-sequence repeats
SES	=	Standard Evaluation System
TAE	=	Tris-acetate-EDTA
i.e.	=	id est (that is)
et al.	=	et alii (and others)
°C	=	Degree Celsius

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Executive Summary

Rice production in the nine coastal upazila's of Bangladesh is predicted to decline by 15.6% due to a 26% increase in salinity in affected areas. At the same time the population of Bangladesh is estimated to increase from 170 to 214 million in the next 20 years (World Bank, 2015). So we urgently need to develop salt-tolerant modern high-yielding rice varieties. However breeding rice for salinity tolerance together with high yield is difficult as the traits are controlled by multiple genes. Moreover, salt tolerance has to be ensured at seedling and reproductive stages, the two most vulnerable stages sensitive to salt. A rice landrace '*Horkuch*' was mapped earlier for target traits and could be used as donor for developing modern high yielding rice variety tolerant to salt stress. As such the present sub-project was undertaken to establish a fluorescence-based quick and easy system for target allele selection toward development of suitable salt tolerant advanced breeding lines/varieties having relatively higher salt tolerance (8-10 dSm^{-1} for both stages of growth) with higher yield compared to previously bred varieties.

Salt tolerant QTL's were identified in recombinant inbred lines (RILs) from a reciprocal population of Horkuch (salt tolerant landrace) and IR29 (salt sensitive). These QTLs were introgressed into commercial high-yielding BRRI varieties (BRRI dhan63, BRRI dhan67 and BRRI dhan74). For this purpose, hybridization followed by generation advancement and fluorescent DNA marker-based fluorescent selection were carried out at Bangladesh Rice Research Institute (BRRI) and Dhaka University (DU), respectively. The activities involved multiple crosses and selections of positive plants with desirable QTLs from the hybrid populations. F_1s with the target QTLs were identified after crossing of the RILs with each of the recipient parents, BRRI dhan63, BRRI dhan67 and BRRI dhan74. The double crossed F_1s containing multiple QTLs were identified with KASP (Kompetitive Allele Specific PCR) markers and backcrossed with respective recipient parents to recover background genomes of the recipient parents. The introgression of these multiple loci was accomplished efficiently in two and half years by fluorescently-tagged DNA-based high-throughput SNP markers. The 'Fluorescent-based KASP' SNP markers were used to identify target genotypes with specific alleles linked to Horkuch salt tolerant QTLs (*RL, Shoot* K^+*, Saltol, FGN+SF*), through real time Polymerase Chain Reaction (RT-PCR) without using gel electrophoreses.

Best donors for QTLs tested and validated were found to be RIL I-14 (*RL* and *Shoot* K^+) and RIL I-71 (*Saltol*, FGN+SF) which was a significant achievement. Allele-specific SNP markers linked to four desired QTLs were identified from the genetic map of Horkuch and IR29. These are, i. Root Length, RL, Chr 2; ii. K^+ content, Chr 3; iii. *Saltol*, Chr 1 (seedling stage), iv. Filled Grain Number, FGN + Spikelet Fertility, SF, Chr 10 (reproductive stage). Allele-specific primers were designed for detection of the SNPs linked to the above QTLs. The design included a tail for

tagging by fluorophore to the allele-specific primer. Two hundred thirty one fluorescent KASP markers were tested for polymorphism in donor RILs and recipient parents. Out of 231 designed markers, eight were selected as foreground marker (FG) based on close proximity and tight linkage with the QTLs, 10 were selected as recombinant markers based on weak or non-linkage to the QTLs and 102 markers were selected as background markers to track rest of the background genome. All these were used for selection of progenies with the desired traits and genetic background. Donor RIL I-14 was identified carrying the RL and K^+ QTLs and I-71 with the *Saltol*, FGN+SF QTLs using KASP markers. The RILs were tested for salt tolerance and yield in the Net house. A total of 17, 11 and 16 BC_2F_2 progenies with ~80% recurrent genome recovery for BRRI dhan63, BRRI dhan67 and BRRI dhan74 were finally selected after the 2nd round of backcrossing with recipients. The selected BC_2F_2 progenies of BRRI dhan63 and BRRI dhan67 contained all four QTLs (RL, K^+, *Saltol*, FGN+SF); however, in the BRRI dhan74 background, seven and nine progenies contained three (RL, *Saltol* and FGN+SF) and two (K^+ and FGN+SF) QTLs, respectively.

All the selected progenies demonstrated excellent plant-type with high yield. The selection procedures could be done efficiently in 2.5 years due to the establishment and use of a highly efficient molecular marker technology, called KASP. This involved SNP DNA markers with fluorescent tags which can be adapted to high-throughput detection of progenies with QTL specific alleles. The selected BC_2F_3 plants will be tested for salinity tolerance parameters such as survival, chlorophyll and Na^+ and K^+ content after salt stress at 120 mM for two weeks at seedling stage. The selected plants will also be tested for yield under 100 mM salt stress at reproductive stage.

PBRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the PBRG sub-project:** DNA marker-assisted breeding for producing highly stress tolerant elite rice varieties for coastal Bangladesh by introgression of multiple salt tolerance loci (QTLs) into commercial cultivars

2. **Implementing organization (s):**
Bangladesh Rice Research Institute (BRRI), Gazipur1701
University of Dhaka, Dhaka-1000, Bangladesh

3. **Name and full address with phone, cell and E-mail of Coordinator, Associate Coordinator, PI/Co-PI (s):**

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*Acted as Coordinator from May 2018 to September 2020
** Acted as Coordinator from October 2020 to October 2021
*** Acted as Principal Investigator from May 2018 to September 2018
**** Acted as Principal Investigator from October 2018 to October 2021

4. **Sub-project budget (Tk.):**
   4.1 **Total: (in Tk. as approved)** 1,41,83,075.00 (Tk. One core forty one lac eighty three thousand seventy five only);
   DU-Component [Tk. 91,02,919.00 (ninety one lakh, two thousand nine hundred nineteen only)]
   BRRI Component [Tk. 50,80,156.00 (fifty lac eighty thousand one hundred fifty six only)]
   4.2 **Latest Revised (if any):** 01 December, 2020 (DU component)
   11 August, 2021 (BRRI Component)
5. **Duration of the sub-project:**
   5.1 **Start date (based on LoA signed):** 8 May, 2018
   5.2 **End date:** 31 October, 2021

6. **Background of the sub-project:**
   Increasing salinity in context of growing population is a great challenge to ensure food security by crop breeders and researchers. Both salinity tolerance and high yield, and their QTLs (Quantitative Trait Loci) are controlled by multiple genes which often segregate in future generations. Moreover, in the case of rice, salt tolerance has to be ensured at seedling and reproductive stages. One solution is to introgress multiple QTLs for salinity tolerance at the two developmental stages into the background of rice with superior agronomic performance. Even so, ensuring the introgression of multiple loci is difficult without DNA-based markers like SNPs (Single Nucleotide Polymorphisms). We derived recombinant inbreed lines (RILs) from reciprocal crosses of IR29 (high yield but sensitive) and Horkuch, a salt tolerant landrace. Genetic mapping identified some of the RILs to have salt tolerance

QTLs like Root length (RL, seedling stage), Potassium concentration (K^+, seedling stage) and Filled grain number with spikelet fertility (FGN+SF, reproductive stage). Another established QTL '*Saltol*' is also available in the F_7 RILs. We aimed to introgress these QTLs into the background of high-yielding BRRI dhan63, BRRI dhan67 and BRRI dhan74. Fluorescent-based Kompetitive Allele Specific PCR (KASP) markers, which can identify the specific allele through real time PCR without running time-consuming gel electrophoreses, are being used for introgression. The hybridization/crossing activities followed by population advancement were done at Bangladesh Rice Research Institute (BRRI) and the fluorescent based DNA marker selection of progenies with the required alleles was carried out at Dhaka University (DU). Firstly, each background parent, BRRI dhan63, BRRI dhan67 and BRRI dhan74 was crossed separately with the two selected donor RILs containing the desired QTLs. The two F_1s were then crossed between themselves, so that all QTLs are then transferred in the double crossed F_1 progenies. Then backcrossing was done twice with recurrent parents. At each stage of crossing and backcrossing, plants with the desired QTLs and genetic background of recipient parents were selected with the help of fluorescent based KASP method.

7. Sub-project general objective (s):

To establish a fluorescent-based quick and easy system for target allele in a breeding program.

8. Sub-project specific objectives (component wise):

DU Component:

A. Phenotyping and physiological screening of selected fixed homozygous reciprocal RIL populations of Horkuch and IR29 under salinity stress at seedling stage.
B. Establishment of efficient SNP-based markers selection system for detection of donor allele specific SNP for validation of the multiple QTLs.
   a. Identification of SNP markers linked to salt tolerant QTLs of Horkuch at seedling and reproductive stages.
   b. Tagging and validating allele-specific SNPs linked to multiple QTLs with fluorescent molecules to establish KASP (Kompetitive Allele Specific PCR).
   c. Identification and validation of RIL parents containing the target QTL's for salt tolerance and high yield under salt-stress.
C. Development of foreground, recombinant and background SNP/SSR markers to be used for marker assisted back crossing
   a. Selection of BC_2F_2 lines with the target QTLs in genetic background of recipient parents, BRRI dhan63, BRRI dhan67 and BRRI dhan74.

BRRI component:

A. Phenotyping and physiological screening of selected reciprocal F_7 RIL populations of Horkuch and IR29 under salinity stress at reproductive stage.
B. Field level trial of best 20 selected F_7 RIL reciprocal population lines in the coastal region.
C. Breeding of highly salt tolerant genotypes (8-10 dSm^{-1} for both stages of growth) through introgression of multiple salinity tolerance QTLs at seedling and reproductive stages into modern BRRI Boro rice varieties to develop lines/varieties suitable for saline coast.

9. Implementing location (s):

1. Plant Biotechnology Laboratory, Department of Biochemistry and Molecular Biology, University of Dhaka.

2. Pant Breeding Division and Plant Physiology Division, Bangladesh Rice Research Institute (BRRI), Gazipur-1701
3. Polder 3 region, Kaliganj, Satkhira.

10. Methodology in brief (with appropriate pictures):

DU Component:

- **Phenotypic and physiological screening of RIL population at seedling stage:**

  The phenotypic and physiological screening for the salinity tolerance at seedling stage was accomplished following Gregorio *et al.* 1997 at Plant Biotechnology Lab Net House, University of Dhaka. Seeds of the selected plants were incubated at 50°C for five days to break dormancy. Then seeds were rinsed several times with distilled water and placed in petri dishes with moistened filter paper and incubated at 37°C for 72 hours for germination.

  The selected 16 RILs, Horkuch parent (tolerant check) and IR29 parent (sensitive check) with 16 replicates (eight for control, eight for stress) were randomized in a complete block design (RCBD) in different floaters containing Yoshida's culture solution (Yoshida *et al.* 1976). After 14 days, 60 mM NaCl (~6 dSm^{-1}) salt was applied with a gradual increment of 20 mM NaCl per day up to 12.0 dSm^{-1}. The electrical conductivity (EC) of the hydroponic solution was measured every day. The pH of the hydroponic solution was checked regularly and maintained pH 5.0 throughout the experiment. The hydroponic solution was being renewed twice a week. After ~2 weeks of salinization, the sensitivity of each seedling was scored when the sensitive checks were almost dead and required data were collected. All plants were individually monitored and physiological parameters like Standard Evaluation System (SES scale of 1-9, Gregorio *et al.* 1997), chlorophyll content, root relative water content, weight and length of root and shoot of the salt-stressed plants were measured.

- **Establishment of efficient SNP based markers system for detection of QTLs in Horkuch and RIL donor:**

  Kompetitive allele specific PCR (KASP) system was used as an efficient SNP-based markers system to detect QTLs in donor lines. Three components are essential for KASP assay: 1) purified DNA sample, 2) primer (two allele specific forward primers and a common reverse primer) and 3) master mix with universal FRET cassette (universal fluorescent tagged primer).

  To design of allele-specific forward primers, 50 bp DNA sequences flanking the SNPs linked to the target QTLs were extracted by aligning with reference sequences. The sequences with the QTL linked-SNPs were obtained by DArT sequencing and genetic mapping (Haque *et al.* 2020). The short read aligner called Bowtie2 was used to align these sequences against reference *Nipponbare*, *Horkuch* and *IR29* genomes. The alignment and results were cross-checked subsequently by Local BLAST using the DArT provided short sequences and 101 bp sequences was extracted (50 bp flanking sequences on both side of each SNP). Additional SNPs surrounding the SNP of interest were coded and marked using IUPAC codes as per the requirement for KASP assay design. These 101 bp sequences with all the additional information of surrounding SNPs and indels were submitted to 3CR Bioscience for designing two allele-specific forward primers. The primers (two allele-specific forward primers and a common reverse primer) were synthesized and obtained in 25 nmole scale from IDT-1st BASE, Singapore. Primers were assembled as follows (Table 1).

Table 1: Assembly of primers for KASP assay

Primer	Final concentration (µM)	Vol required for 100µl assay mix (µL)
Allele-specific primer 1-FAM (100 µM)	12	12
Allele-specific primer 2-HEX (100 µM)	12	12
Common, reverse primer (100 µM)	30	30
PCR grade water	-	46
Total	**-**	**100**

- **DNA extraction:**
  Genomic DNA was isolated from (0.5–1.0 g) pooled leaf tissue of individuals test populations using the modified CTAB method (Doyle and Doyle, 1990) followed by quantification using the nanodrop spectrophotometer (Nanodrop 1000). The quality of the DNA was checked in 0.8% agarose gel in TAE (Tris acetate EDTA) buffer, pH 8.0.

- **Genotyping procedure by KASP:**
  The 96-well plate reaction and 10 µL per well was used in genotyping. Required amount of total reaction mix was added to each DNA sample in the reaction plate (Table 2). Replicated reaction was run for each DNA sample as well as negative and positive controls were also used in each 96 well plate. The plate was sealed with an optically clear seal then be centrifuged briefly to place all liquid at the bottom of the wells.

Table 2: KASP reaction preparation

Component	Volume in µL
DNA (10-12 ng/µL)	5.0
Primer	0.138
Master mix	5.0
Total volume	**10.0**

The genotyping reaction was thermally cycled according to the protocol of 3CR bioscience (Table 3)

Table 3: Thermal cycling profile used in KASP assay

Step	Description	Temperature	Time	No. of cycles
1	Enzyme activation	94	15 Secs	1
2	Denaturation	94	20 Secs	10
	Annealing and Elongation	65-57	60 Secs (drop 0.8° C per cycle)	
3	Denaturation	94	20 Secs	30
	Annealing and Elongation	57	60 Secs	

After thermal cycling is completed, the fluorescent signal was detected by reading the plate in an appropriate qPCR machine. Data analysis and interpretation was done using cluster analysis software. Genotyping master mix reports genotypes with the fluorophore HEX and FAM (Fig. 1).

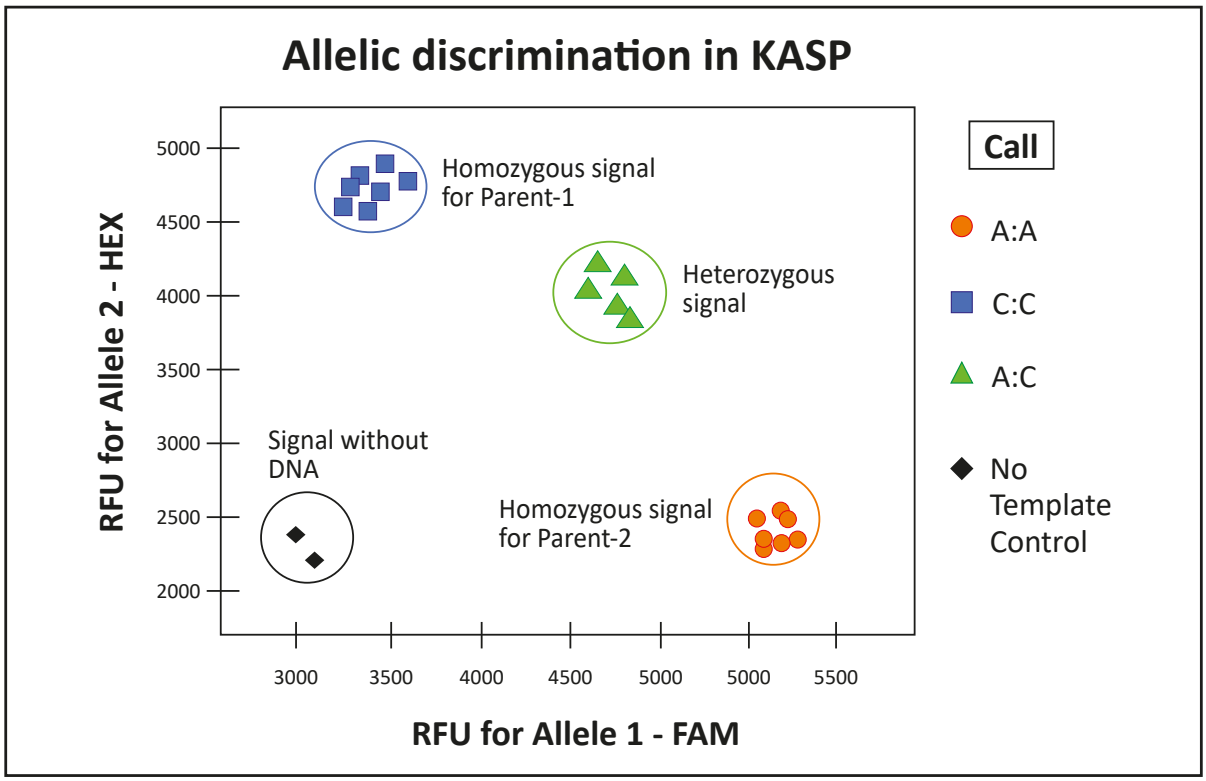

Fig. 1: Diagram of typical genotyping cluster plot data for the KASP genotyping chemistry, where the blue squares represent allele C (Parent 1), the orange circles represent allele A (Parent 2) and the green ones are heterozygous. Black samples at the origin are the no template controls (NTCs)

- **F_1 Confirmation by polymorphic SSR markers:**
  Two RILs, I-14 and I-71 were selected as donor and three high yielding elite rice varieties were taken as recipient parents. The three high yielding varieties are BRRI dhan63, BRRI dhan67 and BRRI dhan74. Total 28 SSR markers have been used to find polymorphism between these donor and recipients. Both I-14 and I-71 were crossed with three varieties- BRRI dhan63, BRRI dhan67 and BRRI dhan74. Crossing was done in Plant Breeding Division of BRRI. Identified polymorphic SSR markers were used to confirm crossing of these F_1 plants.

BRRI component:

- **Advancement of the best progenies:**
  F_6 progenies derived from reciprocal crosses of Horkuch and IR29 with better performance in F_4 and F_5 generation as well as a good combination of seedling and reproductive QTLs were selected. The selected RILs were advanced to F_7 generation using BRRI field facilities at Gazipur. Replication was made to ensure enough seed for further screening and subsequent field trial(s) in the coastal region of Bangladesh.

- **Phenotyping and physiological screening at reproductive stage:**
  This experiment was carried out at Plant Physiology Division net house of BRRI by soil based method (Gregorio *et al.* 1997). Seeds of the selected plants were incubated at 50°C for five days to break dormancy. Then seeds were rinsed several times with distilled water and placed in Petri dishes with moistened filter paper and incubated at 37°C for 72 hours to germinate. Seven replicates (four for control and three for stress) of each 16 selected F_7 plants, Horkuch parent (tolerant check) and IR29 parent (sensitive check) were taken. Twenty one large plastic (12 stresses + nine controls) bowls with a capacity to accommodate six pots were used. Tap water was added to the bowls containing perforated plastic pots filled with fertilized puddle soil. Each bowl contained two pots for the parents and the rest of the pots were randomly assorted for the selected F_7 plants in control and stressed conditions. As one pot contains a single plant and each pot was considered as one experimental unit.

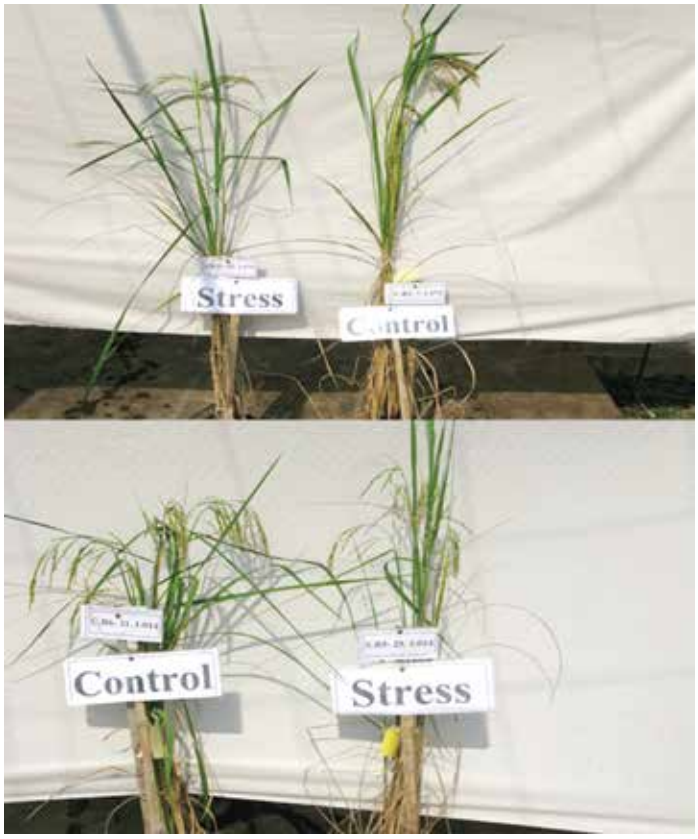

Fig. 2: Stressed (10 dSm^{-1}) plants at reproductive stage

After 30 days of sowing, salinity stress (NaCl) at EC of 10 dSm^{-1} was applied to the bowls by replacing tap water with saline water and maintained by keeping the volume same with water on daily basis until maturity (Fig. 2). All cultural managements i.e. fertilizer, weed and disease-insect managements were carried out following Yoshida *et al.* 1976 and BRRI, 2013. At physiological maturity all plants were harvested and different phenotypic parameters/ traits (plant height, heading date, tiller and effective tiller, panicle length and damage, total spikelet, percent fertility, yield and its components) were measured and recorded. Table 4A and 4B show the phenotypic and physiological properties of the parents and recipients, respectively.

Table 4A: Characteristics of recipient parents (Source: BRRI 2020, Modern Rice Cultivation, 23rd Edition, page 103)

Variety	BRRI dhan63	BRRI dhan67	BRRI dhan74
Season	Boro	Boro	Boro
Crop duration (days)	146	145	147
Grain quality	Extra-long slender	Medium slender and white	Medium bold and white
Plant height (cm)	86	100	95
Yield (t/ha)	6.5	3.8-7.4	7.1
Amylose (%)	25.0	24.6	24.2
Special features	Premium quality high-yielding rice variety, Called 'Sorubalam'	Salt tolerant. It can set grain with 8 dSm^{-1} at salt sensitive stages	Moderately Blast resistant. Zinc (24.2 mg/kg) enriched boro variety

Table 4B: Parameters at reproductive stage under stressed and control conditions of selected RILs (as donors), Horkuch (tolerant control) and IR29 (sensitive control)

Plant ID	Condition	Plant height (cm)	Panicle/ Plant	Filled grain/ Plant	TGW (g)	Spikelet fertility	Yield (g/plant)	QTLs
Horkuch	Control	157.0	5.0	390.2	26.2	78.1	10.3	-
	Stress	150.6	4.1	117.7	21.5	70.0	2.6	
I-14	Control	122.0	4.5	332.0	21.3	77.1	7.1	RL, K^+ conc.
	Stress	117.8	3.2	99.0	13.1	37.5	1.3	
I-71	Control	124.0	4.5	186.0	30.9	56.4	5.7	*Saltol*, FGN+ SF
	Stress	103.2	1.4	8.0	21.6	9.7	0.9	
IR29	Control	110.1	3.3	248.4	20.8	69.3	5.0	-
	Stress	80.2	3.0	37.1	12.5	30.7	0.5	

RL=Root length; FGN=Filled grain number; SF=Spikelet fertility

- **Field trial of selected F_7 RIL population in the coastal region:**
The performance of the selected 16 populations was evaluated in the saline prone area of Kaliganj, Satkhira, Bangladesh (Latitude: 22.453097; Longitude: 89.034659) (Fig. 3). The experiment was conducted following Randomized Complete Block Design (RCBD) with two replications. The unit plot size was 3.0 m^2. On 15 October 2018, the EC level was 6.0 dSm^{-1} after 2-3 days raining due to effect of 'Titli' depression. However, before raining, the water EC was recorded as 8.0-9.0 dSm^{-1} in the field.

Fig. 3: Field trial at Kaliganj, Satkhira (2018-2019)

- **Marker assisted backcrossing:**
  In this study, the Marker Assisted Backcrossing (MABC) approach is used (Neeraja *et al.* 2007 and Collard *et al.* 2008a) to introgress multiple salt tolerance QTLs into cultivated superior varieties such as BRRI dhan63, BRRI dhan67 and BRRI dhan74. Out of 16 F_7 populations, two donor lines (I-71 and I-14) derived from Horkuch and IR29 reciprocal crosses were selected based on phenotype and presence of major QTLs like filled grain number (FGN), spikelet fertility (SF), seedling root length (RL), potassium concentration (K^+) and *Saltol*. BRRI dhan63, BRRI dhan67 and BRRI dhan74 were used as recipient parents.

  Breeding Strategy
  For marker-assisted backcrossing, at first hybridization between donor and recipient was done separately. Two donor lines (I-71 and I-14) were simultaneously crossed with BRRI dhan63, BRRI dhan67 and BRRI dhan74 to produce F_1 seed (BRRI dhan63/I-14 & BRRI dhan63/I-71, BRRI dhan67/I-14 & BRRI dhan67/I-71, and BRRI dhan74/I-14 & BRRI dhan74/I-71) during T. Aman 2018-19.

Fig. 4a: Hybridization block and hybridized parents for double crossing having three QTLs in one background (BRRI dhan63, BRRI dhan67 and BRRI dhan74) from donors (I-71 and I-14), Boro 2018-19

Fig. 4b: Hybridization block and hybridized plants of back crossing, T. Aman 2019-20

Fig. 4c: Hybridization block and hybridized plants of back crossing, T. Aman and Boro 2019-20

Fig. 4d: BC_2F_2 plants from three cross combinations (BRRI dhan63, BRRI dhan67 and BRRI dhan74) in hybridization block, Boro 2020-21

Crossing was done in the hybridization block of Plant Breeding Division at BRRI, Gazipur. In total 316, 133, 108, 123 and 119 F_1 seeds were produced from three different crosses of BRRI dhan63/I-14, BRRI dhan63/I-71, BRRI dhan67/I-14, BRRI dhan67/I-71 and BRRI dhan74/I-14, respectively. Then both positive plants (BRRI dhan63/I-14, BRRI dhan63/I-71, BRRI dhan67/I-14, BRRI dhan67/I-71 and BRRI dhan74/I-14) were selected by polymorphic SSR markers. Positive F_1 plants (BRRI dhan63/I-14 & BRRI dhan63/I-71, BRRI dhan67/I-14 & BRRI dhan67/I-71, and BRRI dhan74/I-14 & I-71) were crossed to develop double crossed F_1 seed (BRRI dhan63/I-14//BRRI dhan63/I-71, BRRI dhan67/I-14//BRRI dhan67/I-71 and BRRI dhan74/I-14//I-71) with combination of three QTLs in a single recipient parent (BRRI dhan63, BRRI dhan67 and BRRI dhan74) during Boro 2018-19 (Fig. 4a). In total 975, 972 and 173 doubled crossed F_1 seeds were produced from double crosses of BRRI dhan63/I-14//BRRI dhan63/I-71, BRRI dhan67/I-14//BRRI dhan67/I-71 and BRRI dhan74/I-14//I-71, respectively. Plants with desired QTLs (Positive plant) were backcrossed with recipient cultivars such as BRRI dhan63, BRRI dhan67 and BRRI dhan74 to generate BC_1F_1 seeds during T. Aman 2019-20 (Fig. 4b). In total 906, 505 and 363 BC_1F_1 seeds were produced from BRRI dhan63/I-14//BRRI dhan63/I-71///BRRI dhan63, BRRI dhan67/I-14// BRRI dhan67/I-71///BRRI dhan67 and BRRI dhan74/I-14//I-71///BRRI dhan74, respectively. The BC_1F_1 seeds from different crosses were used for further backcrossing to produce BC_2F_1 seeds during Boro 2019-20 (Fig. 4c). Seventeen plants with positive alleles (three QTLs) were selected from all combinations of BC_1F_1 plants. Subsequently, out of these 17 BC_1F_1 plants, 10 plants were backcrossed with BRRI dhan63, five plants backcrossed with BRRI dhan67 and the rest two plants backcrossed with BRRI dhan74. In BC_2 generation, total 2344, 1648 and 528 BC_2F_1 seeds were produced from the cross BRRI dhan63/I-14//BRRI dhan63/I-71///2*BRRI dhan63, BRRI dhan67/I-14//BRRI dhan67/I-71///2* BRRI dhan67 and BRRI dhan74/I-14//I-71///2*BRRI dhan74 using the recurrent parents BRRI dhan63, BRRI dhan67 and BRRI dhan74, respectively. Total 1531, 984 and 1024 BC_2F_2 seeds were produced through selfing from the selected 7, 2, 4 BC_2F_1 plants of three different crosses (BRRI dhan63, BRRI dhan67 and BRRI dhan74 background), respectively. The BC_2F_2 plants with target QTLs derived from different crosses were grown in the field during Boro 2020-21 to advance generation towards homozygosity (Fig. 4d). In total 17, 11 and 16 homozygous BC_2F_2 plants were selected with three QTLs of different crosses (BRRI dhan63, BRRI dhan67 and BRRI dhan74 background), respectively and BC_2F_3 seed were produced. The BC_2F_3 seed from different background will be used for physiological screening under salt stress at seedling stage. The marker-assisted backcrossing scheme uses two cycles of crossing back to the recurrent parents followed by selfing, as illustrated in Fig. 5.

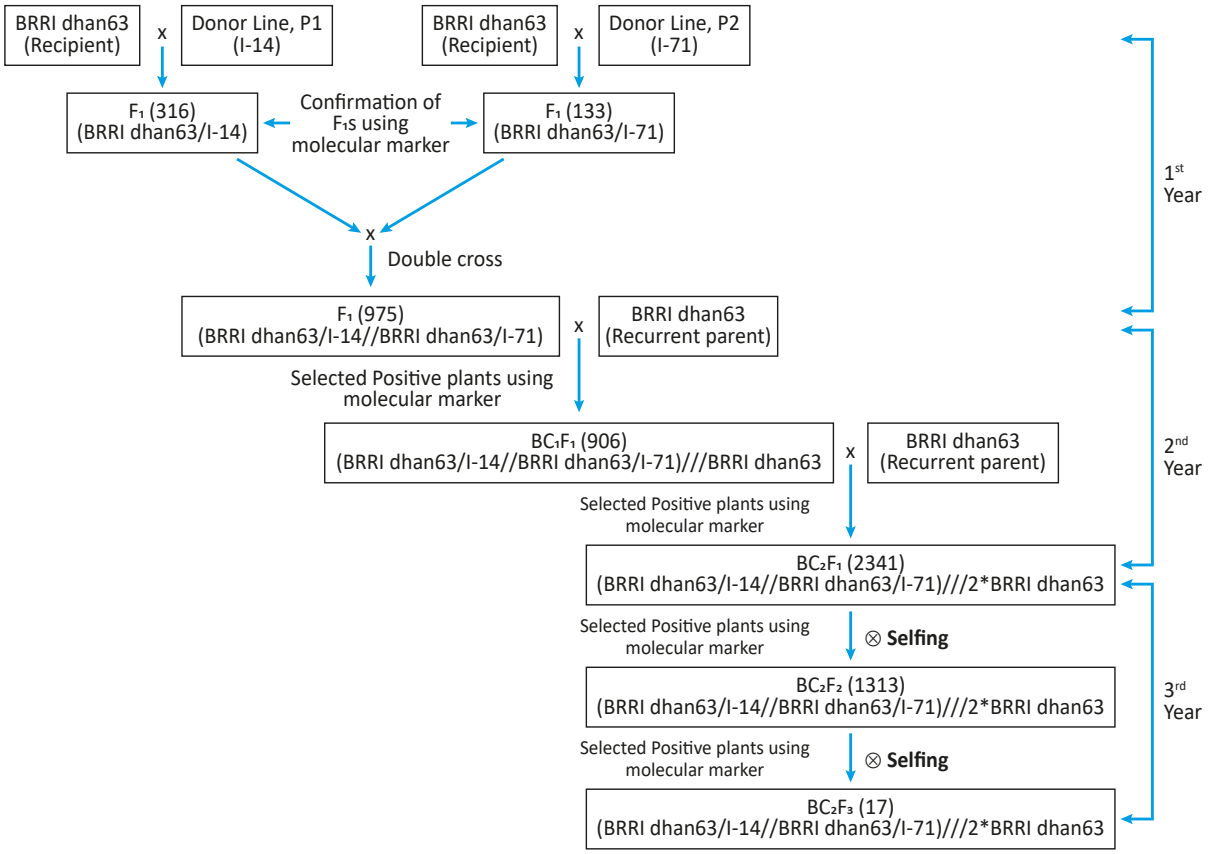

Fig. 5: Schematic diagram for backcrossing with positive plants having three QTLs in one genetic background using recurrent parent (BRRI dhan63) to produce BC_2F_3 seeds. Similar backcrossing scheme also followed with BRRI dhan67 and BRRI dhan74

11. Results and discussion (with appropriate pictures):

DU Component:

- **Phenotypic and physiological screening of RIL population**

Screening of selected fixed homozygous RILs was conducted at seedling stage after subjecting to 12 dSm^{-1} salt stress. All plants were individually monitored and physiological parameters like (chlorophyll content, root relative water content, weight and length of root and shoot of the salt-stressed plants) were measured following Standard Evaluation System (SES scale of 1-9, Gregorio *et al.* 1997). Density plot of root length and SES of RILs are shown in Fig. 6 and 7. RIL I-14 and I-71 were identified as best lines with maximum major QTLs. I-14 had the root length (RL) QTL at chromosome 2 and K^+ QTL at chromosome 3. Whereas I-71 had the *Saltol* at chromosome 1, filled grain number (FGN) and spikelet fertility (SF) QTLs at chromosome 10.

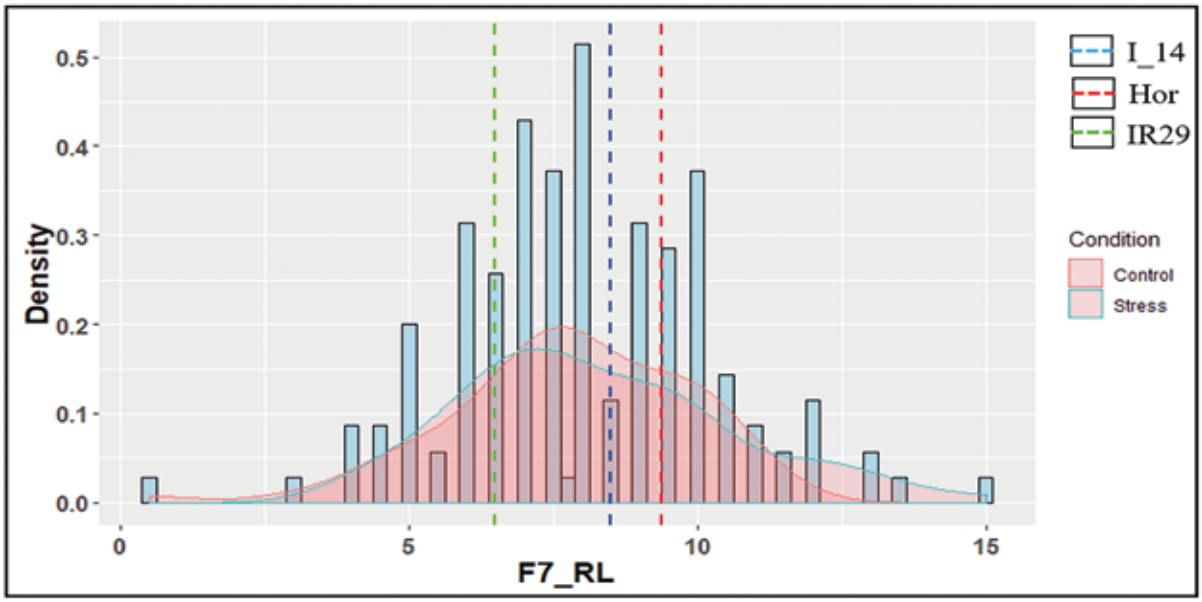

Fig. 6: Density plot at control and stress condition for root length at seedling stage, Here 'Hor' stands for Horkuch

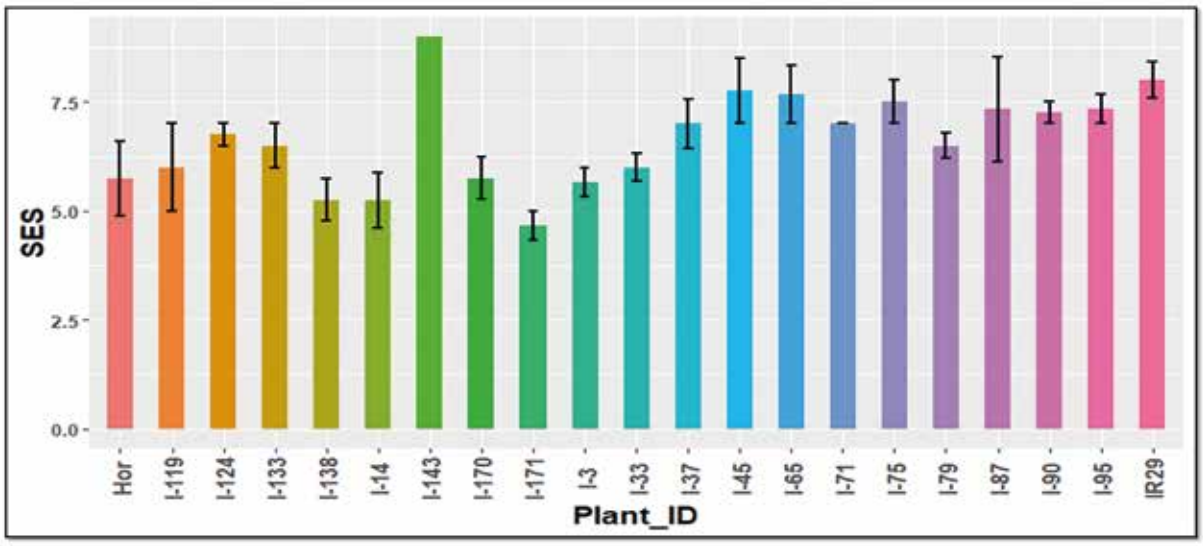

Fig. 7: SES score of RILs at stress condition in seedling stage. Here 'Hor' stands for Horkuch, and 'I-119', 'I-124' etc. are RILs

- **Establishment of efficient SNP based markers system**

In total 378 markers were checked to identify polymorphism between donor and recipients. Eight foreground markers, 10 recombinant markers and 102 background markers were found polymorphic between donor and recipient. Interestingly, the QTL of chromosome 10 (FGN and SF) is from high yielding background. Many markers were tried for Chromosome 10, but no polymorphism was found between donor and recipient in the region of the FGN+SF QTL. So this reproductive stage QTL is already existing in BRRI dhan63, BRRI dhan67 and BRRI dhan74. We identified two foreground markers for each, root length (RL), K^+ and *Saltol* QTLs. All polymorphic markers are shown chromosome wise (Fig.8).

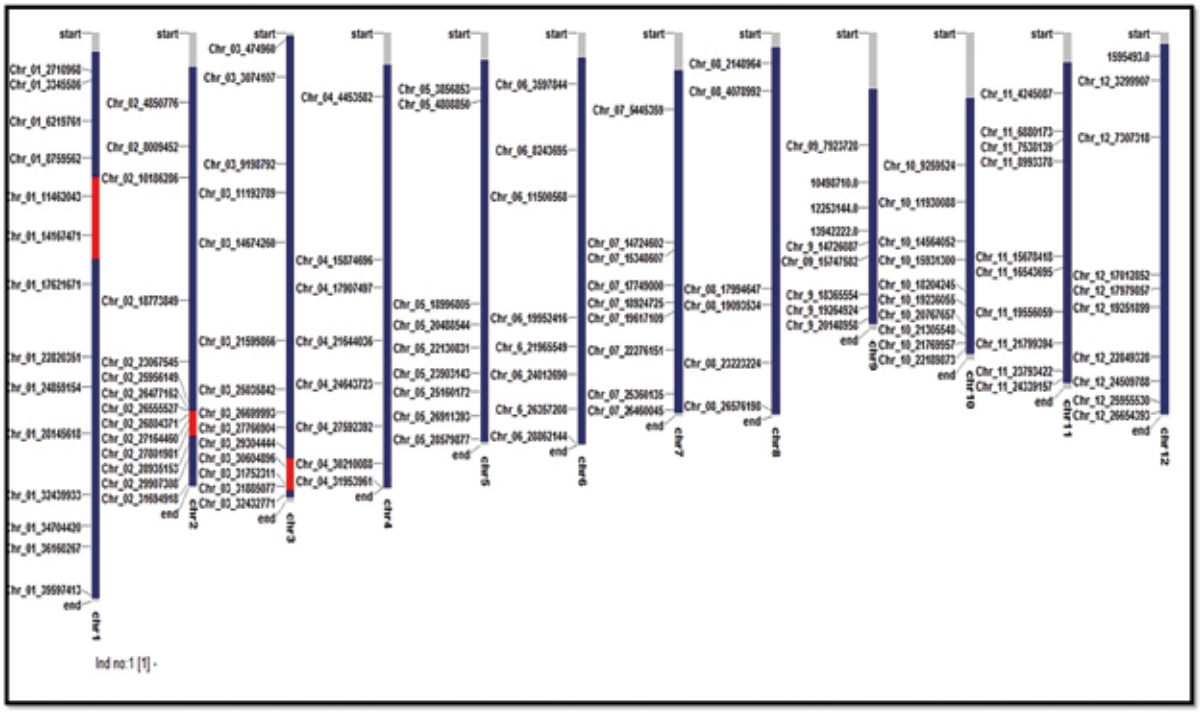

Fig. 8: Efficient polymorphic KASP markers. Foreground positions are marked in red color

- **Identification and validation of RIL parents containing the target QTL's**

The SNP markers designed for KASP technology were used to find out polymorphism between donor and recipients. Results of KASP genotyping assay for foreground marker of RL are presented in Fig. 9. In Fig. 9, Horkuch and I-14 show allele 1, represented in round shapes at bottom right corner. Other plants show allele 2 in triangle shapes. So analyzing the clusters it can be detected that the marker RL_27164460 is very efficient to describe polymorphism between donor I-14 and recipients. Thus all foreground, recombinant and background makers were validated.

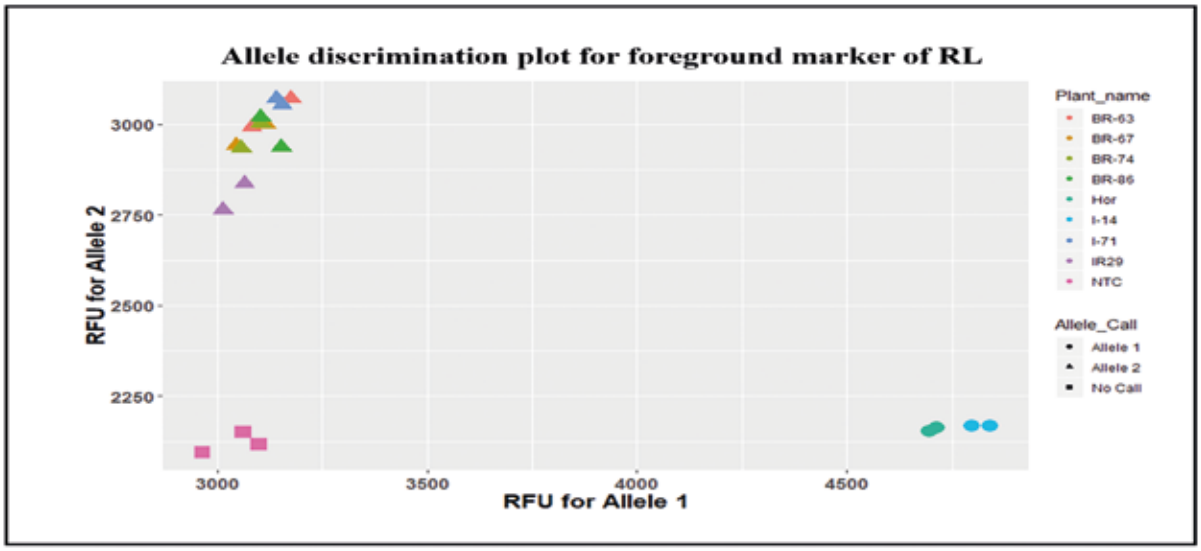

Fig. 9: Allele discrimination plot for foreground marker RL_27164460

- **Marker-assisted selection at each stage of crossing and backcrossing**

  a. F_1 Confirmation by polymorphic SSR markers

  Two separate crosses with selected donors i.e., I-14 and I-71 and three recipient cultivars (BRRI dhan63, BRRI dhan67 and BRRI dhan74) were done to produce two different F_1s for each recipient. DNA of 330 F_1s was isolated by CTAB method. Total 247 F_1 plants were found true F_1 plants (heterozygous) tested with polymorphic SSR markers. The example of BRRI dhan63 is shown in Fig. 10.

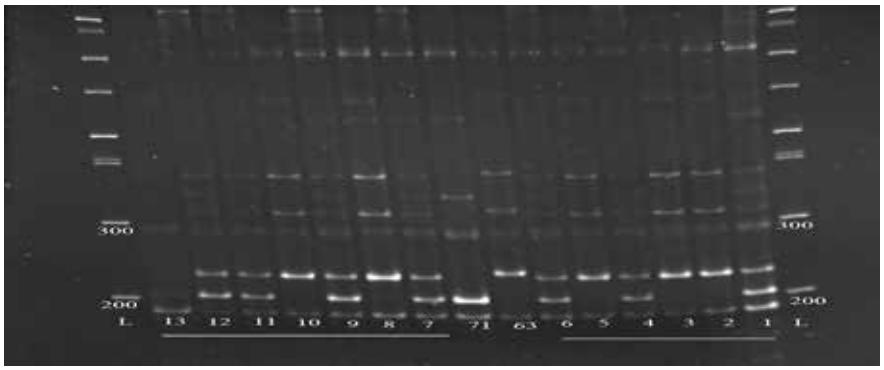

Fig. 10: PAGE run with RM493 for confirmation of F_1 plants from BRRI dhan63/I-71. Here in this figure Lane 63 = BRRI dhan63 and Lane 71 = I-71, and other lanes are for F_1 plants

b. Selection of double crossed F_1 plants with polymorphic SNP markers

A total of 247 F_1 plants were found positive (heterozygous) using SSR markers. Positive BRRI dhan63/I-14 plants were crossed with positive BRRI dhan63/I-71 plants and double crossed F_1 seeds were produced. Similarly double crossed F_1 seeds were also generated for BRRI dhan67 and BRRI dhan74 background. These double crossed F_1 plants were then tested with foreground (SNP) markers for all QTLs by fluorescent based KASP method (Figs.11a and 11b). Among these, 38 plants were found positive (heterozygous) for the three high yielding backgrounds. Ten plants were found with all four QTLs (Table 5). The selected plants were backcrossed to produce BC_1F_1 seeds.

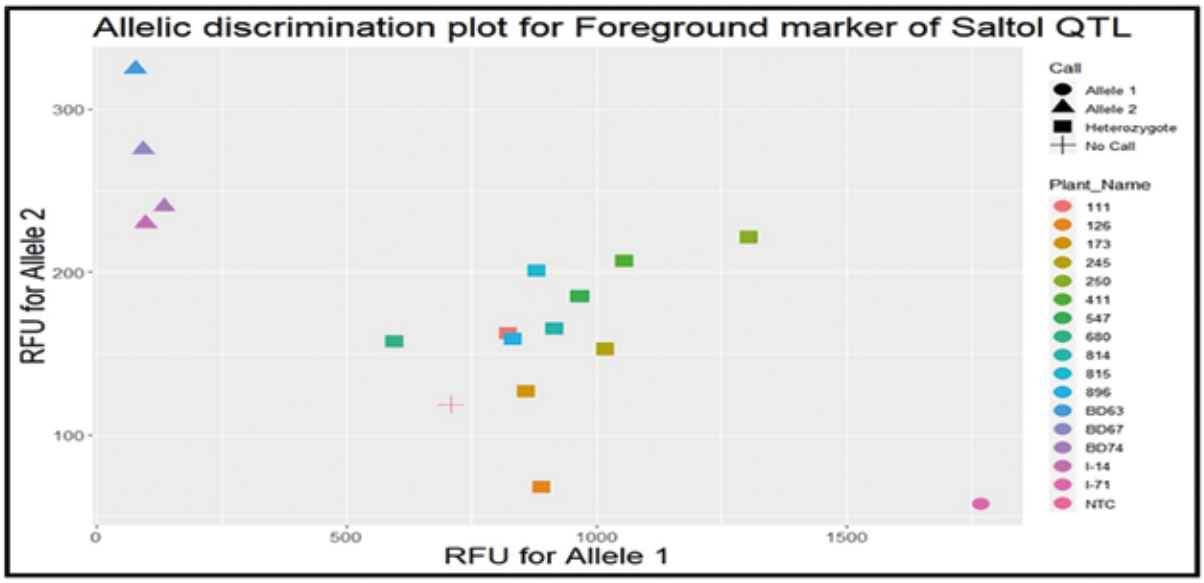

Fig. 11a: Selection of 10 double F_1 plants based on KASP for *Saltol*

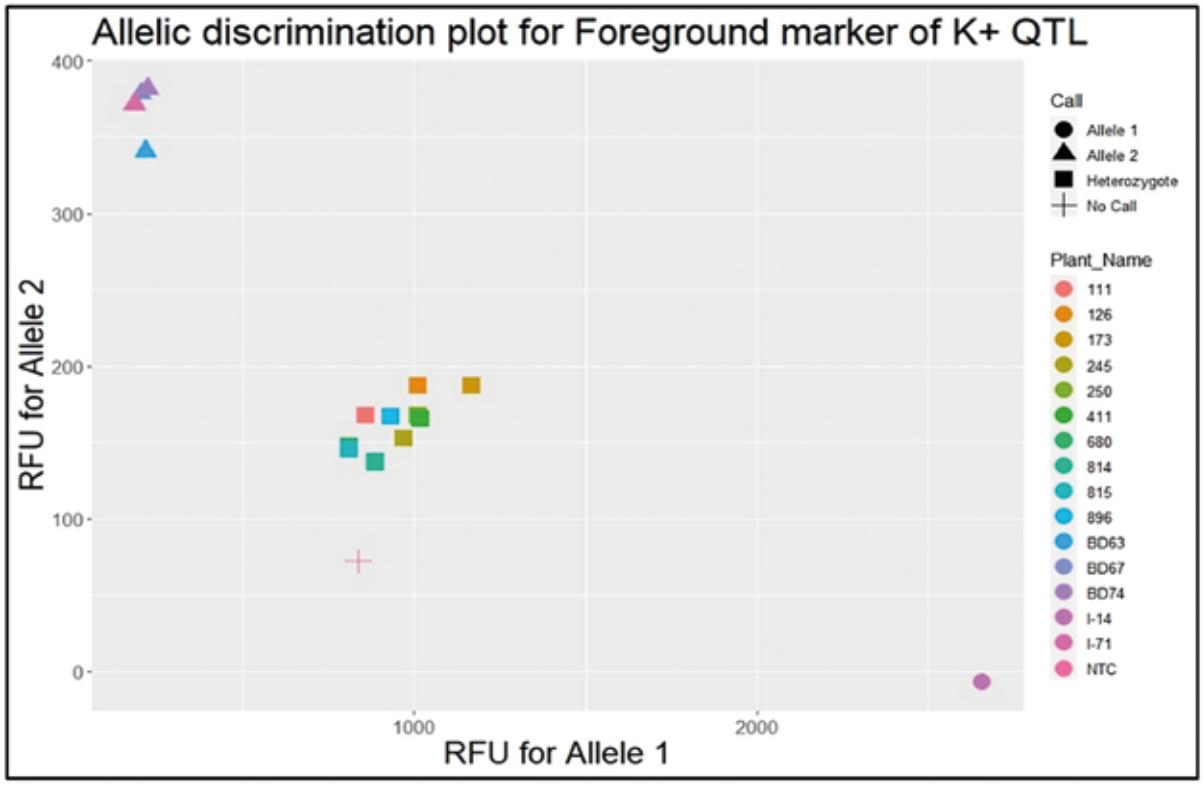

Fig. 11b: Selection of 10 double crossed F_1 plants based on KASP for K^+

Table 5: Best double crossed F_1 plants with all QTLs

Doubled crossed Plant Number	Cross combination	QTLs			
		RL	FGN+SF	K^+ (Potassium)	*Saltol*
111	BRRI dhan63/I-14 //BRRI dhan63/I-71	Hetero	Hetero	Hetero	Hetero
173	BRRI dhan63/I-14 //BRRI dhan63/I-71	Hetero	Hetero	Hetero	Hetero
245	BRRI dhan63/I-14 //BRRI dhan63/I-71	Hetero	Hetero	Hetero	Hetero
250	BRRI dhan63/I-14 //BRRI dhan63/I-71	Hetero	Hetero	Hetero	Hetero
411	BRRI dhan67/I-14 //BRRI dhan67/I-71	Hetero	Hetero	Hetero	Hetero
547	BRRI dhan67/I-14 //BRRI dhan67/I-71	Hetero	Hetero	Hetero	Hetero
680	BRRI dhan67/I-14 //BRRI dhan67/I-71	Hetero	Hetero	Hetero	Hetero
814	BRRI dhan74/I-14//I-71	Hetero	Hetero	Hetero	Hetero
815	BRRI dhan74/I-14//I-71	Hetero	Hetero	Hetero	Hetero
896	BRRI dhan74/I-14//I-71	Hetero	Hetero	Hetero	Hetero

c. Selected positive BC_1F_1 progenies with polymorphic SNP markers

A total of 906, 505 and 363 BC_1F_1 seeds were produced using recurrent parents BRRI dhan63, BRRI dhan67 and BRRI dhan74, respectively. These BC_1F_1 plants were then tested with foreground (Fig. 12), recombinant and background markers in fluorescent based method (KASP). Subsequently, 10, five and two best plants were selected for BRRI dhan63, BRRI dhan67 and BRRI dhan74, respectively. The selected plants were backcrossed with recurrent parents to produce BC_2F_1 seeds.

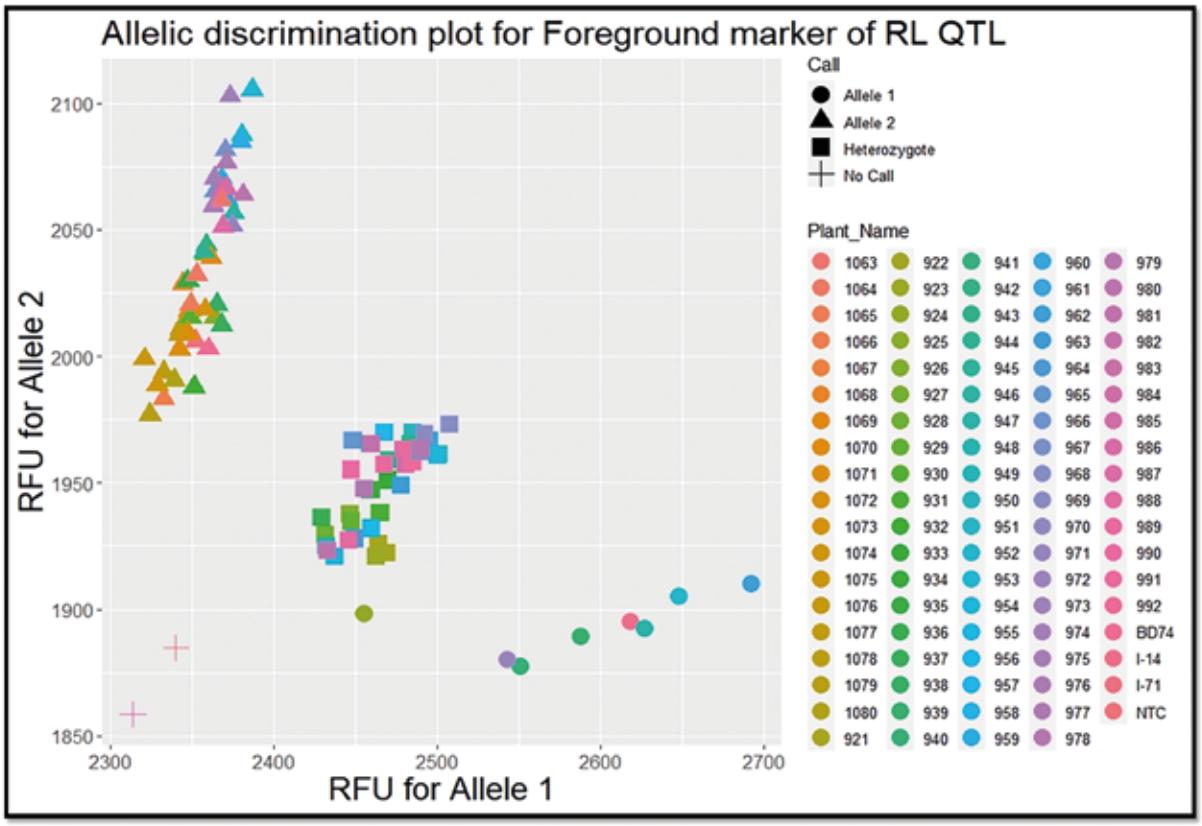

Fig. 12: Selection of BC_1F_1 progenies based on KASP for RL

d. Selection of BC_2F_1 plants

In total 1076 BC_2F_1 plants from the three recurrent parents were tested for foreground QTLs using foreground (SNP) markers and 138 BC_2F_1 plants were selected where QTLs were present (in homozygous donor or heterozygous form). Then background SNP markers were tested in these selected BC_2F_1 plants. All selected 138 BC_2F_1 positive plants with target alleles were examined with 54 background SNP markers. The plants had 56.1 to 78.8% background recovery of recurrent parents and 20.2 to 42.1% of heterozygous allele over 54 background SNP markers (Table 6).

Table 6: Background recovery percentage of best selected BC_2F_1 plants over 54 Background SNP markers

Recipient parent	Plant ID (BC_1F_1)	Plant ID (BC_2F_1)	% donor genome	% recurrent parent genome	% heterozygous genome
BRRI dhan63	107	10	3.5	67.2	29.3
	107	72	1.7	66.7	31.6
	342	202	1.3	66.7	32.1
	342	232	1.9	67.9	32.1
	350	337	1.9	56.6	41.5
	350	349	1.8	61.4	36.8
	350	354	1.8	56.1	42.1
BRRI dhan67	692	428	1.8	60.3	37.9
	692	474	1.8	60.3	37.9
BRRI dhan74	993	816	1.8	65.5	32.8
	993	878	1.8	65.5	32.8
	993	885	1.8	58.6	39.6
	1062	1021	1.0	78.8	20.2

e. Selection of BC_2F_2 plants with the target QTLs and genetic background of recipient parents, BRRI dhan63, BRRI dhan67 and BRRI dhan74

In total 1283, 963 and 1047 BC_2F_2 plants from three recurrent parents BRRI dhan63, BRRI dhan67 and BRRI dhan74, respectively were tested for foreground QTLs using foreground (SNP) markers. Seventeen plants of BRRI dhan63 were selected having all QTLs in homozygous form with 65 to 77% recurrent parent genome over 78 background SNP markers (Table 7). In case of BRRI dhan67, 11 plants were selected having all QTLs in homozygous form with 64 to 81% recurrent parent genome over 77 background SNP markers (Table 8). Sixteen plants of BRRI dhan74 were selected with 69 to 90% recurrent parent genome recovery over 76 background SNP markers (Table 7). Among these 16 plants, seven plants had three QTLs (RL, *Saltol*, FGN+SF) and rest nine plants had two QTLs (K^+, FGN+SF) in homozygous form.

Table 7: Analysis of introgressed segments and recovery percentage of recurrent parent background genome in selected best plants of BC_2F_2 population

Plant ID (BC_2F_2)	Background	Total QTL No.	No. of foreground marker tested	No. of background marker tested	Donor allele (%)	Recipient allele (%)	Hetero (%)	Recovery of recurrent parent genome (%)
ID_99	BD63*	4	8	78	8.3	72.1	4.8	77.33
ID_121	BD63	4	8	78	8.4	62.2	12.7	74.11
ID_137	BD63	4	8	78	9.6	70.7	5	76.74
ID_158	BD63	4	8	78	12.1	64.5	8.6	72.67
ID_182	BD63	4	8	78	9.8	68.5	6.9	76.16

Plant ID (BC_2F_2)	Background	Total QTL No.	No. of foreground marker tested	No. of background marker tested	Donor allele (%)	Recipient allele (%)	Hetero (%)	Recovery of recurrent parent genome (%)
ID_353	BD63	4	8	78	9.8	64.6	10.2	77.06
ID_438	BD63	4	8	78	9.3	63.6	9	77.38
ID_458	BD63	4	8	78	12.4	65.8	6.3	75.29
ID_645	BD63	4	8	78	11.1	67.9	4.6	69.05
ID_675	BD63	4	8	78	6.9	68.2	8.6	74.40
ID_738	BD63	4	8	78	8.5	66.1	9.1	72.02
ID_780	BD63	4	8	78	14.4	66	3.2	69.05
ID_950	BD63	4	8	78	9.7	64.1	8.9	71.34
ID_984	BD63	4	8	78	10.1	64.9	7.5	69.28
ID_1220	BD63	4	8	78	15.1	56.7	8.6	65.85
ID_1252	BD63	4	8	78	14.5	55.3	13.3	66.87
ID_1271	BD63	4	8	78	13.7	51.2	17.9	68.67
ID_1372	BD67**	4	8	77	8.7	55.1	21.4	73.53
ID_1426	BD67	4	8	77	7.9	66.3	11	81.76
ID_1447	BD67	4	8	77	14.6	59.5	11.1	73.53
ID_1488	BD67	4	8	77	14.4	56	14.2	70.24
ID_1500	BD67	4	8	77	21	59	5.2	64.71
ID_1650	BD67	4	8	77	15.7	52.4	16.6	69.64
ID_1685	BD67	4	8	77	11.6	61.3	11.9	76.19
ID_1698	BD67	4	8	77	15.2	51	15.7	71.08
ID_1839	BD67	4	8	77	21.1	49.8	13.8	64.88
ID_2108	BD67	4	8	77	16.9	53.3	14.5	67.26
ID_2128	BD67	4	8	77	8.3	56.9	18.8	74.69
ID_2299	BD74***	3	8	76	16.8	60.8	9.2	70.59
ID_2319	BD74	3	8	76	5.2	68.2	12.4	80.72
ID_2359	BD74	3	8	76	5.8	66.8	14.2	78.82
ID_2391	BD74	3	8	76	5.2	66.7	11.8	79.76
ID_2392	BD74	3	8	76	8.5	63.3	13.2	73.81
ID_2399	BD74	3	8	76	10.7	53.2	17.3	69.51
ID_2403	BD74	3	8	76	6.2	59.4	19.9	77.71
ID_2415	BD74	2	8	76	7.1	69.2	10.1	86.90
ID_2426	BD74	2	8	76	7.6	71.6	7.7	85.29
ID_2432	BD74	2	8	76	11.1	67.5	8.2	85.29

Plant ID (BC_2F_2)	Background	Total QTL No.	No. of foreground marker tested	No. of background marker tested	Donor allele (%)	Recipient allele (%)	Hetero (%)	Recovery of recurrent parent genome (%)
ID_2433	BD74	2	8	76	5.4	69	12.5	86.47
ID_2437	BD74	2	8	76	3.8	69.3	13.2	88.69
ID_2444	BD74	2	8	76	11.5	67.8	7.5	82.94
ID_2446	BD74	2	8	76	13	63.9	9.5	80.95
ID_2448	BD74	2	8	76	11.3	61.9	10.6	78.57
ID_2455	BD74	2	8	76	2.7	73.7	10.5	90.00

*BD63=BRRI dhan63; **BD67=BRRI dhan67; ***BD74=BRRI dhan74

Graphical genotyping of BC_2F_2 plant ID 173-107-10-99 (BRRI dhan63 background), ID 547-692-428-1426 (BRRI dhan67 background) and ID 815-993-878-2319 (BRRI dhan74 background) with foreground, recombinant and background markers are presented in Figs. 13-15.

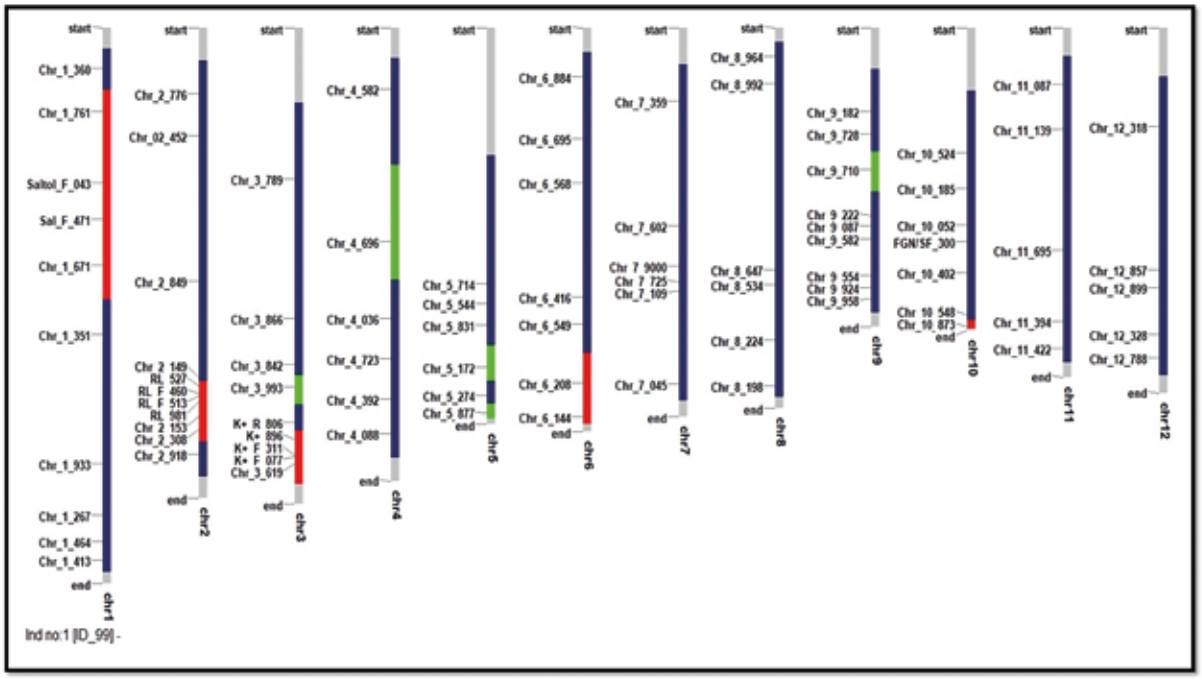

Fig. 13: Graphical genotyping of BC_2F_2 plant ID 173-107-10-99 of BRRI dhan63 background. Blue color represent recipient, red color for donor, green color for heterozygous and ash color for undetermined region

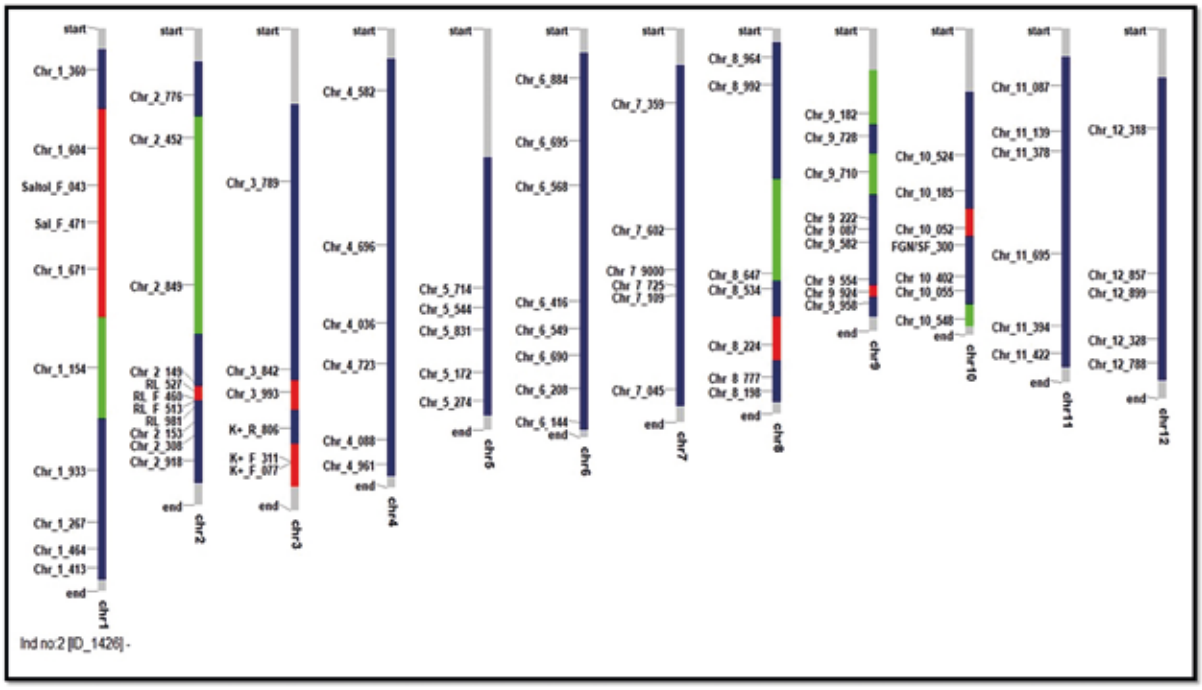

Fig. 14: Graphical genotyping of BC_2F_2 plant ID 547-692-428-1426 of BRRI dan67 background. Blue color represent recipient, red color for donor, green color for heterozygous and ash color for undetermined region

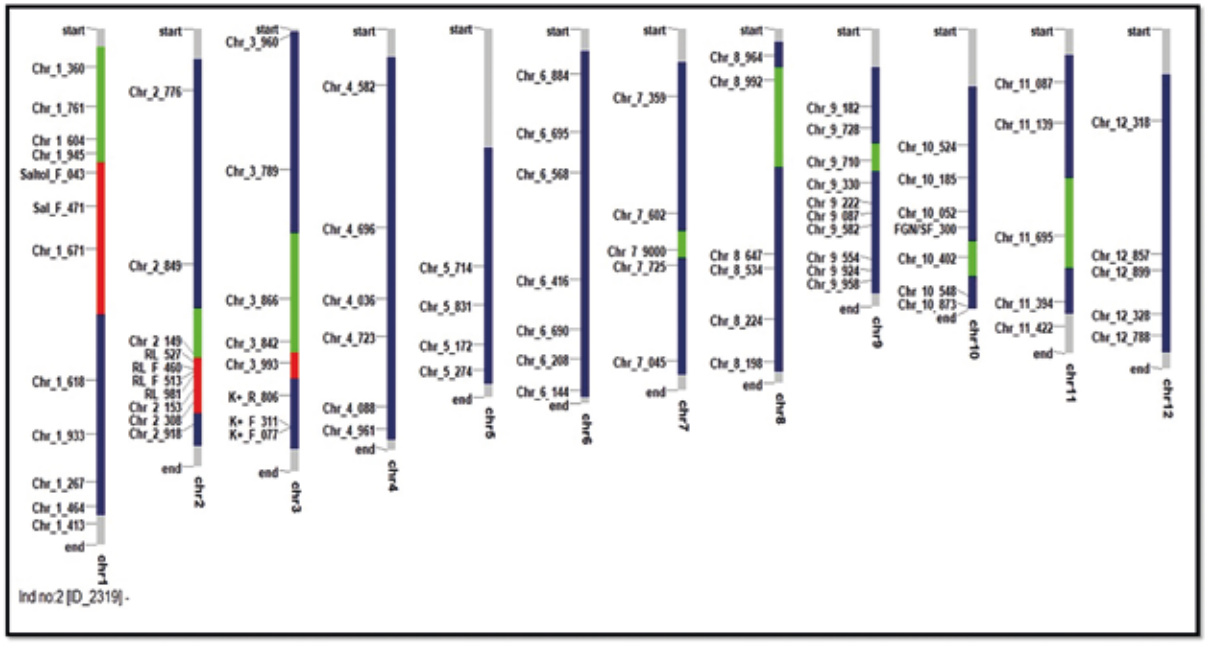

Fig. 15: Graphical genotyping of BC_2F_2 plant ID 815-993-878-2319 of BRRI dhan74 background. Blue color represent recipient, red color for donor, green color for heterozygous and ash color for undetermined region

BRRI Component:

- **Phenotyping and physiological screening of RIL population**

  Screening of selected F_7 RILs for salt tolerance at reproductive stage was analyzed at 10 dSm^{-1} salt stress comparing with control. Some of the results are presented in Figs. 16 and 17. Two best lines (I-14 and I-71) were selected as donor for crossing.

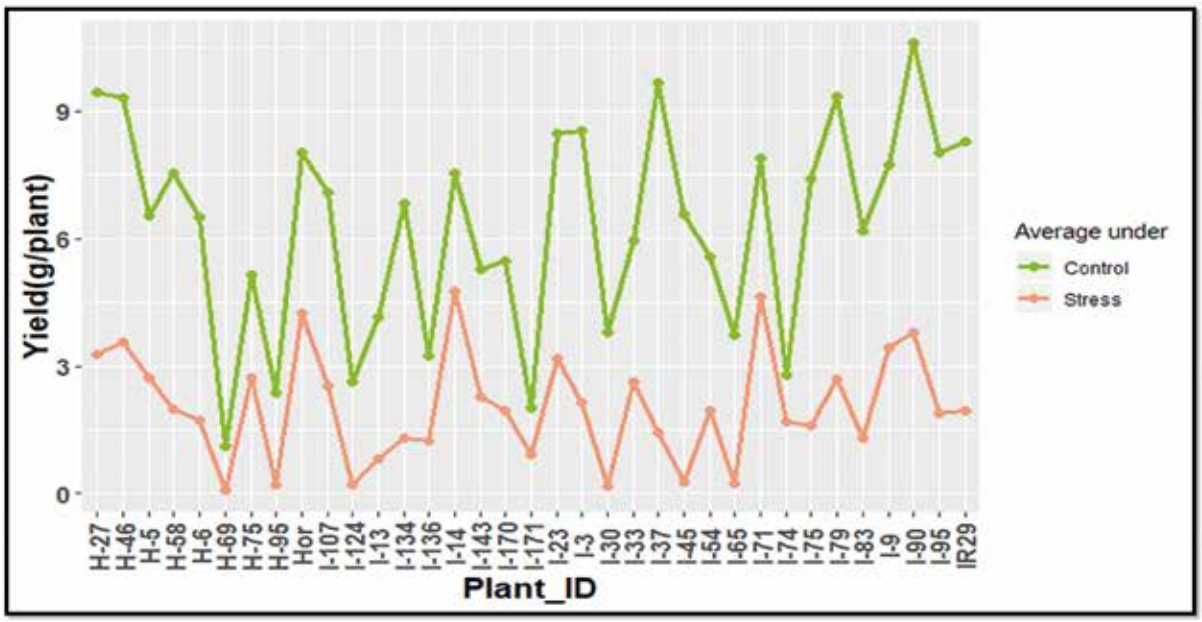

Fig. 16: Yield (g/plant) under control and stressed conditions at reproductive stage

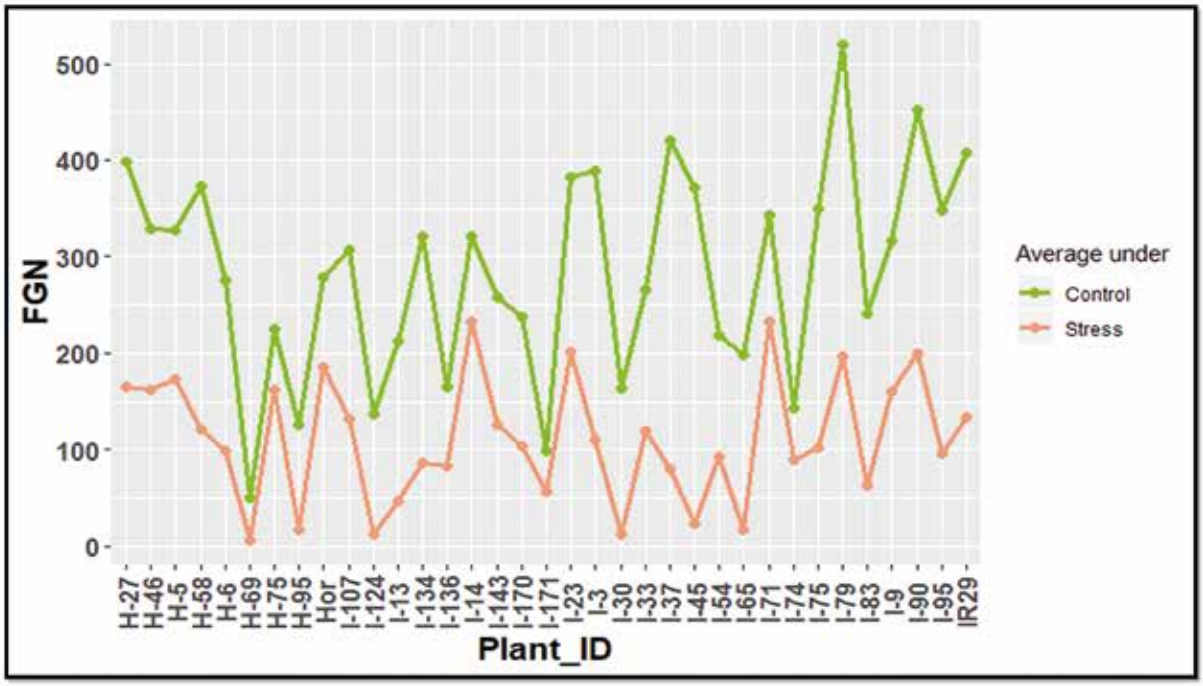

Fig. 17: Filled grain number under control and stressed conditions at reproductive stage

- **Field level trial of selected F_7 RIL population in the coastal region**
  Performance of two best lines is presented in Table 8.

  Table 8: Performance of two selected donors at the saline zone, Kaliganj, Satkhira, 2018

Plant ID	Growth duration (days)	Plant height (cm)	Effective tiller	Moisture content (%)	Yield (t/ha)
I-14	114	80	18	11.8	1.709
I-71	114	142	22	12.2	0.951

- **Crosses with selected donors and single recipient cultivars to produce two different F_1's**
  Two separate crosses with selected donors were made using recipient parents and F_1 seeds were collected from different crosses (BRRI dhan63/I-14, BRRI dhan63/I-71, BRRI dhan67/I-14, BRRI dhan67/I-71 and BRRI dhan74/I-14. Total 316 (BRRI dhan63/I-14), 133 (BRRI dhan63/I-71), 108 (BRRI dhan67/I-14), 123 (BRRI dhan67/I-71) and 119 (BRRI dhan74/I-14) F_1 seeds were produced (Table 9).

  Table 9: List of F_1 seeds from single cross combination, T. Aman 2018-19

Sl#	Recipient parent	Donor parent	Cross combination	No. of F_1 seed
01	BRRI dhan63	I-14	BRRI dhan63/I-14	316
		I-71	BRRI dhan63/I-71	133
02	BRRI dhan67	I-14	BRRI dhan67/I-14	108
		I-71	BRRI dhan67/I-71	123
03	BRRI dhan74	I-14	BRRI dhan74/I-14	119
Total				**799**

- **Crossing between two positive F_1 plants to obtain doubled crossed F_1 seeds with three QTLs from donors in single recipient background**
  Three double crosses were made (BRRI dhan63/I-14//BRRI dhan63/I-71, BRRI dhan67 /I-14//BRRI dhan67/I-71 and BRRI dhan74/I-14//I-71). Positive F_1 (BRRI dhan63/I-14) plants were crossed with positive F_1 (BRRI dhan63/I-71) plants to produce double crossed F_1 (BRRI dhan63/I-14//BRRI dhan63/I-71) seeds with combination of three QTLs in a single recipient parent (BRRI dhan63). Similar double crossed F_1 seeds were also generated for BRRI dhan67 and BRRI dhan74 background. Total 975 (BRRI dhan63/I-14//BRRI dhan63/I-71), 972 (BRRI dhan67/I-14//BRRI dhan67/I-71) and 173 (BRRI dhan74/I-14//I-71) doubled crossed F_1 seeds were produced (Table 10).

Table 10: List of doubled crossed F_1 seeds from three cross combinations, Boro 2018-19

Sl#	Recipient parent	Donor parent	Cross combination	No. of doubled crossed F_1 seed
1	BRRI dhan63	I-14 and I-71	BRRI dhan63/I-14 //BRRI dhan63/I-71	975
2	BRRI dhan67	I-14 and I-71	BRRI dhan67/I-14 //BRRI dhan67/I-71	972
3	BRRI dhan74	I-14 and I-71	BRRI dhan74/I-14//I-71	173
			Total	**2120**

- **Backcrossing of doubled crossed F_1 plants with recipient cultivars (BRRI dhan63, BRRI dhan67 and BRRI dhan74)**

The selected positive doubled crossed F_1 plants were backcrossed with recipient cultivars (BRRI dhan63, BRRI dhan67 and BRRI dhan74) and total 906 (BRRI dhan63/I-14//BRRI dhan63/I-71///BRRI dhan63), 505 (BRRI dhan67/I-14//BRRI dhan67/I-71///BRRI dhan67) and 363 (BRRI dhan74/I-14//I-71///BRRI dhan74) BC_1F_1 seeds were produced (Table 11).

Table 11: List of BC_1F_1 seeds from three cross combinations, T. Aman 2019-20

Recipient parent	Sl#	Plant number	Cross combination	No. of BC_1F_1 seeds
BRRI dhan63	1	16	BRRI dhan63/I-14//BRRI dhan63/I-71///BRRI dhan63	103
	2	27		4
	3	31		23
	4	40		30
	5	111		14
	6	126		8
	8	163		2
	9	173		19
	10	179		21
	11	195		32
	12	202		23
	13	218		45
	14	228		126
	15	232		82
	16	236		7
	17	237		2
	18	245		151
	19	250		4
	20	253		130
	21	259		62

Recipient parent	Sl#	Plant number	Cross combination	No. of BC_1F_1 seeds
	22	276		18
	Sub-total			**906**
BRRI dhan67	1	411	BRRI dhan67/I-14//BRRI dhan67/I-71///BRRI dhan67	35
	2	448		60
	3	498		25
	4	529		75
	5	547		51
	6	591		29
	7	633		8
	8	652		32
	9	680		34
	10	696		126
	11	707		30
	Sub-total			**505**
BRRI dhan74	1	814	BRRI dhan74/I-14// I-71/// BRRI dhan74	165
	2	815		80
	3	845		48
	4	861		23
	5	896		47
	Sub-total			**363**
	Total			**1774**

- **Backcrossing of BC_1F_1 plants for generation of BC_2F_1 seeds with recipient cultivars (BRRI dhan63, BRRI dhan67 and BRRI dhan74)**

  The selected 17 BC_1F_1 plants with positive alleles (four QTLs) were backcrossed with recurrent parents BRRI dhan63, BRRI dhan67 and BRRI dhan74 and total 2341 (BRRI dhan63/I-14//BRRI dhan63/I-71///2*BRRI dhan63), 1648 (BRRI dhan67/I-14//BRRI dhan67/I-71///2*BRRI dhan67) and 528 (BRRI dhan74/I-14//I-71///2*BRRI dhan74) BC_2F_1 seeds were produced (Table 12).

Table 12: List of BC_2F_1 seeds from three cross combinations, Boro 2019-20

Recipient parent	Sl#	Plant ID (doubled crossed F_1)	Plant ID (BC_1F_1)	Cross combination	No. of BC_2F_1 seeds
BRRI dhan63	1	173	99	BRRI dhan63/I-14//BRRI dhan63/ I-71///2*BRRI dhan63	140
	2	173	103		420
	3	173	107		105
	4	173	109		240
	5	228	188		253
	6	232	301		325
	7	245	342		363
	8	245	347		240
	9	245	350		125
	10	245	411		130
	Sub-total				**2341**
BRRI dhan67	1	547	692	BRRI dhan67/I-14//BRRI dhan67/ I-71///2*BRRI dhan67	260
	2	547	694		320
	3	547	700		263
	4	547	715		350
	5	547	716		455
	Sub-total				**1648**
BRRI dhan74	1	815	993	BRRI dhan74/I-14// I-71/// 2*BRRI dhan74	333
	2	896	1062		195
	Sub-total				**528**
Total					**4517**

- **Production of BC_2F_2 seeds**

  The BC_2F_1 seeds from three different crosses were selfed to produce BC_2F_2 population and 1531 (BRRI dhan63/I-14//BRRI dhan63/I-71///2*BRRI dhan63), 984 (BRRI dhan67/I-14// BRRI dhan67/I-71///2*BRRI dhan67) and 1024 (BRRI dhan74/I-14//I-71///2*BRRI dhan74) BC_2F_2 seeds were produced during T. Aman season 2020-21 (Table 13).

Table 13: List of produced BC_2F_2 seeds from three cross combinations, T. Aman 2020-21

Recipient parent	Plant ID (doubled crossed F_1)	Plant ID (BC_1F_1)	Plant ID (Selected BC_2F_1 plants)	Cross combination	No. of BC_2F_2 seeds
BRRI dhan63	173	107	10	BRRI dhan63/I-14//BRRI dhan63/ I-71///2*BRRI dhan63	288
			72		180
	245	342	202		331
			232		420
	245	350	337		84
			349		144
			354		84
	Sub-total				**1531**
BRRI dhan67	547	692	428	BRRI dhan67/I-14//BRRI dhan67/ I-71///2*BRRI dhan67	240
			474		744
	Sub-total				**984**
BRRI dhan74	815	993	816	BRRI dhan74/I-14//I-71/// 2*BRRI dhan74	57
			878		34
			885		66
	896	1062	1021		840
	Sub-total				**1024**
Total					**3539**

- **Selection of BC_2F_2 plants from three different recipient backgrounds to produce BC_2F_3 seeds**

The BC_2F_2 seeds from three different crosses were grown to produce BC_2F_3 generation through selfing during Boro 2020-21. In total 17, 11 and 16 BC_2F_2 plants were selected having all QTLs in homozygous form of three different recipient backgrounds (Table 14). Out of selected 16 BC_2F_2 plants from BRRI dhan74 background, seven plants harbor three QTLs (RL, *Saltol,* FGN+SF) and rest nine plants harbor two QTLs (K^+, FGN+SF) in homozygous condition.

Table 14: List of selected BC_2F_2 plants from three different cross combinations, Boro 2020-21

Sl#	Recipient parent	Donor parent	Cross combination	No. of BC_2F_2 plants
1	BRRI dhan63	I-14 & I-71	BRRI dhan63/I-14//BRRI dhan63/I-71///2*BRRI dhan63	17
2	BRRI dhan67	I-14 & I-71	BRRI dhan67/I-14//BRRI dhan67/I-71///2*BRRI dhan67	11
3	BRRI dhan74	I-14 & I-71	BRRI dhan74/I-14//I-71 ///2*BRRI dhan74	16
Total				**44**

12. Research highlight:

Title of the sub-project: DNA marker-assisted breeding for producing highly stress tolerant elite rice varieties for coastal Bangladesh by introgression of multiple salt tolerance loci (QTLs) into commercial cultivars

Background: Development of salt tolerant high yielding rice varieties is possible through introgression of multiple salt tolerant loci from landraces following marker assisted breeding. SNP markers, which are abundant throughout the chromosome, are good markers for such breeding purposes. But costly detection systems make it inconvenient compared to SSR markers, whereas using the latter markers are inefficient and cumbersome. Fluorescent tagged SNP markers called KASP are cost effective genotyping system, which makes breeding more efficient and less laborious. In this research project this technology has been followed to detect QTL specific donor alleles and recipient alleles for selection of progenies during breeding cycles. Salt tolerant QTLs were identified earlier from a reciprocal crossing population of IR29 and Horkuch. Best recombinant inbred lines (RILs) with target QTLs were chosen as donor for crossing. These QTLs were introgressed into high yielding BRRI dhan63, BRRI dhan67 and BRRI dhan74 separately to enhance their salinity tolerance.

Objectives: Development of DNA marker for detection of multiple salt tolerance QTLs and subsequent transfer into modern rice varieties through marker assisted backcrossing.

Methodology: RILs obtained from reciprocal crosses of Horkuch and IR29 were advanced to F_7 and best lines were identified after screening at seedling and reproductive stages. Two RILs with best agronomic properties having salt tolerant QTLs were used as donors. These salt tolerant QTLs were introgressed into high yielding background of BRRI dhan63, BRRI dhan67 and BRRI dhan74 separately. At first, each recipient/recurrent parent has been crossed separately with the selected donor RILs (I-14 and I-71). The two F_1s are then crossed between themselves and produced doubled crossed F_1s with all target QTLs in single recipient background. Then two times backcrossing were done with recurrent parents. Fluorescent based SNP marker system, KASP had been employed to detect QTLs in F_1 and backcrossed progenies. Moreover, background genome recovery of backcrossed progenies was also detected by the KASP genotyping method.

Key findings:

a) Two RILs (I-14 and I-71) were found best with maximum number of effective QTLs and better agronomic properties and used as donor for crossing.

b) The donor (I-14) was identified having the RL and K^+ QTLs and I-71 with the *Saltol*, FGN+SF QTLs using QTL specific KASP marker.

c) Total eight KASP markers were validated as QTL specific (foreground) markers and 102 as background markers polymorphic between donor and recipient BRRI dhan63, BRRI dhan67 and BRRI dhan74. These were used to select target progenies in each crossed and backcrossed population until the BC_2F_2 generation.

d) Total 17 and 11 plants from BRRI dhan63 and BRRI dhan67, respectively, were identified having all four QTLs (Root length, K^+, *Saltol* and FGN+SF) in BC_2F_2 generation. In case of BRRI dhan74 background, total 16 (seven having three and nine having two QTLs) plants were identified in BC_2F_2 generation.

B. Implementation Status

1. Procurement (Component wise):

DU Component:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Physical (No.)	Financial (Tk.)	Physical (No.)	Financial (Tk.)	
(a) Office equipment	-	-	-	-	-
(b) Lab & field equipment	1	5,50,000.00	1	5,50,000.00	
(c) Other capital items	-	-	-	-	-

BRRI Component: Not Applicable

2. Establishment/renovation facilities:

Not Applicable

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
-	-	-	-	-	-

3. Training/study tour/seminar/workshop/conference organized:

DU Component:

Description	Number of participant			Duration (Days/weeks/months)	Remarks
	Male	Female	Total		
(a) Training	1 PhD student	2 MS students	3	3 year	
(b) Workshop	-	-	-	-	-
(c) Others (if any)	-	-	-	-	-

BRRI Component: Not Applicable

C. Financial and physical progress (Combined & Component wise)

Combined: **Fig in Tk**

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	4606609	4606609	4606609	0	100	
b. Field research/lab expenses and supplies	7867174	7865110	7865110	0	100	
c. Operating expenses	491540	493815	493815	0	100	
d. Vehicle hire and fuel, oil & maintenance	355446	355446	355446	0	100	
e. Training/workshop/ seminar etc.	0	0	0	0	0	
f. Publications and printing	120000	118999	118999	0	100	
g. Miscellaneous	192306	190031	190031	0	100	
h. Capital expenses	550000	550000	550000	0	100	
Total	**1,41,83,075**	**1,41,80,010**	**1,41,80,010**	**0**	**100**	

DU Component:

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	1906501	1906501	1906501	0	100	
b. Field research/lab expenses and supplies	6154129	6152065	6152065	0	100	
c. Operating expenses	182354	184629	184629	0	100	
d. Vehicle hire and fuel, oil & maintenance	200000	200000	200000	0	100	
e. Training/workshop/ seminar etc.	0	0	0	0	0	
f. Publications and printing	0	0	0	0	0	
g. Miscellaneous	109935	107660	107660	0	100	
h. Capital expenses	550000	550000	550000	0	100	
Total	**91,02,919**	**91,00,855**	**91,00,855**	**0**	**100**	

BRRI component:

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
a. Contractual staff salary	2700108	2700108	2700108	0	100	
b. Field research/lab expenses and supplies	1713045	1713045	1713045	0	100	
c. Operating expenses	309186	309186	309186	0	100	
d. Vehicle hire and fuel, oil & maintenance	155446	155446	155446	0	100	
e. Training/workshop/ seminar etc.	0	0	0	0	0	
f. Publications and printing	120000	118999	118999	0	100	
g. Miscellaneous	82371	82371	82371	0	100	
h. Capital expenses	0	0	0	0	0	
Total	**50,80,156**	**50,79,155**	**50,79,155**	**0**	**100**	

D. Achievement of Sub-project by objectives (Tangible form): Technology generated/developed

DU Component:

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
Phenotypic and physiological screening of selected reciprocal fixed RIL populations of Horkuch and IR29 under salinity stress at seedling stage.	1. Advancement of the best progenies having seedling and reproductive QTLs until F_7 generation. 2. Phenotypic and physiological screening of reciprocal F_7 RIL population at seedling stage.	Two RILs (I-14 and I-71) were found best with maximum number of effective QTLs with better agronomic performance.	I-14 and I-71 were found salt tolerant and used as donors in marker assisted backcrossing.
Establishment of efficient SNP-based markers system for detection of donor allele specific SNP for validation of the multiple QTLs.	1. Identification of SNP markers linked to salt tolerant QTL of Horkuch at seedling and reproductive stages. 2. Tagging and validating allele-specific SNPs linked to multiple QTLs with fluorescent molecules to establish KASP (Kompetitive Allele Specific PCR). 3. Identification and validation of RIL parents containing the target QTL's for salt tolerance and high yields under salt stress.	1. RL, K^+, etc. are seedling stage QTLs and ET, FGN, PE, SF, HI etc. are reproductive stage QTLs. 2. Eight KASP markers are validated as QTL specific foreground markers and 102 as background markers. 3. Donor RIL I-14 was identified as carrying the RL and K^+ QTLs and I-71 with the *Saltol*, FGN+SF QTLs.	1. Major QTLs are introgressed into the BRRI dhan63, BRRI dhan67 and BRRI dhan74 background for improving salt tolerance. 2. Polymorphic KASP markers between donor and recipient were used to track QTL specific regions and background in test progenies. 3. I-14 and I-71 are used as donors in marker assisted backcrossing.
Development of foreground, recombinant and background SNP/SSR markers which will be used for marker assisted back crossing.	1. Marker-assisted selection at each stage of crossing and backcrossing, until BC_2F_2 for each of BRRI dhan63, BRRI dhan67 and BRRI dhan74. 2. Selection of BC_2F_2 lines with the target QTLs and genetic background of recipient parents, BRRI dhan 63, BRRI dhan67 and BRRI dhan74.	1. Total 38 plants of double cross F_1, 17 of BC_1F_1, 138 of BC_2F_1 and 44 of BC_2F_2 are selected. 2. Total 17 and 11 plants of BRRI dhan63 and BRRI dhan67, respectively are found with all QTLs. Seven of BRRI dhan74 had three QTLs and nine had two QTLs in BC_2F_2 generation.	1. Polymorphic markers successfully identified best plants in double cross F_1, BC_1F_1, BC_2F_1 and BC_2F_2 progenies. 2. Forty four best plants with desired QTLs and maximum background genome recovery is found.

BRRI component:

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
1. Phenotypic and physiological screening of selected reciprocal F_7 RIL populations of Horkuch and IR29 under salinity stress at reproductive stage.	1.1 Advancement of the best progenies with seedling and reproductive QTLs up to F_7 generation. 1.2. Phenotypic and physiological screening of reciprocal F_7 RIL population at reproductive stage.	Selection of 16 best F_7 RIL population lines.	• Advanced to F_7 generation and seeds of the best progenies are collected and stored. • At reproductive stage, phenotyping and physiological screening was conducted using reciprocal F_7 RIL populations at 10 dSm^{-1} at Plant Physiology Division, BRRI and two genotypes are identified as donor QTLs.
2. Field level trial of best 20 selected F_7 RIL reciprocal population lines in the coastal region.	2.1.Selected 16 F_7 RILs were evaluated at Kaliganj, Satkhira (coastal region of Bangladesh). Salinity level varied from 7.0-9.0 dSm^{-1} during evaluation in coastal region. (Latitude: 22.453097 Longitude: 89.034659)	Selection of two best F_7 RIL population lines.	• Two lines (I-14 and I-71) were found tolerant at field level trial and used as donor QTL parents.
3. Breeding highly salt tolerant genotypes (8-10 dSm^{-1} for both stages of growth) through introgression of multiple salinity tolerance QTLs at seedling and reproductive stages into modern BRRI Boro rice varieties to develop lines/ varieties suitable for saline coast.	3.1. Two separate crosses are made with selected donor and single recipient cultivar to produce two different F_1's.	3.1. Two separate crosses are made with selected donors using recipient parents and F_1 seeds are collected from different crosses (BRRI dhan63/I-14, BRRI dhan63/I-71, BRRI dhan67/I-14, BRRI dhan67/I-71 and BRRI dhan74/I-14).	• Introgression of multiple salt tolerance QTLs into three elite backgrounds of BRRI dhan63, BRRI dhan67 and BRRI dhan74 for higher level of salinity tolerance.

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
	3.2. Crossing between F_1s to obtain three QTLs from donor in a single recipient background.	3.2. Three double crosses are made (BRRI dhan63/I-14 //BRRI dhan63/ I-71, BRRI dhan67/ I-14 //BRRI dhan67/I-71 and BRRI dhan74/ I-14//I-71).	• A total of 17 and 11 plants from BRRI dhan63 and BRRI dhan67, respectively are identified having all four QTLs (Root length, K^+, *Saltol* and FGN+SF) in BC_2F_2. In case of BRRI dhan74 background, 16 (seven having three and nine having two QTLs) plants are identified in BC_2F_2.
	3.3. Crossing of positive plants and recipient cultivars (BRRI dhan63, BRRI dhan67 and BRRI dhan74) through back crossing for generation of BC_1F_1 line.	3.3. BC_1F_1 seeds are produced through back crossing using recipient parents (BRRI dhan63, BRRI dhan67 and BRRI dhan74).	
	3.4. Crossing of positive plants and recipient cultivars (BRRI dhan63, BRRI dhan67 and BRRI dhan74) through back crossing for generation of BC_2F_1 line.	3.4. Positive BC_1F_1 plants are back crossed using recipient parents (BRRI dhan63, BRRI dhan67 and BRRI dhan74) to generate BC_2F_1 seeds.	
	3.5. Generation advancement to BC_2F_2 by selfing.	3.5. Selected BC_2F_1 plants with positive alleles from three cross combinations and selfed to generate BC_2F_2 seeds.	
	3.6. Generation advancement from BC_2F_2 to BC_2F_3.	3.6. Selected BC_2F_2 progenies having three QTLs and selfed to produce BC_2F_3 generation.	
	3.7. Phenotypic characterization of BC_2F_3 plants under salt stress at seedling stage.	3.7. BC_3F_3 progenies having three QTLs will be screened under salt stress at seedling stage.	

E: Information/knowledge generated/policy generated

DU Component:

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
Establishment of fluorescence marker technology, KASP for rapid detection of progenies with desired QTLs	Total 378 SNP markers are designed and tested in donor and recipient lines.	Total eight, 10 and 102 markers are found polymorphic between donor and recipient as foreground, recombinant and background markers, respectively.	These efficient markers are successfully applied in backcrossing program.
Introgression of multiple salt tolerance QTLs for higher level of tolerance	1. In total 837 double crossed plants are tested with QTL specific markers. 2. In total 1068 BC_1F_1 and 1076 BC_2F_1 plants are examined with QTL specific and background markers. 3. In total 1283, 963 and 1021 plants in background of BRRI dhan63, BRRI dhan67 and BRRI dhan74 are examined with QTL specific and background markers.	1. Thirty eight plants of double cross F_1 with all QTLs in heterozygous condition are identified. 2. Seventeen of BC_1F_1, 138 of BC_2F_1 plants are selected. 3. Forty four BC_2F_2 plants are selected with different combination of QTLs with maximum recurrent parents background.	Best selected lines found after second backcrossing would be screened for salt tolerance and subsequently tested in field condition.

BRRI Component:

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
Introgression of multiple salt tolerance QTLs into the background of BRRI dhan63, BRRI dhan67 and BRRI dhan74 for higher level of salt tolerance (8-10 dSm^{-1} for both stages of growth)	1. Two separate crosses are made with selected donor and single recipient cultivar to produce two different F_1's.	1. Three hundred sixteen (BRRI dhan63/I-14), 133 (BRRI dhan63/I-71), 108 (BRRI dhan67/I-14), 123 (BRRI dhan67/I-71) and 119 (BRRI dhan74/ I-14) F_1 seeds are produced.	Forty four BC_2F_2 (17 of BRRI dhan63, 11 of BRRI dhan67 and 16 of BRRI dhan74) plants having all target QTls are selected after second backcrossing. The selected BC_2F_2 plants would be screened for salt tolerance at seedling

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
	2. Crossing between F_1s to assemble three QTLs from donor in single recipient background.	2. Nine hundred seventy five (BRRI dhan63/I-14//BRRI dhan63/I-71), 972 (BRRI dhan67/ I-14//BRRI dhan67/ I-71) and 173 (BRRI dhan74/I-14// I-71) doubled crossed F_1 seeds are produced.	and reproductive stages, and subsequently tested under field condition.
	3. Crossing of positive plants and recipient cultivars through back crossing for generation of BC_1F_1 line.	3. Nine hundred six (BRRI dhan63/I-14// BRRI dhan63/I-71/// BRRI dhan63), 505 (BRRI dhan67/I-14// BRRI dhan67/I-71/// BRRI dhan67) and 363 (BRRI dhan74/ I-14//I-71///BRRI dhan74) BC_1F_1 seeds are produced.	
	4. Crossing of positive plants and recipient cultivars through back crossing for generation of BC_2F_1 line.	4. In total 2341 (BRRI dhan63/I-14//BRRI dhan63/I-71///2* BRRI dhan63), 1648 (BRRI dhan67 /I-14// BRRI dhan67/I-71 ///2* BRRI dhan67) and 528 (BRRI dhan74/I-14//I-71/// 2* BRRI dhan74) BC_2F_1 seeds are produced.	
	5. Generation advancement to BC_2F_2 by selfing.	5. In total 1531 (BRRI dhan63/I-14//BRRI dhan63/I-71 ///2* BRRI dhan63), 984 (BRRI dhan67/I-14// BRRI dhan67/I-71/// 2*BRRI dhan67) and 1024 (BRRI dhan74/ I-14//I-71///2*BRRI dhan74) BC_2F_2 seeds are produced.	

General/specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output	Outcome (short term effect of the research)
	6. Generation advancement from BC_2F_2 to BC_2F_3.	6. In total 17 and 11 plants in recipient background of BRRI dhan63 and BRRI dhan67, respectively are identified having all four QTLs (Root length, K^+, *Saltol* and FGN+SF) in BC_2F_2 generation. In case of BRRI dhan74 background, 16 (seven having three and nine having two QTLs) plants are identified in BC_2F_2 generation.	

F. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/ leaflet/flyer etc.	-	Leaflet: 2 (Bengali and English)	The use of fluorescence-labeled SNP marker in rice breeding: Application of this method for introgression of salt tolerant QTLs ধানের ব্রিডিংয়ে ফ্লুরোসেন্স-লেবেলযুক্ত SNP মার্কার পদ্ধতির ব্যবহার: লবণাক্ততা সহিষ্ণু QTL অনুপ্রবেশে এ পদ্ধতির সফল প্রয়োগ
Journal publication	-	1	Natural variation in growth and physiology under salt stress in rice: QTL mapping in a Horkuch×IR29 mapping population at seedling and reproductive stages. T Haque, SM Elias. S Razzaque, S Biswas, SF Khan, GMNA Jewel, MS Rahman, TE Juenger, ZI Seraj (2020). BioRixv. https://doi.org/10.1101/2020.03.01.971895
News Paper/Popul ar Article	-	-	-
Other publications, if any	-	1	Title: Incorporation of salt tolerant QTLs in the background of high quality commercial rice variety through SNP-based KASP markers 1st Runner-up Yeamin Farabi Chowdhury, MS Student, BMBDU (International poster presentation competition IPPC2020, Jointly organized by National Young Science Academies of Bangladesh, India, Sri Lanka and Thailand)

G. Description of generated Technology/knowledge/policy

i. Technology Fact Sheet

1) **Title of the Technology:** Successful introgression of salt tolerant QTLs (Quantitative Trait Loci) into rice through marker-assisted breeding using KASP genotyping with allele-specific primer and fluorescence-tagged secondary primer.

2) **Introduction:**

The use of DNA-based molecular marker has remarkably improved the selection process of target traits of different crops. Molecular markers have led to many improvements in the accuracy and functionality in selecting traits of interest in breeding. With the help of molecular markers genes or alleles that control various complex abiotic, biotic and yield-related features are very easy to detect. It is also possible to locate the position of the target QTL in the genome, and precisely identify those in breeding populations. Of the various DNA-based molecular markers, SSR marker has been the most widely used due to the ease of its use in any standard molecular laboratory. However, the discovery of SNP (single nucleotide polymorphism) marker in later years and the development of its high throughput detection process have resulted in its increased use due to its abundance and polymorphism. However, due to the unavailability of easy and cheaper methods for its use and testing in a standard molecular laboratory, SNP markers have been rarely used in breeding in our country. Recently, there has been a significant improvement in the development of cheaper methods to detect SNP markers through real-time PCR (RT-PCR) machines, regarded as KASP. Therefore, the undertaken PBRG sub-project of NATP-2 aimed to establish the widespread application of the KASP method for use of SNP markers in rice breeding in Bangladesh. The current sub-project focused to use KASP technology to transfer three salt tolerant QTLs from local salt-tolerant variety, Horkuch into the background of BRRI dhan63, BRRI dhan67 and BRRI dhan74 to elevate tolerance level.

3) **Description of the Technology/Methodology:**

In this process, the fluorescence labeled primer is used to detect the desired allele after an RT-PCR reaction which can be visualized directly on the computer. Therefore, the detection process/result analysis can be performed without using any gel electrophoresis. The KASP genotyping assay uses Kompetitive Allele-Specific PCR to score two alleles of each SNP. In this assay, two allele-specific forward primers and one common reverse primer are used to perform PCR reaction. To detect the two allele-specific forward primers, one of them is labeled to complementary sequence of FAM (Fluorescein amidites) dye and the other is labeled to the complementary sequence of HEX (Hexachloro-fluorescein) dye. The PCR master mix uses Universal FRET (fluorescence resonant energy transfer) cassette and ROX (6-carboxy-X-rhodamine) reference dye. Kompetitive Allele-Specific PCR amplifies and separates specific SNP allele by the competitive binding of the two allele-specific forward primers during the PCR amplification. In this process, the genotype with homozygous for the SNP, the fluorescence signal of any of the two allele-specific forward primers would appear. In contrast, for heterozygous genotype, a mixed-signal of the two dyes would appear. The following figures (Fig. 18, 19, 20) show the position of the homozygous and heterozygous result in Kompetitive Allele-Specific PCR.

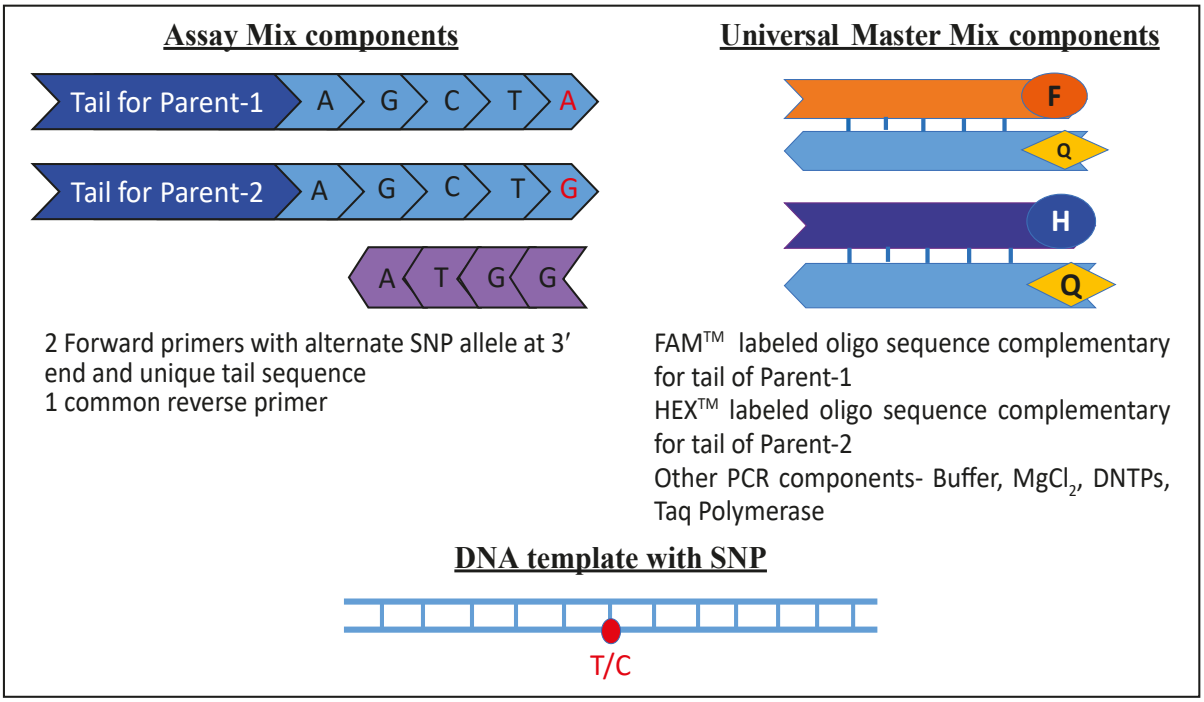

Fig. 18: The components of KASP assay

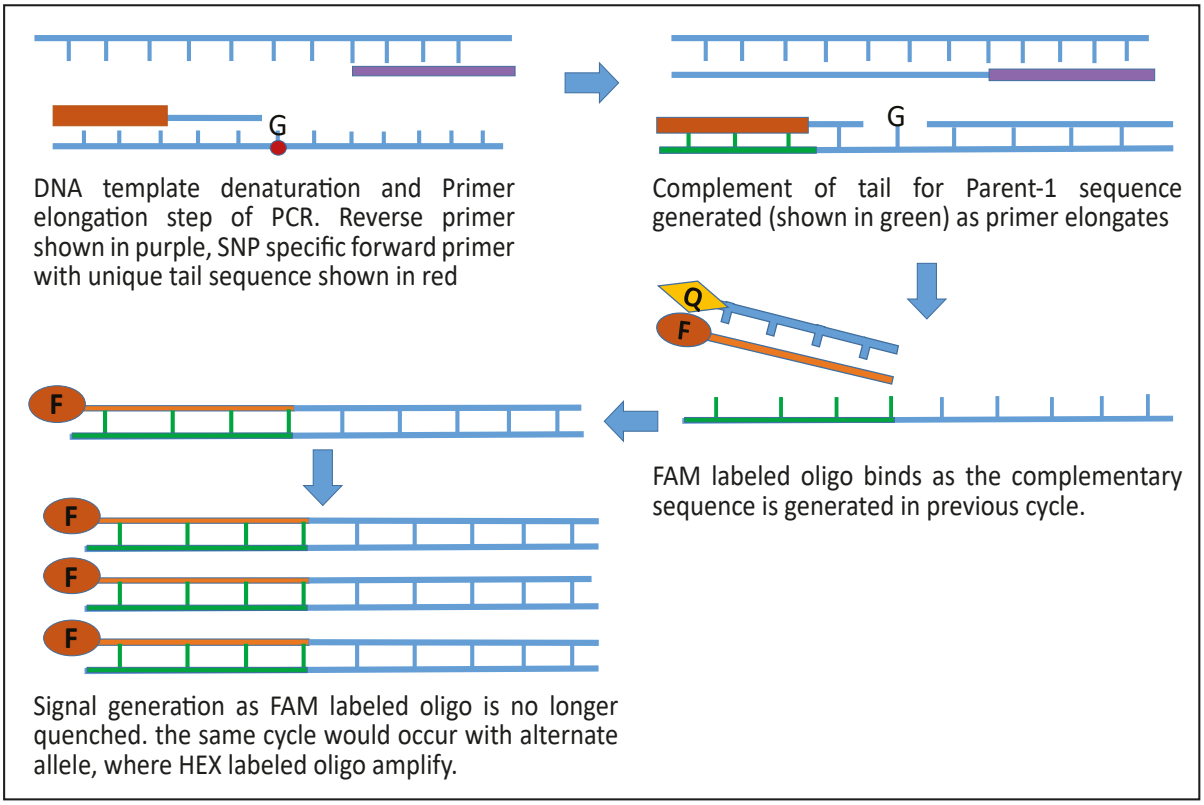

Fig. 19: The principle and steps of KASP assay to detect the genotype of specific SNP using allele-specific primers

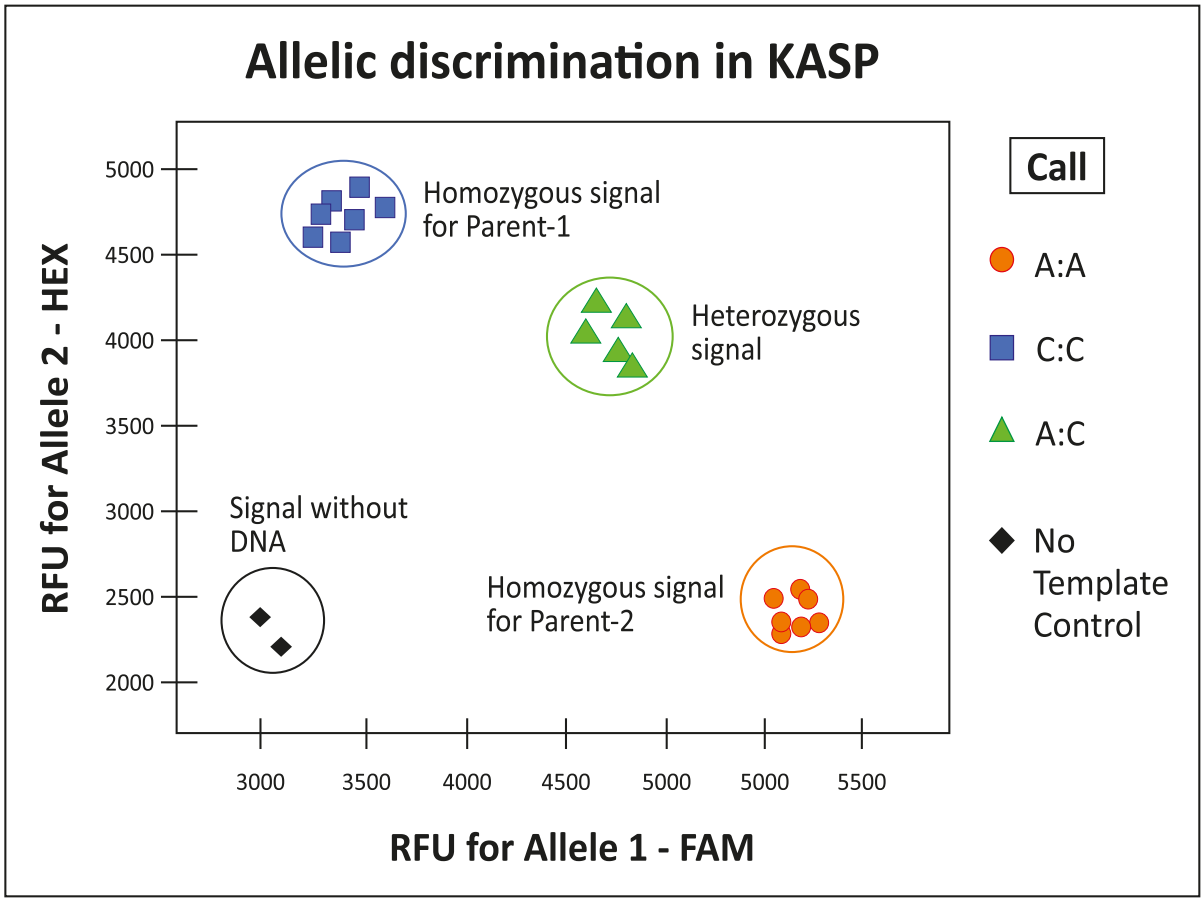

Fig. 20: Different signals generated in KASP assay for amplification of specific SNP using allele-specific primers

For using KASP genotyping in rice breeding, three QTLs (Root Length QTL Chr-2, 27164460 bp; Shoot K^+ QTL Chr-3, 31752311 and 31885077 bp; *Saltol* QTL Chr-1, 11462043 and 14167471 bp) found from the QTL map of Horkuch were selected as the target loci to be introgressed, and BRRI dhan63, BRRI dhan67 and BRRI dhan74 were selected as the recipient parents. The three QTLs were transferred from two RILs (RILS: Recombinant Inbreed Lines) of Horkuch, I-14 (Root length QTL and shoot K^+ QTL) and I-71 (*Saltol* QTL). I-71 also had the yield QTLs, namely, FGN+SF. However the latter was homozygous for all the three recipients. Firstly, each recipient parent was crossed separately with two RILs. By crossing the two F_1s that developed from the cross between themselves, the three QTLs were brought into each background parent's genome. In the Fig. 21, the graphical genotyping image of a cross progeny is shown having the QTLs and background alleles in different colors.

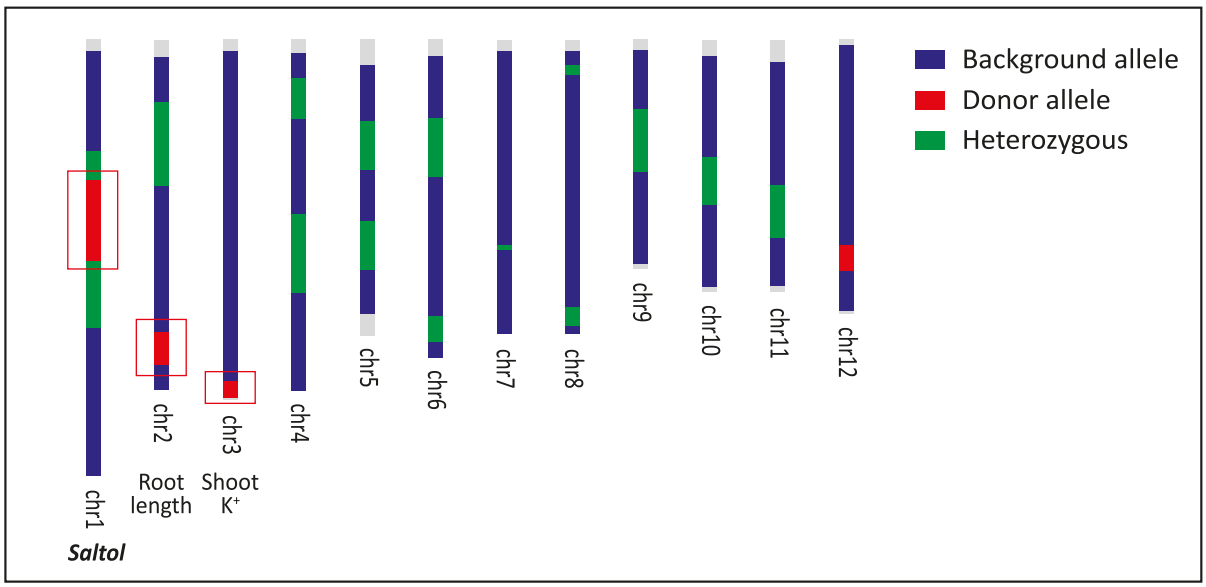

Fig. 21: Graphical genotyping of a progeny having three QTLs from RILs in genomic background of recipient parent presented in different colors

4) **Suitable Area/Location:**
Breeders/researchers can successfully transfer target QTLs/gene easily using fluorescence-labeled SNP marker (KASP genotyping) in a molecular laboratory with quantitative RT-PCR.

5) **Benefit of the Technology:**
   i. The best feature of this technique is that it is a gel-free system, where alleles can be visualized directly in computer software; therefore, it doesn't require the hassle of agarose gel/ PAGE run.
   ii. KASP is a high throughput technology in which multiple populations can be genotyped simultaneously. As a result, many genotyping reactions can be performed rapidly.
   iii. The initial expense of RT-PCR machine and marker designing is relatively high, but the use of the established method in various breeding programs can lessen the cost, time and labor.
   iv. SNP markers are both abundant and highly polymorphic in rice genome due to which the use of these markers is more suitable for mapping of gene/QTL association in the genome. Since this technique significantly lowers the cost of SNP marker development, it has become necessary to use KASP based SNP markers in all aspects of breeding and mapping in contrast to SSR marker which has limitation in its applications.
   v. In the KASP assay, marker designing is relatively technical and may require bioinformatics training. However the markers could be designed with the assistance of service providers or bought from them.
   vi. Researchers using SSR genotyping in breeding programs can easily use KASP for their work.

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ii. **Effectiveness in Policy Support (if applicable):** Not Applicable

H. **Technology/Knowledge generation/Policy Support (as applied)**

i. **Immediate impact on generated technology (commodity & non-commodity)**
Multiple salt tolerant QTLs had been introgressed in high yielding rice background (BRRI dhan63, BRRI dhan67 and BRRI dhan74) within short duration. This method appeared to be less laborious and easy going even after handling a large number of crossing populations. Several advanced lines are also being isolated with a hope of future potential candidate rice variety for the coast and ultimately elevate yield and income of farming community of the areas.

ii. **Generation of new knowledge that help in developing more technology in future**
The KASP method for the first time in Bangladesh is employed to assemble three salt tolerant and one high yield QTLs in commercial varieties towards development of high yielding salt tolerant lines appeared to be quick and less laborious. Further research in this field will bring more efficient, faster and cheaper marker detection for crop improvement in contest of Bangladesh.

iii. **Technology transferred that help increased agricultural productivity and farmers' income**
Salinity is becoming severe day by day, especially in dry season, cultivation of modern varieties are highly impaired in coastal areas. So it is urgent to improve salt tolerance level of high yielding varieties to boost up yield to feed our growing population. Salt tolerance and high yield are multigenic traits. Introgression of salt tolerant QTLs from landraces into commercial cultivars is a sustainable solution. And the marker assisted system in

breeding program has made it possible to develop high level of tolerance in a very short time. With the sub-project, we applied the new method in marker assisted backcrossing for the first time in Bangladesh. We successfully incorporated three salt tolerant QTLs into high yielding backgrounds so that salt tolerance and high yield are available in a single genotype. Advancement of best progenies and subsequent screening will hopefully identify a more salt tolerant version of BRRI dhan63, BRRI dhan67 and BRRI dhan74. Ultimately it is expected to increase agricultural productivity and farmers' income in the salt affected areas of Bangladesh.

iv. **Policy Support:** Not Applicable

I. **Information regarding Desk and Field Monitoring**

i. **Desk Monitoring (description & output of consultation meeting, monitoring workshops/seminars etc.):**

Inception meeting held at BARC on the 18th of September, 2018

Fig. 22: Inception meeting held on 18th September, 2018 at BARC, Farmgate, Dhaka

A six member PMU monitoring team led by Mr. Md. Motiur Rahman, Project Director (PD), (Additional Secretary) NATP-2 visited BRRI to monitor the activities of PBRG sub-projects of Project ID-010 BRRI Component on 2nd March, 2021.

Fig. 23: Project Director, NATP-2 and other members of Monitoring and Evaluation team visited BRRI to monitor PBRG sub-project (ID-010) activities

ii. **Field Monitoring (date & no. of visit, name and addresses of team visit and output): BRRI Component:**

Date & no. of visit	Addresses of Team visit Date(s) of visit	Output
20/3/2019	**PIU-BARC, NATP-2** Dr. Md. Sirajul islam, Consultant, PIU, BARC, NATP-2 	
31/03/2019	**Technical Division/ Unit, BARC** 1. Dr. Md. Aziz Zilani Chowdhury, MD (Crops) 2. Dr. Md. Harunur Rashid, Director, Training 3. Dr. Zakiah Rahman Moni, SSO (TTMU) 4. Dr. Md. Nowsher Ali Sardar, M & E Specialist, PIU-BARC, NATP-2	

Date & no. of visit	Addresses of Team visit Date(s) of visit	Output
30/04/2019	**World Bank Team** 	

iii. **Weather data, flood/salinity/drought level (if applicable) and natural calamities:**
Not Applicable

J. **Sub-project auditing (covers all types of audit performed)**
DU Component:

Types of audit	Major observation/ issues/objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial	None	0	Good	Financial year (2018-2019)
Financial	None	0	Good	Financial year (2019-2020)

BRRI Component:

Types of audit	Major observation/ issues/objections raised; if any	Amount of Audit (Tk.)	Status at the sub-project end	Remarks
Financial	None	0	Good	Financial year (2018-2019)
Financial	None	0	Good	Financial year (2019-2020)

K. Lessons Learned:
The KASP marker detection system appeared to be efficient to trace QTLs in the subsequent generation followed by hybridization. The method seemed promising in introgressing several QTLs of interest in target background genotypes.

L. Challenges (if any):
Buying molecular biology consumables and their subsequent clearance appeared to be cumbersome. Also COVID-19 pandemic driven impacts affected implementation of the sub-project.

M. Suggestions for future planning (if any):

For using KASP assay, marker designing is relatively technical and may require bioinformatics training. However, the markers could be designed with the assistance of service providers or bought from them.

N. References:

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15.07.2021

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Counter signature of the Head of the organization/authorized representative
Date: .....

Seal

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SUB-PROJECT ACTIVITIES

Ministry of Agriculture

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